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Part 1: Reversible and irreversible inhibitors of aldose reductase as probes of the inhibitor binding site, Part 2: Synthesis of permanently charged and permanently uncharged dopamine agonists

Smar, Michael William, Ph.D.
The Ohio State University, 1988

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Part 1: Reversible and Irreversible Inhibitors of Aldose Reductase as Probes of the Inhibitor Binding Site

Part 2: Synthesis of Permanently Charged and Permanently Uncharged Dopamine Agonists

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Michael W. Smar, B.S., R.Ph.

** ** **

The Ohio State University
1988

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Copyright by
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1988
DEDICATION

To Anne and my parents
ACKNOWLEDGEMENTS

I would like to express my sincere thanks to the following individuals for their help throughout the course of my graduate studies:

Dr. Duane D. Miller, for his guidance, support and friendship throughout my graduate career, and for his unending enthusiasm and optimism for the work described herein.

The members of my reading committee, Dr. Robert Brueggemeier, Dr. Norman Uretskey and Dr. Donald Witlak, for their advice in the preparation of this manuscript.

Dr. Robert Curley, for his advice and friendship.

Dr. Peter Kador, Dr. Toshihiro Nakayama, and Dr. Sanai Sato for the biochemical evaluation of the reversible and irreversible aldose reductase inhibitors.

Tahira Forooqui, David Willins and Dr. Norman Uretskey, for their work on the biological studies of the dopamine agonists and for their help in the interpretation of the results.

Undergraduate researcher Ted Crum for his assistance with various aspects of this work.

Jack Fowble, for his help in obtaining and interpreting various spectra, for his assistance with various computer problems, and for his friendship.

My good friends Jeff Ares, Marc Harrold, Kim Markovich, Pui-Ki Li and John Miller, for their often unsolicited but much appreciated advice and for their never ending support.

Paul Campbell, for assistance in using the college computer system, especially for the preparation of this document.

Carol Settles, for her friendship and greatly appreciated assistance throughout my stay at The Ohio State University.

The American Foundation for Pharmaceutical Education, for their generous financial support.

Finally, to Dr. Anne E. Wilson.
VITA


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Publications


Fields of Study

Major Field: Organic Medicinal Chemistry
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Part 1

REVERSIBLE AND IRREVERSIBLE INHIBITORS OF
ALDOSE REDUCTASE AS PROBES OF THE INHIBITOR
BINDING SITE
CHAPTER I
INTRODUCTION

1.1 BACKGROUND

Evidence indicates that as early as 1500 BC the syndrome known today as diabetes mellitus (DM) was understood to be an illness [1]. Proof of the relationship between the pancreas and the development of DM was given in 1899 by Von Merring and Minkowski when they showed that dogs developed a syndrome similar to DM upon removal of their pancreases. In 1921 Banting and Best succeeded in producing the first useful preparation of the antidiabetic hormone insulin from the pancreas [1,2]. The first clinical use of insulin came in 1922 when Banting and Best, at the Toronto General Hospital, treated a 14 year old diabetic boy who was suffering from acute hyperglycemia [3]. Without insulin treatment death was likely to occur within a few weeks [3].

Although traditionally thought of as a singular disease state, DM is actually a group of heterogeneous disorders characterized by one commonality, hyperglycemia [1,4]. In 1984 there were an estimated 5.8 million Americans diagnosed as diabetic, with an additional 4-5 million undiagnosed [4,5]. Diabetes mellitus is currently the seventh leading underlying cause of death in the U.S. [6]. In 1982 DM was itself responsible for 34,583 deaths, or 1.8 percent of all deaths in the
U.S., while in 1978, the most recent year for available data on multiple cause deaths, DM was listed as a contributory cause of 96,847 deaths. These deaths account for an additional 5 percent of all deaths [6]. Encouragingly, there has been a 19% decrease in the overall death rate from DM since 1970, with the current rate standing at 14.9 per 100,000 [6]. There are approximately 600,000 new cases of diabetes diagnosed each year [5].

1.2 TYPES AND TREATMENT OF DIABETES MELLITUS

Four types of DM have been defined by the National Diabetes Data Group. The different types and their major characteristics are listed in Table 1. Type I, or insulin dependent diabetes mellitus (IDDM) is characterized by low levels or the absence of circulating insulin. Type I diabetes accounts for approximately 5-10% of all cases of DM and usually becomes clinically evident in youth. The risk to children of developing IDDM is much greater than the risk of developing such well known childhood diseases as cystic fibrosis, muscular dystrophy, rheumatoid arthritis, leukemia and Hodgkin's disease [7]. Insulin dependent diabetics have lost the ability to synthesize and secrete insulin and, as the name implies, are absolutely dependent on an exogenous source of insulin to maintain their health for the remainder of their lifetime. Until recently insulin dependent diabetics were treated with insulin preparations that contained bovine or porcine insulin [3]. However, recombinant DNA technology has simplified the production of human insulin and, now preparations containing human
insulin are available [8]. Insulin is generally administered by subcutaneous injection by the patient at various doses and times of the day [3]. To eliminate large fluctuations in blood glucose levels caused by this type of treatment, sophisticated computerized pumps have been developed which administer a constant infusion of insulin to the patient. These devices have been developed for those patients that require strict glycemic control [9].
Table 1

Types and characteristics of diabetes

<table>
<thead>
<tr>
<th>Class Name</th>
<th>Former Terminology</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin dependent diabetes mellitus (IDDM, Type I)</td>
<td>Juvenile diabetes</td>
<td>Low or absent levels of circulating endogenous insulin and dependent on injected insulin to prevent ketosis and sustain life. Onset predominantly in youth but can occur at any age. Etiology probably only partially genetic, as only 38% of monozygotic twins are concordant.</td>
</tr>
<tr>
<td>Noninsulin-dependent diabetes mellitus (NIDDM, Type II)</td>
<td>Adult-onset diabetes Maturity-onset diabetes (MODY) Ketosis-resistant diabetes Stable diabetes</td>
<td>Insulin levels may be normal, elevated or depressed. Not insulin-dependent or ketosis-prone under normal circumstances, but may use insulin for treatment of hyperglycemia or during stress conditions. Onset predominantly after age 40, but may occur at any age. Approximately 60% of patients are obese. Etiology probably strongly genetic as almost 100% of monozygotic twins are concordant.</td>
</tr>
<tr>
<td>Gestational diabetes (GDM)</td>
<td>Gestational diabetes</td>
<td>Glucose intolerance that has its onset during pregnancy; virtually all patients return to normal tolerance after parturition. Conveys increased risk for progression to diabetes. Associated with increased risk of congenital abnormalities.</td>
</tr>
<tr>
<td>Other types of diabetes, including those secondary to or associated with Pancreatic disease Hormonal disorders Drug or chemical exposure</td>
<td>Secondary Diabetes</td>
<td>In addition to the presence of the primary condition, hyperglycemia at a level diagnostic of diabetes is present. Causes of hyperglycemia are known for some cases, e.g., pancreatic disease; in other cases an etiological relationship is suspected.</td>
</tr>
</tbody>
</table>
Type II or non-insulin dependent diabetes mellitus (NIDDM) is by far the most common type of DM, accounting for 90-95% of all cases of DM [4]. As indicated in Table 1 on page 5, NIDDM is usually diagnosed later in life, generally after the age of 40. Unlike insulin dependent diabetics, non-insulin dependent diabetics retain the ability to produce and secrete insulin [3]. It is believed that the insulin producing and secreting cells, the β cells, of the pancreas do not respond normally to altered blood glucose levels. Hence, insufficient quantities of insulin are released in response to altered blood glucose levels [3]. For some type II diabetics alteration of diet, weight loss or increased exercise is sufficient treatment for their condition. However, some patients require the administration of oral hypoglycemic agents to correct their hyperglycemia. These agents, shown in Figure 1, are collectively known as the sulfonylureas. The first generation sulfonylureas were introduced in the 1950's while the more potent second generation sulfonylureas have only been made available in the U.S. within the last 4 years [3,10]. Although the exact mechanism of action of the sulfonylureas is unknown, it is known that they can cause the release of insulin from the β cells of the pancreas [3]. Additionally, some studies have shown that peripheral tissues, upon prolonged administration of sulfonylureas, become more sensitive to the effects of circulating insulin [3].
Figure 1: First and second generation sulfonylureas

Gestational diabetes (GDM) occurs in approximately 2-5% of all pregnancies [4]. Ninety eight percent of all GDM patients revert to normal glucose tolerance after parturition. Unfortunately GDM is associated with increased fetal abnormalities [4]. Secondary diabetes accounts for approximately 2% of all cases of DM, and, as the name implies, is secondary to another disease state or syndrome [4]. Generally, the cause of secondary diabetes is known or suspected and may be rectified upon correction of the primary disease or syndrome.
1.3 METABOLIC ABNORMALITIES OF DIABETES MELLITUS

As mentioned previously, the one commonality among all types of DM is hyperglycemia. Independent of the type of DM one suffers from, the lack of sufficient quantities of circulating insulin is the direct cause of the hyperglycemia. Normally, insulin is produced and secreted from the β cells, of the pancreas, in response to varying blood glucose levels [2,11]. Insulin exerts several metabolic effects on a wide variety of tissues, however, it primarily effects 3 target tissues; the liver, adipose tissue and skeletal muscle [11]. Table 2 indicates some of the regulatory effects insulin exerts on the metabolism of carbohydrates, proteins and fats in these target tissues. Although the exact biochemical mechanism of action of insulin is as yet unknown, it is known that insulin interacts with cell surface receptors, forming an insulin-receptor complex. This complex is then internalized [12]. Pharmacologically, insulin acts to increase the facilitated diffusion of glucose into muscle and fat cells. Insulin does not exert this effect on all cells, for example the uptake of glucose into the liver is not effected by insulin [12]. Insulin also acts to increase the uptake of amino acids into muscle tissue. The efflux of sodium (Na⁺) and influx of potassium (K⁺) is also stimulated by insulin, presumably through its actions on sodium-potassium ATPase (Na⁺-K⁺ ATPase) [12].
Table 2

**Summary of insulin's effects on primary target organs**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Carbohydrate</th>
<th>Protein</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>+Glucose transport</td>
<td>+Amino acid uptake</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+Glycogen synthesis</td>
<td>+Protein synthesis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glycolysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>+Glucokinase</td>
<td></td>
<td>-Proteolysis</td>
</tr>
<tr>
<td></td>
<td>+Glycogen synthesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-Phosphorylase</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-Gluconeogenesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adipocytes</td>
<td>+Glucose transport</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+Glycerol synthesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Lipolysis</td>
</tr>
</tbody>
</table>

+ = increase, - = decrease

In addition to its effects on the transmembrane transport of physiologically important ions and molecules, insulin also exerts effects on enzyme pathways important in the cellular metabolism of target tissues. Insulin acts to increase the activity of glycogen synthetase leading to the increased production of glycogen from glucose. Additionally, insulin prevents the breakdown of glycogen in the liver by inhibiting hepatic phosphorylation [13]. Insulin also stimulates the glycolytic pathway, the metabolism of glucose to pyruvate, while inhibiting gluconeogenesis, the production of glucose from amino acids and proteins [13]. In muscle insulin stimulates the production of proteins, while in the liver insulin inhibits protein metabolism. Lipogenesis, the production of fatty acids and
triglycerides, is stimulated by insulin in adipose tissue. Figure 2 briefly outlines the effects of insulin on cellular metabolism [11].

**Figure 2**: Influence of insulin on key enzymes involved in glucose metabolism

Bold solid lines indicate induction, broken lines indicate suppression. 

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-1-P</td>
<td>Glucose-1-phosphate</td>
</tr>
<tr>
<td>G-6-P</td>
<td>Glucose-6-phosphate</td>
</tr>
<tr>
<td>F-6-P</td>
<td>Fructose-6-phosphate</td>
</tr>
<tr>
<td>F-1,6-P</td>
<td>Fructose-1,6-diphosphate</td>
</tr>
<tr>
<td>PEP</td>
<td>Phosphoenolpyruvate</td>
</tr>
<tr>
<td>OA</td>
<td>Oxaloacetate</td>
</tr>
<tr>
<td>PEPase</td>
<td>Fructose-1,6-diphosphatase</td>
</tr>
<tr>
<td>G-6-Pase</td>
<td>Glucose-6-phosphatase</td>
</tr>
<tr>
<td>UDPG</td>
<td>Uridine diphosphate glucose</td>
</tr>
</tbody>
</table>

From the cellular processes outlined above it can be seen that alterations in circulating insulin levels could have serious consequences. Indeed, insufficient levels of circulating insulin, as in the various disease states of DM, can lead to serious metabolic abnormalities. For example, during insulin insufficiency there is a marked decrease in the cellular uptake of glucose in certain cell types. The production of glycogen from glucose is greatly reduced while the rate of gluconeogenesis is abnormally high. These metabolic
derangements manifest themselves as hyperglycemia [11]. If uncorrected, insulin deficiency can lead to serious, sometimes fatal, metabolic imbalances. As shown in Figure 3, under conditions of prolonged hyperglycemia, large quantities of glucose are spilled into the urine, leading to osmotic diuresis [11]. This diuresis can lead to severe electrolyte imbalances, dehydration and possibly death [11]. Generally, electrolyte and fluid imbalances are risk factors for older non-insulin dependent diabetics [14]. Additionally, the increased lipolysis associated with insulin deficiency leads to the overproduction of ketone bodies, which are the products of fatty acid metabolism. Increased production of fatty acids and ketone bodies alters blood pH and generates the condition known as ketoacidosis [11]. Ketoacidosis accounts for approximately 10% of all deaths due to diabetes and is generally only a risk factor for insulin dependent diabetics [14].

![Figure 3: Effects of insulin deficiency. (Modified from [11])](image)
1.4 SECONDARY COMPLICATIONS OF DIABETES MELLITUS

On a daily basis, the metabolic imbalances produced by insulin insufficiency can be easily managed through the use of insulin, oral hypoglycemics, alteration of diet or other means [3,11]. However the increasingly sophisticated treatment of DM, which began over 60 years ago with the discovery of insulin, has generally been unable to alter the development of the tissue damaging complications of DM. These complications or secondary effects of diabetes are largely responsible for the morbidity and mortality of DM [9]. Generally, the secondary complications of DM effect tissues that are not dependent upon insulin to regulate glucose uptake [15,16].

The secondary complications of diabetes are wide ranging. Diabetics suffer from retinopathies, cataracts, nephropathies, angiopathies and neuropathies at rates that are significantly higher than those for non-diabetics [9,15-17]. As an example, diabetes is the leading cause of blindness in the U.S. between the ages of 20 and 74 [18]. Diabetic retinopathy is responsible for approximately 84% of all cases of diabetic blindness [9,18]. Persons with diabetic retinopathy are 29 times more likely to suffer blindness than non-diabetics [18]. Also, diabetics are five times more likely to suffer from cataracts than non-diabetics [17]. Macroangiopathies which develop in medium and large arteries make stroke and coronary artery disease twice as prevalent in diabetics than non-diabetics [9,17]. Diabetics also have a 3 to 4 fold greater chance of suffering from peripheral artery disease and are 5 times more likely than the general public to suffer
from gangrene which leads to amputation [9]. Additionally, diabetics are 17 times more likely to suffer from renal failure than non-diabetics [17]. Overall, the net effect of suffering from the secondary effects of DM is that the average lifespan of a diabetic is only 2/3 that of a non-diabetic [9].

Since these secondary complications are associated with diabetics at a higher rate than with non-diabetics it is not unreasonable to assume that these secondary effects are somehow related to hyperglycemia. As stated earlier, the secondary complications of DM generally effect tissues that are not dependent upon insulin to regulate cellular uptake of glucose. Therefore, the intracellular concentration of glucose will mirror the extracellular concentration [19]. Although the exact mechanism through which hyperglycemia causes tissue damage is not known, a number of biochemical mechanisms have been proposed [20]. These include: an increase in the activity of the polyol pathway leading to elevated intracellular concentrations of sorbitol [15,21], alterations in the metabolism of inositol and its phospholipid derivatives [22,23], abnormal small blood vessel permeability and excessive non-enzymatic glycosylation of proteins [24].

1.5 THE POLYOL PATHWAY

The polyol pathway, Scheme I, is a minor metabolic pathway that consists of the enzymes aldose reductase (AR) and sorbitol dehydrogenase (SD). AR catalyzes the conversion of glucose to sorbitol
and utilizes the cofactor NADPH [15,22,25]. The subsequent conversion of sorbitol to fructose is catalyzed by SD, which utilizes the cofactor NAD$^+$ [15,22,25]. Because hexokinase has a greater affinity for glucose than does AR, under normal physiologic conditions, glucose that enters the cell is rapidly phosphorylated and further metabolized [15,19]. In fact, under normal conditions, the activity of AR and the polyol pathway is so low that intracellular levels of sorbitol are almost undetectable [19,26]. However, under the conditions of hyperglycemia the normal metabolic pathways of glucose metabolism become saturated and the excess glucose is shunted through the polyol pathway [15,19]. In the polyol pathway sorbitol is more rapidly produced than utilized and therefore there is a net increase in the intracellular concentration of sorbitol.

Scheme I

The polyol pathway

First described in 1956, the polyol pathway was originally thought to provide fructose for the normal functioning of sperm in the seminal
vesicles [27]. However, it is now understood that fructose is not necessary for the maintenance of normal spermatozoa [28]. The association between the polyol pathway and diabetic complications was first suggested by van Heyningen when she demonstrated the existence of sorbitol in the lenses of diabetic rats suffering from cataracts [29]. Today, the polyol pathway has been identified in a wide variety of tissues, many of which display the secondary complications of DM [19]. However, the exact physiological role of AR or the polyol pathway has yet to be determined [19].

1.6 DIABETIC COMPLICATIONS AND ALDOSE REDUCTASE

The suspected association between the activation of AR, or the polyol pathway, and diabetic complications was strengthened when Kinoshita demonstrated the adverse effects of AR activation in the rat lens [30,31]. This research led to the development of the osmotic hypothesis of cellular damage leading to sugar cataract formation.

The Osmotic Hypothesis

Activation of AR and the polyol pathway leads to the accumulation of sorbitol in the fiber cells of the lens [29,30]. The high polarity of sorbitol prevents its efficient diffusion out of the fiber cells. An increased intracellular concentration of sorbitol leads to an increased intracellular osmotic potential. Water enters the cell in an attempt to balance the osmotic potential. Lens hydration and cellular swelling follow. Continued swelling leads to changes in cell membrane permeability [15,19,32]. The altered membrane permeability allows for
the diffusion of important intracellular ions and constituents out of the cell [32]. Intracellular concentrations of $K^+$, amino acids, glutathione, inositol and ATP begin to decrease, while the concentrations of $Na^+$ and $Cl^-$ begin to increase [32]. As this process continues the electrolyte imbalance created becomes the predominant factor in cellular swelling [19]. The electrolyte imbalances also cause a decrease and eventual cessation of protein synthesis [33]. At the final stage of these biochemical changes osmotic integrity is lost and most cellular constituents become freely diffusible. Only a few large proteins are prevented from diffusing out of the lens [15,32].

Various histological changes can be associated with the biochemical changes occurring in the lens during polyol pathway activation. Initially, activation of AR and the polyol pathway leads to swelling of the fiber cells of the lens. Eventually, these cells rupture and liquify, forming visible vacuoles [15]. The cataractogenic process, if allowed to continue, proceeds through the cortical cataract stage and finally ends with the formation of an opaque nuclear cataract [15]. The cataractogenic process is reversible if stopped sometime before the formation of the nuclear cataract [15,19,32,34]. The description of the biochemical and histological changes occurring during the cataractogenic process are illustrated in Figure 4.

Traditionally, research of the secondary complications of DM have been difficult because of their long time of onset and associated metabolic complexity. However, research regarding aldose reductase's relationship to diabetic complications has been aided by its broad
substrate specificity and its ease of inhibition [19]. Aldose reductase has the ability to reduce a wide variety of aromatic and aliphatic aldehydes. The sugar galactose, which is a better substrate for AR than is glucose, is used to induce the hyperosmotic effects associated with the secondary complications of DM. The osmotic effects induced by galactose are more dramatic than the effects induced by glucose. The more rapid reduction of galactose to galactitol versus the reduction of glucose to sorbitol is one reason for the more dramatic effects. Additionally, unlike sorbitol, galactitol is not oxidized by sorbitol dehydrogenase and intracellular levels of galactitol remain higher longer [19]. Galactosemia, elevated blood galactose levels, can be induced in animals through the introduction of excessive amounts of galactose in their diets. The use of galactosemic animals has been instrumental in the study of the relationship between AR and the secondary complications of DM.
The development of aldose reductase inhibitors (ARIs) has proven to be a boon for researchers. By studying the secondary complications induced by hyperglycemia and galactosemia and the ability of ARIs to alter the course of the induced secondary effects, researchers have associated AR and the polyol pathway with some of the secondary complications of DM [15,19]. However, before AR can be implicated in the causation of a diabetic complication a number of important criteria must be met [15,35]. These criteria can be summarized as follows:

1. AR must be shown to exist in the affected tissue.
2. The complication should exist in both the galactosemic and diabetic state.
3. The onset should be quicker and the effects more severe in the galactosemic state.
4. At least 2 structurally different ARIs should delay or prevent the onset of the complication.

As stated above, AR and polyol pathway activation have been implicated in the development of some of the secondary effects of DM. Best understood of these implied relationships is that between AR and the generation of diabetic cataract. The four criteria listed above have been met. AR does exist in the lens and it can be inhibited by a number of ARIs. Additionally, the cataractogenic process has been shown to exist in either the galactosemic or diabetic state [29,30]. In fact the relationship between AR and cataractogenesis is so well understood that it is used as an 'acid test' for the development of ARIs. Other secondary complications associated with AR are
keratopathy, microangiopathy, neuropathy, retinopathy and nephropathy [19]. While an understanding of how AR might cause diabetic cataracts exists, an exact mechanism or mechanisms for the generation of the other diabetic complications has eluded researchers. Several excellent reviews regarding the relationship between AR and diabetic complications have been published [15,26,34]. It must be noted that not all diabetic complications can be attributed to AR and the polyol pathway since galactosemic rats, although displaying some of the secondary complications common to diabetes and galactosemia, are in considerably better health than their diabetic counterparts [25].

1.7 ALDOSE REDUCTASE AND ALDOSE REDUCTASE INHIBITORS

Aldose reductase has been isolated from a number of tissues including the lens, placenta, brain, kidney, muscle and seminal vesicles from such diverse sources such as pigs, cows, dogs, rats, rabbits and humans [19]. Studies have revealed that AR is a sulfhydryl containing enzyme, generally appearing as a monomer with a molecular weight of between 28-45kD. Isozymes of AR may exist [19]. Aldose reductase is not a metalloprotein, and there is no evidence of bound phosphate or glycoproteins [19]. Additionally, studies with AR isolated from various sources have shown that there are significant interspecies variations in susceptibility to inhibition [36,37]. Although no specific trends have been detected, human placental aldose reductase (HPAR) is generally less susceptible to inhibition than other ARs [19]. This suggests that inhibitors destined for human use should be screened against human ARs [36].
Using the observation of Hers [27], that rat lens aldose reductase (RLAR) was sensitive to the presence of inorganic anions, Hayman and Kinoshita demonstrated the in vitro inhibition of RLAR by a variety of fatty acids [38]. They determined that chain length, branching and the presence of a carbonyl or second carboxyl group played an important role in the inhibitory potency of these compounds [38]. They also determined that these fatty acids acted as noncompetitive inhibitors indicating that these compounds might be acting at a site distinct from either the active site or cofactor binding site [38]. However, these compounds caused clouding of the lens and work with these compounds was discontinued.

Working in cooperation with Ayerst Research Laboratories, Kinoshita demonstrated the inhibition of RLAR by 3,3 tetramethyleneglutamic acid (TMG) (1) [39]. Although TMG was active in vitro and produced no transparency problems in the lens, it had no activity in vivo [39,40]. The first ARI found to be active in vivo was α,α,α-trifluoro-N-[2-(3-nitropyridyl)]-m-toluidine or AY-20 263, (2) [31]. This compound however, was poorly water soluble and was administered intraocularly as a concentrated DMSO solution. Upon injection, it immediately precipitated and formed a depot source of the drug [38]. The first orally active ARI was 1,3-dioxo-1H-benz[b]isoquinoline-2(3H) acetic acid, Alrestatin, (3) [40]. Although TMG, AY-20 263 and Alrestatin had the ability to inhibit AR in vitro and in the case of AY-20 263 and Alrestatin in vivo, they could only delay not prevent the onset of galactosemic cataracts, presumably due to poor potency [31,40,41].
However, the development and testing of these compounds showed that AR was somehow related to diabetic complications. Moreover, the success of these compounds in delaying the onset of galactosemic cataracts indicated that the inhibition of AR was a plausible route to the treatment of the debilitating complications of DM.

The next major breakthrough in the field of aldose reductase inhibition was the development of racemic 2,3-dihydro-6-fluorospiro (4H-1-benzopyran-4,4'-imidazolidine)-2',5'-dione, (4) [41,42]. Upon resolution of the isomers the inhibitory activity of 4 was found to reside almost entirely in the S isomer, now known as Sorbinil [41]. Sorbinil is a very potent ARI and was the first compound that not only delayed the onset of diabetic and galactosemic cataracts, but actually prevented the formation of the cataracts [43]. Clinical studies demonstrated that treatment with Sorbinil resulted in the improvement of nerve conduction velocities in human diabetics [44]. The results of these studies indicated that inhibition of AR was effective in human diabetics and that there was a reversible component in diabetic nerve damage.
Previous to the development of Sorbinil, most ARIs contained carboxylic acid moieties, like TMG and Alrestatin. The introduction of Sorbinil demonstrated that compounds with other weakly acidic functional groups could inhibit AR. Since the introduction of Sorbinil the development of ARIs has proceeded rapidly, such that numerous compounds of various structural types have been developed as ARIs. Table 3 and Table 4 list several ARIs that have undergone clinical evaluation [45]. As indicated in Table 3 and Table 4 ARIs can be divided into two general classes of compounds; carboxylic acids, Table 3, and hydantoins and related heterocycles, Table 4. As one can see, the structural diversity among ARIs, even within the same class of compounds, is significant. However, some gross structural similarities can be seen. For example, Statil (7), Fr 74366 (8), and AD 5467 (9) share some structural similarities, even though the body of each inhibitor is comprised of a different heterocycle. Tolrestat (5) was developed by Ayerst Labs after it was determined that Alrestatin had poor clinical activity [46,47]. Although not readily apparent, both Tolrestat and Alrestatin contain a similar structural segment as shown in Figure 5.
Among the hydantoins and related heterocycles, the relationship between Sorbinil and Methosorbinil (10) is rather evident. One might also consider the similarity between Alconil (12) and Sorbinil. Alconil could be considered, although somewhat inaccurately due to the nonplanarity of the chroman system of Sorbinil, to be Sorbinil with an additional aromatic ring. Alconil and its analog AL 1576 (13) are 2 of the most potent ARIs known. The presentation of the compounds in Table 3, and Table 4, is by no means meant to be a comprehensive list of active ARIs. Indeed, pharmaceutical companies will make numerous structural analogues of a particular drug of interest. Many more compounds are known to have aldose reductase inhibitory activity, but are not shown for the sake of brevity. However, a number of review articles and patents currently available should satiate the readers hunger for structure activity relationships [15,41,46,48-54].
Table 3

Carboxylic acids as ARIs
Table 4

Hydantoins and related heterocycles as ARIs
A third category of ARIs is comprised of compounds that do not easily fit into either of the two categories discussed above. Natural products such as Quercitin (15), anti-allergy agents like 16 and non-steroidal anti-inflammatory agents such as Sulindac (17), make up this group of compounds and are shown in Table 5 [55,56]. As with the carboxylic acids and hydantoin and related heterocycles this miscellaneous group of compounds shows significant structural diversity.
1.8 THE INHIBITOR BINDING SITE

As stated earlier, kinetic studies indicate that ARIs bind to AR at a site that is distinct from either the cofactor binding site or the active site of the enzyme. ARIs inhibit AR in either a noncompetitive or uncompetitive manner. Although structurally diverse, competition studies between ARIs from different classes and of different structure, indicate that ARIs bind to the same site on AR [45]. Using basic biochemical techniques and the structure activity relationships generated by pharmaceutical companies, much has been learned about the inhibitor binding site of aldose reductase.
The increased activity of Sorbinil versus its antipode indicates that the inhibitor binding site has some stereoselectivity [41,42]. Studies with chiral analogues of Alrestatin (18), showed that the R isomer is the preferred isomer. Additionally, both compounds showed decreased activity relative to Alrestatin, indicating that steric bulk may not be well tolerated, in this area, by the inhibitor binding site [57].

Through the use of biochemical techniques a reactive nucleophilic residue has been detected in the inhibitor binding site [58]. 4-Nitrobromosacophenone (19), when incubated with AR, produced a partial irreversible inhibition. Kinetic studies indicate that 19 alkylates AR at the inhibitor binding site. Amino acid analysis of the hydrolysate suggested that tyrosine was the nucleophilic residue. Furthermore, reaction with the tyrosine specific agent 4-nitrophensylsulfonyl fluoride (20) gave kinetically similar results [58].
Since most ARIs are weakly acidic, it is not unreasonable to question how ARIs are interacting with the inhibitor binding site. That is, are ARIs binding to the inhibitor binding site in their charged ionized form or their uncharged and unionized form? During their research with 1 and 5 positional isomers of hydantoins as ARIs, Inagaki et al. attempted to answer this question [59]. Their results indicated that the inhibitory activity of the 5 substituted hydantoin 21 increased when the pH of the test medium was raised from 6.0 to 7.5. On the other hand, the activity of the 1 substituted hydantoin 22 was decreased as the medium pH was raised. The activity of the non-ionizable hydantoin 23 was unchanged with variations in pH [59]. These results suggest that 5 substituted hydantoins bind to AR in their ionized form while the 1 substituted hydantoins bind in their unionized form. Additionally, De Ruiter et al. have proposed that simple N-benzenesulfonylglycines 24 interact in their ionized forms with an arginine residue, also proposed to be in or near the inhibitor binding site [60].
A model of the inhibitor binding site has been developed by Kador et al., based on information gained from biochemical techniques, structure activity relationships and computational chemistry, Figure 6 [58]. To develop this model, a series of ten ARIs that were available at the time, including Alrestatin and Sorbinil, were assembled into a 'molecular conglomerate' [58]. To assemble this conglomerate the structural similarities of the various ARIs were matched up with one another. Through this type of approach the minimal pharmacophoric requirements for AR inhibition were determined [58]. These requirements are summarized as follows:

1. A primary aromatic group and a carbonyl group separated by 2.8-3.8 angstroms is required for activity (d1 in Figure 6)
2. A secondary aromatic or hydrophobic group centered around 2.8-6.1 angstroms from the carbonyl enhances activity (d2).

3. Two hydroxyl groups one located 2.8-3.8 angstroms (d3) the other 8.0-9.3 angstroms (d4) from the center of the primary aromatic ring also serve to enhance activity.

![Figure 6: Basic pharmacophoric requirements of ARIs](image)

Molecular orbital calculations indicate that the carbonyl at the center of the above pharmacophoric requirements may undergo either reversible nucleophilic attack or participate in the formation of a charge transfer complex [58]. Since tyrosine is suspected to be present in the inhibitor binding site, it is believed that this tyrosine participates in either the reversible nucleophilic attack or, in conjunction with arginine and the carbonyl of the ARI, the formation of a charge transfer complex [58]. However, inhibitors designed to meet these basic pharmacophoric needs were shown to be poor inhibitors of AR [61].
Although there are a large number of ARIs that have shown significant activity experimentally and clinically none are currently available for prescription by physicians. The reasons for this inability to gain entry into the drug market may be manifold. Many ARI are associated with serious side effects [62,63]. With the understanding that diabetes mellitus is a life long disease, it is not unreasonable to assume that patients will be treated with ARIs for the remainder of their lives. As such, it is imperative that the benefit to risk ratio be maximized, that is selectivity, efficacy and potency must be maximized while side effects are minimized. Therefore, a clear and concise understanding of the interactions taking place between inhibitors and aldose reductase at the inhibitor binding site is important for the development of more active and selective inhibitors. Although the previous research stated above has given some understanding of the inhibitor binding site, the intimate interactions taking place between inhibitors and AR during the binding process must be more closely probed.
CHAPTER II
STATEMENT OF THE PROBLEM AND OBJECTIVES

2.1 IRREVERSIBLE INHIBITORS

As noted in the introduction of this document, previous research has suggested the presence of a reactive nucleophile at the inhibitor binding site of aldose reductase. The identification of this reactive nucleophile and the determination of its position within the inhibitor binding site might lead to a better understanding of the interactions taking place between aldose reductase inhibitors and aldose reductase at the inhibitor binding site. A better understanding of the binding interactions taking place at the inhibitor binding site may lead to more active and selective ARIs.

Affinity labeling is a process in which a reactive electrophilic functional group is appended to a compound which is known to have affinity for a particular binding site of interest. Affinity labeling can be used to cause irreversible inhibition of an enzyme. When the compound used has affinity for the active site of an enzyme this type of inhibition is called "active site directed" irreversible inhibition [64]. Regardless of the site of interaction between the enzyme and inhibitor the overall reaction is the same.
As depicted in Figure 7, the inhibitor first forms a reversible enzyme inhibitor complex \([E \cdot I]\). The formation of the reversible enzyme inhibitor complex involves interactions that would normally take place between a reversible inhibitor and the enzyme. However, unlike a reversible inhibitor, which would eventually dissociate from the enzyme, the electrophilic functional group of the inhibitor reacts with a nucleophile on the enzyme and forms a covalent bond, generating the irreversible enzyme inhibitor complex, \([E - I]\). Thus, the enzyme is irreversibly inhibited and is no longer active [64].

A number of groups may function as the reactive electrophile of affinity labels. The \(\alpha\)-halo ketone and sulfonyl fluoride functional groups are two common electrophilic groups that are used in affinity labels. As noted previously, 4-nitrobromoacetophenone \(19\) and 4-nitrophensylsulfonylfluoride \(20\) have been used to cause partial inhibition of AR [58]. Since these compounds probably have no significant affinity for the inhibitor binding site they may not truly be called affinity labels. However, the sulfonyl fluoride and \(\alpha\)-halo ketone functional groups have been used to make true affinity labels of other enzymes [65,66]. Although \(19\) and \(20\) showed partial irreversible inhibition of AR, they gave no indication as to the location of the
reactive nucleophile within the inhibitor binding site. The isothiocyanate functional group has also been used as a reactive electrophile in affinity labels. For example, compound 25 has been used to irreversibly inhibit sweet almond β-glucosidase [67].

![Chemical Structure](image)

25

Ares et al. set about the task of determining if affinity labeling and the resulting irreversible inhibition of AR was possible [68-70]. Using the known reversible inhibitors Alrestatin (3), Sorbinil (4) and Alconil (12), and various electrophilic functional groups, a series of affinity labels was synthesized and tested, Table 6 [68-70]. The results indicated that the isothiocyanato analogues of Alrestatin (26) and Alconil (32) were the two most active irreversible inhibitors, providing 57% and 56% inhibition of rat lens AR (RLAR) respectively. Each compound was tested at a concentration of 10^{-6} M [58]. Equally as important, using various reversible inhibitors, AR could be protected against the irreversible inhibition caused by 26 and 32, indicating that these inhibitors were specifically binding and alkylating AR at the inhibitor binding site [58,69]. Although 26 showed good activity, the corresponding chloroacetamide 27 showed poorer activity, giving only 18% inhibition at 10^{-6} M [58]. Additionally, the racemic Sorbinil analogues 29 and 30 showed very poor activity and were essentially
inactive as irreversible inhibitors [45,68] Interestingly, a comparison of the IC$_{50}$ values of the analogues of Alrestatin 26-28 and Sorbinil 29-31 showed that there was no significant loss of affinity upon addition of the electrophile [68,70]. In other words, the addition of the electrophilic functional group did not appear to significantly alter the binding of the ARIs. Therefore, the low irreversible activity of compounds 27, 29 and 30 was probably not due to a loss of affinity [68,70]. Additionally, since both 26 and 32 contain an isothiocyanate group, the lack of activity of 29 cannot be explained by a lack of reactivity of the electrophile. However, as one can see in Table 6 the spatial relationship between the electrophilic groups and the other functionalities of each inhibitor is different. Therefore, in binding to the inhibitor binding site each series of inhibitors might be expected to place its reactive electrophile in a different area of the binding site. If the electrophile is placed in an area containing a reactive nucleophile, alkylation and subsequent irreversible inhibition would be expected to take place. If, however, the electrophile is placed in an area of the inhibitor binding site that does not contain a reactive nucleophile, then no alkylation and irreversible inhibition will take place. This could be a possible explanation for the inactivity of the racemic Sorbinil analogues 29 and 30.

As mentioned earlier, the chloroacetamide of Alrestatin 27 showed significantly less activity than the isothiocyanate 26. Since the loss of potency does not appear to be the reason for decreased activity of
Table 6

Irreversible inhibitors of aldose reductase

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<td>4</td>
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<td>12</td>
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</tr>
<tr>
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<tr>
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<td>= NHCOCH$_2$Cl</td>
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<td>31</td>
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</tr>
<tr>
<td>28</td>
<td>= NHCOCH$_3$</td>
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27, the explanation for the poor activity of 27 must lie elsewhere. The relative lack of chemical reactivity of the chloroacetamide function may be one reason for the poor activity of 27. It has been demonstrated previously that chloromethylketone derivatives of pyrimidine nucleosides were significantly less active than the corresponding bromo- or iodo- compounds [71].

Steric factors may also play a role in the activity of 27. As illustrated in Figure 8, the isothiocyanato and chloroacetamide functional groups have significant structural differences. The electrophilic carbon of 27 is labeled as C1, whereas the electrophilic carbon of 26 is labeled as C2. The electrophilic portions of the two functional groups occupy different areas in space relative to one another. Therefore, when bound to the inhibitor
binding site the reactive portions of the inhibitors 26 and 27 may lie in different areas of the inhibitor binding site. While 26 may interact more easily with the reactive nucleophile in the inhibitor binding site, the electrophilic portion of 27 may be placed in a position that does not allow for interaction with the reactive nucleophile. Therefore the difference in irreversible inhibitory activity between 26 and 27, may be due to either steric or chemical reactivity differences between the electrophiles.

![Figure 8: Spatial relationship of electrophilic groups of compounds 26 and 27](image)

Through their research Ares et al. demonstrated, quite nicely, that affinity labeling was a reasonable approach to the inhibition of AR [68-70]. We can now begin to ask more detailed questions about the inhibitor binding site and ARIs based on the results generated by Ares
et al. For example, why is the chloroacetamide 27 less active than the corresponding isothiocyanate 26? Is the difference in activity due to the differences in chemical reactivity of the electrophiles, or is it due to different orientations assumed by each electrophile at the inhibitor binding site? Can affinity labels be used to determine if different ARIs are interacting with the inhibitor binding site in a similar fashion? And, can the position of the reactive nucleophile in the inhibitor binding site be more accurately determined using the concept of affinity labeling? The research to be described will attempt to answer these questions.

The role that chemical reactivity plays in the activity of 27 can be tested quite easily by using more reactive electrophiles that are structurally similar to the chloroacetamide group. Obviously, this would include the bromo- and iodoacetamide functional groups. As was mentioned earlier, previous research has shown that within a series of nucleosides the bromo- and iodoacetylketones were more active as affinity labels than was the corresponding chloromethylketone [71]. If the lack of chemical reactivity is the reason for the poor activity of 27, then the more reactive compounds 33 and 34 should provide a greater amount of irreversible inhibition. Based solely on chemical reactivity and leaving group ability one would expect the trend of activity to be I > Br > Cl [72]. Therefore the bromo- and iodoacetamides 33 and 34 respectively were synthesized and tested for their ability to irreversibly inhibit AR.
During attempts to rationalize the inactivity of 26 an interesting observation lead to the desire to synthesize and test compounds 35 - 38. As discussed earlier, it would not be unreasonable to expect each inhibitor to interact with the inhibitor binding site in such a way as to place their respective electrophilic groups in various positions within the inhibitor binding site. Using this assumption, 2 models describing the binding of compounds 26, 29 and 32 to the inhibitor binding site were constructed. Each model was constructed on the premise that functional groups common to each inhibitor with the obvious exception of the isothiocyanate, served the same function at the inhibitor binding site. As shown in Figure 9, the "A" rings of each inhibitor were matched with one another such that the acidic functional groups, the hydantoin of 29 and 32 and the carboxylate of 26 were in close proximity to one another. Although it was not intended, one of the imide carbonyls of 26 is in close proximity to both of the 5' carbonyls of the hydantoin rings of compounds 29 and 32.
This is similar to the positioning of the carbonyls proposed by Kador et al. in the assembly of their 'molecular conglomerate' [58]. The interactions proposed by Kador et al. were not taken into account when model 1 was being constructed. As indicated in Figure 9, after the 'matching' of similar functional groups the isothiocyanate function of each inhibitor occupies a different area in space, where the electrophilic carbons of the isothiocyanates of Alconil, in this model the isothiocyanates of 26 and 32 are in relatively close proximity to one another, possibly explaining their similar activities. The isothiocyanate of 29 in this model appears to occupy an area that is quite distant from the isothiocyanates of either 26 or 32. Based on this model, the lack of irreversible inhibitory activity of 29 is presumably due to the lack of a sufficiently reactive nucleophile in the area of the inhibitor binding site that is occupied by the isothiocyanate of 29.

A second model was assembled in an attempt to move the isothiocyanates of 26 and 32 closer together. This model, as shown in Figure 10, was assembled in the opposite sense of model 1. That is, the 'A' ring of 29 was now overlapped with the 'B' rings of compounds 26 and 32. As in model 1, the electrophilic carbons of the isothiocyanates of Alconil, Alrestatin and Sorbinil are represented by C1, C2 and C3, respectively. Also, the acidic functional groups of each inhibitor were kept in close proximity to one another, and as in model 1 the proposed polarizable carbonyls of the 3 inhibitors were also in close proximity to one another. The isothiocyanates of 26 and
Figure 9: Model 1 depicting proposed binding arrangement of various ARIs

32 are now much closer to one another, while the isothiocyanate of 29 is still quite distant from either. Again this may explain the similar activities of 26 and 32 and the inactivity of 29. However, another and more interesting observation can be made. Upon closer inspection it appears that the isothiocyanates of compounds 26 and 29 overlap the 5
and 6 positions of compound 32. Based on this observation the compounds 36 - 38 were synthesized and tested for their irreversible inhibitory activity. Although, the 8 position of 32 appears to be occupied by one of the imide carboxyls of 26, 35 was synthesized because Kador et al. have suggested that the proposed nucleophile lies in this area of the inhibitor binding site and reversibly interacts with one of the imide carboxyls of 2.

While we can only guess as to how structurally different inhibitors might interact with the same binding site, the positional isomers 35 - 38 should give a more clearly defined answer. Because 35 - 38 are structurally similar they may all be expected to interact with the inhibitor binding in a similar manner. Therefore using the inhibitory activity data of compounds 35 - 38 we may generate more accurate estimates of the position of the proposed nucleophile within the inhibitor binding site. Additionally, compounds 35 - 38 may give a better understanding of how structurally different inhibitors interact with the inhibitor binding site. For example, if the activity of 37 is comparable to the activity of 26 it might suggest that 26 and 32 interact with the inhibitor binding site in a manner similar to that depicted in model 2, (Figure 10). Conversely, the inactivity of 37 would strongly indicate that model 2 is an inaccurate depiction of inhibitor-enzyme interaction.

In summary, the research discussed in this section is concerned with the irreversible inhibition of AR using the concept of affinity labeling. Results from previous research has generated a number of
Figure 10: Model 2 depicting proposed binding interactions of various ARIs

interesting questions. Synthesis and testing of compounds 33 and 34 should answer questions about the importance of chemical reactivity and structure of electrophiles used in affinity labeling. Also, the inhibitory activities of compounds 35 - 38 may yield an understanding as to how various structurally different ARIs interact with the
inhibitor binding site of AR. The activities of compounds 35 - 38 may also indicate where in the inhibitor binding site the proposed nucleophile resides.

\[ R_1 \quad R_2 \quad R_3 \quad R_4 \]

\[ 35 = \text{NCS} \quad \text{H} \quad \text{H} \quad \text{H} \]
\[ 36 = \text{H} \quad \text{NCS} \quad \text{H} \quad \text{H} \]
\[ 37 = \text{H} \quad \text{H} \quad \text{NCS} \quad \text{H} \]
\[ 38 = \text{H} \quad \text{H} \quad \text{H} \quad \text{NCS} \]

2.2 REVERSIBLE INHIBITORS

While the first section of this chapter dealt with the use of irreversible inhibitors of AR to describe characteristics of the inhibitor binding site of AR, this section will deal with the use of reversible inhibitors and how they might be used to provide additional information about the inhibitor binding site of AR. The concept of isosteric replacement was used to design a series of reversible inhibitors that may lend some insight into the spatial and structural requirements of that portion of the inhibitor binding site that interacts with the acidic functional group of various ARIs.
The concept of isosterism was introduced in 1919 by Langmuir in an attempt to rationalize the physical similarities of non-isomeric molecules [73,74]. Isosteres were defined by Langmuir to be compounds or molecules that had the same number and arrangement of electrons. Isosteres that were isoelectronic, that is carried the same net charge, would possess similar physical properties [74]. For example, \( \text{N}_2 \) and \( \text{CO} \), each with 14 electrons and no net charge, have similar physical properties [74]. The term bioisosterism was introduced in the 1950's to describe the ability of structurally similar functional groups to produce similar or antagonistic biological activities when introduced into a drug molecule [75]. Today, isosteres, or more commonly bioisosteres, may be considered as functional groups or molecules which have similar chemical or physical properties and produce broadly similar biological activity [76]. Bioisosterism was at one time a rather empirical understanding of the effects of functional group substitution upon biological activity, however, it is now considered part of the spectrum of quantitative structure activity relationships (QSAR) [76].

Isosteric substitutions may be of 2 types; classical and non-classical. Classical isosteres are functional groups that are structurally and electronically similar. Examples of classical isosteres are shown in Table 7. Using classical isosterism the oral hypoglycemic carbutamide (39) was progressively modified to yield tolbutamide (40) and chlorpropamide (41) [65]. Tolbutamide and chlorpropamide possess extended half lives and reduced toxicities.
relative to carbutamide [64]. Non-classical isosteres are defined as functional groups that are not structurally or electronically similar. Carboxylic acids, hydantoins and tetrazoles are all weak organic acids but they are structurally and electronically different and are therefore considered non-classical isosteres.

\[
R_{39} = \text{NH}_2 \\
R_{40} = \text{CH}_3 \\
R_{41} = \text{Cl}
\]

Table 7

Functional groups used as isosteric replacements

\[
\begin{align*}
\text{—N—} & \quad \text{—O—} & \quad \text{—F—} \\
\text{—CH—} & \quad \text{—NH—} & \quad \text{—OH—} \\
& \quad \text{—NH}_2 & \quad \text{—NH}_2 \\
& \quad \text{—CH}_3 & \quad \text{—CH}_3 \\
& \quad \text{—S—} & \quad \text{—CH}_3
\end{align*}
\]

Regardless of the type of isosteric replacement being made, consideration must be given to the functional group parameters that are being changed during isosteric substitution [75]. These include, size,
shape, electronic distribution, solubility (lipid, water), pKa, chemical reactivity and metabolism, and hydrogen bonding capacity. It is unlikely that any one isosteric substitution will leave all of these parameters unchanged. Indeed, the goal of bioisosterism is to alter a drug's activity or disposition through subtle changes in the above listed parameters. The isostere that alters the functional group parameters most important in determining a drug's activity, in a desirable fashion, will be the most effective isostere. Generally, how an isosteric substitution will effect a drug's activity and the parameters cited above is unknown. The determination of an isostere's effect on activity becomes part of the structure activity relationships for a given set of compounds [75].

The biological activity resulting from an isosteric substitution will depend on the role the modified portion of the drug plays in determining the biological activity of the parent molecule. Additionally, it must be mentioned that what may appear to be a useful isosteric substitution in one set of compounds may not necessarily result in a useful substitution in another [75]. In addition to its use in developing more potent and less toxic drugs, isosterism can be used to develop enzyme inhibitors from known substrates and receptor antagonists from known agonists. Isosterism can also be used to alter a drug's pharmacokinetic profile, that is, its absorption, excretion and metabolism.

The use of the tetrazole moiety as a non-classical isosteric replacement for the carboxylic acid moiety has provided some
interesting examples of the effects of isosteric replacement upon a drug's biological activity. Nicotinic acid (42) is known to lower blood lipid levels [64,77-79]. Although its exact mechanism of action is unknown, nicotinic acid is known to inhibit the lipolysis of triglycerides in adipose tissue [77]. The tetrazole analogue 43 was tested for its ability to inhibit lipolysis in vitro. The results showed that 43 was approximately 3,000 times less active than nicotinic acid [77]. However, when tested in vivo, 43 was found to be equipotent with nicotinic acid [79]. Moreover, 43 had a significantly longer duration of action than nicotinic acid. Metabolism studies showed that 43 is not metabolized as rapidly or as extensively as nicotinic acid [78,79]. Apparently the alteration of normal metabolism gives 43 its increased in vivo activity [77-79].

The amino acid L-ornithine (44) is decarboxylated by the enzyme ornithine decarboxylase (ODC) to yield the diamine putrescine [80]. Putrescine functions as a precursor to the diamines spermine and spermidine, which appear to have essential functions in normal cellular mechanisms, especially cellular division [80]. The tetrazole analogue of L-ornithine (45) was found to be a competitive inhibitor of ODC,
with an affinity for the enzyme that was similar to that of the natural substrate [80].

As mentioned earlier, what appears to be a successful isosteric substitution in one series of compounds may not necessarily prove successful in another. Such is the case with γ-amino butyric acid (GABA) (46) and its analogues 47 and 48 [81,82]. While the endogenous GABA is active as an inhibitory neurotransmitter, both the tetrazole 47 and hydantoin 48 were devoid of any significant GABAergic activity [81,82].

As indicated above, the carboxylic acid, tetrazole and hydantoin functional groups are considered non-classical isosteres [76]. These isosteric groups differ significantly in size, structure, pKa’s and charge distribution, as indicated in Figure 11 and Figure 12.
Structural similarities of these functional groups are highlighted in Figure 11.

![Figure 11: Structural similarities of the carboxylic acid, hydantoin and tetrazole moieties](image)

The pKa's of structurally related tetrazoles and carboxylic acids are roughly similar, with carboxylic acid being slightly stronger acids \([64,83]\). Hydantoins however, are much less acidic. The pKa of Sorbinil (4) is 8.7, while the pKa of a structurally similar carboxylic acid 49 is 4.9 \([84]\).

![49](image)

The differences in pKa's may not be significant in determining inhibitory activity since 5 substituted hydantoins have been shown to increase in inhibitory activity with increasing pH, indicating that 5 substituted hydantoins are binding to the inhibitor binding site in
their ionized state [59]. Although the differences in pKa's may not be important at the level of the inhibitor binding site, compounds that contain either a hydantoin, carboxylic acid or tetrazole moiety would be expected to have significantly different pharmacokinetic parameters based on the differences in the pKa's of these functional groups. The differences in the pharmacokinetic parameters alone, of compounds containing these functional groups, might account for the differences in activity of these compounds, without significantly altering drug-binding site interactions. Therefore, assessment of the results of isosteric substitutions must be approached with caution.

These isosteres also differ in their delocalization of charge in their ionized states, Figure 12. The carboxylate can distribute its charge over three atoms, while the ionized hydantoin may distribute its charge over the five atoms that are part of the imide portion of the hydantoin ring. The charge density of the tetrazoles is more diffuse than either the carboxylate or hydantoin since the tetrazole can delocalize its charge over its entire ring structure.

![Figure 12: Comparison of the charge delocalizations of the carboxylate, hydantoin and tetrazole moieties](image)

Figure 12: Comparison of the charge delocalizations of the carboxylate, hydantoin and tetrazole moieties
During their research involving the development of Sorbinil, Sarges et al. synthesized and tested a large number of structurally similar inhibitors that contained either a carboxylic acid or hydantoin ring [41, 86]. The carboxylate analogs were always less active in vitro and were often significantly less active in vivo. Sarges et al. also synthesized and tested the hydantoin analogue of Alrestatin 50 and found it to be about 8 fold less active than Alrestatin in vivo.

With the above observations in mind the reversible inhibitors shown in Table 8 were synthesized to determine if the hydantoin ring of Alconil and its analogs could be replaced by other weakly acidic functional groups. The inhibitors in the series 51, 54, 57 and 58 were designed to provide some information about the possible location of a proposed cationic residue in the inhibitor binding site [60]. Fluorine substitution has been shown to have a significant effect on the inhibitory activity of Alconil and structurally related ARIs [53].
Therefore, compounds in the series 51 - 53 and 54 - 56 were synthesized and tested to determine if fluorine exerted similar effects on the isosterically substituted inhibitors. The anti-allergy compounds 59 and 60 have similar inhibitory activities against AR. However, the tetrazole or carboxylate moiety is not believed to play a role in the determination of the inhibitory activity of these compounds [58]. The compounds 61, 62 and 63 were designed to test the influence of the tetrazole moiety on inhibitory activity when placed in a position of a known inhibitor believed to play an important role in determining biological activity. In summary, the concept of bioisosteric substitution was used to design a series of novel reversible ARIs. These inhibitors were designed to test the spatial, structural and electronic requirements of the inhibitor binding site of AR.
Table 8

**Novel reversible inhibitors of aldose reductase**

<table>
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<th>R</th>
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<tbody>
<tr>
<td>51</td>
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<td>52</td>
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<tr>
<td>56</td>
<td>F</td>
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![Chemical structures](image)
CHAPTER III
RESULTS AND DISCUSSION

This chapter is divided into two main sections. The first section involves a discussion of the synthesis of the irreversible and reversible inhibitors. The second section discusses the results of the biological testing of these inhibitors. The chemistry section is additionally divided into 2 parts, the first of which discusses the chemistry of the irreversible inhibitors. The later portion of the chemistry section discusses the chemistry of the reversible inhibitors. The section of biological results will be subdivided in a similar manner.

3.1 CHEMISTRY

3.1.1 Synthesis of irreversible inhibitors

Compound 27 has been previously synthesized by Ares using 64 as the immediate precursor [68]. Acetylation using chloroacetic anhydride and subsequent hydrolysis of the methyl ester yielded the desired product, 27. Since the target compounds, 33 and 34, were expected to be more chemically reactive than the chloroacetamide, the possible displacement of either the bromine or iodine during ester hydrolysis was seen as a potential complication in this synthetic sequence. Additionally, the
procedure of Ares for the chloroacetylation of 64 required the overnight incubation of 64 with an excess amount of chloroacetic anhydride and base in methylene chloride [68]. An alternate method was sought to shorten the reaction time, increase the yield and avoid possible complications of ester hydrolysis. Scheme II, shows this alternate route, which involves ester hydrolysis before acetamide formation and the alteration of acetylation conditions. Although previously prepared the synthesis of 27 by this route was used as a "test case" for the synthesis of the bromo- and iodoacetamide analogues, 33 and 34 respectively.

Compound 64, synthesized by the method of Ares [68], was saponified to yield 65. At a reaction temperature of 0°C, using pyridine as a solvent, 4-dimethylaminopyridine (DMAP) as a catalyst and 5 equivalents of chloroacetic anhydride as the acetylating agent, the reaction was complete within one hour. Before the reaction was worked up a small amount of dilute acid was added to hydrolyze any mixed anhydride that may have formed. Although the reaction time had been significantly reduced the yield was not greatly increased over that achieved by Ares [68]. When these reaction conditions were employed for the synthesis of the bromoacetamide 33, only the pyridinium bromide 66 was isolated, as identified by nuclear magnetic resonance (NMR) and mass spectrometry (MS). Shortening of the reaction time to 15 minutes, and lowering of the reaction temperature to -20°C yielded a 3:2 mixture of 33 and 66. It rapidly became apparent that pyridine was not a suitable solvent for the acetylation reaction. Tetrahydrofuran (THF) proved to be a more
suitable solvent. Both the bromo- and iodoacetamides, 33 and 34 respectively, were synthesized using 5 equivalents of the appropriate $\alpha$-haloacetic anhydride and DMAP in THF at 0°C for approximately 1 hour, as shown in Scheme III.

Scheme II

Synthesis of the chloroacetamide analogue of Alrestatin 27
Scheme III

Synthesis of the bromo- and iodoacetamide analogues of Alrestatin 33 and 34

Scheme IV outlines the proposed synthesis of the amino spirohydantoins 71 - 74, which are the immediate precursors to the desired isothiocyanates 35 - 38. These spirohydantoins were seen to arise from the four commercially available amino-9-fluorenones 67 - 70, via the Bucherer-Borgs reaction [87]. Compounds 72 and 74 are known compounds having been previously synthesized by the Alcon company during the development of Alconil and other structurally related ARIs [53]. Compounds 72 and 74 were synthesized in accordance with the literature procedures, except that the reaction times were increased from 24 to 48 hours. The attempted synthesis of 71 or 73, utilizing conditions similar to those used for the synthesis of 72 or 74, failed to yield any detectable amount of the desired products and resulted in the isolation of only unreacted starting materials, 67 or 69.
Scheme IV

Synthesis of the amino-spirohydantoins 72 and 74, and attempted synthesis of 71 and 73

Structurally, 3 substituted 9-fluorenones are similar to 4 substituted benzaldehydes, as shown in Figure 13. Previously, it had been shown that the rate of cyanohydrin formation involving 4 substituted benzaldehydes was dependent upon the ring substituent present at the 4 position [88,89]. The electron releasing dimethylamino and methoxy substituents were found to have an inhibitory effect on the rate of cyanohydrin formation. In the 4 position of benzaldehyde the electron releasing substituents are in direct conjugation with the reactive carbonyl and through conjugation act to
increase the electron density of the carbonyl function. The increased electron density of the carbonyl makes it less electrophilic and less susceptible to nucleophilic attack by cyanide anion. Conversely, 4-nitrobenzaldehyde, with its strongly electron withdrawing nitro group in direct conjugation with the reactive carbonyl, was found to undergo cyanohydrin formation at a significantly higher rate than benzaldehyde [89]. Since 3-amino-9-fluorenone is structurally similar to 4 substituted benzaldehydes and the first step in the Bucherer-Bergs reaction is believed to involve cyanohydrin formation, it is not unreasonable to suggest that the inability of 69 to participate in the Bucherer-Bergs reaction may be related to the electron releasing ability of the amino group in direct conjugation with the carbonyl of 3-amino-9-fluorenone. This hypothesis could be easily tested since 3-nitro-9-fluorenone is commercially available. Indeed, when subjected to Bucherer-Bergs conditions, similar to those used for the amines above, 75 was successfully converted to the corresponding spirohydantoin 76, Scheme V. The yield for this reaction was reasonably high, 63%, and in direct contrast to a report by the Alcon company that nitrated fluorenones were poor substrates for the Bucherer-Bergs reaction [54]. Catalytic reduction of 76, as shown in Scheme V, completed the synthesis of 3-aminospiro[9H-fluoren-9,4'-imidazolidine]-2',5'-dione, (73).

The inability to produce the amino hydantoin 71 from 1-amino-9-fluorenone (67) was also believed to result from the increased electron density of the carbonyl due to the electron
Figure 13: Structural comparison of 4 substituted benzaldehydes and 3 substituted 9-fluorenone.

Scheme V

Synthesis of 3-aminospiro[9H-fluoren-9,4'-imidazolidine]-2',5'-dione (73)

releasing ability of the amine substituent. If this were the case 1-nitro-9-fluorenone (77) should prove reactive under Bucherer-Bergs conditions, as was 3-nitro-9-fluorenone (75), and yield the
corresponding nitro spirohydantoin \(82\), which could be catalytically reduced, in a manner similar to that of \(76\), and yield the desired amino spirohydantoin \(71\). Unfortunately, 1-nitro-9-fluorenone (77) is not commercially available and had to be synthesized. A search of the literature revealed that the first synthesis of 77 was performed by Chase and Hey in 1952 [90]. They reported the total synthesis of 77 from o-toluic acid, which required 7 steps to complete. Since 1-amino-9-fluorenone (67) is readily available, a route to 77 involving the direct conversion of 67 into 77 was sought.

The first attempted synthesis of 77 directly from 67 utilized diazonium chemistry as shown in Scheme VI. Formation of the diazonium fluoroborate and reaction with aqueous sodium nitrite (NaNO₂) in the presence of finely divided copper metal catalyst, according to the procedure of Starkey, produced unreacted starting material and a small amount of 9-fluorenone (78), but none of the desired product [91]. An alternate procedure was attempted, which utilized the diazonium sulfate and a mixture of copper salts as a catalyst, Scheme VI [92]. Although the desired compound, 77, was produced, the yield was poor. Additionally, a small amount of 9-fluorenone was produced and unreacted starting material was recovered. Attempts at increasing the yield by altering the reaction conditions were largely unsuccessful, with yields generally in the range of 20 - 30%. Attempts at synthesizing 77 via diazonium chemistry were abandoned.

A review of the literature, uncovered another paper dealing with the synthesis of 1-nitro-9-fluorenone. In their paper, Huntress et
al., outlined 3 synthetic sequences, one of which involved the conversion of 1-amino-9-fluorenone to 1-nitro-9-fluorenone via its diazonium sulfate salt [93]. They reported a yield of 34% for this reaction. They also reported their inability to convert the diazonium fluoroborate to the desired 77. Interestingly, Huntress et al. cited two literature procedures which they followed in their synthesis of 77 via diazonium chemistry [91,92]. These same two articles were found independently, and their reaction procedures utilized by this researcher, in his attempts to synthesize 77. Huntress et al. also outlined two total syntheses of 77, however these synthetic sequences
required a number of steps to complete and overall gave poor yields [93].

The oxidation of the amine 67 to yield 77 directly was seen as an alternative to diazonium chemistry. The first attempt at oxidation of the amine to a nitro group, Scheme VII, utilized m-chloroperbenzoic (MCPBA) as the oxidant. On a small scale (2.5 mmol) oxidation with MCPBA produced about 35% of the desired 77. However, upon scale up the yield dropped to approximately 20%. Oxidation with glacial acetic acid and 30% H₂O₂ was also attempted, however this produced a large number of side products [94]. Approximately 35% of a crude product was isolated but upon TLC analysis was found to contain 4-5 compounds. It became apparent that a direct route to 77 from 67 was not going to be easily found.

Scheme VII

Synthesis of 1-nitro-9H-fluoren-9-one (77) from 1-amino-9H-fluoren-9-one (67) via oxidation

Another route to 77 was envisioned, as outlined in Scheme VIII. Previously, 1-nitro-9-fluorene (81) has been synthesized from
1-amino-9-fluorenone (67) by Yost et al. [94]. Yost et al. first reduced the carbonyl of 67 using Wolff-Kishner chemistry and then oxidized the amine, of the resulting 1-amino-9-fluorene (80), using maleic anhydride and 90% H2O2 to give 1-nitro-9-fluorene (81). 1-Nitro-9-fluorenone (77) was envisioned to arise from the oxidation of 81 using known chemistry. Reduction of 67 was performed according to the literature procedure [94], however, the oxidation of amine 80 to the nitro compound 81 was accomplished with MCPBA instead of maleic anhydride and 90% H2O2. Unlike the oxidation of amine 67 the oxidation of 80 was rapid, clean and high yielding. Undoubtedly, the presence of the ketone in 67 complicated the oxidation of the amine. The oxidation of the fluorene 81 to the fluorenone 77 was easily accomplished using sodium dichromate in glacial acetic acid [96]. These oxidizing conditions have been previously used to synthesize 9-fluorenone-2-carboxylic acid from 2-acetylfluorene [96].

As Scheme IX indicates, 77 was successfully converted to the nitro-spirohydantoin 82 via the Bucherer-Borges reaction. The successful completion of this reaction indicated, as did the successful synthesis of 76 from 75, that the electron releasing amino group in direct conjugation with with the carbonyl of the fluorenone ring system was probably responsible for lack of reactivity of compound 67 under Bucherer-Borges conditions. Catalytic reduction, using conditions similar to those for the reduction of 76, gave the desired product 71.

With the 4 spirohydantoins amines, 71 - 74 successfully synthesized, the synthesis of the desired isothiocyanates, 35 - 38 could begin.
Scheme VIII

**Synthesis of 1-nitro-9H-fluoren-9-one (77)**

Reaction of compound 77 with MCPBA in refluxing dichloromethane produced the desired 1-nitro-9H-fluoren-9-one (81).

Scheme X outlines the synthesis of the isothiocyanates 36 - 38 from the corresponding amines 72 - 74. Reaction of amines with thiophosgene is known to produce isothiocyanates in high yields [97]. Reaction of compounds 72, 73 and 74 with a 20 to 35% excess of thiophosgene, in refluxing acetone, produced the desired isothiocyanates 36, 37 and 38 respectively. However, under similar reaction conditions 71 was never completely converted to the corresponding isothiocyanate 35. Extended reaction times did not lead to increased conversion. Because the hydantoins are poorly soluble in almost all organic solvents,
Scheme IX

Synthesis of 1-aminospiro[9H-fluoren-9,4'-imidazolidine]-2',5'-dione (71)

separation of the desired product from side products or unreacted starting material is difficult to accomplish by either recrystallization or chromatography. Therefore, essentially complete conversion to product is desired to simplify the purification of the product. An alternate procedure, that would allow for the more complete conversion of 71 to the desired 35, was sought.
Thiophosgene and isothiocyanates are stable enough in aqueous solutions to allow for the synthesis of isothiocyanates under aqueous conditions [97]. Previously, it has been shown that aromatic amino acids could be converted to the corresponding isothiocyanates under slightly basic conditions [97]. The first attempt at making 35 under aqueous conditions involved the addition of a sufficient quantity of 30% KOH to a suspension of 71 in water to cause dissolution. A large excess of thiophosgene was then added. After a short period of time a solid precipitated, and after 1 hour this solid was collected. Analysis by TLC and NMR indicated the presence of 2 compounds; unreacted starting material and presumably product. During the reaction of thiophosgene with an amine 2 moles of HCl are generated for
every mole of amine and thiophosgene that react. Apparently, during the reaction, the HCl generated, not surprisingly, neutralized the hydantoin salt, causing it to precipitate and essentially preventing any further isothiocyanate formation. Therefore, during the next attempt at the synthesis of **35**, after addition of the thiophosgene and the formation of a solid, a small amount of 30% KOH was added to dissolve the solid. After dissolution of the solid, thiophosgene was added again. The addition of 30% KOH and thiophosgene were continued until conversion of the amine **71** to **35** was complete. Using these reaction conditions, as outlined in Scheme XI, the desired isothiocyanate **35** was produced.

**Scheme XI**

_Synthesis of 1-isothiocyanatospiro[9H-fluoren-9,4'-imidazolidine]-2',5'-dione (35)_

![Chemical structure](image)

3.1.2 Synthesis of reversible inhibitors

Scheme XII outlines the synthesis of the fluorenylidene acetic acids **51** - **53** and the fluorenyl acetic acids **54** - **56**. 9-Fluorenone (**78**) and 2-fluoro-9-fluorenone (**83**) are commercially available, while
2,7-difluoro-9-fluorenone (84) was synthesized via the decomposition of the corresponding diazonium fluoroborate salt as previously described by Ares [68]. The production of 85, as shown in Scheme XII, has been previously described by Bergmann and Solomonovic [98]. Saponification of the fluorenylidene acetate esters 85 - 87 was accomplished using 30% KOH and a 1:1 mixture of ethanol and acetonitrile as a solvent system. Catalytic hydrogenation of the fluorenylidene acetic acids using 5% Pd/C as catalyst, under basic conditions, afforded, after acidification, the fluorenyl acetic acids 54 - 56. As indicated in Scheme XII, the mono-fluorinated compounds 88 and 52 were produced as a mixture of E and Z isomers. These isomers could not be separated by low pressure liquid chromatography, however, they could be separated by high pressure liquid chromatography (HPLC). During the workup of the separated isomers of 86, which required the removal of large amounts of solvent, the isomers reisomerized. The reisomerization after the separation of the isomers happened twice. Further attempts at the separation of the isomers was contingent upon the biological activity of 52.

The outline for the synthesis of the fluorenylidene and fluorenyl tetrazoles, 61 and 62 respectively, is shown in Scheme XIII. This sequence closely follows that used for the synthesis of the fluorenylidene and fluorenyl acetic acids shown previously. Compound 88 was synthesized from 9-fluorenone in a manner similar to that of the fluorenylidene ester 85. The synthesis of the desired tetrazole 58 was proposed to follow the sequence 88 → 61 → 62 as shown in Scheme XIII.
Scheme XII

Synthesis of fluorenylidene and fluorenyl acetic acids, 51 - 53 and 54 - 56

However, numerous attempts of the synthesis of 61 using well known techniques previously cited in the literature proved futile.

Initial attempts of the synthesis of 61 utilized the procedures of Arnold and Thatcher [99]. Anhydrous aluminum chloride (AlCl₃), sodium azide (NaN₃) and 88 were refluxed in dry THF for 24 hours. However, after 24 hours no appreciable amount of product 58 could be isolated. A search of the literature revealed that Nohara et al. successfully synthesized allylic tetrazoles from the corresponding nitriles using
Scheme XIII

Synthesis of fluorenylidene and fluorenyl tetrazoles (59) and (60)

Conditions similar to Arnold and Thatcher's [100]. Trans-cinnamonic nitrile (92), which is structurally similar to 68, was used in a test reaction to determine if a structurally similar nitrile could be transformed into the corresponding tetrazole using these reaction conditions. The tetrazole 93 is a known compound but has been synthesized by an alternate route [101]. Scheme XIV outlines the synthesis of compound 93. The successful synthesis of 93 indicated that the reaction conditions of Nohara et al. could be used to synthesize tetrazoles of this structural type. However, while the
reactions of Nohara et al. generally required a few hours for completion, the synthesis of 93 from trans-cinnamonic acid required 4 days. When these reaction conditions were extended to the synthesis of 61 no significant amount of the desired tetrazole could be isolated, even after 4 days of reaction.

Scheme XIV

Synthesis of trans 5-(2-phenylethenyl)tetrazole (93)

Trialkyltin azides have also been shown to be useful in the synthesis of tetrazoles [102-104]. A tetrazole containing peptide has been synthesized from the corresponding nitrile using tributyltin azide in THF at 80°C sealed in a glass reaction vessel for 4 days [102]. However, no significant amount of the desired product 61 could be isolated when these reaction conditions were employed. A large amount of unreacted starting material was recovered. The reaction conditions of Finnegan et al. have also been used to produce a number of tetrazoles [105-107]. The reaction conditions of Finnegan et al. utilize NaN₃, NH₄Cl, as a reaction catalyst, and DMF as a solvent at
elevated temperatures to synthesize tetrazoles from the corresponding nitriles [105]. When these conditions were applied to the synthesis of 61 a black tarry residue was isolated which upon TLC analysis indicated the presence of a large number of side products and unreacted starting material.

The reaction between nitriles and AlCl$_3$/NaN$_3$ and tributyltin azide is summarized in Figure 14. In each reaction a large stable intermediate is formed, which is hydrolyzed, with acid, during the workup procedure [74,77]. Although the reaction between trans-cinnamonic nitrile and AlCl$_3$/NaN$_3$ was successful, the formation of the tetrazole 61 may be retarded or prevented by the increased steric bulk provided by the additional aromatic ring of the fluorene ring system. The synthesis of 61 was temporarily abandoned, and may be resumed depending on the biological activity exhibited by the tetrazoles 62 and 63.

\[
\text{R-C} = \text{N} + (\text{Bu})_3\text{SnN}_3 \rightarrow \text{H}^+ \rightarrow \text{R-C} = \text{N} - \text{Sn(Bu)}_3
\]

\[
\text{R-C} = \text{N} + \text{Al(N}_3)_2 \rightarrow \text{H}^+ \rightarrow \text{R-C} = \text{N}
\]

*Figure 14: Summarized mechanism of tetrazole formation*
Since 61 proved difficult to synthesize an alternate route to 62 was devised. Catalytic reduction of the double bond of 88 generated the fluorenyl acetonitrile 89, which unlike 88 was conveniently transformed to the corresponding tetrazole 62 using reaction conditions that had failed previously for the synthesis of 61. The increased reactivity of 89 relative to 88 may be related to the increased flexibility afforded by the reduction of the double bond of 88. Since 89 is more flexible than 88, it may avoid the steric interactions that may be responsible for the poor reactivity of 88.

Compound 94, 9-fluorenecarbonitrile, was prepared from commercially available 9-fluorenecarboxylic acid (57) as shown in Scheme XV. Formation of the acid chloride by reaction with oxalyl chloride and reaction of the acid chloride with anhydrous ammonia, in ether, generated 9-fluorenecarboxamide, as previously described [108]. The amide was dehydrated through the actions of phosphorous oxychloride (POCl$_3$) in DMF at 80°C. 9-fluorenecarbonitrile has been previously synthesized by an alternate route, beginning with fluorene and continuing through 9-formylfluorene, the oxime and ending with the dehydration of the oxime to yield 9-fluorenecarbonitrile [109]. The route shown in Scheme XV was chosen because the reactions leading to 9-fluorenecarboxamide were rapid and high yielding and because the starting material (57) was already at hand. Reaction of the nitrile 94 with AlCl$_3$/Na$_3$ in refluxing THF for 3 days gave the desired tetrazole 63.
Scheme XVI outlines the synthesis of (±)spiro(cyclopropane-1,9'-fluoren)-2-carboxylic acid (58). Compound 58 has been previously synthesized by the Smith, Kline and French company for use as a synthetic intermediate in the synthesis of antipsychotics [110]. The synthesis of 58, by SK&F was accomplished by the room temperature decomposition of 95 in the presence of ethyl acrylate in ether over an 18 hour period [110]. Subsequent isolation of the cyclopropyl ethyl ester and hydrolysis yielded 58 [110]. In the alternate sequence chosen, the procedure of Baltzly et al. was used for
the synthesis of diazofluorene 95 [111]. Although the procedure used for the synthesis of 95 was similar to that of the SK&F procedure, a slightly different procedure was used for the synthesis of 58. Compound 95 was dissolved in a small amount of toluene and added to a refluxing solution of ethyl acrylate. Removal of the volatile organics left the cyclopropyl ethyl ester, which without further purification was saponified using 30% KOH. The acid 58 was isolated after acidification of the reaction mixture.

Scheme XVI

Synthesis of (±)spiro(cyclopropane-1,9'-fluorene)-2-carboxylic acid (58)

3.2 BIOLOGICAL RESULTS

The isolation and purification of rat lens aldose reductase (RLAR) has been previously described in the literature [69]. The inhibitory activity of the irreversible and reversible inhibitors discussed previously were determined by Drs. Peter Kador, Toshihiro Nakayama and Sanai Sato, at the National Eye Institute, National Institutes of Health [45]. Before the determination of irreversible inhibitory activity reactive compounds, such as 2-mercaptoethanol and glycerol
that are used to stabilize the enzyme during purification, are removed by passing the purified enzyme through a Pharmacia PD-10 column equilibrated with 0.1 M phosphate buffer at pH 7.4. To determine the activity of the irreversible inhibitors 27 and 33 - 38 the purified enzyme was incubated with the appropriate inhibitor for 5 minutes at room temperature in 0.1 M phosphate buffer at pH 7.4. This mixture was then applied to a Pierce GF-5 desalting column and eluted with 0.1 M phosphate buffer, pH 7.4, containing 10 mM 2-mercaptoethanol. The effluent was collected in glass tubes, containing 0.1 mL of a 1.0 mg/mL solution of BSA. Enzyme activity was assayed by monitoring the decrease of NADPH spectrophotometrically, at 340 nm, using 10 mM DL glyceraldohyde as substrate. Inhibitory activity was determined by comparing the activity of RLAR incubated in the presence of inhibitor to that of similarly treated RLAR in the absence of inhibitor. The experimental techniques for the determination of irreversible activity of ARIs have been described in the literature [45]. The concentrations of inhibitors needed to cause a 50% inhibition of enzyme activity (IC50) were estimated from least square regressions of the Marquart-Leveberg iterative curve fitting algorithm of dose response curves. The techniques used for the determination of IC50 values has also been described in the literature [45,55].
3.2.1 Irreversible Inhibitors

Table 9 shows the IC<sub>50</sub> values determined for the inhibitors 27, 28, 33 and 34. As expected the bromo- and iodoacetamides, 33 and 34 respectively, were significantly more active than the chloroacetamide, 27. Compound 34 inhibited RLAR by 89% at a concentration of 10<sup>-4</sup> M, while the bromoacetamide 33 caused a 46% inhibition of RLAR at 10<sup>-4</sup> M. Also, as shown in Table 9, the IC<sub>50</sub> values for haloacetamides were not very different indicating that the increased activity of the bromo- and iodoacetamides was not due to an increase in potency. As anticipated the trend of activity was shown to follow iodo- > bromo- > chloroacetamide. These results indicate that the poor activity of 27 was probably due to a lack of chemical reactivity of the electrophile.

Table 9

<table>
<thead>
<tr>
<th>Cmpd.</th>
<th>Apparent IC&lt;sub&gt;50&lt;/sub&gt; (uM)</th>
<th>Irreversible Activity % Inhibition (10&lt;sup&gt;-4&lt;/sup&gt;M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>27</td>
<td>0.6</td>
<td>18</td>
</tr>
<tr>
<td>33</td>
<td>0.7</td>
<td>48</td>
</tr>
<tr>
<td>34</td>
<td>0.5</td>
<td>89</td>
</tr>
</tbody>
</table>

The reversible and irreversible activities of the isothiocyanates 35 - 38 are shown in Table 10. As shown, the reversible activity of compounds 36 - 38 are not appreciably different. However, the
4-isothiocyanato analogue, 38, is much less active as an irreversible inhibitor than either 36 or 37. Additionally, the IC\textsubscript{50} for compound 35 is much larger than that of any other irreversible inhibitor tested. This data may indicate that the inhibitor binding site is poorly tolerant of steric bulk at this position of fluorene based ARIs. A comparison of the IC\textsubscript{50} values of compounds 36 - 38 and 32 shows that, in this series of compounds fluorine exerts little effect on either the reversible or irreversible inhibitory activity. The irreversible activity of 35 has yet to be determined.

Protection studies designed to determine the specificity of irreversible inhibition of the irreversible inhibitors presented above are currently being conducted. Without the knowledge of the specificity of alkylation, comments about binding interactions, such as those described in Figure 9 on page 42 and Figure 10 on page 44, or the location of the proposed nucleophile at the inhibitor binding site, cannot be made.

Table 10

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Apparent IC\textsubscript{50} (uM)</th>
<th>Irreversible Activity % Inhibition (10\textsuperscript{-4}M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>0.50</td>
<td>56</td>
</tr>
<tr>
<td>35</td>
<td>189.1</td>
<td>N.D.</td>
</tr>
<tr>
<td>36</td>
<td>0.23</td>
<td>56</td>
</tr>
<tr>
<td>37</td>
<td>0.09</td>
<td>54</td>
</tr>
<tr>
<td>38</td>
<td>0.28</td>
<td>21</td>
</tr>
</tbody>
</table>
3.2.2 Reversible Inhibitors

Table 11, Table 12 and Table 13 show the IC₅₀ values determined for the spirohydantoins 12, 13 and 96 and the novel inhibitors 51 - 61 and 63 and 59. In almost every case, the activity of the spirohydantoins was greater than that of either the carboxylic acids or tetrazoles. Table 11 shows the effects of increasing fluorine substitution on the inhibitory activity of a series of fluorenylidene- and fluorenylacetic acid analogues, compounds 51 - 53 and 54 - 56 respectively. As with the spirohydantoins, 96, 12 and 13, the activities of the fluorenylidene- and fluorenylacetic acids were increased with increasing fluorine substitution. However, the effects of fluorine substitution were much more significant for the fluorenylacetic acid analogues 54 - 56. Interestingly, the activity of the fluorenylacetic acids, in the series 54 - 56 closely parallels the activity of the corresponding spirohydantoins 96, 12 and 13. Although as a group, the fluorenylacetic acids are approximately 15 fold less active than the corresponding spirohydantoins, compound 56 is slightly more active than the spirohydantoin 96.

Table 11
Effects of fluorine substitution on inhibitory activity of compounds 51 - 53 and 54 - 56

<table>
<thead>
<tr>
<th>Cmpd.</th>
<th>IC₅₀ (uM)</th>
<th>Cmpd.</th>
<th>IC₅₀ (uM)</th>
<th>Cmpd.</th>
<th>IC₅₀ (uM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td>0.857</td>
<td>51</td>
<td>88.0</td>
<td>54</td>
<td>15.0</td>
</tr>
<tr>
<td>12</td>
<td>0.043</td>
<td>52</td>
<td>54.8</td>
<td>55</td>
<td>3.44</td>
</tr>
<tr>
<td>13</td>
<td>0.024</td>
<td>53</td>
<td>15.5</td>
<td>56</td>
<td>0.431</td>
</tr>
</tbody>
</table>
Table 12 shows the effects of spatial orientation of the carboxylate on inhibitory activity. The fluorenlylacetic acid derivative 54 was the most active of this series of compounds. The spirocyclopropyl analogue 58 had very poor inhibitory activity, and as such was able to provide only 36% inhibition at a concentration of 100μM. These data indicate that a specific spatial orientation of the acidic functional group is necessary for optimum inhibitory activity, which is probably best achieved by the hydantoin moiety.

Table 12

<table>
<thead>
<tr>
<th>Compd.</th>
<th>IC₅₀ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td>0.857</td>
</tr>
<tr>
<td>51</td>
<td>88.00</td>
</tr>
<tr>
<td>54</td>
<td>15.00</td>
</tr>
<tr>
<td>57</td>
<td>180.0</td>
</tr>
<tr>
<td>58</td>
<td>100 (36%)</td>
</tr>
</tbody>
</table>
As shown in Table 13, the isosteric substitution of tetrazole ring for a carboxylate moiety had a profound effect on the activity of the fluorenlylacetic acid 54 while having little effect on the activity of 9-fluorenecarboxylic acid 57. The tetrazole 62 was almost 100 fold less active than the parent carboxylic acid 54. Interestingly, the difference in activity between 57 and its tetrazole analogue 63 was much smaller, each compound having an IC$_{50}$ value of approximately 200µM. Although a significant amount of activity was retained when a carboxylic acid moiety was substituted for the hydantoin ring system, inhibitory activity was virtually abolished when the carboxylic acid moiety was subsequently substituted with a tetrazole ring system. The poor activity of the tetrazole 62, relative to the parent carboxylate 54, may be due to electronic or steric differences between the two functional groups.

Table 13
Comparison of the reversible inhibitory activity of compounds 54, 57, 62, 63 and 96

<table>
<thead>
<tr>
<th>Cmpd.</th>
<th>IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td>0.857</td>
</tr>
<tr>
<td>54</td>
<td>15.00</td>
</tr>
<tr>
<td>57</td>
<td>180.0</td>
</tr>
<tr>
<td>62</td>
<td>1360</td>
</tr>
<tr>
<td>63</td>
<td>213.8</td>
</tr>
</tbody>
</table>
Based on the data presented above, a number of conclusions regarding the irreversible and reversible inhibition of RLAR can be made.

1. The increased activity of the Bromo- and Iodosacetamides, 33 and 34 respectively, indicates that the poor activity of 27 was due to a lack of chemical reactivity of the electrophile. The activity of these inhibitors was found to follow the trend I > Br > Cl.

2. Data shown in Table 10 on page 81 suggests that the nucleophile proposed to exist in the inhibitor binding site of AR may be localized, and may lie in an area accessible to the isothiocyanates of compounds 36 and 37. However, since compounds 35 - 38 show differences in potency, based on their IC₅₀ values, the differences in irreversible activity might simply be a result of different inhibitor binding site affinities displayed by each compound. Therefore, an accurate explanation of the differential activity of these compounds cannot be made.

3. The poor potency displayed by 35 indicates that steric bulk may not be well tolerated at the one position of the fluorene ring system, by the inhibitor binding site.

4. The fluorenylacetic acid derivatives consistently showed greater reversible activity than any of the other novel reversible aldose reductase inhibitors synthesized.

5. The substitution of the tetrazole ring system for a carboxylate moiety was largely unsuccessful. The reasons for the large decrease in the activity upon substitution of the carboxylate of 54 with a tetrazole ring, to yield 62 are not readily apparent.
The difference in both steric and electronic properties of a tetrazole ring relative to a carboxylate moiety may play a role in the poor activity of 62.
CHAPTER IV
EXPERIMENTAL

Melting points are uncorrected and were determined with a Thomas-Hoover melting point apparatus. IR spectra were obtained with a Beckman 4230 infrared spectrophotometer. NMR spectra were obtained at The Ohio State University College of Pharmacy, with either an IBM NR/250 FTNMR (250 MHz), IBM AF/270 FTNMR (270 MHz) spectrometers or at the Ohio State University Chemical Instrumentation Center with a Bruker AM-500 (500 MHz) spectrometer. Mass spectra were obtained with either a Kratos MS25RFA mass spectrometer at The Ohio State University College of Pharmacy or at the Ohio State University Chemical Instrumentation Center with a Kratos MS-30 mass spectrometer. Anhydrous THF was produced by distillation over sodium using benzophenone as an indicator for dryness. Diethyl ether was stored over sodium. Anhydrous diglyme and DMF were purchased from the Aldrich Chemical Company in Sure-Seal bottles and were used without further purification. Chemical analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tn. All analytical results for the indicated elements were within ± 0.4% of the theoretical values.
To a suspension of 64 [68] (1g, 3.5mmol) in 50mL of a 1:2 mixture of MeOH/CH$_3$CN was added 20mL of 30% aqueous KOH. The reaction was allowed to continue for 2 hours, with stirring, at room temperature. During the reaction the starting material passed into solution. Upon completion of the reaction, the organics were removed at reduced pressure and the resulting basic solution was acidified to pH 5 with concentrated HCl. The resulting suspension was allowed to stand for 5 minutes and the solids were collected by filtration. The solids were dried in vacuo and recrystallized from MeOH to yield 0.85g (90%) of 65 as a yellow solid. mp 290-291°C (lit. mp [52] 292°C). $^1$H NMR (DMSO d$_6$, 250 MHz) $\delta$ 13.0 (broad s, 1H, D$_2$O exchangeable,), 8.09-8.05 (m,2H, ArH), 7.97 (d, $J$ = 2.3 Hz, 1H, ArH ortho to NH$_2$), 7.62 (dd, 1H, ArH), 7.30 (d, $J$ = 2.3 Hz, 1H, ArH), 6.03 (broad s, 2H, D$_2$O exchangeable,), 4.69 (d, 2H, CH$_2$COOH).
To a suspension of 65 (0.3 g, 1.1 mmol) in 10 mL pyridine at 0°C was added, at one time, a mixture of chloroacetic anhydride (0.95 g, 5.5 mmol) and 5 mg 4-dimethylaminopyridine (DMAP) in 2 mL anhydrous THF. The reaction was allowed to continue with stirring at 0°C for approximately 1 hour. Upon the completion of the reaction 100 mL of water was added and allowed to stir at room temperature for 20 minutes. At this time 100 mL CH$_2$Cl$_2$ was added and the aqueous layer was acidified with concentrated HCl. The neutralized acid 27 fell out of solution, but was not soluble enough in CH$_2$Cl$_2$ or other organic solvents to allow for efficient extraction. Solid 27 formed at the water/organic interface and was collected by filtration. The organic layer was dried and evaporated at reduced pressure to yield a solid that was combined with the solid collected by filtration. These combined solids were dried in vacuo and recrystallized from acetone, to yield 0.255 g (67%) of 27 as a light yellow solid, mp 250-251°C (lit [68] 251°C).
To a suspension of 65 (0.3 g, 1.1 mmol), in 10 mL anhydrous THF at 0°C was added a mixture of bromoacetic anhydride (1.44 g, 5.5 mmol), 5 mg DHAP in a small amount of THF. This mixture was allowed to stir for 1 hour at 0°C, after which time approximately 20 mL of water was added, to hydrolyze any mixed anhydride that may have formed. This mixture was allowed to stir at room temperature for about 20 minutes. The resulting mixture was extracted with a large amount of ethyl acetate (3 \times 200 mL). The ethyl acetate extracts were combined, dried over magnesium sulfate (MgSO₄), filtered and evaporated under reduced pressure leaving a light yellow solid. A small amount of 33 collected at the organic/water interface. The solids were collected by filtration and added to the solids isolated by extraction. The combined solids were recrystallized from acetone yielding 0.375 g (87%) of 33 as a light yellow solid.

dp 259-260°C. $^1$H NMR (DMSO d$_6$, 250 MHz) δ 13.1 (broad s, 1H, D₂O exchangeable), 11.03 (s, 1H, D₂O exchangeable), 8.79 (d, J = 1.9 Hz, 1H, ArH, ortho to acetamide), 8.6 (d, J = 1.9 Hz, 1H, ArH, ortho to
acetamide), 8.44 (apparent d, J = 7.7 Hz, 1H, ArH), 8.40 (apparent d, J = 7.7 Hz, 1H, ArH), 7.87-7.81 (apparent t, J = 7.7 Hz, 1H, ArH), 4.72 (s, 2H, CH$_2$COOH), 4.15 (s, 2H, CH$_2$Br). IR (KBr) cm$^{-1}$: 3600-2700 (COON). Mass spectrum, m/z: 391.997 (theoretical 391.983) (H$^+$, 81Br), 390.0005 (theoretical 389.985) (H$^+$, 71Br). Analysis for C$_{16}$H$_{11}$N$_2$O$_5$Br; calculated: C, 49.13; H, 2.83; N, 7.16; found: C, 49.13; H, 2.99; N, 6.78.

5-Iodoacetamido-1,3-1H-benz[d]isoquinoline-2(3H)-acetic acid (34)

The procedure for the synthesis of 34 was similar to that used for the synthesis of 33. Thus, a solution of iodoacetic anhydride (0.654 g, 1.85 mmol) and 5 mg DMAP in a small amount of THF was added to a rapidly stirred suspension of 65 (0.1 g, 0.37 mmol) in THF at 0°C. The reaction mixture was allowed to stir for an additional hour at 0°C, at which time 10 mL water was added. This mixture was allowed to stir at room temperature for an additional 15 minutes, after which time the mixture was extracted with a large amount of ethyl acetate (3 x 100 mL). The combined ethyl acetate extracts were dried (MgSO$_4$) and evaporated under reduced pressure to provide a light yellow solid. The solid that formed at the organic/water interface was collected by
filtration and combined with the solids from the extraction process. The combined solids were dried in vacuo and recrystallized from acetone to yield 0.13g (80%) of 34 as a light yellow solid. dp 222-223°C. 

$^1$H NMR (DMSO d$_6$, 250 MHz) δ 13.1 (broad s, 1H, D$_2$O exchangeable), 10.97 (s, 1H, D$_2$O exchangeable), 8.77 (d, J = 2.0 Hz, 1H, ArH ortho to acetamide), 8.58 (d, J = 2.0 Hz, 1H, ArH, ortho to acetamide), 8.47-8.41 (m, 2H, ArH) 8.38 (apparent t, J = 7.8 Hz, 1H, ArH), 4.72 (s, 2H, CH$_2$COOH), 3.91 (s, 2H, CH$_2$I). IR (KBr) cm$^{-1}$: 3650-2700 (COOH). Mass spectrum, m/z: FAB 439 (M$^+$ +H). Analysis for C$_{16}$H$_{11}$N$_2$O$_5$I; calculated: C, 43.86; H, 2.53; N, 6.39; found: C, 43.92; H, 2.60; N, 6.14.

3-Nitrospiro[9H-fluoren-9,4'-imidazolidine]-2',5'-dione (76)

![Chemical structure of 3-Nitrospiro[9H-fluoren-9,4'-imidazolidine]-2',5'-dione](image)

Commercially available 3-Nitro-9-fluorenone 75 (2.2 g, 9.8 mmol), potassium cyanide (KCN) (1.5 g, 23.0 mmol), ammonium carbonate ((NH$_4$)$_2$CO$_3$) (4.36 g, 45 mmol) and 40 mL absolute ethanol were added to a 300 mL Parr stainless steel reaction bomb. The bomb was sealed and maintained at a temperature between 105-110°C in an oil bath for 48 hours. After 48 hours the vessel was cooled to room temperature, opened and the contents diluted with 100 mL water. The mixture was
cautiously acidified, in a hood, with HCl. The solids were collected by filtration. The filtrate must be rebasified before disposal! The solids collected above were dissolved in approximately 20 mL 1N NaOH and filtered. The dark brown filtrate was acidified, to pH 2, with concentrated HCl. The suspension was allowed to stand at 0°C overnight. The solids were collected by filtration, dried in vacuo and recrystallized twice from acetone/H2O to yield 1.8 g (61%) of 76 as a tan solid. dp 300 - 305°C. 1H NMR (DMSO d6, 250 MHz) δ 11.41 (s, 1H, D2O exchangeable), 8.79 (d, J = 2.1 Hz, 1H, ArH ortho to nitro), 8.73 (s, 1H, D2O exchangeable), 8.21 (dd, 1H, J = 2.1 Hz, J = 8.32 Hz, 1H, ArH ortho to nitro), 8.18-8.15 (m, 1H, ArH), 7.8 (d, J = 8.32 Hz, 1H, ArH meta to nitro), 7.57-7.53 (m, 3H, ArH). IR (KBr) cm⁻¹: 1785 (C=O), 1720 (C=O), 1560 (NO2), 1390 (NO2). Mass spectrum, m/z: 295 (M+), 224 (base). Analysis for C15H9N3O4·0.5H2O; calculated: C, 59.21; H, 3.31; N, 13.81; found: C, 59.33; H, 3.27; N, 13.91.

3-Aminospiro[9H-fluoren-9,4'-imidazolidine]-2',5'-dione (73)

![Diagram of 3-Aminospiro[9H-fluoren-9,4'-imidazolidine]-2',5'-dione (73)](image.png)

Compound 76 (0.4 g, 1.4 mmol), 50 mL of absolute ethanol and 120 mg of 5% palladium on carbon were added to a Parr hydrogenation bottle and hydrogenated for 4 hours at 40 psi and room temperature. The catalyst
was removed by filtration through a Celite pad. The resulting brown liquid was concentrated and water was added until the cloud point was reached. The mixture was allowed to stand at -10°C overnight. The resulting solid was collected by filtration and dried in vacuo yielding 0.16 g (43%) of 73 as a tan solid. dp = 280°C. $^1$H NMR (DMSO d$_6$, 250 MHz) δ 11.0 (s, 1H, D$_2$O exchangeable), 8.40 (s, 1H, D$_2$O exchangeable), 7.64 (apparent d, J = 7.4 Hz, 1H, ArH), 7.45-7.26 (m, 3H, ArH), 7.05 (d, J = 8.1 Hz, 1H, ArH meta to NH$_2$), 6.95 (d, J = 1.96 Hz, 1H, ArH ortho to NH$_2$), 6.54 (dd, 1H, J = 8.1 Hz, J = 1.96 Hz, ArH ortho to NH$_2$), 5.39 (s, 2H, D$_2$O exchangeable, NH$_2$). IR (KBr) cm$^{-1}$: 3405 (NH$_2$), 1790 (C=O), 1725 (C=O). Mass spectrum, m/z: 265 (M$^+$), 194 (base). Analysis for C$_{15}$H$_{11}$N$_3$O$_2$·0.25H$_2$O; calculated: C, 66.78 H, 4.30; N, 15.58; found: C, 66.68; H, 4.20; N, 15.48.

1-Nitro-9H-fluorene (81)

![Diagram of 1-Nitro-9H-fluorene]

To a rapidly stirred solution of 85% m-Chloroperbenzoic acid, MCPBA, (8.4 g, 41.5 mmol) in 125 mL of CH$_2$Cl$_2$ was added 1-amino-fluorene (80) [95] (1.5 g, 8.3 mmol). The reaction was allowed to continue for 2 hours, at room temperature, after which time, 100 mL of 10% NaOH was added. The layers were separated and the aqueous layer was extracted.
with CH$_2$Cl$_2$ (3 x 100 mL) and the organic extracts were combined and washed with 10% NaOH (1 x 200 mL). The layers were separated and the organic layer was dried (MgSO$_4$) and evaporated under reduced pressure to yield 1.4 g (80%) of 81 as a light yellow solid. mp 102-102.5°C (lit. mp [95] 104-106°C). $^1$H NMR (Acetone d$_6$, 250 MHz) $\delta$ 8.26 (apparent d, J = 7.48 Hz, 1H, ArH), 8.14 (dd, J = 8.22 Hz, J = 0.9 Hz, 1H, ArH), 7.95 (m, 1H, ArH), 7.70-7.64 (m, 2H, ArH), 7.44-7.40 (m, 2H, ArH), 4.35 (s, 2H, CH$_2$).

1-Nitro-9H-fluoren-9-one (77)

![1-Nitro-9H-fluoren-9-one](image)

Compound 81 (1.2 g, 5.7 mmol) was dissolved in 30 mL glacial acetic acid with heating. After 81 had completely dissolved, sodium dichromate dihydrate (Na$_2$Cr$_2$O$_7$-2H$_2$O) (10 g, 33.5 mmol) was added over a period of 30 minutes. After the addition of Na$_2$Cr$_2$O$_7$-2H$_2$O was complete the mixture was heated to a gentle reflux for 1.5 hours. Water (300 mL) was added after the reaction mixture had cooled, and this mixture was extracted with ethyl acetate (2 x 200 mL). The combined ethyl acetate extracts were washed with 10% NaOH (2 x 50 mL), dried (MgSO$_4$) and evaporated under reduced pressure to provide 1 g (78%) of 77 as a bright yellow solid. mp 186-188°C (lit. mp [90] 187-188°C). $^1$H NMR
The synthesis of 82 was performed in a manner similar to that for the synthesis of 76. Thus, 1-Nitro-9-fluorenone (77) (1 g, 4.4 mmol), KCN (0.687 g, 10.4 mmol), (NH₄)₂CO₃ (2 g, 20.8 mmol) and 40 mL of absolute ethanol were placed in a 300 mL stainless steel reaction bomb. The bomb was sealed and heated to a temperature between 105-110°C for 48 hours. After cooling the bomb was open and the contents diluted with about 30 mL water. The diluted reaction mixture was cautiously acidified with concentrated HCl and filtered. The filtrate must be basified before discarding. The resulting solids were dissolved in a small amount of base (10 mL 30% KOH) and filtered. The resulting filtrate was acidified with concentrated HCl and allowed to stand overnight at 0°C. The solids were collected by filtration, dried in vacuo and recrystallized from acetone to yield 0.91 g (70%) of 82 as a tan solid. dp 324-325°C. ¹H NMR (DMSO d₆, 250 MHz) δ 11.43 (s, 1H, D₂O exchangeable), 8.42 (s, 1H, D₂O exchangeable), 8.37 (dd, J = 7.7
Hz, J = 0.8 Hz, 1H, ArH), 8.09 (dd, J = 8.1 Hz, J = 0.8 Hz, 1H, ArH), 8.03 (apparent d, J = 7.4 Hz, 1H, ArH), 7.83 (apparent t, J = 7.85 Hz, 1H, ArH), 7.60-7.45 (m, 3H, ArH). IR (KBr) cm⁻¹: 1785 (C=O), 1725 (C=O), 1545 (NO₂), 1400 (NO₂). Mass spectrum, m/z: 295 (M⁺). Analysis for C₁₅H₁₉N₃O₆; calculated: C, 61.02; H, 3.07; N, 14.23; found: C, 60.84; H, 3.14; N, 14.07.

1-Aminospiro[9H-fluoren-9,4'-imidazolidine]-2',5'-dione (71)

A mixture of 82 (0.8 g, 2.7 mmol), 120 mg of Pd/C and 50 mL of absolute ethanol were hydrogenated for 8 hours at 40 psi. The product was not appreciably soluble in ethanol or acetone and the workup procedure used in the synthesis of 73 had to be altered. The reaction mixture was gently warmed on a steam bath and filtered. The filtered solids were a mixture of product and catalyst. Therefore, the solids were dissolved in a small amount of 30% KOH and filtered. The filtrate was acidified and the resulting solids collected by filtration. The ethanol filtrate was evaporated under reduced pressure and the resulting solids were combined with the solids collected by filtration. The combined solids were dried in vacuo and recrystallized twice from absolute ethanol yielding 0.58g (81%) of 71 as a tan solid. dp
324-325°C. ¹H NMR (DMSO d₆, 250 MHz) δ 11.28 (s, 1H, D₂O exchangeable), 8.43 (s, 1H, D₂O exchangeable), 7.76 (apparent d, J = 7.4 Hz, 1H, ArH), 7.46-7.31 (m, 3H, ArH), 7.22-7.12 (m, 2H, ArH), 6.65 (apparent d, J = 7.5 Hz, 1H, ArH), 4.77 (s, 2H, D₂O exchangeable, NH₂).

IR (KBr) cm⁻¹: 3360 (NH), 3240 (NH). Mass spectrum, m/z: 265 (M⁺), 194 (base). Analysis for C₁₅H₁₁N₃O₂·0.25H₂O; calculated: C, 66.78; H, 4.24; N, 15.59; found: C, 66.79; H, 4.21; N, 15.45.

2-Isothiocyanato-spiro[9H-fluoren-9,4'-imidazoline]-2',5'-dione (36)

Compound 72 (0.13 g, 0.49 mmol), was prepared by the method of York [53], with the exception that the reaction time was lengthened to 48 hours from 24 hours, was added to 15 mL of acetonitrile and heated with stirring to reflux temperature. Thiophosgene (52 µL, 0.68 mmol) was rapidly added and the reaction was allowed to continue for 1 hour. After completion of the reaction the mixture was cooled, concentrated and allowed to stand at -10°C overnight. The resulting solids were collected by filtration and recrystallized from acetonitrile yielding 0.15 g (86%) of 36 as a light yellow solid. dp = 325-327°C. ¹H NMR (DMSO d₆, 250 MHz) δ 11.28 (s, 1H, D₂O exchangeable), 8.59 (s, 1H, D₂O exchangeable), 7.93 (apparent t, 2H, ArH), 7.75 (d, J = 1.8 Hz, 1H, ArH
ortho to NCS), 7.53 (dd, J = 8.0 Hz, J = 1.9 Hz, 1H, ArH ortho to NCS & H), 7.50-7.47 (m, 2H, ArH), 7.36 (m, 1H, ArH). IR (KBr) cm⁻¹: 2140 (NCS). Mass spectrum, m/z: 307 (M⁺), 236 (base). Analysis for C₁₆H₉N₃O₂S; calculated: C, 62.53; H, 2.95; N, 13.67; found: C, 62.44; H, 2.89; N, 13.42.

4-Isothiocyanato[9H-fluoren-9,4'-imidazolidine]-2',5'-dione (38)

The precursor amine 74 was prepared according to the literature procedure, with the exception that the reaction time was increased from 24 to 48 hours [53]. Compound 74 (0.1 g, 0.38 mmol) was added to 20 mL acetone and was heated with stirring to reflux temperature. Thiophosgene (35 µL, 0.46 mmol) was rapidly added and then reaction was allowed to continue for 1 hour. After completion of the reaction the mixture was cooled, concentrated and allowed to stand at -10°C overnight. The resulting solid was collected by filtration and dried in vacuo to yield 0.095g (82%) of 38 as a white powder. mp > 335°C. 

¹H NMR (DMSO d₆, 250 MHz) δ 11.33 (s, 1H, D₂O exchangeable), 8.65 (s, 1H, D₂O exchangeable), 7.94 (apparent d, J = 7.6 Hz, 1H, ArH) 7.63-7.41 (m, 6H, ArH). IR (KBr) cm⁻¹: 2140 (NCS). Mass spectrum, m/z: 307 (M⁺), 236 (base). Analysis for C₁₆H₉N₃O₂S; calculated: C, 62.53; H, 2.95; N, 13.67; found: C, 62.37; H, 2.91; N, 13.67.
Amino 73 (0.13 g, 0.49 mmol) was added to acetone and heated to reflux temperature with stirring. Thiophosgene (52 µL, 0.66 mmol) was rapidly added and the reaction was allowed to continue for 1 hour at reflux temperature. After completion of the reaction, the mixture was cooled, concentrated and allowed to stand at -10°C overnight. No significant amount of solid was produced and therefore a small amount of water was added until the cloud point was reached. The mixture was again allowed to stand at -10°C overnight. The resulting solid was collected by filtration and dried in vacuo. The dried solid was recrystallized from acetone/H₂O yielding 0.135 g (88%) of 37 as a light yellow solid. dp = 305-307°C. ^1H NMR (DMSO d₆, 250 MHz) δ 11.28 (s, 1H, D₂O exchangeable), 8.60 (s, 1H, D₂O exchangeable), 8.04 (d, J = 1.9 Hz, 1H, ArH), 7.94 (apparent d, J = 7.94 Hz, 1H, ArH), 7.57-7.36 (m, 5H, ArH). IR (KBr) cm⁻¹: 2040 (NCS). Mass spectrum, m/z: 307 (M⁺), 236 (base). Analysis for C₁₆H₉N₃O₂S·0.25H₂O; calculated: C, 61.62; H, 3.07; N, 3.48; found: C, 61.75; H, 3.06; N, 13.20.
**1-Isothiocyanatospiro[9H-fluoren-9,4'-imidazolidine]-2',5'-dione (35)**

![Chemical Structure](image)

Amine 71 (0.1 g, 0.38 mmol) was added to 5mL of H₂O at room temperature and a solution of 30% KOH was slowly added with stirring until 71 completely dissolved. A large excess of thiophosgene (0.1mL, 1.3 mmol) was rapidly added. A short while after the addition of thiophosgene a solid began to form. The solid was dissolved by the careful addition of 30% KOH, and thiophosgene (0.1 mL) was again added. This sequence was repeated until the conversion of 71 to 35 was complete. Upon completion of the reaction the mixture was acidified with HCl and the resulting solid was collected by filtration. The solid is dried in vacuo and recrystallized twice from acetone/H₂O to yield 0.9 g (76%) of 35 as a tan solid. d.p 334-335°C. ¹H NMR (DMSO d₆, 250 MHz) δ 11.53 (s, 1H, D₂O exchangeable), 8.60 (s, 1H, D₂O exchangeable), 7.95-7.89 (m, 2H, ArH), 7.61-7.39 (m, 5H, ArH). IR (KBr) cm⁻¹: 2105 (NCS). Mass spectrum, m/z: 307 (M⁺), 236 (base).

Analysis for C₁₆H₉N₃O₂S·0.25H₂O: calculated: C, 61.63; H, 3.19; N, 13.35; found: C, 61.98; H, 3.07; N, 13.47.
Compound 85 [98] (0.5 g, 2.0 mmol), was dissolved in 10 mL of a 1:1 mixture of MeOH/CH$_3$CN at room temperature with stirring. To this solution was added 2 mL of a 30% KOH solution. The reaction was complete within approximately 1 hour. The reaction mixture was acidified with 10% HCl and allowed to stand at 0°C overnight. The resulting solids were collected by filtration, washed with water and dried in vacuo to yield 0.41 g (93%) of 51 as a bright yellow solid. mp 225-226°C (lit. mp [98] 222°C, [112] 223.5-224.5°C). $^1$H NMR (MeOH d$_4$, 250 MHz) δ 8.74 (apparent d, J = 7.87 Hz, 1H, ArH), 7.75 (apparent d, J = 7.6 Hz, 1H, ArH), 7.71-7.66 (m, 2H, ArH), 7.43-7.31 (m, 2H, ArH), 7.30-7.23 (m, 2H, ArH), 6.85 (s, 1H, C=CH[(COOH)].
9H-Fluorenencetic acid (54)

Compound 85 (0.5 g, 2.0 mmol), was dissolved in 10 mL of a 1:1 mixture of MeOH/CH₃CN at room temperature with stirring. To this solution was added 2 mL of a 30% KOH solution. The reaction was complete within approximately 1 hour. The reaction mixture was acidified and the solid collected by filtration. The solid 51 was dissolved in 10 mL 10% NaOH, and added to a Parr bottle containing 50 mg Pd/C. This mixture was hydrogenated at 40 psi and room temperature for 4 hours. The catalyst was removed by filtration through a Celite pad. The resulting filtrate was acidified and allowed to stand for a few minutes. The solid was collected by filtration and dried in vacuo to yield 0.33 g (85%) of 54 as a white solid. mp 129-130°C (lit. mp [112,113] 131-132°C). ¹H NMR (MeOH d₄, 250 MHz) δ 7.76 (apparent d, J = 7.1 Hz, 2H, ArH), 7.56 (apparent d, J = 7.5 Hz, 2H, ArH), 7.38-7.25 (m, 4H, ArH), 4.31 (t, J = 7.2 Hz, 1H, CH₂CH₂), 2.71 (d, J = 7.2 Hz, CH₂COOH).
A solution of triethyl phosphonoacetate (2.24 g, 10.0 mmol) in 5 mL diglyme was added to a stirred suspension of NaH (60% dispersion, 0.6 g, 15.0 mmol) in 20 mL diglyme, under argon. After 1 hour a solution of 2-fluoro-9-fluorenone 83 (2.0 g, 10.0 mmol) in 10 mL diglyme was added to the mixture. The resulting mixture was heated to reflux temperature and maintained at that temperature for an additional 8 hours, after which time the reaction mixture was cooled, and diluted with 150 mL H₂O. The resulting solution was extracted with ethyl acetate (3 x 150 mL). The combined ethyl acetate extracts were dried (MgSO₄) and evaporated at reduced pressure to yield a solution of 86 in diglyme (approx. 20 mL). The excess diglyme was removed overnight through the use of a high vacuum pump. The resulting solid was purified by flash column chromatography, (5% EtOAc/Hexane). The combined fractions from the column were evaporated under reduced pressure yielding 1.75 g (65%) of 86 as a bright yellow solid. mp 58-68°C. ¹H NMR (DMSO d₆, 250 MHz) δ 8.62 (apparent d, J = 7.7 Hz, 1H, ArH), 8.56 (dd, J = 10.7 Hz, J = 2.4 Hz, 1H, ArH), 8.1 (apparent d, 1H, ArH), 7.99-7.78 (m, 5H, ArH), 7.46-7.44 (m, 2H, ArH), 7.34-7.31 (m, 4H,
ArH), 7.05 (s, 1H, C=CH(COOH)), 4.30 (q, J = 7.1 Hz, 4H, CH₂CH₃), 1.3 (t, J = 7.1 Hz, 6H, CH₂CH₃). IR (KBr) cm⁻¹: 1720 (C=O). Mass spectrum, m/z: 268 (M⁺). 223 (base), 196. Analysis for C₁₇H₁₃O₂F; calculated: C, 76.11; H, 4.88; found: C, 76.47; H, 5.24.

2-Fluoro-9H-fluoren-9-ylideneacetic acid (52)

\[
\begin{align*}
\text{COOH} \\
\text{F} \\
\end{align*}
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Compound 86 (0.5 g, 1.86 mmol) was dissolved in 10 mL of a 1:1 mixture of EtOH/CH₃CN with stirring. 2 mL of 30% KOH was added and the reaction was stirred for an additional hour at which time the volatile organic solvents were removed under reduced pressure. The resulting aqueous solution is acidified and allowed to stand overnight at 0°C. The resulting solid is collected by filtration, dried in vacuo and recrystallized from CH₃CN yielding 0.385 g (86%) of 52 as a mixture of the E and Z isomers. mp 202-204°C. ¹H NMR (DMSO d₆, 250 MHz) δ 13.14 (broad s, 1H, D₂O exchangeable, COOH), 8.6 (apparent d, J = 7.7 Hz, 1H, ArH), 8.52 (dd, J = 10.7 Hz, J = 2.4 Hz, 1H, ArH), 8.5-7.4 (m, 6H, ArH), 7.48-7.41 (m, 2H, ArH), 7.36-7.23 (m, 4H, ArH), 7.05 (s, 1H, C=CH(COOH)), 7.02 (s, 1H, C=CH(COOH)). IR (KBr) cm⁻¹: 1695 (C=O). Mass spectrum, m/z: 240 (M⁺, base). Analysis for C₁₅H₉O₂F; calculated: C, 74.99; H, 3.77; found: C, 74.62; H, 3.76.
2-Fluoro-9H-fluoreneacetic acid (54)

Compound **86** (0.5 g, 1.86 mmol) was dissolved in 10 mL of a 1:1 mixture of EtOH/CH₂CN and 2 mL 30% KOH was added. Upon completion of hydrolysis the volatile organics were removed under reduced pressure. The resulting aqueous solution was added to a Parr bottle containing 50 mg of Pd/C and about 30 mL of H₂O. The resulting mixture was hydrogenated at 40 psi for 3 hours at room temperature. The catalyst was removed via filtration through a pad of Celite and the filtrate was acidified. The resulting suspension was allowed to stand at 0°C overnight. The solid was collected by filtration dried in vacuo and recrystallized from ethyl acetate and hexane to yield 0.375 g (83%) of racemic **55** as a white crystalline material. mp 114-115°C. **¹H NMR** (MeOH d₄, 250 MHz) δ 7.76-7.70 (m, 2H, ArH), 7.56-7.51 (m, 1H, ArH), 7.37-7.22 (m, 3H, ArH), 7.12-7.05 (m, 1H, ArH), 4.32 (apparent t, JHHvic = 7.2 Hz, JHHzvic = 7.2 Hz, 1H, CHCH₂), 2.80 (dd, J = 7.2 Hz, JHHzgem = 16.3, 1H, CHCH₂COOH), 2.64 (dd, J = 7.2 Hz, J = 16.3, 1H, CHCH₂COOH). **IR (KBr) cm⁻¹**: 1700 (C=O). **Mass spectrum, m/z**: 242 (M⁺), 196 (base). Analysis for C₁₅H₁₁O₂F; calculated: C, 74.37; H, 4.58; found: C, 74.40; H, 4.66.
A solution of triethyl phosphonoacetate (1.91 g, 8.3 mmol) in 5 mL diglyme was added to a stirred suspension of NaH (60% dispersion, 0.332 g, 8.3 mmol) in 20 mL diglyme under argon. After 1 hour a solution of 84 in 10 mL diglyme was added and the mixture was heated to reflux temperature and maintained at this temperature for an additional 8 hours. Upon completion of the reaction period the mixture was cooled and diluted with 150 mL of water. This mixture was extracted with ethyl acetate (3 x 150 mL). The combined extracts were dried (MgSO₄) and evaporated under reduced pressure to yield a solution of 87 in diglyme. The diglyme was removed through the use of a vacuum pump overnight. The resulting solid was purified using flash column chromatography, (5% EtOAc/hexane). The combined fractions from the column were evaporated under reduced pressure yielding 1.34 g (67%) of 87 as a bright yellow solid. mp 87-89°C. ¹H NMR (Acetone d₆, 250 MHz) δ 9.12 (dd, J = 10.5 Hz, J = 2.5 Hz, 1H, ArH), 8.25-8.18 (m, 3H, ArH), 7.69-7.65 (m, 2H, ArH), 7.43 (s, 1H, C=CH(CO₂H)), 4.78 (q, J = 7.1 Hz, 2H, CH₂CH₃), 3.23 (t, J = 7.1 Hz, 3H, CH₂CH₃) IR (KBr) cm⁻¹: 1730 (C=O). Mass spectrum, m/z: 286 (M⁺, base), 241, 214. Analysis
for C_{17}H_{12}O_2F_2; calculated: C, 71.33; H, 4.23; found: C, 71.26; H, 4.28.

2,7-Difluoro-9H-fluoren-9-ylideneacetic acid (53)

Compound 87 (0.5 g, 1.86 mmol) was dissolved in 10 mL of a 1:1 mixture of EtOH/CH_3CN with stirring. To this solution was added 2 mL of 30% KOH and the reaction was stirred for an additional hour at which time the volatile organic solvents were removed under reduced pressure. The resulting aqueous solution was acidified and allowed to stand overnight at 0°C. The resulting solid was collected by filtration, dried in vacuo and recrystallized from ethyl acetate/hexane yielding 0.38 g (84%) of 53 as a yellow solid. mp 234-235°C. 1H NMR (DMSO d_6, 250 MHz) δ 13.24 (broad s, 1H, D_2O exchangeable), 8.43 (dd, 1H, ArH), 7.89-7.78 (m, 3H, ArH), 7.35-7.22 (m, 2H, ArH), 7.09 (s,1H, C=CH(COOH)). IR (KBr) cm⁻¹: 1705 (C=O). Mass spectrum, m/z: 258 (H^+, base). Analysis for C_{15}H_{9}O_2F_2; calculated: C, 69.77; H, 3.12; found: C, 69.52; H, 3.12.
2,7-Difluoro-9H-fluoren-9-yiacetic acid (56)

Compound 87 (0.5 g, 1.75 mmol) was dissolved in 10 mL of a 1:1 mixture of EtOH/CH₃CN and 2 mL 30% KOH was added. Upon completion of hydrolysis, about 1 hour, the volatile organics were removed under reduced pressure. The resulting aqueous solution was added to a Parr bottle containing 30 mg of 5% Pd/C and about 30 mL of H₂O. The resulting mixture was hydrogenated at 40 psi for 3 hours at room temperature. The catalyst was removed via filtration through a pad of Celite and the filtrate was acidified. The resulting suspension was allowed to stand at 0°C overnight. The solid was collected by filtration dried in vacuo and recrystallized from ethyl acetate and hexane to yield 0.375 g (85%) of 56 as a white crystalline material, mp 159-161°C. ¹H NMR (DMSO d₆, 250 MHz) δ 12.5 (broad s, 1H, D₂O exchangeable), 7.85 (dd, J = 8.4 Hz, J = 5.2 Hz, 1H, ArH, meta to F), 7.42 (dd, J = 9.4 Hz, J = 2.2 Hz, 1H, ArH, ortho to F), 7.20 (ddd, J = 9.4 Hz, J = 8.4 Hz, J = 2.2 Hz, 1H, ArH, ortho to H & F) 4.27 (t, J = 7.0 Hz, 1H, CH₂CH₂), 2.82 (d, J = 7.0 Hz, 2H, CH₂COOH) IR (KBr) cm⁻¹: 1700 (C=O); Mass spectrum, m/z: 260 (M⁺), 214 (base); Analysis for C₁₅H₁₀O₂F₂; calculated: C, 69.24; H, 3.87; found: C, 68.84; H, 3.85.
**5-(2-Phenylethenyl)tetrazole (93)**

![Chemical Structure]

To 10 mL of THF cooled to 0°C, under argon, were added in order \( \text{AlCl}_3 (0.454 \text{ g, } 3.4 \text{ mmol}), 92 (0.2 \text{ g, } 1.6 \text{ mmol}) \) and \( \text{NaN}_3 (0.442 \text{ g, } 6.8 \text{ mmol}) \). After addition of the above reagents the ice bath was removed and the reaction mixture was heated to reflux temperature with stirring and maintained at this temperature for 4 days. After 4 days the mixture was cooled to room temperature and, in a hood, approximately 10 mL of 10% HCl was added. Water (10 mL) is added to the mixture and the THF layer is separated from the aqueous layer. The aqueous layer was extracted with ethyl acetate (20 mL), which was combined with the THF layer. The combined organic layers were washed with water (30 mL), dried (\( \text{MgSO}_4 \)) and evaporated under reduced pressure to yield 0.2 g (78%) of 93 as a white solid. mp 177°C (lit. mp 175°C). \(^1\)H NMR (Acetone d\(_6\), 250 MHz) \( \delta \) 7.75 (d, \( J = 16.8 \text{ Hz}, 1H, \text{CH}=\text{C}) \), 7.71-7.68 (m, 2H, ArH), 7.45-7.42 (m, 3H, ArH), 7.33 (d, \( J = 16.8 \text{ Hz}, 1H, \text{CH}=\text{CH}) \).
A solution of diethyl cyanomethylphosphonate (1.77 g, 10 mmol) in 5 mL diglyme was added to a stirred suspension of NaH (60% dispersion, 0.4 g, 10 mmol) in 20 mL diglyme under argon. After 1 hour a solution of 78 (1.8 g, 10 mmol) in 10 mL diglyme was added and the mixture was heated to reflux temperature and maintained at this temperature for an additional 8 hours. Upon completion of the reaction period the mixture was cooled and diluted with 150 mL of water. This mixture was extracted with ethyl acetate (3 x 150 mL). The combined extracts were dried (MgSO₄) and evaporated under reduced pressure to yield a solution of 88 in diglyme. The diglyme was removed through the use of a vacuum pump overnight. The resulting solid was recrystallized from acetone yielding 1.82 g (90%) of 88 as a bright yellow solid. mp 109-110°C (lit. mp [115] 109-111°C) ¹H NMR (Acetone d₆, 250 MHz) δ 8.34 (dd, J = 8.7 Hz, J = 0.8 Hz, 1H, ArH), 7.91-7.77 (m, 3H, ArH), 7.57-7.31 (m, 4H, ArH), 6.63 (s, 1H, C=CH(CN)). IR (KBr) cm⁻¹: 2210 (CN); (lit. IR [115] (Nujol) cm⁻¹: 2190).
9H-Fluorenesacetonitrile (89)

To 50mL absolute ethanol in a Parr bottle was added 50mg of 5% Pd/C and 88 (0.5g, 2.5 mmol). This mixture was hydrogenated at 30 psi and room temperature for 24 hours. The catalyst was removed by filtration through a Celite pad (2x) and the filtrate was evaporated under reduced pressure to yield 0.415 g (82%) of 89 as a white solid. mp 134-135°C (lit. mp [116] 134-136°C). $^1$H NMR (DMSO d$_6$, 250 MHz) δ 7.90 (dd, $J = 6.7$ Hz, $J' = 1.7$ Hz, 2H, ArH), 7.73 (apparent d, $J = 7.4$ Hz, 2H, ArH), 7.47-7.37 (m, 4H, ArH), 4.24 (t, $J = 5.8$ Hz, 1H, CHCH$_2$CN), 3.32 (d, $J = 5.8$ Hz, 2H, CHCH$_2$CN).
5-(9H-Fluoren-9-ylmethyl)tetrazole (62)

To 10 mL of THF cooled to 0°C, under argon, were added in order AICI₃ (0.214 g, 1.6 mmol), B9 (0.15 g, 0.73 mmol) and NaN₃ (0.21 g, 3.2 mmol). After addition of the above reagents the ice bath was removed and the reaction mixture was heated to reflux temperature with stirring and maintained at this temperature for 4 days. After 4 days the mixture was cooled to room temperature and, in a hood, approximately 10 mL of 10% HCl was added. Water (10 mL) was added to the mixture and the THF layer was separated from the aqueous layer. The aqueous layer was extracted with CH₂Cl₂ (2 x 25 mL), which was combined with the THF. The combined organic layers were extracted with 10% NaOH (2 x 25 mL). The basic aqueous layers were combined, acidified and extracted with ethyl acetate (2 x 50 mL). The ethyl acetate extracts were combined, dried (MgSO₄) and evaporated under reduced pressure to yield 0.1 g (55%) of 58 as a white solid. mp 219-220°C. ¹H NMR (DMSO d₆, 250 MHz) δ 16.2 (broad s, 1H, D₂ exchangeable), 7.86 (apparent d, J = 7.5 Hz, 2H, ArH), 7.36 (ddd, J = 7.5 Hz, J = 1.3 Hz, 2H, ArH), 7.28-7.23 (m, 4H, ArH), 4.47 (t, J = 7.4 Hz, 1H, CHCH₂), 3.40 (d, J = 7.4 Hz, 2H, CHCH₂) IR (KBr) cm⁻¹: 3200-2300 (tetrazole nucleus). Mass spectrum,
m/z: 248 (M+), 165 (base). Analysis for C$_{15}$H$_{12}$N$_4$: calculated: C, 72.56; H, 4.87; N, 22.57; found: C, 72.32; H, 4.99; N, 22.34.

9H-Fluorene-9-carbonitrile (94)

9-Fluorencarboxamide was synthesized according to the procedures of Hoyng et al., with the exception that a small amount of benzene was used to aid in the dissolution of 9-fluorenecarboxylic acid during the reaction with oxalyl chloride, and the subsequent amide formation using anhydrous NH$_3$ was performed in diethyl ether instead of dioxane [108]. Therefore, 9-fluorenecarboxamido (0.8 g, 3.8 mmol) was dissolved in anhydrous DMF (10 mL). To this solution was rapidly added 3 mL POCl$_3$. The resulting orange mixture was gently heated to 80° and maintained at this temperature for an additional 30 minutes. The reaction mixture was poured onto crushed ice and neutralized with NH$_4$OH. The resulting solid was collected by filtration, dried in vacuo and purified by flash column chromatography, (5% EtOAC/Hexane). The fractions from the column were combined and evaporated under reduced pressure to yield 0.5 g (68%) of 94 as a white solid. mp 149-150°C (lit. mp [109] 151-152°C). $^1$H NMR (CDCl$_3$, 250 MHz) δ 7.77 (apparent d, J = 7.7 Hz, 2H, ArH), 7.71 (d, J = 7.6 Hz, 2H, ArH), 7.51-7.44 (m, 2H, ArH), 7.42 (ddd, J = 7.4 Hz, J = 1.3 Hz, 2H, ArH).
5-(9H-Fluoren-9-yl)tetrazole (63)

To 20 mL THF under argon, was added AlCl₃ (0.21 g, 1.6 mmol), NaN₃ (0.31 g, 4.8 mmol) and 94 (0.3 g, 1.6 mmol). This mixture was heated to reflux temperature, with stirring, and maintained at this temperature for 3 days. Upon cooling 10 mL of 10% HCl was added and the layers separated. The aqueous layer was extracted with ethyl acetate (2 x 100 mL). The ethyl acetate and THF layers were combined and extracted with 10% NaOH (2 x 50 mL). The basic aqueous layers were combined and acidified and extracted again with ethyl acetate (2 x 100 mL). The final ethyl acetate extracts were combined, dried (MgSO₄) and evaporated under reduced pressure to yield 0.325 g (87%) of 63 as a white solid. dp 257-259°C. ¹H NMR (DMSO d₆, 250 MHz) δ 16.35 (broad s, 1H, D₂O exchangeable), 8.00 (apparent d, J = 7.4 Hz, 2H, ArH), 7.50-7.30 (m, 6H, ArH), 5.78 (s, 1H, CH-tetrazole). IR (KBr) cm⁻¹: 3200-2300 (tetrazole). Mass spectra, m/z: 234 (M⁺), 165 (base). Analysis for C₁₄H₁₀N₄; calculated: C, 71.78; H, 4.30; N, 23.92; found: C, 71.54; H, 4.54; N, 24.00.
To 10 mL of refluxing ethyl acrylate was added, over a period of 45 minutes, a solution of 95 [111], (1 g, 5.2 mmol) in 10 mL toluene. Upon completion of the addition, the reaction mixture was cooled and the excess ethyl acrylate and toluene were evaporated under reduced pressure. The resulting solid was dissolved in 10 mL of MeOH and 5 mL 30% KOH was added. This mixture was heated at 65°C for 12 hours. The reaction mixture was cooled and the volatile organics were removed under reduced pressure. The remaining basic aqueous solution was acidified and extracted with CH₂Cl₂ (3 × 150 mL). The CH₂Cl₂ extracts were combined, dried (MgSO₄) and evaporated under reduced pressure to yield a solid which was recrystallized from MeOH/water to give 0.9 g (73%) of racemic 61 as a white solid. mp 212-214°C (lit. mp [110] 217-219°C). ¹H NMR (MeOH d₄, 270 MHz) δ 7.85-7.80 (m, 2H, ArH), 7.52 (dd, J = 7.7 Hz, J = 0.9 Hz, 1H, ArH), 7.38-7.13 (m, 5H, ArH), 2.74 (dd, Jvic = 8.3 Hz, Jvic = 7.5 Hz, 1H, CHCOOH), 2.31 (dd, Jvic = 7.5 Hz, Jgem = 5.0 Hz, 1H, CHH-CHCOOH), 2.14 (dd, Jvic = 8.3 Hz, Jgem = 5.0 Hz, 1H, CHH-CHCOOH).
Part 2

SYNTHESIS OF PERMANENTLY CHARGED AND PERMANENTLY UNCHARGED DOPAMINE AGONISTS
CHAPTER V
INTRODUCTION

5.1 BACKGROUND

Dopamine (DA) [97] is one of three endogenous catecholamine neurotransmitters, norepinephrine (NE) [98] and epinephrine (EPI) [99] being the other two. Dopamine was first synthesized in 1910 [117], however the actual biological function of DA, that of neurotransmitter, wasn't elucidated until the late 1950's [118-119]. Prior to the understanding of the neurotransmitter role of DA, dopamine was thought to simply serve as a biosynthetic precursor to the other biogenic amines NE and EPI. Dopamine was also known to possess weak biological activity similar to that of NE and EPI [118-121]. The observation by Blashko that DA was present in greater amounts than either NE or EPI in certain sympathetic nerve pathways, was inconsistent with the idea that DA was simply a biosynthetic precursor to NE and EPI [118]. This lead Blashko to suggest that DA might serve a regulatory role of its own in certain nerve pathways [118]. Indeed, a short time later, DA was shown to function as a neurotransmitter in the central nervous system (CNS) [119,120]. Although the neurotransmitter role of DA in the CNS has been firmly established, some doubt remains as to whether DA serves a true neurotransmitter role in the periphery [122].
The biosynthesis of DA and the other catecholamines, begins with the hydroxylation of the essential amino acid L-tyrosine yielding L-
dihydroxphenylalanine (L-DOPA) [123]. This reaction is catalyzed by
the enzyme tyrosine hydroxylase and is the rate limiting step in the
biosynthesis of the catecholamines [123]. Decarboxylation of L-DOPA by
L-aromatic amino acid decarboxylase, commonly known as DOPA
decarboxylase, yields dopamine [123]. Hydroxylation of DA by
dopamine β-hydroxylase yields the second catecholamine neurotransmitter
norepinephrine [123]. Methylation of norepinephrine leads to the third
biogenic amine epinephrine [123]. The catecholamines are synthesized
in the adrenal medulla and catecholaminergic nerves. The biosynthetic
pathway of the catecholamines is shown in Figure 15.

After release of dopamine from the presynaptic neuron into the
synaptic cleft, the actions of dopamine may be terminated in two ways.
The major method of dopamine inactivation is reuptake into the
presynaptic nerve terminals [124]. There are 2 reuptake mechanisms; a
high affinity sodium dependent uptake mechanism and a low affinity
sodium independent uptake mechanism. The high affinity sodium dependent
uptake mechanism is commonly referred to as the "uptake₁" mechanism,
while the low affinity uptake mechanism is referred to as the "uptake\textsubscript{2}" mechanism \cite{124,125}. The actions of dopamine may also be terminated through enzymatic degradation, as shown in Figure 16. The two principal enzymes involved in the metabolism of dopamine are monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT) \cite{123}. The metabolism of dopamine may follow one of two routes, both of which end in the production of homovanillic acid (HVA) \cite{123}. Oxidation of dopamine by MAO, a mitochondrial enzyme, yields dihydroxyphenylacetaldehyde, which is further oxidized to dihydroxyphenylacetic acid \cite{123}. Homovanillic acid is produced from
dihydroxyphenylacetic acid through the actions of catechol-O-methyltransferase [123]. Alternately, methylation by COMT and subsequent oxidation by MAO and aldehyde dehydrogenase also yields HVA [123].

Figure 16: Metabolism of dopamine. (modified from [123])

5.2 LOCATION AND DISTRIBUTION OF DOPAMINE

As mentioned earlier, DA is known to function as a neurotransmitter in the CNS. Although distributed throughout the brain, dopamine is found in large amounts in four major areas of the brain. The nigrostriatal complex accounts for approximately 80% of all dopamine found in the brain [124]. Dopamine is also found in the median
eminence, the nucleus accumbens and the inner nuclear layer of the retina [124]. Dopaminergic nerve pathways have been extensively studied in the brain [124,126,127]. Cell bodies of DA-containing nerves are concentrated in an area of the brain known as the substantia nigra. The most clearly defined dopaminergic pathway projects from the substantia nigra to the caudate nucleus and is known as the nigrostriatal pathway [124]. The mesolimbic system has cell bodies located in the midbrain area and terminals in the nucleus accumbens and olfactory tubercle or forebrain area [128]. The cerebral cortex is also known to contain an extensive network of dopaminergic neurons [129].

Although still unresolved, accumulating evidence is clarifying the role of dopamine in the periphery [130]. A number of peripheral tissues have been shown to elicit responses to the administration of dopamine, which can be specifically blocked by the administration of dopamine receptor antagonists [130]. The dog kidney, for example, has been shown to respond to dopamine in a biphasic manner; vasoconstriction followed by vasodilatation [130]. The vasoconstriction can be blocked by α-adrenergic antagonists, indicating dopamine's ability to act as an adrenergic agonist. However, the vasodilatation phase is blocked by administration of the dopamine antagonist sulpiride [130]. The cardiovascular system has also been shown to contain dopamine receptors and to respond to the administration of dopamine [131].
5.3 DOPAMINE RECEPTOR SUBTYPES

The identification and classification of dopamine receptors has been an area of debate for a number of years [124,132]. Dopamine receptors have been classified according to physiological [133,134], or pharmacological and biochemical criteria [133,135,136]. Theories about the classification of DA receptors have ranged from the idea that there is only 1 type of dopamine receptor, to suggestions that there are as many as four receptors [124,137-139]. The large amount and often conflicting data generated by radioligand binding studies of the mid-1970's has, no doubt, contributed to the confusion in this area [124,132]. Today, it is generally accepted that there are 2 major classes of dopamine receptors; D₁ and D₂, as defined by Kebabian and Calne in 1979 [133]. Table 14 outlines the pharmacological and biochemical criteria used by Kebabian and Calne to define two different DA receptors [133].

Dopamine receptors found to be positively linked to adenylate cyclase were defined as D₁ [133]. Activation of this receptor causes an increase in the synthesis of cyclic AMP (cAMP) by adenylate cyclase [140]. The dopamine sensitive adenylate cyclase linked receptor has been found in a wide variety of neural tissues, ranging from gastropods [141], to human brain [142]. The bovine parathyroid is defined as the tissue that contains the prototypical D₁ receptor [133]. However, although the D₁ receptor has been found in a wide variety of tissues, only in the bovine parathyroid, is the activation of adenylate cyclase known to cause a physiological response [132]. Activation of the D₁
receptor on the bovine parathyroid stimulates the release of parathyroid hormone [132].

Table 14

**Kebabian and Calne’s criteria for dopamine receptor classification**

<table>
<thead>
<tr>
<th>Type</th>
<th>(D_1)</th>
<th>(D_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclase Linked?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Location of Prototypic receptor</td>
<td>Bovine Parathyroid</td>
<td>Anterior Pituitary</td>
</tr>
<tr>
<td>Dopamine Potency</td>
<td>(\mu M)</td>
<td>(nM)</td>
</tr>
<tr>
<td>Apomorphine Activity</td>
<td>Partial Agonist/Antagonist</td>
<td>Agonist (nM)</td>
</tr>
<tr>
<td>Ergot Alkaloids</td>
<td>Antagonist (nM)</td>
<td>Agonist ((\mu M))</td>
</tr>
<tr>
<td>Selective Antagonists</td>
<td>None Known</td>
<td>Metoclopramide Sulpiride</td>
</tr>
</tbody>
</table>

As originally defined, \(D_2\) receptors were those dopamine sensitive receptors that were believed to have no association with adenylate cyclase [133]. However, recent research has shown that \(D_2\) receptors may also be linked to adenylate cyclase but in an inhibitory fashion [132,143]. Therefore, activation of \(D_2\) receptors may have no effect on the synthesis of cAMP or it may have an inhibitory effect on the synthesis of cAMP by adenylate cyclase. The prototypical \(D_2\) receptor was defined as those receptors that were found on the mammotrophs of the anterior pituitary of the rat [133]. Activation of the \(D_2\) receptors on the anterior pituitary of the rat causes an inhibition of the release of prolactin [133]. Dopamine \(D_2\) receptors are found on both pre- and postsynaptic nerve terminals [133,143,144].
In addition to biochemical data, Kebabian and Caine used pharmacological data to classify the dopamine receptors, as shown in Table 14 on page 124 [133]. Dopamine, the endogenous ligand was shown to have differential potency for either receptor subtype, being active in the nM range at D₂ receptors and in the pM range at D₁ receptors [133]. Additionally, apomorphine was shown to have different effects at each receptor subtype [133]. Apomorphine is a potent, full agonist at D₂ receptors, while at D₁ receptors it exhibits partial agonist or antagonist activity [133]. The ergot alkaloids were also found to have different effects at each receptor subtype [133]. The ergots lisuride, lergotrilo and bromocriptine were found to be potent D₂ agonists [133,145]. When tested against isolated cells of the anterior pituitary, bromocriptine was found to be ten times more potent than dopamine [145]. Also, lisuride and lergotrilo lack agonist activity at the D₁ receptor [146,147], while bromocriptine has antagonistic activity at the D₁ receptor [148]. Additionally, some dopamine antagonists were found to selectively inhibit the D₂ receptor [149,150]. Metoclopramide has shown the ability to increase prolactin secretion, which can be reversed by bromocriptine [149]. Conversely, neither metoclopramide or sulpiride were able to block dopamine sensitive adenylate cyclase [151]. Interestingly, the neuroleptics, such as chlorpromazine, show little or no selectivity for either receptor subtype [151].

At the time of the development of the 2 dopamine receptor theory, selective D₁ and D₂ agonists and D₁ antagonists were unknown. Since
that time however, a large amount of research has resulted in the
development of selective agonists and antagonists of each receptor
subtype, Table 15 [152]. The development of selective agonists and
antagonists reinforced the understanding that dopamine receptors could
be limited to two types.

Table 15

Selected dopamine receptor agonists and antagonists

<table>
<thead>
<tr>
<th>Agonists</th>
<th>Antagonists</th>
</tr>
</thead>
<tbody>
<tr>
<td>SK6F 38393</td>
<td>D1</td>
</tr>
<tr>
<td>LY-171555</td>
<td>D2</td>
</tr>
<tr>
<td>Dopamine</td>
<td>D1/D2</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td></td>
</tr>
</tbody>
</table>

Selected dopamine receptor agonists and antagonists

Note: The table contains chemical structures of selected dopamine receptor agonists and antagonists.
Since its introduction, however, the 2 dopamine receptor theory has not remained unchanged. It is now understood that both D₁ and D₂ receptors may exist in two interchangeable states, those of high affinity and those of low affinity [143,153,154]. In both receptor systems a ternary complex is formed between the agonist, receptor and a guanine nucleotide binding protein [143]. Dopamine D₁ receptors are associated with a stimulatory nucleotide binding protein (Nₛ), while D₂ receptors are associated with an inhibitory nucleotide binding protein (N₁) [143]. The formation of the ternary complex with Nₛ leads to the activation of adenylate cyclase, while formation of a ternary complex with N₁ leads to the inhibition of adenylate cyclase [143]. The ternary complexes can be dissociated through the actions of guanidino triphosphate, divalent cations and monovalent cations [143].

The D₁/D₂ nomenclature of dopamine receptors has generally been limited to dopamine receptors in the CNS. Peripheral receptors are denoted as DA₁ and DA₂ [132]. Biochemically and pharmacologically DA₁ and DA₂ receptors in the periphery parallel their counterparts in the CNS. That is, DA₁ receptors are similar to D₁ receptors biochemically and pharmacologically. The same can be said for DA₂ receptors and D₂ receptors. However, although there appears to be no striking pharmacological difference between DA₂ and D₂ receptors [155], the same does not appear to be true for DA₁ and D₁ receptors [132,156]. Until this discrepancy is clarified, the distinction between central and peripheral dopamine receptors will continued to be made by some researchers [155]. Other researchers have erased this distinction and
now consider central and peripheral receptors to be one and the same [143]. Table 16 summarizes some of the information discussed above [143].

**Table 16**

**Characteristics of** $D_1$ **and** $D_2$ **receptors**

<table>
<thead>
<tr>
<th>$D_1$ (Syonyms D-1, DA in the periphery)</th>
<th>$D_2$ (Syonyms D-2, DA in the periphery)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agonist stimulation leads to increased cAMP formation followed by adenylate cyclase activation and downstream signaling pathways</td>
<td>Agonist stimulation leads to decreased cAMP formation</td>
</tr>
<tr>
<td>Receptor/adenylate cyclase interaction is mediated by a guanine nucleotide-binding regulatory protein, $N_d$.</td>
<td>Receptor binding affinity of agonists is dependent on degree of association with $N_d$. This is regulated by GTP and by divalent cations.</td>
</tr>
<tr>
<td>$R_n$ represents the $D_1$ dopamine receptor in association with $N_d$.</td>
<td>$R_n$ represents the $D_2$ dopamine receptor in association with $N_d$.</td>
</tr>
<tr>
<td>Agonist affinity for $R_n$ correlates with agonist potency in stimulating cyclic AMP production.</td>
<td>Agonist affinity for $R_n$ correlates with agonist potency</td>
</tr>
<tr>
<td>Selective agonist: SKF 33393</td>
<td>Selective agonist: SKF 33393</td>
</tr>
<tr>
<td>Selective antagonist: SCH23390</td>
<td>Selective antagonist: SCH23390</td>
</tr>
<tr>
<td>Location and function of $D_1$ receptors</td>
<td>Location and function of $D_2$ receptors</td>
</tr>
<tr>
<td>Central nervous system: post synaptic to dopamine neuron terminals and dendrites in CNS (e.g., striatum, nucleus accumbens, olfactory tubercle, substantia nigra, etc.). Function: unclear.</td>
<td>Pituitary gland: anterior lobe mammotroph. Function: inhibit cyclic AMP and prolactin release. May also regulate Ca$^{2+}$ channels and phosphoinositide turnover.</td>
</tr>
<tr>
<td>SKF33393 stimulates grooming and some types of stereotypic motor behavior patterns in rodents. SCH23390 can block these behaviors and reduce catalepsy. SCH23390 can also block behaviors stimulated by $D_2$ agonists in normal rats. This suggests a functional interaction between $D_1$ and $D_2$ dopamine receptors on the same neurons, or alternatively, between interconnected neuronal systems activated by $D_1$ and $D_2$ dopamine receptors, respectively. $D_1$ dopamine receptors are reduced in Huntington's chorea, increased in some Parkinson's disease patients, and may be decreased in schizophrenia where there linkage with adenylate cyclase may be modified.</td>
<td>Substantia nigra: dopamine neuron soma. Function: inhibit dopamine neuron firing.</td>
</tr>
<tr>
<td></td>
<td>Carotid body. Function: depress spontaneou chemosensory discharge.</td>
</tr>
<tr>
<td></td>
<td>Chemosensitive trigger zone. Function: emesis.</td>
</tr>
<tr>
<td></td>
<td>Activation of CNS $D_1$ dopamine receptors leads to increased motor activity and stereotyped motor behavior. In humans, psychosis with stereotyped behavior and thinking patterns can also develop. $D_2$ and mixed $D_1/D_2$ dopamine receptor antagonists are antipsychotic and can produce Parkinson's disease-like rigidity in humans or catalepsy in animals. CNS $D_1$ dopamine receptors are increased in schizophrenia and in patients with Parkinson's disease who are not treated with L-dopa. $D_2$ dopamine receptors are decreased in the striatum of patients with Huntington's chorea.</td>
</tr>
</tbody>
</table>
5.4 LOCATION AND FUNCTION OF DOPAMINE RECEPTORS

As shown in Table 16 on page 128, both D₁ and D₂ receptors serve numerous functions [143]. The regulatory functions dopamine exerts via D₁ and D₂ receptors suggest a number of clinically significant applications of peripheral and central dopamine receptor agonists. Indeed, the major clinical application of centrally acting dopamine agonists is in the treatment of Parkinson's disease [157].

Parkinsonism is a disease state that is associated with an imbalance of the normal dopaminergic and cholinergic interactions in the striatum [158]. As noted in Table 16 on page 128, D₂ receptors innervate cholinergic interneurons and produce an inhibitory effect on the release of acetyl choline (ACh) [143,158]. The destruction of dopamine nerve tracts in the nigrostriatal pathway results in the loss of the regulatory effect of dopamine upon the cholinergic interneurons. This loss of the regulatory effect of dopamine in clinically manifested as the symptoms of Parkinson's disease, which include muscular tremor and rigidity, bradykinesia, and postural defects [158]. Centrally acting dopamine agonists, such as bromocriptine, have been shown to be useful in the treatment of Parkinsonism [158]. Dopamine is ineffective as a centrally acting agonist because under physiological conditions it exists mainly in the protonated state and is therefore too polar to cross the blood brain barrier. Additionally, dopamine is rapidly metabolized by MAO in the blood. Although L-DOPA is pharmacologically inert, it is transported past the blood brain barrier by an amino acid uptake mechanism. As shown in Figure 15 on page 120, L-DOPA is the
immediate biosynthetic precursor to dopamine. Once in the brain L-DOPA in rapidly decarboxylated by dopa decarboxylase, yielding dopamine, which can then activate dopaminergic receptors [158]. Therefore, L-DOPA is commonly used in the treatment of Parkinson's disease. Unfortunately the decarboxylation of L-DOPA is not limited to the CNS, L-DOPA is also rapidly decarboxylated in the periphery. To compensate for the loss of L-DOPA administered, due to peripheral decarboxylation, either large dose of L-DOPA must be administered, or a peripheral dopa decarboxylase inhibitor, such as carbidopa, must be concomitantly administered with the L-DOPA [158]. In practice the latter approach is often used.

As previously mentioned, D2 receptors are known to reside on the anterior lobe of the pituitary. Activation of these receptors inhibits the release of prolactin. Bromocriptine has been used successfully in the treatment of hyperprolactinemia [159]. Bromocriptine has also been used in the treatment of acromegaly associated with the hypersecretion of growth hormone from pituitary tumors [159]. Although not useful for the treatment of Parkinson's disease, dopamine has been used in the treatment of various types of shock [159]. During an episode of shock, administration of an adrenergic elevates blood pressure by causing peripheral vasoconstriction. However, adrenergics also cause vasoconstriction of the renal vasculature, decreasing the flow of blood to the kidneys, which may lead to kidney failure. Because it has weak adrenergic activity, dopamine can be administered during an episode of shock to elevate blood pressure. However, unlike the adrenergics
dopamine causes vasodilatation of the renal vasculature, which maintains blood flow to the kidneys. Because of its ability to enhance renal perfusion, while elevating blood pressure, dopamine is well suited for use during episodes of shock [159]. As noted in Table 16 on page 128, D₁ and D₂ receptors are located throughout the periphery and appear to have effects on the cardiovascular system. It has been suggested that activation of these receptors may provide alternate treatment regimens than those currently available for a host of diseases, such as hypertension, congestive heart failure and angina pectoris [124].

5.5 DOPAMINE AGONISTS

In the years since the understanding of dopamine's role as a neurotransmitter and the realization that correction of abnormal dopamine function might lead to new treatment regimens for certain disease states, a large number of novel dopamine agonists have been developed. In addition, the desire to understand how dopamine interacts with its receptors has also inspired research into the development of novel dopamine agonists. A large amount of pharmacological data has been generated during the development of these novel dopamine agonist. However, many different test systems have been used to evaluate the pharmacological activity of these compounds. Therefore, direct comparison of pharmacological results is difficult and should be approached with caution.
The development of novel dopamine agonists has followed three general approaches: simple alterations of dopamine, rigidification of dopamine, and simplification of complex naturally occurring dopamine agonists, such as apomorphine and the ergot alkaloids [160]. These approaches to the development of novel dopamine agonists has yielded compounds from a wide variety of chemical classes including, phenethylamines, aminotetralins, aminindans, benzoquinolines, benzazepines, aporphines and the ergot alkaloids [121,124,160]. The following sections will describe approaches that have been taken towards the development of novel dopamine agonists.

5.6 SIMPLE MODIFICATIONS OF DOPAMINE

5.6.1 Phenethylamines

A number of attempts have been made at altering the activity of dopamine through simple alterations of its structure. Alteration of the length of the ethylamine side chain, or alkylation of the side chain generally leads to a drastic reduction or abolition of agonist activity [121]. With the exception of N-methyl dopamine (100, Epinino), all N-monoalkylated derivatives of dopamine are poorly active as dopamine agonists [121]. Epinine (100) was shown to be equipotent with dopamine in stimulating DA sensitive adenylate cyclase and in displacing 3[H]-spiperone from D2 binding sites [161,162]. Tertiary amines have shown dopaminergic activity with the N,N-dipropyl analogue 101 being the most active [121]. Interestingly, in a series of unsymmetrically disubstituted tertiary amines the "propyl effect" was
observed [163]. The presence of one propyl group appeared to confer near maximum activity, even if the other substituent was as large as a phenylethyl group. This phenomena is not simply related to hydrophobicity since higher and lower homologues do not follow any specific order of activity [124]. It has been suggested that the dopamine receptor possesses a hydrophobic cleft with a geometry that uniquely accommodates the N-propyl group [163,164].

Alterations of the catechol moiety of dopamine have led to some interesting structure activity relationships. The monophenolic m-tyramine (102) and its dipropyl analog 103 were found to be potent dopaminergics [161,165]. These results indicate that the para hydroxyl group may not be necessary for receptor activation. Interestingly, when the 'di-meta' resorcinol analogue was tested, it was found to be devoid of agonist activity [166].
Fluorinated analogues of dopamine 105 - 107 have been synthesized and tested for their dopamine agonist activity [167,168]. In the dog renal vascular assay for D<sub>1</sub> activity, the 2- and 5-fluoro derivatives, 105 and 106 respectively, were equipotent with dopamine, while the 6-fluoro analogue 107 was 4 times less potent than dopamine [167]. In binding studies, all three compounds were equipotent with dopamine in displacing <sup>3</sup>H-spiperone, while 106 was more potent in displacing <sup>3</sup>H-apomorphine [168]. It has been suggested that the fluorination of the catechol moiety alters the acidity of the phenolic groups [169]. However, no general trend of activity has been displayed. Interestingly, the site of methylation by COMT is altered by ring fluorination [170].
5.7 RIGIDIFICATION OF DOPAMINE

Dopamine is a flexible molecule and as such may exist in a number of conformations. Figure 17 shows the angles $\theta$ and $\phi$ that are used in describing the conformations of dopamine [121]. Early experiments using computational chemistry indicated that dopamine could exist in one of three preferred conformations, the anti or the two gauche conformations, as shown in Figure 18 [121,171,172]. In the anti, or fully extended trans conformation, the values for $\theta$ and $\phi$ are $180^\circ$ and $90^\circ$ respectively, while the values for $\theta$ and $\phi$ in the two gauche conformers are $\pm 60^\circ$ and $90^\circ$ respectively. X-ray crystallographic analysis of dopamine has shown that in the crystal state, dopamine exists in the anti conformation [173]. Additionally, Cannon has suggested that dopamine may exist in either the $\alpha$ or $\beta$ conformation, as shown in Figure 18 [174,175]. With the understanding that the energetically favored conformation, whether determined computationally or by x-ray crystallography, is not necessarily the preferred conformation assumed by dopamine at the receptor site, the above
discussion is merely of suggestive value [121]. Therefore a number of structurally distinct molecules have been designed to determine what conformations, if any, of dopamine are preferred by dopamine receptors. Rigidification of the dopamine skeleton may be accomplished in either of two ways. The ethylene portion of the ethylamine side chain can be incorporated into a ring leaving the nitrogen exocyclic, or the nitrogen may be incorporated into a heterocyclic ring [121]. Examples of both approaches will be discussed below.

![Dihedral angles used to describe the conformations of dopamine](image.png)

**Figure 17:** Dihedral angles used to describe the conformations of dopamine
A series of compounds in which the ethylene portion of the ethylamine side chain has been incorporated into the tetrinal ring system are known collectively as the aminotetralins. One of the first aminotetralins synthesized was 2-Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (A-6,7-DTN, 108), where R,R' = H [121,176]. The positional isomer, A-5,6-DTN (109), where R,R' = H, was synthesized a few years later by Cannon et al. [127,177]. Although these compounds are rigidified analogues of dopamine, with 108 representing a rigid β rotamer and 109 a rigid α rotamer, these compounds also represent simplified analogues of isopomorphine 110 and apomorphine 111, respectively. Compound 109, where R,R' = Me, is commonly referred to as "M7". An x-ray crystallographic study showed that the catechol ring system of 109 was nearly coplanar with the ethylamine side chain [178].
Derivatives of 108 and 109, where R and R' were various combinations of H, methyl, ethyl and propyl groups, showed potent peripheral and central dopaminergic activity [179,180]. Attempts to establish general patterns of SAR between analogues of 108 and 109 have been unsuccessful [181]. Apomorphine (111) has been shown to be a potent central and peripheral dopamine agonist, so that the activity exhibited by 109 is not surprising. However, isonpomorphine (110) is inactive as a dopaminergic and therefore the activity of 108 is somewhat surprising. Upon separation of the enantiomers, the agonist activity was found to reside primarily in one isomer in each series of compounds [182]. As shown in Figure 19, the active isomers of the analogues of 108 and 109 are of opposite stereochemistry [182]. A rationalization for this apparent difference in stereochemical requirements of 108 and 109 is also shown in Figure 19 [182]. As illustrated, 108 is believed to interact with the receptor in an inverted fashion, such that the 7-OH now occupies the same area of the receptor as the 5-OH of 109. Additionally, when displayed in this way, the stereochemistry of each compound appears to be the same [182]. This receptor model has also been used to explain the inactivity of isonpomorphine 110. It has been suggested that a site of steric intolerance exists at the receptor site that prevents isonpomorphine 110 from assuming the same orientation as its simplified analogue 108 [182]. To test their hypothesis, Freeman et al. synthesized the 5- and 8-propyl analogues of 108, compounds 112 and 113 respectively [182]. It was thought that compound 112 would be less active than the isomeric 113. If 108 was binding to
the receptor as depicted in Figure 19, then the 5-propyl analogue 112, due to increased steric bulk, would not be able to assume the correct orientation required for receptor activation. [182] As theorized, 112 was significantly less active than 113 [182].
Figure 19: Receptor model rationalizing the activity of 108 and inactivity of 110

The monophenolic 2-aminotetralins, 114 and 115, corresponding to rigidified α and β rotamers of m-tyramine have been synthesized and tested [181]. Generally, the 5 hydroxy isomers were more active than the 7 hydroxy isomers, with the di-N-propyl analogs of 114 being the most active [183,184]. Not surprisingly the mono hydroxylated compounds 114 and 115 follow the same stereochmical trends as their dihydroxylated counterparts [182]. However, unlike the inactive resorcinol 104, the di-"meta" tetralin 116 did show some dopaminergic activity [185].
5.7.2 Aminoindans

The aminoindan analogues of 117 and 118, where R,R' = combinations of H, Methyl, Ethyl or Propyl, have shown some interesting SAR. While the symmetrically dialkylated analogues of 117 showed dopaminergic activity, none of the analogues of 118 showed any significant dopaminergic activity [177]. As with the tetralins the "meta" hydroxyl seemed to be of significance. In a series of CNS assays 119 showed significantly greater dopaminergic activity than did 120 [186]. When the enantiomers of 119 were separated, the R isomer was found to be 100 times more active than its antipode. Thus 119 shares the same relative stereochemistry as the active isomer of apomorphine (111) and the aminotetralin 109 [187].
5.7.3 Benzoquinolines

The octahydrobenzo[g]quinolines are a class of semi-rigid dopamine analogues, that can exist in 2 conformers, since the B/C rings can be either cis or trans fused. Dopaminergic activity generally resides in the trans conformer. In this ring system the dopamine moiety is held in an almost perfect anti conformation [121]. The tertiary analogues of 121, where R = methyl, ethyl or propyl, were potent dopamine agonists [181,188]. Interestingly, similar to the aminotetralins but unlike the aminotetralins, the β rotamer analogues, 122, where R = H, methyl, ethyl or propyl, were found to be devoid of dopaminergic activity [189]. The resorcinol derivative 123 like its aminotetralin counterpart 116, showed weak dopaminergic activity [190]. The isomeric octahydrobenzo[f]quinolines, 124-126 where R₄ = H, methyl, ethyl, or
propyl, showed the same requirements for a trans fused ring system as 121. As with 122, the isomeric analogues, 125, were inactive as dopamine agonists [189]. However, unlike 123 the resorcinol derivative 126 was inactive as a dopamine agonist.

5.7.4 Miscellaneous semi-rigid dopamine analogues

Semi-rigid cis and trans cyclopropyl 127, 128 and cyclobutyl 129 - 132 analogues of dopamine have also been synthesized and tested for their dopaminergic activity [191,192]. Both the cis and trans cyclopropyl analogues, 127 and 128 respectively showed no dopaminergic activity in the dog renal artery test [191], even though the trans
Isomer closely resembles the fully extended trans conformation of dopamine. It has been suggested that, as with the methyl group of α-methyl dopamine, the cyclopropyl ring locks the aromatic ring in a plane that is perpendicular to the plane of the ethylamine sidechain [191]. As such, the aromatic ring can not assume either the α or β conformation that appears necessary for dopaminergic activity.

The trans cyclobutyl analogues 129 and 130 showed the ability to displace [3H]-dopamine from dopamine receptors, with the tertiary amine being more potent than the primary amine [192]. Both cis isomers 131 and 132, were decidedly less potent than the trans isomers. A conformational analysis showed that for the trans isomers the angle θ varied from 110° to 160°, while for the cis isomers the angle θ varied between 10° and -30° [192]. The poor activity of the trans isomers may be explained by the inability of these isomers to achieve an anti conformation. On the other hand, the cis isomers exist in almost a fully eclipsed conformation, which is probably responsible for their very poor binding activity.
5.7.5 Nitrogen heterocycles

The rigidification of dopamine by the incorporation of portions of the ethyl amine side chain into heterocyclic structures has provided compounds with significantly altered dopaminergic activity. The tetrahydroisoquinolines 133 and 134 have shown poor dopaminergic activity in a variety of test systems [160]. Interestingly, when the reduced portion of the tetrahydroisoquinoline ring was expanded, to give the benzazepine 135, significant dopaminergic activity was observed in both D1 and D2 test systems [193]. Compound 135 also showed significant α-adrenergic activity [193]. Introduction of a phenyl ring at the 1 position yielded SKF-38393 (136), a compound that
showed increased selectivity for dopamine receptors [193]. Not only did 136 show selectivity for dopamine receptors, it also had the ability to discriminate between D1 and D2 receptors, being more active in D1 test systems [193]. Compound 136 was the first agonist with significant receptor selectivity that would allow for the in vivo analysis of the effects of selective receptor activation [194]. Compound 136 was shown to cause renal vascular dilatation in dogs [194].

Extensive structure activity relationships have been generated by the Smith, Kline and French company during their development of benzazepines as D1 selective agonist [124]. The SAR of the benzazepines can be summarized as follows; 1) the phenyl group at the 1
position markedly enhances dopaminergic activity, 2) the 7,8 catechol is absolutely necessary for agonist activity, 3) additional substitutions of the 1,2,4 or 5 position decreases or abolishes activity, 4) substitution of the phenyl ring has variable affects on activity, 5) alkylation of the nitrogen decreases D₁ selectivity, 6) substitution at the 6 position generally enhances activity, and 7) substitution at the 1 position with groups other than phenyl decreases or abolishes activity [124,193]. Compound 137 is one of the most potent D₁ selective agonists known [193]. As noted above, an intact catechol moiety is necessary for agonist activity. Replacement of the 7-OH with chlorine, along with N-methylation, generated a potent and selective D₁ receptor antagonist, 138 [193]. Upon separation of the enantiomers, for the compounds 136, 137 and 138, the dopaminergic agonist or antagonist activity resided almost entirely in the R isomer [124,193].

![Diagram of compound 138](image)

A slightly different approach to the rigidification of dopamine, in which the ethylamine side chain was incorporated into a six-membered ring that was separate from the aromatic ring of dopamine, led to the
production of a series of compounds known as the 3 phenyl piperidines [195]. One of the most active compounds of the series was 3-(3-hydroxyphenyl)-N-propyl piperidine (139), also known as 3-PPP [195]. Compound 139 was originally purported to be a selective presynaptic agonist, however, upon separation of the enantiomers the true pharmacology of 3-PPP was revealed [121]. The (+) isomer of 3-PPP was shown to act as both a pre- and postsynaptic agonist of D$_2$ receptors [197,198]. The pharmacology of (-) 3-PPP appears to be much more complex. Agonist, antagonist or mixed agonist-antagonist activities have been detected for (-) 3-PPP in various test systems [197,198]. When tested in vitro for D$_1$ receptor affinity (+) 3-PPP has weak agonist activity, while (-) 3-PPP acted as a weak antagonist [197].
5.8 SIMPLIFICATION OF NATURALLY OCCURRING DOPAMINE AGONISTS

The aporphines and ergot alkaloids represent two classes of naturally occurring compounds that exhibit dopamine agonist activity. A large amount of research has been conducted in an attempt to define the dopaminergic pharmacophore in each class of compounds. The next two sections will discuss the results of simplification of the aporphine and ergot structures, respectively.

5.8.1 Aporphines

Apomorphine (APO) (111) is the most studied compound of the aporphine family [121]. As discussed previously, the pharmacophore of APO was shown to be A-5,6-DTN, or "H7" (109). The aminotetralins and the octahydrobenzo[g]quinolines are simplified analogues of APO, and as such, the SAR of these compounds is strikingly similar. One unreconciled difference in the SAR of these compounds is the dopaminergic activity displayed by the aminotetralin, 108, while both isoapomorphine and the octahydrobenzo[g]quinoline 122 display no significant dopaminergic activity. Although, as previously stated, the difference in activity between 108 and 110 has been rationalized as being due to a steric effect at the receptor site, compound 122 should not suffer the same steric interaction as isoapomorphine. It has been suggested that the inactivity of 122 is due to a steric interaction with a different portion of the receptor. As with other dopamine agonists, N-propyl substitution enhances activity [121]. The mono-hydroxylated analogues of propyl norapomorphine 140 and 141 have been shown to have dopaminergic activity, with the 11-OH derivative,
being more active than the 10-OH analogue, 141 [199]. These results, along with the results obtained for other mono-hydroxylated compounds, indicate that the "meta" hydroxyl plays an important role in the determination of dopamine agonist activity.

![Ergot alkaloid structure](image)

5.8.2 Ergot alkaloids

The ergot alkaloids represent a class of compounds that are structurally different from classical dopamine agonists. The central dopaminergic activity of the ergots lergotrile (142), pergolide (143), lisuride (144) and bromocriptine (145) have been well established and reviewed [121,124]. A number of attempts have been made to rationalize the dopaminergic activity of the ergots. Structural comparisons are often made between apomorphine and the ergots since both are large polycyclic structures and share similar pharmacological profiles. The portions of the ergots that have been proposed to be the active pharmacophore are shown in Figure 20 [200]. One of the earliest
attempts at rationalizing the activity of the ergots was made by Nichols [200]. Noting the importance of stereochemistry on the activity of both apomorphine and the ergot alkaloids, the dopaminergic activity in both series of compounds resides in the R enantiomer, Nichols proposed that the pyrrole ethylamine portion of the ergots was the active pharmacophore [201]. As depicted in Figure 21, Nichols proposed that when the stereochemistry and the basic amine of the ergot alkaloids was matched with the corresponding portions of APO, (1 vs 2), the pyrrole ring would serve the same function as the intact catechol ring of apomorphine at the receptor [201]. Camerman et al. in a structural comparison of the x-ray structures of bromocriptine and apomorphine, suggested that bromocriptine and apomorphine should be matched such that the electronegative elements are overlayed upon one another [202]. Thus, Camerman et al. suggested that the indole N-H should be matched with the 11-OH of apomorphine and that the basic amines of both compounds should be matched, (2 vs 3) [202]. The stereochemical requirements of each compound were also considered [202]. The model generated was similar to that of Nichols, with the exception that the ergot structure is slightly twisted, relative to apomorphine, such that the indole N-H of bromocriptine and the 11-OH of APO overlap. As with the discussion of the conformations of dopamine these rationalizations are merely of suggestive value.
Figure 20: Proposed pharmacophores of the ergot alkaloids. (modified from [200])
A series of simplified ergots and their analogues, as shown in Figure 22, were prepared by Bach et al. in an attempt to determine the active dopaminergic pharmacophore of the ergot alkaloids [203]. The simplified ergots synthesized were tested using two in vivo techniques [203]. All of the compounds synthesized, with the exception of the pyrrole ethylamine 149 showed dopaminergic activity [203]. The inactivity of 149 was reasoned to be due to rapid metabolism of 149 by MAO, since in the same test system dopamine is also inactive. As with other dopamine agonists the tertiary amines were found to be more more active than either the primary or secondary amines [203]. The propyl analogues generally showed the most activity in a series. The tricyclic analogues 146, 148 and 151 were more active than the bicyclic derivatives 147 or 150 [203]. Most interesting though was the...
observation of potent dopamine activity when the pyrrole rings of the bi- and tricyclic analogues 147 and 148 were replaced by pyrazole rings, as in compounds 150 and 151 [203]. The activity of the linear bi- and tricyclic structures suggested that the pyrazole ring might function as an isostere of the pyrrole ring [203].

In addition to dopamine agonist activity, the ergots are known to possess adrenergic and serotonergic activity [203]. As mentioned earlier, the ergot alkaloids are also selective D₂ receptor agonists [203]. However, the simplified ergot derivatives appeared to be highly selective agonists, with decreased effects on other receptor systems [203]. Not surprisingly, separation of the enantiomers showed that the dopaminergic activity was almost entirely present in the R isomer [204]. The stereochemistry of 151 corresponds to the stereochemistry of the active intact ergots [205].

Although the pyrazole ring appeared to serve as an isostere in the simplified ergots, when this isosteric substitution was attempted with the more structurally complex ergot alkaloids, it failed [205].

The results from Bach's research would seem to indicate that the pyrrole ethylamine, or at least the tetrahydroisoindole, portion of the ergots was the active pharmacophore [203]. It has been suggested, however, that the indole N-H serves as an isostere for the "meta" hydroxyl of dopamine agonists, including apomorphine [206,207]. To demonstrate this point a series of 4-indole ethylamines were synthesized and were found to have significant dopaminergic activity [206,207]. These compounds could be considered as the pyrrole
Figure 22: Simplified ergots and their analogues isosteres of m-tyramine. Although, at one time it appeared the pharmacophore of the ergots had been determined, this last body of information has once again clouded the issue.
5.9 SUMMARY

From the structure activity relationships discussed in the preceding sections a number of observations about dopamine agonists can be made:

1. An intact catechol function appears to be of prime importance for $D_1$ activity, while the existence of the "meta" hydroxyl, or some isostere, is apparently sufficient to impart $D_2$ activity upon a compound.

2. Analysis of the large amount of data available on the activity of rigid dopamine analogues indicates that the fully extended trans, or anti conformation is preferred for activity. Additionally, it is apparent that the aromatic ring and the ethylamine side chain should be coplanar or nearly so. Although more analogues with dopaminergic activity appear to have the $\alpha$ rotameric form rather than the $\beta$ rotameric form, conclusions about the preferred rotamer at the receptor site are difficult to make.

3. The distance between the "meta" hydroxyl group and the amine seems to be of little importance since agonists have been found in which the interatomic distance, between the hydroxyl and the amine, has varied from as little as 0.55 nm to the maximum of 0.74 nm.

4. Both $D_1$ and $D_2$ receptors appear to be highly stereoselective, with activity in a series of agonists or antagonists generally restricted to one isomer.

Although a large amount of work has been done in the area of dopamine agonist SAR, a number of questions remain unanswered. Generally, with the exception of alkylation or incorporation into a
ring system, little has been done to the basic amine of dopamine. One question that remains unanswered is whether dopamine interacts with its receptors in a charged ammonium form or in an uncharged amine form. Additionally, since the activity of certain dopaminergics can be altered through changes in the catechol moiety, such as with the benzazepines, will alterations of the basic amine lead to changes in the activity of known agonists? Answers to these questions may give us a better understanding of how dopamine interacts with its receptors and lead to the development of new classes of dopamine agonists or antagonists.
CHAPTER VI

STATEMENT OF PROBLEMS AND OBJECTIVES

As illustrated in the preceding chapter, a large amount of information about the structure activity relationships (SAR) of dopamine and dopamine agonists has been generated during attempts to understand how dopamine interacts with its receptors and at producing potent and selective dopamine agonists. Generally, alterations in the structure of dopamine or related agonists have been limited to the catechol ring or the ethylamine side chain. For example, alteration of catechol function of the benzazepines has a profound effect on the activity of these dopamine agonists. While the catechol 136 is a selective D₁ agonist, the structurally similar mono-hydroxylated analogue 138 is a potent and selective D₁ antagonist [193]. Further evidence for the importance of the catechol moiety in determining dopamine agonist activity is seen in the difference in activity between 2-amino-5-hydroxytetralin (114) (R = H) and A-5,6-DTN (109). While 114 has both D₁ and D₂ activity, introduction of the "para" hydroxyl group significantly increases the D₁ activity, while having little effect on the D₂ activity [122]. Alteration of the conformation of an agonist has also been shown to have a profound effect on dopaminergic activity, as shown by the selectivity exhibited by the benzazepines. However,
with the exception of alkylation few attempts have been made to alter agonist activity through changes in the basic amine functional group.

One of the first attempts at altering the activity of dopamine through changes in the basic amine moiety involved the bioisosteric substitution of a permanently charged dimethylsulfonium functionally for the basic amine of dopamine [208]. Compound 153 exhibited both in vivo and in vitro activity as a direct acting dopamine agonist, however it was less potent than dopamine [208,209]. The activity of 153 indicated that alterations could be made to the amine portion of dopamine with retention of agonist activity. Additionally, the activity of the permanently charged dimethylsulfonium 153 suggested that the basic amine of dopamine interacted with dopamine receptors in its charged ammonium form.

\[ \text{HO-S(Me)}_2^+I^- \quad \text{HO-SMe} \]

153  154

The question of how the basic amine of dopamine and related agonists interacts with dopamine receptors has been an area of debate for many years. Some have suggested that the amine is in its neutral uncharged amine form [210-213], while others have theorized that the amine is in its charged ammonium form, when agonists are bound to dopamine receptors [139,160]. A number of researchers have also suggested that
the lone pair set of electrons associated with the basic amine plays an important role in the binding of agonists to dopamine receptors [163,214]. Since most dopamine agonists are either primary, secondary or tertiary amines, and, as such, exist in an equilibrium between their ionized ammonium and their unionized amine form at physiological pH, definite conclusions about the ionization state of the amine cannot be made. While the sulfonium 153 was suggested to be an isostere of the ammonium form of the basic amine, the uncharged sulfide 154 was suggested to be an isostere for the uncharged form of the amine [215]. When tested, the uncharged sulfide 154 was found to be inactive, reinforcing the theory that the basic amine of dopamine agonists is in its charged ammonium form when bound to the receptor [215,216].

The bicososteric substitution of the dimethylsulfonium group for an amine has been extended to other known dopamine agonists [217]. The dimethylsulfonium analogues of A-6,7-DTN (155) and α-methyldopamine (156) were synthesized and tested for their dopamine agonist activity [217]. Both 155 and 156 showed agonist activity. However, unlike the amine analogues, where A-6,7-DTN is more active than either dopamine or α-methyldopamine, in the same in vitro experiments all three sulfonium analogues tested, 153, 155, and 156 showed approximately the same activity [217]. Additionally, the selenide 157 and the selenonium 158 analogues have been synthesized and tested for their agonist activity [217]. Since selenium has a larger atomic radius than either sulfur or nitrogen it was thought that the selenonium and selenide would yield some information about the steric bulk and charge density requirements
of dopamine receptors [212,216]. As with the sulfide and sulfonium analogues, the permanently charged dimethyl selenonium 158 showed agonist activity while the uncharged selenide 157 was inactive [216,218,219]. The trimethylammonium analogue 159 of dopamine was also tested and was found to have agonist activity with a potency that was similar to the of the sulfonium 153 and the selenonium 158 analogues [216,219]. When compared to dopamine (DA) and dimethyl dopamine (DMDA) the range of potency was found to follow the trend DMDA > DA > N(Me$_3$)$_2$+I$^-$, S(Me)$_2$+I$^-$, Se(Me)$_2$+I$^-$ >> SHMe, SeMe [219]. The D$_2$ activity of the permanently charged analogues 153, 155, 156 and 158 has been well established [208,209,217,219], however, their ability to activate D$_1$ receptors is unclear and presently under investigation [220].
Interestingly, the idea of sulfonium/ammonium bioisosterism has been applied to drug molecules other than dopamine agonists [221]. The sulfonium analogue of levorphanol \textbf{160} has recently been synthesized and tested for its analgetic activity [221]. When tested in the CNS, compound \textbf{160} was shown to be as potent as levorphanol [221]. However, when tested in the guinea pig ileum assay, \textbf{160} was found to be a potent antagonist, in contrast to levorphanol, which is a potent agonist.

Although the research previously presented regarding permanently charged dopamine agonists answered a few important questions about agonist/receptor interactions, it also generated new questions. For example, the permanently charged agonists previously discussed are all
analogues of non-selective dopamine agonists. The question left to answer is, can the basic amine of selective dopamine agonists be replaced by either the permanently charged sulfonium or uncharged sulfide moiety with retention of activity? If so, would the activity exhibited by the selective agonists parallel that of the non-selective agonists? Additionally, if the sulfonium or sulfide analogues of selective agonists showed activity would it affect their selectivity?

The partial ergot analogue 150 was chosen as the model D2 selective agonist. It was anticipated that the sulfide analogue of 147 would be lipophilic and poorly soluble in the biological test media used. Therefore, since pyrazoles can be isolated as water soluble salts 150 was chosen as the model compound. The dimethylsulfonium 161, sulfide 162 and trimethylammonium 163 analogues of 150 were synthesized and tested to determine whether the activity of the D2 selective agonist analogues would follow the same trend of activity as the non-selective agonists. Additionally, the sulfonium and sulfide analogues, 161 and 162 respectively, represent compounds in which both the catechol and the basic amine moieties have been substituted with bioisosteres.
In addition to determining the range of activity of analogues of known D₂ selective agonists, an explanation for the inactivity of 149 was sought. As mentioned previously, the only compound that did not show dopamine agonist activity, in a series of simplified ergot derivatives, was 149 [203]. Since the simplified ergots were tested in vivo, it was suggested that rapid metabolism by MAO was responsible for the inactivity of 149, because dopamine is also inactive in the in vivo tests used [203]. A tertiary amine derivative was not reported, nor was 149 tested in the presence of an MAO inhibitor. Therefore, the tertiary amine analogue 164, which would not serve as a substrate for MAO was synthesized and tested to determine if the inactivity of the pyrrole ethylamine 149 was in fact due to metabolism by MAO. Additionally, the trimethylammonium 165, sulfide 166 and dimethylsulfonium 167 analogues were prepared to determine if the bioisosteric replacement of the basic amine would be successful in this series of compounds and if the activity of these compounds would follow the same trend as that of other dopamine agonists.
One possible explanation for the observed trend of activity may be related to the differences in lipophilicity between the permanently charged analogues and the parent amines. It may be that the permanent positive charge of the analogues alters their interactions with lipophilic membrane components near the receptor, such that the effective concentration of the drug in the vicinity of the receptor is decreased [222]. Additionally, a difference in the modes of binding of the permanently charged compounds, versus the parent amines, may be responsible for the consistently observed trend of activity [222].

Both the amines of DA and DMDA may be protonated at physiological pH. When protonated DA and DMDA acquire not only a positive charge but also contain a hydrogen that, as theorized, may lead to the formation of a reinforced ionic bond, as shown in Figure 23 [222]. The permanently charged analogues cannot acquire a proton, which could enter into
H-bonding, and therefore simply interact with an anionic site on the receptor via an ionic bond [222]. The permanently uncharged analogues are inactive presumably because they are not charged nor do they have the ability to acquire a proton and become charged. Thus, the permanently uncharged analogues cannot enter into an ionic bond or a reinforced ionic bond at the receptor site.

Figure 23: Proposed ionic and reinforced ionic bonds of dopamine agonists at dopamine receptors. (modified from [222])

The guanidino analogue of dopamine 168, which exists primarily in its protonated form, under physiological pH, was synthesized to determine if a permanently charged compound containing a proton would have increased affinity for dopamine receptors, because of its ability to enter into a reinforced ionic bond. The mono-substituted thiourea 169 was originally selected as a permanently uncharged compound with H-bonding capacity, that would allow for the assessment of the importance of H-bonding in determining dopamine agonist activity. Due to synthetic difficulties 169 was not tested for its dopamine agonist
activity. However, the disubstituted 170 was synthesized and tested for its dopamine agonist activity. Compound 170, like 169, is permanently uncharged and has the ability to hydrogen bond. Any effects the additional methyl group might have on activity are unknown at this time. Although the uncharged sulfide and selenide were shown to be inactive, these compounds do not have the ability to hydrogen bond, therefore it is necessary to determine if H-bonding alone is sufficient to impart receptor affinity to a compound.

Because of the variable distance requirements seen for dopamine agonist, between the meta hydroxyl and basic amine (0.55 nm - 0.74 nm), it has been suggested that the protonated amine of dopamine agonists interacts with the resonance extremes of a carboxylate residue at the receptor site, since amino acids have also shown this type of binding
ability, Figure 24 [223,224]. Therefore, if the guanidino portion of 168 is able to assume the correct orientation at the dopamine receptor, it may be able to hydrogen bond to both oxygens of the proposed carboxylate residue, as shown in Figure 25. If 168 can bind in this way it may exhibit increased affinity for the dopamine receptor. The thiourea 170 also has the ability to enter into a double hydrogen bond with the carboxylate, but since it is uncharged it may simply bind to the receptor without causing activation, thus acting as an antagonist. The activity of compounds 168 and 170 may lend some insight as to the requirements for receptor affinity and activation. For example, if 168 shows activity as an agonist while 170 shows antagonist activity, then it might be said that a positive charge is required for receptor activation, while H-bonding ability is important for receptor affinity.

**Figure 24:** Proposed interaction between the basic amine of dopamine agonists and a carboxylate residue of dopamine receptors. (modified from [122])
Although compound 168 has been reported in the literature, as the sulfate salt, it was tested for its ability to inhibit MAO, but has never been tested for its dopamine agonist activity [225]. However, compounds structurally similar to 168 have been tested for their dopamine agonist activity [226,227]. The imino-imidazolidine 171 (DPI) was originally described as a potent dopamine agonist [228]. However, more recent research has shown that the apparent dopaminergic activity expressed by 171 is due to α-adrenergic agonist activity [229-231]. The structurally related imidazoline 172, was found to have weak dopaminergic activity in the rabbit artery assay, but in vitro it showed no activity against dopamine sensitive adenylate cyclase [226]. Interestingly, it was proposed that the poor activity of 172 was due to the fact that with a pKa of 11.32, 172 would exist in the ammonium form at physiological pH and be unable to interact with dopamine receptors [226].

**Figure 25:** Suggested interaction of the guanidine moiety of 168 with a carboxylate residue of dopamine receptors.
Although structurally related analogues of 168 have shown poor dopamine agonist activity, a systematic assessment of the activity of compounds such as 168 and 170 has not been attempted. Therefore, the determination of the dopaminergic activity of compounds such as 168 and 170 is the first step in developing the structure activity relationships for compounds of this type.
CHAPTER VII
RESULTS AND DISCUSSION

This chapter is divided into two sections. The first section is devoted to a discussion of the chemistry involved in the synthesis of the proposed dopamine agonists. The second section is a discussion of results of biological testing of these compounds.

7.1 CHEMISTRY

While the synthesis of compound 149 has been previously reported [203,232], the procedure utilized for its production could not be easily altered to allow for the synthesis of the desired sulfide 166 or sulfonium 167 analogues. Although the tertiary amine 164 and the quaternary ammonium 165 might have been produced from the primary amine 149, a procedure was sought that would allow for the synthesis of all of the desired compounds from one common intermediate.

As shown in Scheme XVII, this approach began with the synthesis of compound 174. The synthesis of 174 required the acetylation of pyrrole at the three position. Generally, preparatively useful amounts of 3 substituted pyrroles cannot be obtained by direct electrophilic substitution of pyrrole, since substitution at the more reactive 2 position is preferred [233,234]. As such, procedures have been
developed that allow for the synthesis of 3 substituted pyrroles in useful quantities [233]. Introduction of substituents at either the 1 or 2 position of pyrrole are used to direct further substitution of pyrrole, such that upon removal of the substituent at the 1 or 2 position, the resulting product has been effectively substituted at the 3 position [233]. Early attempts at the synthesis of three substituted pyrroles, by our group, failed to produce any useful quantities of the appropriately substituted pyrrole [235]. Therefore, an alternate approach was chosen. Rokach et al. have shown that 1-phenylsulfonylpyrrole (173) can be specifically acetylated at the 3 position with a variety acid chlorides and anhydrides, in high yields [236]. They also demonstrated that the phenylsulfonyl directing group could be easily removed by base hydrolysis [236]. Thus, using the procedures of Rokach et al., 1-phenylsulfonylpyrrole (173) was specifically acetylated at the 3 position using chloroacetic anhydride and AICl₃, producing the desired intermediate 174 in good yield [236]. With the intermediate 174 in hand the synthesis of the target compounds was straightforward.

Reaction of 174 with a 40% aqueous solution of dimethylamine and subsequent hydrolysis of the directing group gave the dimethyl amino ketone, 175, which was isolated as the hydrochloride. After basification, reduction with lithium aluminum hydride (LAH) in refluxing ether, gave the desired tertiary amine 164, as the free base. Attempts to isolate 164 as the hydrochloride resulted in the production of an extremely hygroscopic material that was not easily purified.
However, isolation as the oxalate salt produced 164 as a white, easily purified solid.

Scheme XVII

Synthesis of 3-pyrrole ethylamine analogues 164 - 167

Interestingly, when the amine oxalate was placed in D₂O, for the purpose of obtaining a ¹H-NMR spectrum, the aromatic portion of the NMR spectrum did not appear as expected, Figure 26. The unexpected NMR spectrum could be due to two things; either the pyrrole ring was altered during the reduction of the ketone, or, since pyrroles are
known to protonate under acidic conditions [237,238], the NMR spectra observed could have been due to proton-deuterium exchange, catalyzed by the one unionized carboxylate of the oxalate salt. Indeed, when the NMR spectrum of 164 was obtained using the aprotic solvent, DMSO, the aromatic portion of the spectra appeared as expected, Figure 26. Additionally, a broadband decoupled $^{13}$C spectrum of 164 in $D_2O$ was obtained. As shown in Figure 27, the aromatic portion of the spectrum indicates the existence of 4 carbons. The sharp singlet represents the quaternary carbon at the 3 position pyrrole ring. The 3 triplets seen in this area of the spectra represent the three deuterated carbons of the pyrrole ring. A mass spectrum of the compound isolated from the $D_2O$ NMR solvent also indicated the presence of deuterium substitution. None of the other pyrrole derivatives, when placed in $D_2O$, exhibited this exchange phenomena. However, a small amount of 20% DCl added to a solution of 165 in $D_2O$, caused the rapid and complete exchange of deuterium for the hydrogens, of the pyrrole ring, as evidenced by $^1H$-NMR. Within a short period of time the $D_2O$ solution of 165 had turned a dark brown color and contained a precipitate, suggesting polymerization and decomposition had taken place.

The quaternary ammonium 165 was synthesized from 175 without the isolation of the intermediate 164. Reduction of 175 with LAH in refluxing ether as described earlier, gave the free base form of amine 164, which was treated with excess methyl iodide, yielding the desired 165.
Figure 26: $^1$H-NMR spectra of 164 in D$_2$O and DMSO (aromatic region)

Treatment of 174 with sodium methanethiolate, in CH$_3$CN, afforded the ketone sulfide 176 in one step. The unexpected hydrolysis of the phenylsulfonyl directing group conveniently eliminated the need for the hydrolysis step in this reaction sequence. Reduction of the ketone of 176 using conditions similar to those stated above gave the desired sulfide 166. Treatment of the sulfide 166 with excess methyl iodide, in accordance with the procedures of Chang, gave the desired dimethyl sulfonium iodide 167 [235].
Figure 27: Aromatic region of the $^{13}$C spectrum of 164 in D$_2$O

The key intermediate in the synthesis of the tertahydroindazoles 161 and 162, was the ketone sulfide 179. The synthesis of 179 has been previously described by Gray et al. [239]. Their reported synthesis, as summarized in Scheme XVIII, began with the synthesis of 4-tosyloxy cyclohexanone ethylene ketal (178), from hydroquinone (177) [239, 240]. Formation of the intermediate sulfide ketal was achieved by bubbling methyl mercaptan through a solution of the tosylate ketal in DMF, which also contained Na, and allowing this mixture to stand at room temperature, in the dark, for 116 hours [239]. The sulfide ketone 179 was generated upon hydrolysis of the ketal using glacial acetic acid at 60°C for 16 hours [239].

The commercial availability of the mono-ethylene ketal of 1,4 cyclohexanedione (180) simplified the production of 179 considerably,
Scheme XVIII

Reported synthesis of 4-thiomethyl cyclohexanone (179) by Gray

as shown in Scheme XIX. Reduction of the ketone with NaBH₄, in absolute methanol at room temperature, provided the ketal alcohol, which without further purification was tosylated using known procedures, yielding 178 [241]. Interestingly, when the tosylate was treated with a 4 fold excess of sodium methanethiolate in DMSO, the displacement of the tosylate was rapid; the reaction was complete within an hour. Deketalization, to give the desired ketone sulfide 179, was preformed according to the procedure of Gray et al. [239].

The pyrazole 181 was synthesized according to procedures of Booher et al. [242]. Thus, in anhydrous THF the sulfide ketone 179 was treated with potassium tert-butoxide and ethylformate. After 2 hours, anhydrous hydrazine was added to the mixture and the pH was adjusted to 9 with 10% HCl. This reaction mixture was allowed to react for an additional two hours. Work up of the reaction mixture yielded the pyrazole 181, which was isolated as the oxalate salt 162. Since the pyrazole ring system contains a basic and nucleophilic amine, it was
thought that to avoid alkylation of the pyrazole ring, the pyrazole sulfide 181 would have to be reacted with methyl iodide in its salt form. The production of the dimethyl sulfonium analogue 161 followed the same procedure as that of the sulfide 162 except that the sulfide pyrazole was not isolated as the oxalate. Instead, the reaction mixture was extracted with a small amount of 10% HCl. The acidic extract was concentrated and an excess amount of methyl iodide was added. Methanol was added as a cosolvent to provide a homogeneous reaction mixture. The reaction mixture was allowed to stir at room temperature for 5 days. A yellow solid, presumably the dimethyl sulfonium iodide pyrazole HCl, was isolated after work up of the dark red-brown reaction mixture. This solid proved very difficult to purify. Attempts to recrystallized the solid from methanol and ether or THF consistently resulted in the production of a dark red-brown solution. Therefore the product was purified using ion-exchange
chromatography. Using a cation exchange resin, Amberlite IR-120(plus), and eluting with 6N HCl, the product was isolated as the dimethylsulfonium chloride pyrazole HCl, 161. Unlike the yellow solid isolated from the reaction mixture, the product from the ion-exchange purification step was a white solid which proved to be more easily recrystallized. By $^1$H-NMR analysis alkylation was shown to be specific for the sulfide.

Scheme XX

Synthesis of tetrahydroindazole sulfonium chloride 161 and sulfide 162
The dimethylamine tetrahydroindazole 150 was synthesized according to the procedure of Bach et al. [203]. Basification and treatment of 150 with excess methyl iodide gave the specifically alkylated trimethylammonium iodide 163, Scheme XXI. Unlike the alkylation of the sulfide 181, the alkylation of 150 was rapid; a solid formed within minutes of addition of methyl iodide. Additionally, alkylation was specific for the secondary amine, indicating that perhaps the sulfide pyrazole 181 could have been alkylated as the free base pyrazole 181.

Scheme XXI

\[
\text{Synthesis of tetrahydroindazole ammonium iodide 163}
\]

\[
\begin{align*}
\text{150} & \quad \xrightarrow{1) \text{NaHCO}_3} \quad \text{163} \\
\text{2) xs Mel} & \\
\end{align*}
\]

Although the pharmacology of the sulfate salt of 168 has been presented in the literature [225], accounts of the synthesis could not be found. Therefore, literature procedures for the synthesis of structurally similar guanidines were used as guidelines for the synthesis of 168 [243]. Treatment of an aqueous solution of 3,4-dimethoxyphenethylamine with a 2 fold excess of S-methylisothiouronium sulfate and then with a saturated solution of
sodium bicarbonate, gave the guanidine carbonate 182, as shown in Scheme XXII. Cleavage of the 3,4-dimethoxy protecting groups with 48% HBr gave the desired guanidine catechol as the hydrobromide salt, 168, which has not been previously described.

Scheme XXII

Synthesis of N-[2-(3,4 dihydroxyphenyl)ethyl]guanidine HBr (168)

While the synthesis of 168 was straightforward, the synthesis of the desired thiourea 169 proved more complex. There are a number of procedures available for the synthesis of thioureas [244-248]. Initially the synthesis of the 169 was attempted using Si(NCS)$_4$, as outlined by Neville and McGee, since they had shown this procedure to be applicable to a wide range of aryl, aralkyl and alkyl amines [247,249]. Thus, treatment of 3,4-dimethoxyphenethylamine with Si(NCS)$_4$ in accordance with the procedures of Neville and McGee, as
shown in Scheme XXIII, resulted in the isolation of a tan solid, which
was presumed to be the desired product 169. However, upon IR analysis
the anticipated C=S absorptions located between 1550-1400 cm\(^{-1}\) and
1300-1100 cm\(^{-1}\) [250] were not observed, but rather, a sharp absorbance
was present at 2090 cm\(^{-1}\), suggesting that the compound isolated was not
the desired thiourea 169.

A search of the literature revealed an article in which Gillion et
al. reported their inability to convert \(t\)-butylamine and benzylamine
into the corresponding thioureas using the procedures of Neville and
McGee, even though Neville and McGee reported the successful synthesis
of these thioureas [251]. Instead of the desired thioureas, Gillion et
al. isolated \(t\)-butylammonium thiocyanate and benzylammonium thiocyanate
[251]. Gillion et al. also reported that alterations of several
reaction conditions failed to yield any of the desired thioureas [251].
In each case, only the thiocyanate salts of the starting amines were
isolated [251]. The synthesis of N-benzylthiourea was attempted to
confirm either the unsuccessful results of Gillion et al. or the
successful results of Neville and McGee. Following the reaction
conditions of Neville and McGee, benzylammonium thiocyanate was
isolated, as verified by melting point [251,252], thus confirming the
results of Gillion. The IR spectra of benzylammonium thiocyanate
showed strong absorptions at 2050 cm\(^{-1}\) and 2150 cm\(^{-1}\), suggesting that
the compound presumed to be the thiourea 169 was the ammonium
thiocyanate 183.
Confirmation of the structure of 183 was sought through its synthesis by an alternate method. The reaction of aryl, aralkyl and alkylamines with various cyanate salts, such as sodium, potassium or ammonium cyanate, or cyanic acid is known to produce the appropriately substituted ureas [244,252]. Similarly, arylamines when treated with thiocyanate salts produce the corresponding thioureas [248,253]. However, when aralkyl or alkyl amines are treated with thiocyanate salts only the ammonium thiocyanates are isolated [253]. Thus, the ammonium thiocyanate was produced as shown in Scheme XXIV. The product from the reaction in Scheme XXIV was shown to have similar spectral data as that of the product from Scheme XXIII. Additionally, their melting points were identical. $^{13}$C-NMR indicated that the product from Scheme XXIV contained a carbon with a chemical shift corresponding to a thiocyanate salt [254]. Through a comparison of melting point and spectral data it appears that the product isolated from the reaction
shown in Scheme XXIII was in fact the ammonium thiocyanate 183 and not the desired thiourea 169.

Scheme XXIV

**Synthesis of 2-(3,4-dimethoxyphenyl)ethylanmine Thiocyanate (183)**

![Reaction Scheme](image)

Unlike the monosubstituted thiourea 169, the N,N'-disubstituted thiourea 170 was produced efficiently and in good yield. As shown in Scheme XXV, dopamine HCl (92) was dissolved in absolute ethanol and 0.95 equivalents of triethyamine were added. Addition of a slight excess of methylisothiocyanate and refluxing the resulting solution for 1/2 hour, in accordance with the procedure of Durant et al., gave the unsymmetrically substituted thiourea 170, as a light brown oil [255]. The product was purified by flash column chromatography, and the resulting colorless oil was triturated with ether, over a period of a few hours, at room temperature, to give the desired product 170, as a white solid.
Scheme XXV

Synthesis of N-methyl-N'-(2-(3,4-dihydroxyphenyl)ethyl)thiourea (170)

\[
\begin{align*}
\text{HO} & \quad \text{NH₂} & \quad \text{Me-HCS} \\
97 & \rightarrow & \text{HO} \\
\text{OH} & \quad \text{HO} & \quad \text{ HO} & \quad \text{HO} & \quad \text{Me}
\end{align*}
\]

7.2 BIOLOGICAL RESULTS

The dopaminergic activities of compounds 150 (R = Me), 161 - 165, 167 and 168 were determined by assaying their ability to inhibit the \(K^+\)-evoked release of \(^3H\)acetylcholine from rat striatal slices. As mentioned earlier, the inhibition of cholinergic output in the striatum is mediated by \(D_2\) receptors and therefore the ability to inhibit the \(K^+\)-evoked release of \(^3H\)acetylcholine is a measure of \(D_2\) agonist activity. The determination of pharmacological activity was performed by Tahira Farooqui and David Willins under the guidance of Dr. N. J. Uretsky, of the Department of Pharmacology, in the College of Pharmacy, at The Ohio State University. The experimental techniques utilized in generating the following data have been discussed previously in the literature [208,209,216-219].

Table 17 shows the results of the experiments performed to determine the agonist activity of compounds 164, 165 and 167. Apomorphine (111)
is known to be a potent dopamine agonist and therefore was used as a control. As the data indicates, apomorphine caused an approximately 60% inhibition of the stimulated release of 3H-acetylcholine, at a concentration of 0.3 µM. The effects of apomorphine were reversed by the administration of sulpiride, a D2 selective antagonist, indicating that apomorphine is a direct acting dopamine agonist. On the other hand, compounds 164, 165 and 167 showed no significant activity as dopamine agonists. The sulfide 166 could not be tested for activity due to its poor solubility in the biological test media. The inability of compound 164 to act as a dopamine agonist indicates that the inactivity of the parent amine 149 is not due to metabolic inactivation by MAO, as previously suggested in the literature [203]. The inactivity of 164 is rather perplexing since the relationship between 164 and the bicyclic pyrrole 147 and pyrazole 150 is similar to the relationship between dopamine 97 and aminotetralin analogues, such as 108 and 109. That is, compounds 108 and 109 are conformationally restricted analogues of dopamine, while compounds 147 and 150 may be considered conformationally restricted analogues of compounds 149 and 164. Yet, while dopamine 97 and the aminotetralins 108 and 109 are active as dopamine agonists, only the rigid bicyclic pyrroles and pyrazoles, 147 and 150 respectively, act as dopamine agonists. Since none of the compounds in the series 164, 165 and 167 showed activity, comments about the ability of the sulfonium moiety to serve as an isostere in these compounds cannot be made.
### Table 17

**Inhibition of the K⁺-evoked release of ³H-acetylcholine by compounds 164 - 167 and apomorphine (111)**

<table>
<thead>
<tr>
<th>Cmpd.</th>
<th>Conc. (µM)</th>
<th>Compound Alone</th>
<th>Compound + Sulpiride</th>
</tr>
</thead>
<tbody>
<tr>
<td>164</td>
<td>300</td>
<td>89.3 ± 9.2(8)</td>
<td>93.5 ± 16.1(8)</td>
</tr>
<tr>
<td>165</td>
<td>300</td>
<td>99.9 ± 11.6(9)</td>
<td>102.3 ± 4.7(9)</td>
</tr>
<tr>
<td>166</td>
<td>---</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>167</td>
<td>300</td>
<td>75.3 ± 2.5(7)</td>
<td>80.3 ± 4.7(7)</td>
</tr>
<tr>
<td>111</td>
<td>0.3</td>
<td>39.7 ± 4.9(4)</td>
<td>104.4 ± 11.4(5)</td>
</tr>
</tbody>
</table>

*% Control = \( \frac{(\text{Release of } ³H-Ach \text{ in Presence of Cmpd.}) \times 100}{(\text{Release of } ³H-Ach \text{ in Absence of Cmpd.})} \)

Compound 150 (R = Me), showed dopamine agonist activity, inhibiting the stimulated release of ³H-acetylcholine by approximately 50% at a concentration of 100 µM. This was not unexpected since 150 has been previously shown to act as a potent dopamine agonist [203]. The quaternary ammonium 163 also showed agonist activity. As expected, compound 163 showed significantly decreased potency relative to the tertiary amine 150. However, what was not expected was the apparent lack of agonist activity of 161. At concentrations up to 300 µM compound 161 showed no significant agonist activity. The reasons for the lack of activity of 161 are not readily apparent. Previously, it has been shown that the difference in activity between the trimethylammonium analogue of dopamine 159 and the dimethylsulfonium
analogue 153 was approximately 25 fold [219]. If compounds 161 and 163 were to act in an analogous fashion, then the concentration of 161 needed to elicit an agonist response would approach 7.5 mM. The activity of 161 has not been determined at concentrations greater than 300 μM. The sulfide 162 was also devoid of agonist activity, however the lack of activity of 162 was expected, based on previous experiments with other sulfide analogs of dopamine agonists [216,218,215].

As mentioned previously, 161 is a compound which incorporates 2 bioisosteres in the same molecule. The apparent lack of activity may be due to the incorporation of two isosteres in the same compound. For example, it is known that the aminotetralin 108 causes a 50% inhibition of the K+‐evoked release of 3H‐acetylcholine at a concentration of 0.01 μM, while the sulfonium analogue 155 is significantly less active [217]. Additionally, 108 is more active than the bicyclic pyrazole 150, where R = Me. Therefore, a loss of potency is associated with the isosteric substitution of either a pyrazole ring for the catechol moiety, or a sulfonium for an amino functionality. Since a loss of potency is seen when these isosteric substitutions are made separately, the apparent lack of activity of 161 may be due to a significant decrease in potency upon incorporation of 2 isosteres within the same compound. The agonist effects of 163 were blocked by sulpiride, indicating that 163 is a direct acting dopamine agonist.

As indicated in Table 19, the guanidino compound 168 showed dopamine agonist activity. The agonist activity of 170 is currently under investigation. The effects of 168 could be blocked by the antagonist
Table 18

Inhibition of the K⁺-evoked release of ³H-acetylcholine by compounds 150, 161, 163

<table>
<thead>
<tr>
<th>Cmpd.</th>
<th>Conc. (µM)</th>
<th>Compound Alone</th>
<th>Compound + Sulpiride (2µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>161</td>
<td>100</td>
<td>79.0 ± 5.8(6)</td>
<td>N.D.</td>
</tr>
<tr>
<td>161</td>
<td>300</td>
<td>89.6 ± 5.8(10)</td>
<td>N.D.</td>
</tr>
<tr>
<td>163</td>
<td>300</td>
<td>60.1 ± 2.3(6)</td>
<td>82.2 ± 7.8(4)</td>
</tr>
<tr>
<td>150</td>
<td>30</td>
<td>48.4 ± 2.5(3)</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

(R=Me)

* %Control = \[
\frac{\text{(Release of ³H-Ach in Presence of Cmpd. X 100)}}{\text{(Release of ³H-Ach in Absence of Cmpd.)}}
\]

Sulpiride indicating that 168 is a direct acting agonist. Since the activity of 170 has not been determined, the importance of a positive charge for determining dopamine agonist activity, in this series of compounds cannot be addressed. However, 168 was significantly less potent than the quaternary ammonium analogue of dopamine 159. This was somewhat unexpected since it was theorized that the guanidino compound 168, because it contains both a positive charge and protons, would be able to enter into a reinforced ionic bond and have increased potency relative to compounds such as 159, that are only able to participate in ionic bonding. There are 2 significant differences between compounds 159 and 168 that must be addressed. First, the ability of compound 168 to delocalize its charge over a larger area may be one reason for the
decreased activity of 168. The more diffuse charge density of 168 may be responsible for a weaker ionic interaction taking place at the receptor site and hence a lower potency. Additionally, the guanidine functional group is rather large, and 168 may be considered to be structurally similar to N-isopropyl dopamine. As mentioned previously, with the exception of N-methyldopamine (Epinine, 100), all monosubstituted analogues of dopamine are essentially devoid of agonist activity [121]. Therefore the decreased potency of 168 may be related to its structural similarity to an N-monosubstituted dopamine analogue. Because of the lack of a definitive answer about the decreased activity of 168, conclusions about the importance of reinforced ionic binding at the receptor site cannot be made. Research in this area will continue, to determine if reinforced ionic binding is indeed an important agonist-receptor interaction.

Table 19

Inhibition of the K⁺-evoked release of ³H-acetylcholine by compound 168

<table>
<thead>
<tr>
<th>Cmpd.</th>
<th>Conc. (µM)</th>
<th>Compound Alone</th>
<th>Compound + Sulpiride (2µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>168</td>
<td>300</td>
<td>62.0 ± 4.5(10)</td>
<td>94.0 ± 5.3(10)</td>
</tr>
</tbody>
</table>

* %Control = \[
\frac{\text{(Release of } ^3\text{H-Ach in Presence of Cmpd. X 100)}}{\text{(Release of } ^3\text{H-Ach in Absence of Cmpd.)}}
\]
In summary based on the data presented above, a number of conclusions can be made:

1. The inactivity of compounds 164, 165 and 167 indicates that the inactivity of the parent amine 149 may not be due to metabolic inactivation by MAO, as previously suggested in the literature [203].

2. The activity of 163 reinforces the idea that dopamine agonists interact with D$_2$ receptors in their charged ammonium forms. Although this had been previously demonstrated for non-selective dopamine agonists, this is the first report of the activity of permanently charged D$_2$ selective agonists.

3. The inactivity of 161 is not easily explained. However, incorporation of two isosters into the same agonist and the decrease in potency associated with each separate isosteric substitution may be responsible for the apparent lack of activity of 161.

4. The activity of 166, which exists predominantly in its charged form under physiological conditions, suggests that a positive charge is important in determining dopamine agonist activity. However, since the activity of 170 has not been determined a definitive statement about the importance of a positive charge, for determining agonist activity, cannot be made. The decreased activity of 168 relative to other permanently charged analogues, such as 159, may be related to steric or electronic differences between these compounds. The agonist or antagonist activity of 170 has yet to be determined.
The general information concerning instrumentation, solvent preparation and elemental analysis provided in the introduction of chapter 4 is also applicable to this chapter. Additionally, dry acetonitrile and methylene chloride were obtained by distillation from phosphorus pentoxide. Hexane was distilled prior to use.

3-Chloroacetyl-1-(phenylsulfonyl)pyrrole (174)

![Chemical Structure]

1-(Phenylsulfonyl)pyrrole was prepared in accordance with the procedure outlined by Rokach et al., with the exception that acetonitrile was used as the solvent instead of DMF [236]. Thus, to a stirred solution of chloroacetic anhydride (12.37 g, 72 mmol) and AlCl₃ (19.2 g, 144 mmol) in 200 mL CH₂Cl₂, under argon, was slowly added a solution of 1-(phenylsulfonyl)pyrrole (5.0 g, 24 mmol) in 100 mL of CH₂Cl₂. After the addition was complete, the reaction mixture was
allowed to stir at room temperature for an additional 2 hours. The resulting mixture was poured onto 200 g of ice, and the aqueous and organic phases were separated. The aqueous layer was extracted with CH₂Cl₂ (3 X 100 mL) and the combined organic extracts were washed with 5% NaHCO₃ (2 X 100 mL), water (100 mL), dried (MgSO₄) and evaporated under reduced pressure leaving a solid, which was recrystallized from hexane/ethyl acetate to yield 5.8 g (86.5 %) of 174 as an off white solid. Occasionally the reaction mixture, the CH₂Cl₂ extracts, and the resulting crude solid were colored, generally purple. A majority of the color could be effectively removed by filtering a solution of the product in CH₂Cl₂ or ethyl acetate through a short column of silica gel. Evaporation of the filtrate and recrystallization of the resulting solid gave an off white solid as reported above. mp 101 - 102°C. ¹H NMR (CDCl₃, 250 MHz) δ 7.92-7.88 (m, 2H ArH), 7.82 (apparent t, J = 2.0 Hz, 1H, ArH), 7.69-7.62 (m, 1H, ArH), 7.58-7.51 (m, 1H, ArH), 7.15 (dd, J = 3.3 Hz, J = 2.0 Hz, 1H, ArH), 6.70 (dd, J = 3.3 Hz, J = 2.0 Hz, 1H, ArH), 4.40 (s, 2H, CH₂Cl). IR (KBr) cm⁻¹: 1685 (C=O). Mass Spectrum m/z: 283 (H⁺), 234, 77 (base). Analysis for C₁₂H₁₀N₂O₃S; calculated: C, 50.80; H, 3.50; N, 4.93; S, 11.30; found: C, 50.51; H, 3.71; N, 4.97; S, 11.67.
To a stirred solution of 174 (1.0 g, 3.5 mmol) in 10 mL dry acetonitrile was added a 40% aqueous solution of dimethyl amine (2.0 mL, 16.0 mmol). The mixture was allowed to stir for 2 hours at room temperature, at which time the mixture was concentrated under reduced pressure. A solution of 1.2 g of KOH in 25 mL of methanol was added to the oil and this mixture was allowed to stir at room temperature for an additional 5 hours. After 5 hours, water (100 mL) was added and the resulting mixture was extracted with CH$_2$Cl$_2$ (3 x 100 mL). The combined organic extracts were dried (MgSO$_4$) and evaporated under reduced pressure to give an oil which was dissolved in a small amount of ether. Dry HCl gas was bubbled through the ether solution. Care must be taken when adding HCl gas to the ether solution. Overzealous addition of HCl resulted in the production of a brown resinous material, which made product isolation difficult. Initial addition of HCl gas led to the precipitation of a white solid, presumably product. At the first sign of the formation of a brown color addition of the HCl gas was halted. The resulting precipitate was collected by filtration and recrystallized from MeOH/ether to yield 0.374 g (57%) of 175 as a white
solid.  \( \text{dp 193-196}\degree C. } \)\(^{1}\text{H NMR (D}_2\text{O, 270 MHz) } \delta 7.53 \text{ (apparent t, } J = 1.7 \text{ Hz, 1H, ArH), 6.96 (dd, } J = 3.1 \text{ Hz, } J = 1.7 \text{ Hz, 1H, ArH), 6.80 (dd, } J = 3.1 \text{ Hz, } J = 1.7 \text{ Hz, 1H, ArH), 4.48 (s, 2H, CH}_2\text{N), 2.81 (2, 6H, N(CH}_3\text{)_2). IR (KBr) cm}^{-1}: 1655 \text{ (C=O). Mass spectra m/z: 152 (H}^+ - \text{HCl), 58 (base). Analysis for C}_8\text{H}_{13}\text{N}_2\text{OCl; calculated: C, 50.93; H, 6.94; N, 14.85; found: C, 51.07; H, 6.90; N, 14.78. \)\(^{3}\text{-[2-(Dimethylamino)ethyl]pyrrole Oxalate (164)} \)

\[
\begin{align*}
\text{N(Me)}_2 & \\
\cdot \text{(COOH)}_2 & \\
\text{H} & 
\end{align*}
\]

Compound 175 (0.25 g, 1.3 mmol) was dissolved in a solution of NaHCO\(_3\) and extracted with CH\(_2\text{Cl}_2\) (2 x 25 mL). The CH\(_2\text{Cl}_2\) extracts were dried (HgSO\(_4\)) and evaporated under reduced pressure to leave a colorless oil, which was dissolved in a small amount of anhydrous ether. The ether solution was then added to a suspension of lithium aluminum hydride (LAH) (0.25 g, 6.5 mmol) in refluxing ether, over a period of 10 to 15 minutes. The reaction was allowed to continue for 1 hour at reflux temperature with stirring. After 1 hour the mixture was allowed to cool in an ice bath. The excess LAH was inactivated through the cautious addition of water. The resulting white solids were removed by filtration and washed with ether. The filtrate was concentrated at reduced pressure, and a solution of oxalic acid
dihydrate ((COOH)$_2$-2H$_2$O) (0.164 g, 1.3 mmol) in a small amount of methanol was added. The mixture was allowed to stand at -10°C overnight. The resulting solids were collected by filtration and dried in vacuo to yield 0.25 g (84%) of 164 as a white solid, mp 114-115°C. 

$^1$H NMR (DMSO d$_6$, 270 MHz) $\delta$ 6.67 (dd, $J = 2.5$ Hz, $J = 4.5$ Hz, 1H, ArH), 6.61 (m, 1H, ArH), 5.93 (dd, $J = 2$ Hz, $J = 4.5$ Hz, 1H, ArH), 3.17-3.11 (m, 2H, CH$_2$), 2.79-2.71 (m, 2H, CH$_2$), 2.74 (s, 6H, N(CH$_3$)$_2$). IR (KBr) cm$^{-1}$: 3240 (NH, pyrrole). Mass spectrum m/z: 138 (M$^+$ - (COOH)$_2$), 80 (base). Analysis for C$_{10}$H$_{16}$N$_2$O$_4$; calculated: C, 52.62; H, 7.07; N, 12.27; found: C, 52.35; H, 6.85; N, 12.20.

3-[2-(Trimethylammonium)ethyl]pyrrole Iodide (165)

![Chemical Structure]

Compound 175 (0.25 g, 1.3 mmol) was reduced as reported above, however, the resulting free base amine was not isolated as the oxalate, but rather, 5 mL of methyl iodide was added to the clear colorless oil. This mixture was allowed to stand at -10°C for 12 hours. The excess methyl iodide was evaporated under reduced pressure and the resulting solids were washed with CH$_2$Cl$_2$ and collected by filtration to yield 0.322g (85%) of 165 as a white solid, mp 195-197°C. $^1$H NMR (D$_2$O, 270 MHz) $\delta$ 6.73-6.71 (m, 1H, ArH), 6.67-6.65 (m, 1H, ArH), 6.04-6.02 (m,
1H, ArH), 3.40-3.34 (m, 2H, CH₂), 3.02 (s, 9H, N(CH₃)⁺I⁻), 2.91-2.85 (m, 2H, CH₂). IR (KBr) cm⁻¹: 3305 (broad, NH pyrrole). Mass spectrum (FAB) m/z: 153 (H⁺ + H, base). Analysis for C₉H₁₄N₂I: calculated: C, 38.59; H, 6.12; N, 9.99; found C, 38.47; H, 6.04; N, 9.99.

(Methylthio)methyl pyrrol-3-vl ketone (176)

A solution of 174 (5.0 g, 1.76 mmol) in 50 mL of CH₂Cl₂ was added to a solution of sodium methanethiolate [256] (4.8 g, 5.8 mmol) in 100 mL of MeOH, with stirring. The mixture was allowed to stir for an additional 2 hours at room temperature, after which time 150 mL of water was added and the resulting mixture extracted with CH₂Cl₂ (3 X 200 mL). The combined organic extracts were dried (MgSO₄) and evaporated under reduced pressure leaving a yellow solid which was recrystallized from CH₂Cl₂ to yield 2.2 g (80%) of 176 as a white solid. mp 135-137°C (lit. mp [235] 135-137°C.). ¹H NMR (CDCl₃, 270 MHz) δ 7.50-7.47 (m, 1H, ArH), 6.80-6.77 (m, 1H, ArH), 6.68-6.65 (m, 1H, ArH), 3.55 (s, 2H, CH₂SMe), 2.15 (s, 3H, SCH₂).
Compound 176 (0.5 g, 3.2 mmol) was dissolved in 20 mL of anhydrous THF and added to a refluxing suspension of LAH (0.5 g, 13 mmol) in ether, over a period of 15 minutes. The reaction was allowed to continue for 1 hour at reflux, after which time the reaction mixture was cooled in an ice bath and the excess LAH inactivated through the cautious addition of water. The resulting white solids were removed by filtration and washed with ether. The filtrate was evaporated under reduced pressure to yield 0.25 g (55%) of 166 [235] as a clear colorless oil. bp 85°C at 0.25 mm Hg. 1H NMR (CDCl3, 270 MHz) δ 7.20 (dd, J = 4.5 Hz, J = 2.5 Hz, 1H, ArH), 6.63-6.62 (m, 1H, ArH), 6.11 (dd, J = 4.5 Hz, J = 2.5 Hz, 1H, ArH), 2.80-2.69 (m, 4H, CH2CH2SMe), 2.13 (s, 3H, SCH3).
3-(2-(Dimethylsulfonium)ethyl)pyrrole Iodide (167)

Compound 167 was synthesized according to the procedure of Chang [235]. Thus, 166 (0.141 g, 1.0 mmol) was added to 3 mL methyl iodide and allowed to stand at -10°C overnight. The excess methyl iodide was evaporated under reduced pressure, leaving a solid, which was recrystallized from MeOH/ether to yield 0.25 g (88%) of 167 as a white solid. dp 79°C (lit. dp [235] 79°C). $^1$H NMR (CD$_3$OD, 270 MHz) δ 6.72 (d, J = 2.1 Hz, 2H, ArH), 6.09 (apparent t, J = 2.1 Hz, J* = 2.1 Hz, 1H, ArH), 3.55 (t, J = 7.1 Hz, 2H, CH$_2$), 3.05 (t, J = 7.1 Hz, CH$_2$), 2.79 (s, 6H, S(CH$_3$)$_2$).

4-Tosyloxy-cyclohexanone ethylene ketal (178)

To a solution of NaBH$_4$ (4.0 g, 106 mmol) in 50 mL of absolute methanol was added a solution of 1,4-cyclohexanedione mono-ethylene
ketal (10.0 g, 64 mmol) in 15 mL of absolute MeOH, with stirring at 0°C. Upon completion of addition, the reaction was allowed to continue for 1 hour, with stirring, at room temperature. Water was cautiously added to inactivate the excess NaBH₄. After inactivation, the resulting mixture was extracted with CH₂Cl₂ (3 x 200 mL). The combined organic extracts were dried (MgSO₄) and evaporated under reduced pressure, leaving a clear colorless oil, to which was added a solution of tosyl chloride (24.4 g, 128 mmol) in 50 mL dry pyridine. The resulting mixture was allowed to stand at 0°C for 12 hours, after which time the mixture was added to 200 g of ice. The mixture was allowed to stand for a few hours at 0°C. The resulting white solids were collected by filtration and washed with water. The collected solids were dried in vacuo yielding 18.0 g (90%) of 178 as a white solid, which was used without further purification. mp 69-70°C (lit. mp [239] 69-71°C). ¹H NMR (CDCl₃, 270 MHz) δ 7.81 (d, J = 8.5 Hz, 2H, ArH), 7.32 (d, J = 8.5 Hz, 2H, ArH), 3.95-3.88 (m, 4H, OCH₂CH₂O), 2.45 (s, 3H, Ph-CH₃), 1.89-1.77 (m, 6H), 1.60-1.52 (m, 2H).
4-Thiomethyl cyclohexanone (179)

To a solution of 178 (4.5 g, 14 mmol) in 20 mL DMSO was added, in portions, with stirring at room temperature, sodium methanethiolate (4.0 g, 57 mmol) [256]. The reaction mixture was stirred at room temperature for 1 hour, at which time 200 mL of water was added. The resulting mixture was extracted with CH₂Cl₂ (3 x 200 mL). The combined CH₂Cl₂ extracts were dried (MgSO₄) and evaporated under reduced pressure, leaving a yellow oil, presumably the intermediate sulfide ketal. Deketalization was accomplished using the procedure of Gray et al. [239]. Thus, to the above isolated oil was added 10 mL of 90% acetic acid. The mixture was heated to 60°C for 16 hours, at which point the mixture was cooled and diluted with water (200 mL). The resulting mixture was extracted with CH₂Cl₂ (3 x 200 mL). The combined CH₂Cl₂ extracts were dried (MgSO₄) and evaporated under reduced pressure, leaving a slightly brown oil, which was purified by flash column chromatography (10% EtOAc/Hexane). Evaporation of the resulting organic fractions, under reduced pressure gave 1.5 g (74%) of 179 [239] as a clear colorless oil. bp 75-78°C at 0.65 mm Hg. ¹H NMR (CDCl₃, 270 MHz) δ 3.05-2.99 (m, 1H, C(1H)-SHMe), 2.57-2.50 (m, 2H), 2.50-2.18...
(m, 4H), 2.14 (s, 3H, SCH₃), 1.95-1.86 (m, 2H). Mass spectrum m/z: 144 (H⁺, base) (lit. Mass spectrum m/z: [239] 144 (H⁺, base)).

5-(Methylthio)-4,5,6,7-tetrahydroindazole Oxalate (162)

The general procedure of Booher et al. was followed in the synthesis of 162 [242]. Thus, a solution of ethyl formate (2.3 mL, 28 mmol) and 179 (1.0 g, 7 mmol) in 15 mL anhydrous THF was rapidly added to a solution of potassium tert-butoxide (1.57 g, 14 mmol) in 15 mL THF under argon at 0°C. The reaction mixture was maintained at 0°C for 15 minutes, with stirring, and then at room temperature for an additional 2 hours. After 2 hours had elapsed anhydrous hydrazine (2.66 mL, 84 mmol) was added and the reaction mixture was adjusted to pH 9 with 10% HCl. The mixture was allowed to continue stirring at room temperature for an additional 2 hours. To the brown mixture was added 50 mL cold 10% NaOH and the mixture was extracted with diethyl ether (3 x 100 mL). The ether extracts were combined, dried (MgSO₄) and concentrated under reduced pressure. To the concentrated ether solution was added a solution of oxalic acid dihydrate ((COOH)₂·2H₂O) (0.8 g) in a small amount of MeOH and the mixture was allowed to stand overnight at -10°C. The solids were collected by filtration and dried in vacuo to yield
0.45 g (25%) of 162 as a white solid. mp 141-142°C. ¹H NMR (D₂O, 270 MHz) δ 7.65 (s, 1H, ArH), 3.09-3.00 (m, 1H, CH-SMe), 2.84 (dd, J = 16.1 Hz, J = 5.0 Hz, H₄), 2.78-2.57 (m, 2H, H₇), 2.45 (dd, J = 16.1 Hz, J = 7.2 Hz, H₄), 2.10-2.00 (m, 1H, H₆), 1.98 (s, 3H, SCH₃), 1.87-1.77 (m, 1H, H₆). IR (KBr) cm⁻¹: 3600 - 2100 (pyrazole). Mass spectrum m/z: 168 (M⁺ - (COOH)₂), 120 (base). Analysis for C₁₀H₁₄N₂O₄S; calculated: C, 46.50; H, 5.46; N, 10.85; found: C, 46.37; H, 5.52; N, 10.67.

Dimethyl(4,5,6,7-tetrahydroindazol-5-yl)sulfonium chloride, Hydrochloride (161)

The free base pyrazole was synthesized in a manner similar to that outlined above for the synthesis of 162. Thus, a solution of ethyl formate (4.5 mL, 56 mmol) and 179 (2.0 g, 14 mmol) in 15 mL anhydrous THF was rapidly added to a solution of potassium tert-butoxide (3.1 g, 28 mmol) in 15 mL THF under argon at 0°C. The reaction mixture is maintained at 0°C for 15 minutes, with stirring, and then at room temperature for an additional 2 hours. To the reaction mixture was added anhydrous hydrazine (3.0 mL, 95 mmol) and the reaction mixture was adjusted to pH 9 with 10% HCl. The mixture was allowed to continue stirring at room temperature for an additional 2 hours. To the brown
mixture was added 50 mL cold 10% NaOH and the mixture was extracted with diethyl ether (3 x 100 mL). The combined ether extracts were extracted with 10% HCl (50 mL) and the aqueous solution was concentrated under reduced pressure. To this solution was added 20 mL of methyl iodide and a quantity of MeOH necessary to create a homogeneous mixture. The reaction mixture was stirred at room temperature for 5 days. The resulting dark brown solution was evaporated to dryness under reduced pressure to yield a brown solid that was dissolved in a small amount of water and applied to the head of an ion-exchange column (20 g, Amberlite IRA-120(plus)). The column was washed with several volumes of water until the eluant was colorless. The product was eluted with 6N HCl. The column fractions containing the desired product were combined and evaporated to dryness under reduced pressure. The resulting solid was recrystallized from EtOH/ether to yield 0.5 g (14%) of 161 as a white solid. mp 157-161°C.  

$^1$H NMR (D$_2$O, 250 MHz) δ 7.74 (s, 1H, ArH), 3.79 (m, 1H, CH$_5$(Me)$_2$Cl$^+$), 3.13 (dd, J = 5.0 Hz, J = 15.9 Hz, 1H, H$_4$), 3.00-2.70 (m, 3H), 2.84 (s, 3H, S(CH$_3$)Me$^+$Cl$^+$), 2.8 (s, 3H, S(CH$_3$)Me$^+$Cl$^+$), 2.43 (m, 1H, H$_6$), 2.08 (m, 1H, H$_6$). IR (KBr) cm$^{-1}$: 3590-3200 (b, pyrazole). Mass spectrum (FAB) m/z: 183 (H$^+ -$HCl,Cl), 169 (base). Analysis for C$_9$H$_{16}$N$_2$S$^+$Cl$^-$: 0.5H$_2$O; calculated: C, 40.87; H, 6.46; N, 10.32; found: C, 40.91; H, 6.48; N, 10.60.
Trimethyl(4,5,6,7-tetrahydroindazol-5-yl)ammonium iodide (163)

Compound 150 (R = H) (0.15 g, 0.63 mmol) [203] was dissolved in 10 mL H₂O and basified with NH₄OH. The solution was extracted with a 3:1 mixture of CH₂Cl₂ and iPrOH (3 X 50 mL). The resulting organic extracts were combined, dried (MgSO₄) and evaporated under reduced pressure to leave an oil, which was dissolved in 10 mL methyl iodide. The mixture was allowed to stand at -10°C for 12 hours at which time the excess methyl iodide was evaporated under reduced pressure. The resulting yellow solid was recrystallized from EtOH to yield 0.14 g (72%) of 163 as a white solid, mp 222-224°C. ¹H NMR (D₂O, 250 MHz) δ 7.35 (s, 1H, ArH), 3.56 (m, 1H, H₅), 3.10 (m, 1H, H₄), 3.01 (s, 9H, N(CH₃)₃), 2.80 (m, 1H, H₇), 2.77-2.52 (m, 2H, H₇ & H₄), 2.36 (m, 1H, H₆), 1.80 (m, 1H, H₆). IR (KBr) cm⁻¹: 3300-3000 (pyrazole). Mass spectrum (FAB) m/z: 180 (H⁺ - I, base). Analysis for C₁₀H₁₈N₃I; calculated: C, 39.10; H, 5.90; N, 13.68; found: C, 38.99; H, 5.95; N, 13.43.
N-[2-(3,4 Dimethoxyphenyl)ethyl] guanidine carbonate (182)

To a solution of 3,4 dimethoxyphenethylamine (2.0 g, 11 mmol) in 10 mL H₂O was added S-methylisothiouronium sulfate (6.14 g, 22 mmol) [257]. The mixture was slowly warmed to 100°C with stirring. Methyl mercaptan gas evolved was passed through a Chlorox trap. The reaction was allowed to stir at 100°C for an additional 2 hours at which time the mixture was concentrated under reduced pressure. The concentrated mixture was cooled to ice bath temperature and 30mL of a saturated solution of NaHCO₃ was added. The mixture was allowed to stand at 0°C for 12 hours. The solids were collected by filtration and dried in vacuo to yield 1.73 g (55%) of 182 as a white solid. NOTE: 182 was insoluble in most deuterated solvents, used for NMR analysis. However, the addition of a small amount of DCl to a suspension of 182 in D₂O allows for the dissolution of 182 in D₂O and its analysis by ¹H NMR.

mp 156-160°C. ¹H NMR (D₂O + DCl, 250 MHz) δ 6.64 (d, J = 8.1 Hz, 1H, ArH, ortho to OMe and II), 6.60 (d, J = 1.6 Hz, 1H, ArH, ortho to OMe and CH₂), 6.53 (apparent d, J = 8.1 Hz, 1H, ArH, ortho to H and CH₂), 3.54 (s, 3H, OCH₃), 3.52 (s, 3H, OCH₃), 3.11 (t, J = 6.7 Hz, 2H, CH₂), 2.51 (t, J = 6.7, 2H, CH₂). IR (KBr) cm⁻¹: 1690, 1635 (C=N).; Mass
spectrum m/z: 224 (H^+ - HCO_3^-), 223, 152 (base). Analysis for C_{12}H_{19}O_5N_3; calculated: C, 50.52; H, 6.71; N, 14.73; found: C, 50.57; H, 6.74; N, 14.25.

N-[12-(3,4-Dihydroxyphenyl)ethyl]guanidine Hydrobromide (168)

![Image of the chemical structure of N-[12-(3,4-Dihydroxyphenyl)ethyl]guanidine Hydrobromide (168)]

Compound 162 (0.5 g, 1.75 mmol) was dissolved in 5 mL of 48% HBr and heated to 100°C and maintained at this temperature for 4 hours. The resulting mixture was cooled and evaporated to dryness through the use of a vacuum pump. The resulting solids were triturated with ether, and the solids were collected by filtration to yield 0.425 g (88%) of 168 as a white solid. mp 140-142°C. \(^1\)H NMR (D_2O, 250 MHz) \(\delta\) 6.73 (d, J = 8.1 Hz, 1H, ortho to OH and H), 6.75 (d, J = 1.8 Hz, 1H, ortho to OH and CH_2), 6.68 (dd, J = 8.1 Hz, J = 1.8 Hz, ortho to H and CH_2), 6.25 (t, J = 6.6 Hz, 2H, CH_2), 2.62 (t, J = 6.6 Hz, 2H, CH_2). IR (KBr) cm\(^{-1}\): 3600-3000 (catechol). Mass spectrum m/z: 195 (H^+ - Br), 136. Analysis for C_9H_{14}N_3O_2Br; calculated: C, 39.15; H, 5.11; N, 15.22; found: C, 38.98; H, 5.38; N, 15.24.
N-Methyl-N'-[2-(3,4-dihydroxyphenyl)ethyl]thiourea (170)

\[
\begin{align*}
&\text{HO} \\
&\text{HO} \\
&\text{C} \\
&\text{Me} \\
&\text{S} \\
&\text{N} \quad \text{C} \\
&\text{H} \quad \text{H} \\
&\text{O} \\
&\text{O}
\end{align*}
\]

Triethylamine (0.71 mL, 5.04 mmol) was added to a suspension of dopamine hydrochloride (1.0 g, 5.30 mmol) in 10 mL of absolute ethanol. The resulting solution was warmed to reflux temperature, at which point methylisothiocyanate (MITC) (0.44 g, 6.0 mmol) was rapidly added. The MITC was decolorized with carbon before use. After the addition of the MITC the mixture was allowed to reflux for an additional hour, at which point the mixture was cooled and diluted with CH₂Cl₂ (200 mL). The solution was washed with 2N HCl (2 x 100 mL), dried (MgSO₄) and evaporated under reduced pressure yielding a light brown oil, which resisted all attempts at crystallization. The oil was purified by flash column chromatography (40% EtOAc/hexane) and the collected organic fractions were evaporated under reduced pressure to yield a clear colorless oil, which when triturated with ether, at room temperature for a period of approximately 6 hours, produced a white solid. The solid was collected and dried to yield 0.85g (71%) of 170. mp 94-96°C. \( ^1\text{H} \) NMR (Acetone d₆, 250 MHz) \( \delta 7.70 \) (very broad s, D₂O exchangeable), 6.88 (broad s, D₂O exchangeable, 1H), 6.82 (broad s, D₂O exchangeable, 1H), 6.72 (d, \( J = 8.0 \text{ Hz} \), 1H, ArH, ortho to H and OH),
6.71 (d, J = 2.0 Hz, 1H, ArH, ortho to OH and CH$_2$), 6.54 (dd, J = 8.0 Hz, J = 2.0 Hz, ArH, ortho to H and CH$_2$), 3.65 (m, 2H, CH$_2$), 2.92 (d, J = 4.5 Hz, 3H, -NCH$_3$), 2.72 (t, J = 7.7 Hz, 2H, CH$_2$) IR (KBr). cm$^{-1}$: 1565 (s, C=S), Mass spectrum m/z: 226 (M$^{+}$), 123 (base). Analysis for C$_{10}$H$_{14}$N$_2$O$_2$S; calculated: C, 53.08; H, 6.24; N, 12.38; found: C, 53.43; H, 5.98; N, 12.42.

2-(3,4-Dimethoxyphenyl)ethylamine Thiocyanate (183)

![2-(3,4-Dimethoxyphenyl)ethylamine Thiocyanate (183)]

Concentrated HCl (0.5 mL) was added to a solution of 3,4-dihydroxyphenethylamine (1 g, 5.5 mmol) and potassium thiocyanate (0.6 g, 6.05 mmol) in 10 mL water. The mixture was heated to reflux and maintained at this temperature for 3 hours, at which time the mixture was cooled and concentrated under reduced pressure. The concentrated solution was allowed to stand at 0°C for 12 hours and the resulting solids were collected by filtration and dried in vacuo to yield 1.0 g (76%) of 183 as white crystals. mp 164-165°C. $^1$H NMR (MeOH d$_4$, 250 MHz) $\delta$ 6.92 (d, J = 8.2 Hz, 1H, ArH, ortho to OMe and H), 6.89 (d, J = 1.9 Hz, 1H, ArH, ortho to OMe and CH$_2$), 6.82 (dd, J = 8.2 Hz, J = 1.9 Hz, 1H, ArH, ortho to H and CH$_2$), 3.84 (s, 3H, OCH$_3$), 3.80 (s, 3H, OCH$_3$), 3.16 (t, J = 7.5 Hz, 2H, CH$_2$), 2.89 (t, J = 7.5 Hz, 2H,
CH₂. IR (KBr) cm⁻¹: 2090 (s, "SCN). Mass spectrum m/z: 181 (M⁺ -SCN), 152 (base). Analysis for C₁₁H₁₆NO₂S; calculated: C, 54.98; H, 6.71; N, 11.66; found: C, 55.00; H, 6.67; N, 11.54.


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