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Tantishaiyakul, Vimon, Ph.D.

The Ohio State University, 1990

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PART 1: SYNTHESIS OF IODOCATECHOLAMINE DERIVATIVES AS ADRENERGIC STIMULANTS AND THROMBOXANE A₂ ANTAGONISTS

PART 2: SYNTHESIS OF IRREVERSIBLE INHIBITORS OF ALDOSE REDUCTASE

DISSERTATION

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

By
Vimon Tantishayakul, B.S., M.S.

* * * *

The Ohio State University

1990

Dissertation Committee:
Duane D. Miller, Ph.D.
Robert W. Brueggemeier, Ph.D.
Dennis R. Feller, Ph.D.
Donald T. Witiak, Ph.D.

Approved by
Duane D. Miller, Adviser
College of Pharmacy
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1990
DEDICATION

To my parents, sisters and brothers
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VITA

October 8, 1956 ............... Born - Banpong, Ratchaburi, Thailand

March 1980 .................. B.S. Pharmacy, Chulalongkorn University, Bangkok, Thailand

April 1980 - present ........ Instructor, department of pharmaceutical chemistry, Faculty of Pharmacy, Prince of Songkla University

June 1985 .................. M.S. Pharmacy, Chulalongkorn University, Bangkok, Thailand

Aug.1987 - Aug. 1989 ........ Graduate Research Associate, College of Pharmacy, The Ohio State University

September 1989 - present .... Graduate Research Associate, College of Pharmacy, The Ohio State University

Publications


Fields of Study

Major Field: Synthetic Medicinal Chemistry (Pharmacy)
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PART 1
SYNTHESIS OF IODOCATECHOLAMINE DERIVATIVES AS ADRENERGIC STIMULANTS AND THROMBOXANE A₂ ANTAGONISTS
CHAPTER I  
INTRODUCTION

1.1 BACKGROUND

The nervous system is one of the major means by which body functions are controlled and integrated. It relies primarily on rapid electrical transmission of information over nerve fibers. However, between nerve cells, and nerve cells and their effector cells, signals are usually carried by chemical rather than electrical impulses. Chemical transmission takes place through the release of small amounts of transmitter or "neuromediator" substances from the nerve terminals into the region of the synaptic cleft by diffusion and it then activates or inhibits the postsynaptic cells by binding to a specialized receptor. The nervous system of vertebrates is divided (as shown in Figure 1)\(^1\) into the central nervous system (CNS), comprising the brain and the spinal cord, and the peripheral nervous system (PNS), which includes all nervous tissue outside the brain and spinal cord.

1.2 THE ADRENERGIC NEURONAL SYSTEM

The adrenergic system, or the sympathetic nervous system, is found both peripherally and centrally.\(^2\) The three naturally occurring catecholamines: epinephrine, norepinephrine, and dopamine play important roles as brain and peripheral neurohumoral transmitters.
Figure 1: Nervous system.

Epinephrine was discovered first as the hormone released from the adrenal medulla in response to a variety of physiological or environmental stresses. Norepinephrine was later identified as the neurotransmitter released from peripheral sympathetic nerve endings. Dopamine was the last of the three to be considered as having a physiological role.

1.2.1 BIOSYNTHESIS OF CATECHOLAMINES

The biosynthetic pathway of dopamine, norepinephrine, and epinephrine from tyrosine is shown in Figure 2. The optically active substrate L(-)-tyrosine is converted to L(-)-dopa by the enzyme tyrosine hydroxylase. This enzyme requires a tetrahydrobiopterin coenzyme, O2, and Fe2+. The enzyme itself is inhibited by a variety of catechols, including dihydroxyphenylalanine (DOPA), or norepinephrine so it is regulated by end-product feedback inhibition. This transformation is the rate-limiting step in the synthesis of the catecholamines, since the rate of
hydroxylation of tyrosine is slower than the subsequent enzymatic conversion.

L(-)-dopa is converted to dopamine by dopa decarboxylase. The enzyme requires pyridoxal phosphate as a cofactor. The conversion of dopamine to (-)-norepinephrine is catalyzed by dopamine β-hydroxylase. This enzyme is located in the membranes of storage vesicles and norepinephrine is thus synthesized in the vesicle. Dopamine β-hydroxylase, a mixed function oxygenase, is a copper-containing protein that uses O₂ and ascorbic acid. Finally, phenylethanolamine-N-methyltransferase uses S-adenosylmethionine to catalyze the formation of epinephrine from norepinephrine.

It appears that each type of adrenergic cell contains only those biosynthetic enzymes that are active in the elaboration of the chemical messenger that is released from it.⁴⁻⁵ In mammals, where norepinephrine serves as the mediator in the sympathetic system, phenylethanolamine-N-methyltransferase is absent. Phenylethanolamine-N-methyl transferase is located primarily in the adrenal medulla which is the main site of epinephrine synthesis.

In Figure 2, the alternate pathways in the biosynthesis of catecholamines is shown by dashed arrows. These pathways have not been found to be of physiological significance in humans. However, tyramine and octopamine may accumulate in patients treated with monoamine oxidase inhibitors.³
Figure 2: Biosynthesis of catecholamines (modified from reference 3)
1.2.2 METABOLISM OF CATECHOLAMINES

Metabolism is one mechanism which terminates the action of norepinephrine, epinephrine and dopamine. These catecholamines can be metabolized by several enzymes including catechol-O-methyltransferase (COMT), monoamine oxidases (MAO), and conjugating enzymes. The aldehyde products produced by the action of MAO may be reduced to an alcohol by aldehyde reductase or oxidized to the acid by aldehyde dehydrogenase. Alcohol dehydrogenase may reverse the action of aldehyde reduction. The amine products of O-methylation can be substrates for MAO and conjugate enzymes; the deaminated catechols can also be O-methylated by COMT. The major metabolic pathway of catecholamines is shown in Figure 3.

COMT is a magnesium-dependent enzyme which catalyzes the transfer of the methyl group from S-adenosylmethionine to one of the hydroxyl groups (usually the hydroxy group in position 3) of a catechol. In vivo, O-methylation occurs preferentially with (+)-norepinephrine (unnatural isomer) rather than the (-)-isomer. This may be due to the rapid uptake of the (-)-isomer by the nerve ending, so that only a small fraction of the substrate is available for methylation by the enzyme.

MAOs are mitochondrial enzymes which catalyze the oxidation of amines, presumably via an intermediate imine, to their corresponding aldehydes. There are at least two types of MAO, MAO-A and MAO-B. MAO-A is more active in deaminating 5-hydroxytryptamine, and MAO-B is more active towards benzylamine and phenylethylamine.

Phenolsulfotransferases (PST) are enzymes which catalyze the formation of sulfoconjugated derivatives. There are at least two forms of PST, the P (or TS) and M (or TL) forms. The P form has a greater
Figure 3: Metabolic pathway of catecholamines (modified from reference 7)
affinity for phenol but the M form has higher affinity for catecholamines. Glucuronide formation is also an important means of metabolizing the catecholamines. The transferase is responsible for the reaction in which active glucuronic acid (uridine diphosphoglucuronic acid) is converted to the phenolic hydroxide of catecholamine metabolites.

There are other metabolic pathways in addition to the one shown in Figure 3. N-Demethylation and β-dehydroxylation of catecholamines have also been demonstrated to take place. A pathway of minor importance is the shortening of the phenylethylamine side chain leading to the formation of 3,4-dihydroxy benzoic acid and 4-hydroxy-3-methoxybenzoic acid (vanillic acid). This conversion is most likely to occur with those compounds bearing a β-hydroxyl group. A small proportion of the amines are excreted as N-acetyl derivatives. Other minor metabolic reactions are 4-O-methylation and 3,4-di-O-methylation of the catecholamines. A possible metabolite of dopamine might be the very unstable compound 2,4,5-trihydroxyphenethylamine (6-hydroxydopamine). Dopamine can also condense with aldehyde to form tetrahydroisoquinoline derivatives. At last, epinephrine can be oxidized to adrenochrome.

1.2.3 THE STORAGE OF CATECHOLAMINES

Catecholamine storage utilizes synaptic vesicles of different sizes in different organs. Eight three types of vesicles have been found in the varicosities: large dense-cored vesicles (750-1000 Å), small dense-cored vesicles (450-500 Å) and small electron translucent vesicles (450-500 Å). Nonterminal axons have only the large type of vesicles. The largest ones, called chromaffin granules, are found in the adrenal medulla and
store catecholamines as their ATP complexes, in a proportion of 4:1, in association with the acidic protein chromogranin. In contrast, norepinephrine:ATP molar ratios of 6.8-12.1 have been found in other large vesicles. This type of storage can keep the neurotransmitter in a high concentration within the vesicles and protect it from enzymatic oxidation by MAO.

1.2.4 THE RELEASE OF CATECHOLAMINES

The release of catecholamines has been studied mainly in the adrenal medulla. Neuronal stimulation releases acetylcholine which allows the influx of Ca\(^{2+}\) and triggers fusion of the chromaffin cell membrane with the secretory vesicle, resulting in exocytosis of the entire vesicle contents, including all of the vesicle proteins. It is not clear whether neurotransmitter release in noradrenergic varicosities follows the same mechanism. There are two principal ways in which norepinephrine might be released: (1) norepinephrine can first pass from the vesicle into the cytosol (soluble part of the cytoplasm), and from there pass across the cell membrane; or (2) the vesicle might release its contents directly into the extracellular space. Some hypothetical mechanisms of release related to these two main mechanisms have been proposed. However, exocytosis seems to be the most probable mechanism. Newly synthesized norepinephrine has been found to be released preferentially. This suggests that norepinephrine exists in more than one pool in the sympathetic neuron. The release and turnover of catecholamines are subject to complex regulation. Although the activity of the sympathetic nervous system is ultimately controlled by the central nervous system, it is likely that the
terminal networks of peripheral neurones are susceptible to control by other factors. The most important type of which is modulation by presynaptic receptors. In addition to the autoreceptors, there are a variety of presynaptic receptor sites in noradrenergic nerve endings; muscarinic inhibitory receptors, dopamine inhibitory receptors, enkaphalin inhibitory receptors, prostaglandin inhibitory receptors, adenosine inhibitory receptors, angiotensin II facilitatory receptors, and nicotinic facilitatory receptors. Some of these receptors are not located in all noradrenergic nerve endings. These receptors can play an important role in modifying a sympathetic neurotransmission in the PNS by their agonists.

1.2.5 THE UPTAKE OF CATECHOLAMINES

The idea that catecholamines might be taken up into tissue binding sites was first suggested by Burn. Uptake but not enzymatic destruction is the principal mechanism for deactivation of released catecholamines. However, catecholamines can be metabolized by intracellular enzymes subsequent to uptake. Several different uptake processes exist for the catecholamines in animal tissues. The process by which catecholamines are transported from the extracellular space across the axonal membranes of adrenergic nerves is called uptake 1 (neuronal uptake). A further mechanism exists to promote the transfer of free catecholamines from the axoplasm into the membrane bound storage vesicle in adrenergic nerves. Catecholamines are transported across the membranes of smooth muscle and various other postsynaptic cells by another process known as uptake 2 (extraneuronal uptake). Uptake 2 normally occurs in tissues innervated by the sympathetic nervous system.
The existence of uptake 1 sites in noradrenergic neuron both in
the PNS and in the CNS is well established. Epinephrine has an affinity for
uptake 1 approximately one half that of norepinephrine. Uptake 1 is a
stereochemically selective process, with an affinity for the (-)-isomer
of norepinephrine being about five times higher than that for the (+)-
isomer. In mammalian tissues, many other phenolic derivatives of β-
phenylethylamine are accumulated in sympathetic nerves by uptake 1,
including metaraminol, α-methylnorepinephrine, α-methylepinephrine,
tyramine, octopamine, and α-methylyramine. It appears that the necessary
structural requirements for uptake 1 are (1) absence of bulky N-
substituent groups (isoproterenol is not a substrate); (2) absence of
methoxyl groups on the aromatic ring (normetanephrine is not a substrate);
(3) presence of at least one phenolic hydroxyl group (amphetamine,
phenylethylamine, phenylethanolamine and norephedrine are not
substrates).¹³ Uptake 1 appears to result from the activity of a membrane
carrier system requiring metabolic energy.

The uptake of catecholamines into the storage vesicles seems to be
due to a membrane transport mechanism and associated with the Mg²⁺-
dependent ATPase activity present in the vesicle membrane. This uptake
is quite different from uptake 1. It is inhibited by reserpine which is
ineffective as an uptake 1 inhibitor. The uptake has a relatively low
affinity for the catecholamines, it is energy requiring and
stereochemically selective for (-)-norepinephrine.¹⁴

At low perfusion concentrations of catecholamines, uptake 1 is the
main mechanism for taking up the catecholamines. A high perfusion
concentrations, however, an accumulation of catecholamines occur and the
mechanism responsible for this is uptake 2. Uptake 2 is saturable and has a much higher capacity ($V_{\text{max}}$) compared to uptake 1. The proportion of these two uptakes also varies with the density of adrenergic innervation, i.e. uptake 1 dominates in densely innervated tissues. Uptake 2 is different from uptake 1 in its substrate specificity: uptake 2 does not have stereochemical specificity for (+)- or (-)-norepinephrine or epinephrine; it has a higher affinity for epinephrine than norepinephrine; isoproterenol is a better substrate for uptake 2 than epinephrine, while it is not taken up by uptake 1. Uptake 2 is rapidly followed by intracellular metabolism by MAO and COMT. Uptake 2 is thus described as a process of "uptake-and-metabolism".

1.2.6 SUBCLASSIFICATION AND SIGNAL TRANSDUCTION OF ADRENERGIC RECEPTORS

The adrenergic receptors have been classified in a number of ways based on their: (1) anatomical properties (the location of the receptors); (2) pharmacological properties (the relative potencies of selective agonists and antagonists); or (3) biochemical mechanism correlated to the pharmacological properties. In 1948, Ahlquist proposed two major receptor subtypes as $\alpha$ and $\beta$ according to their pharmacological basis. In 1967, Lands subdivided the $\beta$-receptor into $\beta_1$- and $\beta_2$-subtypes on the basis of their pharmacological characteristics (their differential affinities for isoproterenol, epinephrine and norepinephrine). Langer subdivided $\alpha$ receptors into $\alpha_1$ and $\alpha_2$ (the postsynaptic and presynaptic $\alpha$-adrenergic receptors, respectively) on the basis of their anatomical localization. The anatomical subdivision is not a useful definition of subtypes because some of the postsynaptic adrenergic receptors have the
pharmacological properties of an $\alpha_2$-receptor. It has also been found that $\alpha_1$-adrenergic receptors can exist on presynaptic nerve terminals.\textsuperscript{19} In general, the pharmacological approach is the most useful method for subclassification of the receptors.

Figures 4 and 5\textsuperscript{20-23} show compounds that are selective $\alpha_1$- and $\alpha_2$-adrenoceptor agonists and antagonists. These compounds can be used for differentiation between the $\alpha_1$- and $\alpha_2$-adrenoceptors. Alpha 1-adrenergic receptors are generally defined as receptors which are blocked by the competitive antagonist prazosin (5), irreversibly blocked by the alkylating agent phenoxybenzamine (8), and stimulated by agonists such as phenylephrine(1), methoxamine (2) and more selective amidephrine (3). The definition of $\alpha_1$ versus $\alpha_2$ subtypes is currently based on the antagonists prazosin and yohimbine (13).\textsuperscript{21} The selectivity for $\alpha_2$-adrenoceptor has been shown to be: imiloxan (RS 21361, 15) > rauwolscine (14) > yohimbine.

The presence of $\alpha_1$- and $\alpha_2$-adrenoceptors at the postjunctional (the term suggested to use to cover receptors which are located on target organs and accessible to circulating agonists, but may not be closely associated with the "synapse" between nerve and muscle or nerve and nerve\textsuperscript{23}) neurons in vasculature of many mammalian species is now accepted, and both subtypes mediate vasoconstriction.\textsuperscript{24-25} In vivo, the postjunctional vascular $\alpha_2$-adrenoceptors are located predominantly extrasynaptic, while postjunctional vascular $\alpha_1$-adrenoceptors exist primarily within the synaptic area. The differentiation between prejunctional and postjunctional $\alpha_2$-adrenoceptors has been demonstrated by Ruffolo et al.\textsuperscript{26} using SK&F-104078 (17) which is a potent antagonist of postjunctional $\alpha_2$-adrenoceptors and at least 100-fold weaker at most
Alpha₁-adrenoceptor agonists:

1. \( \text{HO-CH(OH)CH}_2\text{NHCH}_3 \)

2. \( \text{OCH}_3-\text{CH(OH)CH}_2\text{NHCH}_3 \)

3. \( \text{CH}_3\text{SO}_2\text{NH-CH(OH)CH}_2\text{NHCH}_3 \)

4. \( \text{NHCH}_3 \)

Alpha₁-adrenoceptor antagonists:

5. \( \text{CH}_3\text{O-N-N-CO-F} \)

6. \( \text{OCH}_3-\text{CH}_2\text{(CH}_3\text{)}\text{NHCH}_2\text{CH}_2\text{O} \)

7. \( \text{CH}_3\text{O-CH(OH)CH}_2\text{NHCH}_2\text{CH}_2\text{OH} \)

8. \( \text{CH}_3-\text{OCH}_2\text{CH}_2\text{(CH}_3\text{)}\text{NCH}_2\text{CH}_2\text{Cl} \)

**Figure 4:** Alpha₁-adrenoceptor agonists and antagonists
Alpha₂-adrenoceptor agonists:

\[ \text{Figure 5: Alpha}_2\text{-adrenoceptor agonists and antagonists} \]
prejunctional receptors. Both $\alpha_1$- and $\alpha_2$-adrenoceptors can be demonstrated in the CNS; however, it appears that these receptors are mainly $\alpha_2$ adrenoceptors. These $\alpha$-adrenoceptors play a role in regulation of central adrenergic outflow to the cardiovascular system. The stimulation of these receptors result in a reduction in sympathetic outflow and an increase in vagal tone and reductions in both systemic vascular resistance and heart rate.

Most of the $\beta$-adrenoceptors are postsynaptic receptors. Recently, it has been proposed that there is a positive feedback control through presynaptic $\beta$-adrenoceptors which exist on most peripheral noradrenergic nerve terminals and on catecholaminergic nerve terminals in some areas of the central nervous system. The activation of these receptors results in the facilitation of transmitter release during the nerve stimulation. The peripheral presynaptic $\beta$-adrenoceptors are activated by circulating epinephrine or epinephrine taken up and released as a cotransmitter with norepinephrine rather than norepinephrine itself. Attempts to elucidate the subtypes of presynaptic $\beta$-adrenoceptors give inconsistent results. Evidences indicate the coexistence of presynaptic $\beta_1$- and $\beta_2$-adrenoceptors in several tissues but the presynaptic $\beta$-adrenoceptors are predominantly of $\beta_2$-subtype. It has been suggested that increased activation of these receptors by epinephrine may play a role in the development of essential hypertension. The antihypertensive action of $\beta$-antagonists may be in part due to blockade of these presynaptic $\beta$-receptors.

Alpha$_1$-adrenoceptors are mainly found at postsynaptic sites mediating the physiological action by raising the cytosolic Ca$^{2+}$. Stimulation of $\alpha_1$-adrenoceptors by agonists causes activation of membrane-bound
phospholipase C (PLC) in which an unidentified guanine nucleotide regulatory protein (G protein) is involved. The activated phospholipase C catalyzes the breakdown of phosphatidylinositol 4,5-biphosphate into two second messenger molecules: inositol 1,4,5-triphosphate (1,4,5-IP$_3$) and 1,2-diacylglycerol (DG). 1,4,5-IP$_3$ may be able to release Ca$^{2+}$ from intracellular pools: endoplasmic reticulum, which is the major source of activator Ca$^{2+}$, mitochondria, and the inner plasma membrane. Calcium binds to calmodulin and the Ca$^{2+}$-calmodulin complex interacts with a variety of enzymes and other cellular proteins altering their activities and leading to the various physiological responses (Figure 6). 1,4,5-IP$_3$ can be dephosphorylated to free inositol, which has no Ca$^{2+}$ mobilizing activity, or phosphorylated by inositol triphosphate kinase to inositol 1,3,4,5-tetrakisphosphate (1,3,4,5-IP$_4$), which may function as a second messenger that regulates the entry of Ca$^{2+}$ across the membrane. The mobilization of Ca$^{2+}$ from intracellular stores of 1,4,5-IP$_3$ may respond to the rapid phase of smooth muscle contraction, while the opening of the slow Ca$^{2+}$ channels by 1,3,4,5-IP$_4$ may apply to the tonic phase.$^{31}$ The complicating factor is that some of $\alpha_1$ agonists primarily activate Ca$^{2+}$ influx, while others are also able to promote an intracellular mobilization of Ca$^{2+}$ via the breakdown of phosphatidylinositol. It has recently been found that activation of $\alpha_1$-adrenoceptor can directly open a slow Ca$^{2+}$ channel without the involvement of inositol lipids.$^{32}$ Diacylglycerol (DG), the other second messenger molecule, can stimulate protein kinase C to phosphorylate a variety of specific proteins. The DG/protein kinase C pathway is proposed to modulate various aspects of the Ca$^{2+}$ signalling pathway to give an integrated and highly versatile receptor mechanism which is employed
to control many cellular processes including secretion, metabolism, contraction, neuronal excitability and cell growth.

It is suggested that activation of presynaptic α₂-adrenoceptors can negatively modulate norepinephrine release by decreasing cAMP level and subsequently attenuate Ca²⁺ influx (Figure 7). Binding of ligands to the presynaptic α₂-adrenoceptor is believed to stabilize the interaction of the receptor with a guanine nucleotide binding inhibitory protein, Gᵢ or Nᵢ, and promote the release or exchange of GDP for GTP. The binding of GTP to Gᵢ promotes dissociation of the α-subunit from a complex of the β- and γ-subunits of Gᵢ. It is thought that an inhibitory action of the α-subunit of Gᵢ on the catalytic moiety of adenylate cyclase inhibits the enzyme activity. Pertussis toxin abolishes the inhibitory effects of α₂-adrenoceptor agonists on adenylate cyclase because it catalyzes the ADP-ribosylation of a specific cysteine side chain on the α-subunit of Gᵢ; this covalent modification locks Gᵢ in the GDP form (inactivated form). It seems that central presynaptic α₂-adrenoceptors are also coupled to a Gᵢ and this leads to an inhibition of neurotransmitter release by decreasing the cAMP dependent-enhancement (phosphorylation) of the opening of N-type Ca²⁺ channel. Whereas stimulation of presynaptic α₂-adrenoceptors is likely to limit the influx of extracellular Ca²⁺, activation of postsynaptic α₂-adrenoceptors in vascular smooth muscle promotes the influx of Ca²⁺ without mobilization of intracellular Ca²⁺ through the breakdown of phosphatidylinositol. There is no evidence showing that α₂-adrenoceptor stimulation affects cAMP levels in vascular smooth muscle.

Both β₁- and β₂-adrenoceptors can couple to adenylate cyclase but it has been demonstrated that in ventricular myocardium, β₂-receptors are
Figure 6: Signal transduction mechanism to stimulation of α₁-adrenergic receptor
(modified from reference 31)
more efficiently coupling to adenylate cyclase than β₁-receptors. The biochemical event in β-adrenergic stimulation is shown in Figure 8. Binding of agonist to the receptor promotes the coupling between the receptor and Gₛ. This causes the exchange of GDP bound to the α-subunit of Gₛ by GTP. GTP binding to Gₛ destabilizes the receptor-Gₛ complex and Gₛ itself dissociates into α and β. The α-subunit then interacts with the catalytic subunit of adenylate cyclase to form the active enzyme complex and stimulates cAMP synthesis. The production of cAMP promotes the activation of protein kinase which can lead to the intracellular events that are responsible for the physiological responses produced by β-adrenergic stimulants. cAMP can also cause the relaxation of muscle possibly by inhibition of Ca²⁺ entry into the cells, or increasing sequestration of Ca²⁺ into intracellular storage, or reducing the affinity of myosin light chain kinase for Ca-calmodulin when the kinase is phosphorylated.

In conclusion, α₁-adrenoceptors stimulate phosphoinositol turnover and cause both the entry of extracellular Ca²⁺ and the release from intracellular stores. Alpha₂-adrenoceptors inhibit adenylate cyclase activity, whereas β₁- and β₂-adrenoceptors activate adenylate cyclase.

There is no general basis for subclassification of these adrenoceptors. The proliferation of subclassification is shown mainly for α₁-adrenoceptors. McGrath and Wilson have debated the rational of classifying α-adrenergic receptors. They raised a lot of examples of the diversity of α₁-adrenoceptor classification as follows:

1. Alpha₁ was proposed by Coates to identify the α₁-receptors which are activated by idanidine (Sgd 10175, 4), particularly sensitive
Figure 7: Signal transduction mechanism to stimulation of presynaptic $\alpha_2$-adrenoceptors (modified from reference 31)

Figure 8: Signal transduction mechanism to stimulation of $\beta_1$- and $\beta_2$-adrenoceptors (modified from reference 41)
to phenoxybenzamine (§) and separate from other α₁-adrenoceptors where norepinephrine, not idanidine, is active.

2. Alpha₁a and α₁b were proposed by McGrath²⁴ in 1982. Alpha₁a is activated by imidazolines or low concentrations of phenethylamines. This subtype was later found to be susceptible to Ca²⁺ antagonists and thus it is associated with activity via Ca²⁺ channel opening. Alpha₁b is activated only by high concentrations of phenethylamines. This subtype releases Ca²⁺ from intracellular stores which might be involved in phosphoinositol turnover.

3. Alpha₁H and α₁L⁶⁵ were identified based on prazosin's variable antagonism of contractile responses in the pulmonary artery of the rabbit.

4. Alpha₁A and α₁B were proposed by Morros and Cresse based on variable competition by phentolamine and WB 4101 (§) at [³H]prazosin binding sites.⁴⁶

5. Alpha₁a and α₁b were proposed by Han⁴⁷. Alpha₁a is described as the subtype which has high affinity for WB 4101 (§), and does not employ phosphoinositol hydrolysis and activates muscle contraction by opening dihydropyridine-sensitive Ca²⁺ channels. Alpha₁b mediates dihydropyridine-resistant contraction and is involved in the release of intracellular Ca²⁺ via the phosphoinositol pathway.

Bevan et al.⁴⁸,⁴⁹ have proposed the 'variable receptor affinity' hypothesis based on the varying affinity of norepinephrine for α-adrenoceptors in different tissues. They suggested that this variation in affinity can cause the variation in sensitivity and this does not provide a basis for receptor type subdivision. They also found that prazosin and yohimbine affinities can vary in vascular α-adrenoceptors.
The factors causing this variation are differences in receptor chemical structure, microenvironment and intracellular components.

Figure 9 shows the traditional classification of adrenoceptors which is well accepted. Bylund\textsuperscript{21} has proposed the classification of these receptors according to their biochemical properties as shown in Figure 10. In the proposed scheme, there are three major subtypes ($\alpha_1$, $\alpha_2$, and $\beta$) which couple to different G proteins and each subtype is further subdivided into two or more additional subtypes. He subdivided $\alpha_1$-adrenoceptors into $\alpha_{1A}$ and $\alpha_{1B}$ as proposed by Morrow and Creese.\textsuperscript{46} Alpha$_2$-adrenoceptors were subdivided into $\alpha_{2A}$ and $\alpha_{2B}$ as proposed by Bylund\textsuperscript{50} and Nahorski\textsuperscript{51} based on the studies that $\alpha_{2A}$ (found in human platelets) has a relatively low affinity for prazosin (S) while the $\alpha_{2B}$ (found in neonatal lungs) has a high affinity for prazosin. Bylund also proposed the $\alpha_{2C}$ subtype as the receptor having a high affinity for prazosin like $\alpha_{2B}$ but having the $K_i$ (antagonist affinity for binding) ratio of prazosin to yohimbine closer to $\alpha_{2A}$.

Hirst and Neild\textsuperscript{52-53} have found an excitatory effect of norepinephrine which is resistant to phentolamine ($\alpha_1$- and $\alpha_2$-adrenoceptor antagonists), tolazoline and prazosin; they have suggested the term gamma-receptors.

1.2.7 PROPERTIES OF ADRENERGIC RECEPTORS

The properties of adrenergic receptors have been recently reviewed.\textsuperscript{54-55} Each subtype of adrenergic receptors appears to be an integral membrane glycoprotein. Utilizing phenoxybenzamine (8) as a specific affinity reagent, it was demonstrated that $\alpha_1$-adrenoceptor of the rat liver has a molecular weight 85,000. Radiation inactivation
Figure 9: Traditional classification of adrenoceptors (modified from reference 21)

Figure 10: Proposed classification of adrenoceptors (modified from reference 21)
studies indicated that the $\alpha_1$-receptor has a molecular weight of 160,000 suggesting that $\alpha_1$-receptor may exist as a dimer as shown in Figure 11.

The properties of $\beta$-adrenoceptors are much better known. Some homology exists between $\beta_1$- and $\beta_2$-adrenoceptors. Both contain a methionine or cysteine residue in the ligand binding site as well as intramolecular disulfide bonds. $\beta_1$-adrenoceptors have a molecular weight of about 65,000-70,000 and the receptors are most likely monomers as shown in Figure 12. $\beta_2$-adrenoceptors have a molecular weight of 55,000-63,000. It is suggested that the $\beta_2$-adrenoceptor in the membrane is a dimer of two identical subunits (Figure 13). The mammalian $\beta_2$-receptor was elucidated based on the cDNA sequence. The topography of the receptor appears to contain seven clusters of hydrophobic amino acids which represent transmembrane spanning regions (Figure 14).56

1.2.8 STRUCTURE-ACTIVITY RELATIONSHIPS OF ADRENERGIC RECEPTORS

1.2.8.1 STRUCTURE-ACTIVITY RELATIONSHIPS OF $\alpha$-ADRENOCEPTORS AGONISTS

This section will discuss the structure-activity relationships of two major classes of $\alpha$-adrenoceptor agonists, the phenethylamines and the imidazolines. Many exceptions to this generalization exist today, the phenethylamines and the imidazolines are either nonselective or show selectivity for the $\alpha_1$-adrenoceptor and the $\alpha_2$-adrenoceptor, respectively. The phenethylamines are characterized as full agonists, while imidazolines are often partial agonists. This is because phenethylamines have low affinity (the ability of an agonist to bind to, or attach to, the receptor) and high efficacy (or intrinsic activity which is the ability of an agonist to stimulate or activate the receptor subsequent to
Figure 11: Dimeric $\alpha_1$-adrenoceptor (modified from reference 56)

Figure 12: Proposed model for $\beta_1$-adrenoceptor (modified from reference 55)
Figure 13: Proposed model for β₂-adrenoceptor (modified from reference 55)
Figure 14: Proposed topography of the mammalian β₂-adrenoceptor. There are seven transmembrane helices that are coupled to G proteins. Arrows indicate serine and threonine residues, potential sites of regulatory phosphorylation by receptor kinase. P is potential sites of phosphorylation by cAMP dependent protein kinase. There are at least two potential N-linked glycosylation sites near N-terminal. (modified from reference 56)
binding), while the imidazolines are high in affinity and low in efficacy. There is considerable evidence suggesting that imidazolines and phenethylamines interact differently with \( \alpha \)-adrenoceptors as shown in Table 1.\(^{57} \)

Both groups show differences when assessing the applicability of the Easson-Stedman hypothesis. The Easson-Stedman hypothesis\(^{58} \) proposes that three functional groups (a basic nitrogen, an alcoholic hydroxyl group, and an aromatic group) of a sympathomimetic amine are involved in the binding to an adrenergic receptor. This hypothesis explains why the R (-)-isomer of an optically active phenethylamine is more active than the S (+)-isomer or the corresponding desoxy derivative, which are equally active to each other. In the (+)-isomer of norepinephrine the alcoholic hydroxyl group is oriented in the wrong position or absent in the desoxy derivative, therefore only two-point interaction is expected. In the (-)-isomer; however, the correct stereochemical configuration is obtained and three-point interaction is possible as shown in Figure 15.\(^{59} \) Easson-Stedman hypothesis is valid for phenethylamines but not for imidazolines.

The structure-activity relationships of phenethylamines and imidazolines at \( \alpha_1 \)- and \( \alpha_2 \)-adrenoceptors can be described as follows:\(^{57,59-60} \)

1. **Aromatic Hydroxyl Substitution**

The existence of an aromatic hydroxyl group(s) affects affinity of the phenethylamines for \( \alpha_1 \)-adrenoceptors, but has little effect on efficacy. This substituent has also been shown to affect affinity of the imidazolines for the \( \alpha_1 \)-adrenoceptor, but to a much lesser degree than that observed for the phenethylamines. The efficacy of the imidazolines
is critically dependent on this substituent. For both the phenethylamines and imidazolines, the rank order of potency for \( \alpha_1 \)- and \( \alpha_2 \)-adrenoceptor agonist is:

\[
3,4\text{-dihydroxy} > 3\text{-hydroxy} > 4\text{-hydroxy} > \text{nonphenolic}.
\]

However, the decrease in activity of the phenethylamines is due primarily to the reduction in affinity, while the decrease in activity observed for the imidazolines is essentially from reduction in efficacy.

2. **Benzylic Hydroxyl Substitution**

This substituent increases the affinity for the phenethylamines at both \( \alpha_1 \)- and \( \alpha_2 \)-adrenoceptors by more than 100-fold with the R\((-\) configuration being optimum, while it decreases the affinity for the imidazolines by up to 10-fold.

3. **Substitution at the \( \alpha \)-Carbon Atom**

This substituent can produce impressive effects in the agonist activity of the phenethylamines. The methyl group substitution at the \( \alpha \) carbon atom of phenethylamines can increase the activity at \( \alpha_2 \)-adrenoceptors, but no change, or a decrease in activity is shown at \( \alpha_1 \)-adrenoceptors.

4. **Substitution at the Nitrogen Atom**

This substitution of phenethylamines can slightly increase the activity at both \( \alpha_1 \)- and \( \alpha_2 \)-adrenoceptors but large substituent decrease the activity at both subtypes.

5. **Substitution on the Aromatic Ring**

Replacement of the catechol of epinephrine to 3,4-dichloro or 3,4-difluoro analogs leads to a reduction in \( \alpha_1 \)-adrenergic activity.\(^5^9\)

The substitution of fluorine for hydrogen on the aromatic ring of
Table 1

Deviation between phenethylamines and imidazolines for agonist activity

<table>
<thead>
<tr>
<th></th>
<th>Phenethylamines</th>
<th>Imidazolines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Easson-Stedman hypothesis</td>
<td>applicable</td>
<td>not applicable</td>
</tr>
<tr>
<td>stereochemical demands</td>
<td>high</td>
<td>low</td>
</tr>
<tr>
<td>subtype selectivity</td>
<td>$\alpha_1$</td>
<td>$\alpha_2$</td>
</tr>
<tr>
<td>intrinsic efficacy</td>
<td>high</td>
<td>low</td>
</tr>
<tr>
<td>agonist profile</td>
<td>full agonists</td>
<td>partial agonists</td>
</tr>
<tr>
<td>calcium utilization</td>
<td>intra and extracellular</td>
<td>extracellular</td>
</tr>
<tr>
<td>time course of response</td>
<td>rapid</td>
<td>slow</td>
</tr>
<tr>
<td>aromatic hydroxyl substitution</td>
<td>increase affinity</td>
<td>increase efficacy</td>
</tr>
<tr>
<td>benzylic hydroxyl substitution</td>
<td>increase affinity</td>
<td>decrease affinity</td>
</tr>
</tbody>
</table>

Figure 15: Model representation of the Easson-Stedman hypothesis

A = amine binding site
H = hydroxyl binding site
P = phenyl binding site
phenethylamines\textsuperscript{61-63} (norepinephrine, epinephrine) gives changes in adrenergic receptor subtype specificity. The 2-fluoro isomer is a selective \( \beta \)-adrenergic agonist, while the 6-fluoro isomer is a selective \( \alpha \)-adrenergic agonist.

Activity of phenylaminoimidazolines (the basic nucleus of clonidine-like imidazoline) at \( \alpha_2 \)-adrenergic receptors is increased by the addition of a chlorine atom at either the 2, 3 or 4 position, with the greatest activity observed with the 2-chloro derivative. The activity is further enhanced by the addition of the second chlorine atom with the highest activity for the 2,6-dichloro derivative (clonidine). Decrease in \( \alpha_2 \)-adrenergic receptor affinity is observed with the replacement of both chlorine atoms by fluorine or bromine atom. A methyl or ethyl group can be substituted for the halogen with little change in affinity for the \( \alpha_2 \)-adrenergic receptor.\textsuperscript{59}

6. Substitution on Carbon or Nitrogen Bridge

Replacement of the nitrogen atom with either a carbon or sulfur atom produces a very minor decrease in activity but replacement with oxygen abolishes \( \alpha_2 \)-agonist activity.\textsuperscript{59} Substitution of the nitrogen bridge with either an allyl or cyclopropylmethyl group abolishes \( \alpha \)-adrenergic agonist activity.

7. Substitution on the Imidazoline Ring

For optimum affinity at the \( \alpha_2 \)-adrenoceptor, the imidazoline ring should not be substituted. Replacing one of the imidazoline nitrogen with a carbon, oxygen, or sulfur results in a reduction in affinity for the \( \alpha_2 \)-adrenergic receptor with greatest reduction resulting from the oxygen replacement.
1.2.8.2 STRUCTURE-ACTIVITY RELATIONSHIPS OF $\beta_2$-ADRENOCEPTORS AGONISTS

The exploration for selective $\beta_2$-adrenoceptor agonists is important for the treatment of bronchial asthma without side effects. Epinephrine was the first drug used as a bronchodilator, followed by ephedrine and isoproterenol (18, a pure $\beta$-adrenergic receptor agonist). Isoproterenol acts on both $\beta_1$- and $\beta_2$-adrenoceptors and can produce a number of side effects in addition to its bronchodilator activity. In general, some $\beta_2$-adrenergic agonists are catechols [such as isoproterenol (18), isoetharine (19), rimiterol (20) and trimetoquinol (21)] and some are resorcinols [such as metaraminol (22), terbutaline (23), fenoterol (24)]. The modification of the catechol ring can increase the $\beta_2$-activity indicated by the $\beta_2/\beta_1$ index. The $\beta_2/\beta_1$ index can increase when a 3-hydroxy group is substituted by the following functional groups: hydroxymethyl (albuteral, 25), sulfonamide (soterenol, 26) or methylamino (27). An $\alpha$-dihydroquinolone 28 is 23,000 times more active than isoproterenol and extremely selective for the $\beta_2$-adrenoceptor. Tertiary amines abolish activity. A large N-substituent of secondary amines is required for $\beta_2$ activity. The $\beta_2$-agonists are shown in Figure 16.

1.2.9 PHYSIOLOGICAL ROLE OF ADRENERGIC AGONISTS

Physiological roles of $\alpha$-adrenoceptors are reasonably well established and is summarized as shown in Table 2. $\alpha_2$-adrenoceptors play a prominent role in the cardiovascular system. $\alpha_2$ agonists, such as clonidine (9), guanfacine (10) and azepexole, are used clinically as antihypertensive agents because they activate central postsynaptic $\alpha_2$-adrenoceptor (located in the brainstem) causing the inhibition of
Figure 16: Structure of $\beta_2$-adrenoceptor agonists.
sympathetic outflow and enhancement of parasympathetic outflow; this produces vasodilation and bradycardia. The peripheral prejunctional $\alpha_2$-adrenoceptor does not have a major contribution to the hypertensive effect. These receptors play a physiological role in inhibition of neurotransmitter release. Alpha$_2$-adrenoceptors can mediate the inhibition of vasopressin-induced sodium and water reabsorption in the cortical, and possibly medullary, collecting tubule and duct, leading to a natriuresis and diuresis, while possibly sparing potassium. Presynaptic $\alpha_2$-adrenoceptors can inhibit the contraction of gastric smooth muscle (produced by vagal stimulation) by the reduction in acetylcholine release. These $\alpha_2$-adrenergic receptors may be located on the intramural parasympathetic ganglia in addition to being present on the postganglionic cholinergic neurons.\(^{69}\)

In general, $\alpha$-adrenergic receptors cause the contraction of both vascular and nonvascular smooth muscles. Both postjunctional $\alpha_1$-adrenoceptors and $\alpha_2$-adrenoceptors (being present in large arteries\(^{68}\)) can produce artery vasoconstriction. The relaxation of smooth muscle and vasodilation of vascular smooth muscle is a $\beta_2$-adrenergic property (which interestingly causes contraction of skeletal muscle). Many smooth muscles can possess the dual capacity to contract or to relax after exposure to $\alpha$- or $\beta$-adrenergic stimulants; however, the predominant response appears to be a property of the particular smooth muscle. The action of adrenergic receptors on nonvascular smooth muscle is summarized and shown in Table 3.\(^{66}\)

The prominent actions of $\beta_1$- and $\beta_2$-adrenergic receptors are summarized in Table 4.\(^{70}\) Beta$_1$-adrenoceptors can mediate the increases
### Table 2

**Actions of α-adrenoceptors**

<table>
<thead>
<tr>
<th></th>
<th>α₁</th>
<th>α₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased cardiac contractility</td>
<td>Hypotensive effect via central mechanism</td>
<td></td>
</tr>
<tr>
<td>Contraction of smooth muscle</td>
<td>Inhibition of postganglionic norepinephrine release</td>
<td></td>
</tr>
<tr>
<td>Decreased cardiac automaticity¹</td>
<td>Arterial constriction</td>
<td></td>
</tr>
<tr>
<td>Increased secretion of ACTH</td>
<td>Inhibition of vasopressin action</td>
<td></td>
</tr>
<tr>
<td>Inhibition of renin release</td>
<td>Inhibition of intestinal water and electrolyte secretion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stimulation of platelet aggregation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inhibition of insulin release</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inhibition of lipolysis</td>
<td></td>
</tr>
</tbody>
</table>

¹ decreasing in the old and increasing in the young
² presynaptic α₂-adrenergic receptors

in rate and contraction force of the heart. There is evidence²⁰ suggesting that α₁-adrenoceptors can also mediate positive inotropic effects but at a lower extent than those produced by the β-adrenoceptor. However, the role of α₁-adrenoceptors may be more prominent when the α₁ agonists or antagonists are used pharmacologically or in the pathological conditions such as ischemia with the change in the numbers of α- and β-adrenoceptors to favor α-mediated responses to endogenous catecholamines. Activation of β₂-adrenoceptors causes relaxation of bronchiolar smooth muscle and decreased airway resistance. This effect is clinically used for the
treatment of bronchial asthma. Activation of $\alpha_1$-adrenoceptors can cause vasoconstriction of vessels in the upper respiratory tract mucosa; this decongestant effect in nasal and bronchiolar mucosa is clinically useful.

Table 3

<table>
<thead>
<tr>
<th>Adrenoceptors responses on nonvascular smooth muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>receptor type</td>
</tr>
<tr>
<td>--------------------------------</td>
</tr>
<tr>
<td>Bronchiolar smooth muscle</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>motility</td>
</tr>
<tr>
<td>sphincters</td>
</tr>
<tr>
<td>Genitourinary tract</td>
</tr>
<tr>
<td>bladder wall</td>
</tr>
<tr>
<td>sphincter</td>
</tr>
<tr>
<td>uterus, pregnancy</td>
</tr>
<tr>
<td>Metabolic function</td>
</tr>
<tr>
<td>liver</td>
</tr>
<tr>
<td>Eye</td>
</tr>
<tr>
<td>iris, radial muscle</td>
</tr>
<tr>
<td>ciliary muscle</td>
</tr>
</tbody>
</table>
Table 4

Action of \( \beta \)-adrenoceptors.\(^{70} \)

<table>
<thead>
<tr>
<th>( \beta_1 )</th>
<th>( \beta_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac stimulation:</td>
<td>Bronchodilation</td>
</tr>
<tr>
<td>chronotropic</td>
<td>Vasodilation</td>
</tr>
<tr>
<td>inotropic</td>
<td>Inhibition of histamine release</td>
</tr>
<tr>
<td>Lipolysis</td>
<td>Skeletal muscle tremor</td>
</tr>
<tr>
<td></td>
<td>Gluconeogenesis</td>
</tr>
<tr>
<td></td>
<td>Glycogenolysis</td>
</tr>
</tbody>
</table>

1.3 **THROMBOXANE \( A_2 \) (TXA\(_2\))**

Thromboxane \( A_2 \) (TXA\(_2\)), discovered in 1975 and previously called rabbit aorta contracting substance, is a potent vasoconstrictor and inducer of platelet aggregation. TXA\(_2\) is a prostanoid derived from arachidonic acid via the cyclooxygenase pathway as shown in Figure 17.\(^{71} \)

Arachidonic acid is release from tissue phospholipids mainly by enzyme phospholipase \( A_2 \). The combined activity of phospholipase \( C \), diacylglyceride, and monoglyceride lipase can also release arachidonic acid from phosphatidylinositol. The arachidonic acid cascade can be summarized as follows:\(^{71} \)

1. Cyclooxygenase converts arachidonic acid to unstable cyclic peroxides (PGG\(_2\) and PGH\(_2\), collectively called prostaglandin endoperoxides). PGH\(_2\) can be converted to TXA\(_2\) by thromboxane synthetase and to prostacyclin by prostacyclin synthetase. Prostacyclin has the biological effects opposite to those of TXA\(_2\). Prostaglandin endoperoxide isomerase converts PGH\(_2\) to prostaglandins PGD\(_2\), PGE\(_2\), and PGF\(_{2\alpha}\). All of these enzymes can be
referred to as prostaglandin synthetase complex. The unstable TXA₂ (half-life = 30 seconds) is converted to the inactive TXB₂.

2. Lipoxygenase converts arachidonic acid to 5-hydroperoxyeicosatetraenoic acid (HPETE) which is enzymatically transformed further to the leukotrienes A₄, B₄, C₄, D₄, and E₄.

3. A cytochrome P-450 monooxygenase system can epoxidize the double bonds of arachidonic acid to the corresponding mono-epoxide derivatives of the fatty acid (EPETEs). The epoxide can be transformed into the corresponding dihydroxy derivatives by the action of epoxide hydrolases.

Figure 17: Arachidonic acid cascade.
The EPETEs have the ability to cause the release of calcium from liver microsomes and the release of pituitary hormones from the hypothalamus.

$\text{TXA}_2$ is synthesized and released by activated platelets. However, other cells and tissues including: polymorphonuclear leukocytes, macrophages, fibroblasts, spleen, iris, conjunctiva, lung, umbilical artery, pulmonary artery, and kidney can produce $\text{TXA}_2$.

1.3.1 PLATELET FUNCTION

Platelets are round or oval discs 2 to 4 microns in diameter generated in the bone marrow from megakaryocytes. The membrane surface is coated with glycoproteins. The membrane contains large amount of phospholipids which can activate the blood-clotting system. Platelet function can be summarized as follows:

1. **Adhesion to an injured vessel wall** In response to vessel wall injury platelets rapidly attach to the site of exposed subendothelium, become activated, and spread along the injured surface. The attachment involves platelet membrane glycoprotein Ib, von Willebrand factor, and calcium.

2. **Aggregation** Platelet aggregation can be activated by many stimuli including: ADP, thrombin, epinephrine, bacteria, serotonin, fatty acid, arachidonic acid, and $\text{TXA}_2$. When platelets are activated, the surface of platelets become sticky and the platelets aggregate together. Aggregation involves the interaction of membrane glycoproteins IIb and IIIa with fibrinogen and thrombospondin. The latter two proteins act as bridge ligands forming the cross-bridges between adjacent platelets necessary for platelet-platelet attachment or aggregation.
3. **Shape change and granule centralization (internal contraction)**

The early phenomenon in the response to the stimuli is a change in platelet shape, from normal disc shape to a rounded form, an extension of pseudopods. The granules are redistributed toward the center. Closely surrounding the centrally packed granules is a constricted band of microtubules and a contracting web of platelet actin and myosin filaments. The interaction of actin with a 260,000 dalton actin-binding protein is suggested to be important for pseudopod formation, while actin-myosin interaction is required for the centralization of platelet granules and constriction of the microtubule ring. Actin-myosin interaction is regulated by the level of cytoplasmic calcium. Calcium interacts with calmodulin to activate a protein kinase which phosphorylates myosin light chain and this phosphorylated myosin light chain initiates the actin-myosin interaction.

4. **Secretion**

The contents of platelet granules are secreted when platelets are activated. In general, a stronger stimulus is required for secretion than for aggregation. The released contents include: a) ADP, ATP, pyrophosphate, calcium and/or magnesium, and serotonin from the dense granules; b) a number of proteins including platelet-derived growth factor, platelet factor 4, low affinity platelet factor 4, β-thromboglobulin, factor V, factor VIII-related antigen, albumin, thrombospondin, fibrinogen, fibronectin, antiplasmin, α₁-antitrypsin, α₂-macroglobulin, permeability factor, bactericidal factor, chemotactic factor, and glycosaminoglycans from the α-granules; and c) some acid hydrolyses from the lysosomal granules.²² A strong stimulus such as thrombin can cause the secretion of most of the dense and α-granules...
contents, but only partial secretion of the lysosomal granules. Secreted products such as ADP and serotonin can augment the extent of platelet aggregation. Secretion requires labilization of the granules and the interaction of platelet contractile proteins. There might be a specific mechanism for granule labilization (fusion of granule membranes with the plasma membrane) so that granule contents can be extruded. It is suggested that the phosphorylated 47,000-dalton protein (47P), either by protein kinase C or diglycerides, is responsible for labilizing platelet granules. It was found that calcium flux and myosin light-chains potentiate secretion. This suggested that the labilization process may be driven by an internal platelet contraction which squeezes the granule contents out through the channels of the surface-connected canalicular system. 73

5. Clot The clotting process initiates by the formation of thrombin from prothrombin by the action of prothrombin activator. Prothrombin activator can be formed by two pathways: 74 1) the extrinsic pathway which starts with trauma to the vascular wall and surrounding tissues; 2) the intrinsic pathway which begins with trauma of the blood itself. Both pathways generate a number of active plasma proteins called blood-clotting factors. The extrinsic mechanism is shown in Figure 18. 74 This pathway begins when blood contacts traumatized vascular walls or extravascular tissues. The mechanism involves the activation of factor X to form activated factor X by the action of tissue thromboplasnin and factor VII. Activated factor X can form prothrombin activator by the action of factor V. The mechanism of intrinsic pathway is shown in Figure 19 74. When thrombin is formed it activates fibrinogen to form fibrin monomer which
polymerizes to give fibrin threads. Fibrin threads can entrap blood cells and platelets to form blood clot as shown in Figure 20.74

6. **Clot retraction** In a few minutes after a clot is formed, it begins to contract and express most of the fluid (serum) from the clot. Platelets contribute to clot contraction by activating the platelet actin and myosin which are the contractile proteins. The clot retraction pulls the edges of the broken blood vessel together providing hemostasis (prevention of blood loss).

In 1962, Born and O'Brien described a simple photometric measurement of platelet aggregation; when an agonist was added to a suspension of platelets, the light transmittance of the suspension increased as the platelets aggregated.75 With the proper concentration of many agonists, the increase in light transmittance occurs in two distinct phases (biphasic aggregation). The second phase aggregation is the result of a platelet regulatory process that is initiated by incomplete activation by the initial agonist. Second-phase aggregation is always accompanied by secretion of the contents in platelets and by synthesis of prostanoids, especially TXA\(_2\). With a higher concentration of an agonist, a full aggregation response can be observed even though prostanoid synthesis and secretion are inhibited. The term primary and secondary (instead of first phase and second phase) are used to refer to aggregation alone (reversible aggregation) and aggregation with secretion (irreversible aggregation).76 Essentially, all platelets aggregate during the initial phase. The second phase represent consolidation into large and more dense aggregates, rather than aggregation of more platelets.75
Figure 18: Extrinsic mechanism for formation of prothrombin activator. (modified from reference 74)
Figure 19: Intrinsic mechanism for formation of prothrombin activator. (modified from reference 74)

Figure 20: Conversion of fibrinogen to fibrin threads.
1.3.2 BIOCHEMICAL ROLE OF THROMBOXANE A₂

TXA₂ plays an important role in platelet function including stimulation of platelet aggregation, change in platelet shape, platelet granule centralization, platelet granule labilization, and secretion of platelet granules. The mechanism of platelet aggregation (shown in Figure 21) requires the binding of fibrinogen to the platelet surface. It is possible that stimulation of fibrinogen binding may be related to an effect of intracellular calcium flux or the direct action of TXA₂. The concept that a decrease in cAMP is a significant factor in causing platelet aggregation has recently been questioned because a number of compounds which inhibit platelet adenylate cyclase do not promote platelet aggregation.

The mechanism to induce platelet secretion is summarized in Figure 22. The sequence of events depends on calcium flux initiating phosphorylation of myosin light chain and actin-myosin interaction to induce platelet granule centralization. The mechanism of TXA₂-induced calcium flux whether direct or indirect (via phosphoinositide breakdown by the action of phospholipase C) is uncertain. Moreover, TXA₂ stimulates granule labilization, one of the important steps in granule secretion. This requires the phosphorylation of 47P. The mechanism of TXA₂-induced phosphorylation of 47P whether through direct activation of protein kinase C or by an indirect mechanism (via phosphoinositide breakdown) is uncertain.

For agonists such as epinephrine, thromboxane production is necessary for both granule centralization and granule labilization, with a lesser contribution to aggregation as shown in Figure 23. For ADP
Figure 21: Mechanism of platelet aggregation.

Figure 22: Mechanism of platelet secretion.
which can directly stimulate aggregation and granule centralization, TXA₂ is required for 47P phosphorylation, granule labilization, and secretion, and it makes only small contributions to aggregation (second wave only) and granule centralization (also shown in Figure 23). For agonists like collagen, thrombin, and platelet-activating factor which stimulate the phosphoinositide breakdown, the role of TXA₂ is to amplify this effect (shown in Figure 24). While, at low concentration of agonists this effect may be very important, at high concentration of agonists the production of TXA₂ has little influence on the overall effect. The effect of low concentrations of collagen is different from that of low concentrations of thrombin since there is only a single wave pattern to the aggregation response. The primary wave of thrombin could be due to a direct effect of thrombin on calcium flux independent of phospholipase C-induced phosphoinositide breakdown. Alternatively, collagen might induce more phospholipase A₂ activation relative to phospholipase C activity so that thromboxane production is more important for a single-wave collagen response.

1.3.3 PATHOPHYSIOLOGICAL ROLES OF THROMBOXANE A₂

The pathophysiological roles of TXA₂ has been reported as follows:

1. Renal effects

The release of TXA₂ in the kidneys of young hypertensive rats is relatively high compared with the modest release observed in older hypertensive rats. This observation is consistent with the finding that there is an increase in TXA₂ production in isolated glomeruli from hypertensive rats. These observations suggest that TXA₂ may have an effect
Figure 23: Role of TXA$_2$ in the effect of epinephrine and ADP

Figure 24: Role of TXA$_2$ in the effect of thrombin and collagen
on a primary change leading to the increased renal vascular resistance and subsequently to the development of hypertension.\textsuperscript{67}

2. Cardiovascular disorders

The proaggregatory and vasoconstrictor properties of TXA\textsubscript{2} have been implicated in the etiology of many cardiovascular pathologies. Due to the short half-life of TXA\textsubscript{2}, it is more convenient to measure TXB\textsubscript{2}, a stable metabolite of TXA\textsubscript{2}, as a marker of TXA\textsubscript{2}. A number of methods are used to measure TXB\textsubscript{2} including radioimmunoassay, and gas chromatography-mass spectrometry. The concentration of TXB\textsubscript{2} in plasma has been found to be increased in conditions of cardiopulmonary bypass, unstable angina, and variant myocardial angina. However, Sobel et al.\textsuperscript{72} reported that the increase in TXB\textsubscript{2} might not agree with an increased incidence of angina. Increased platelet aggregation and TXA\textsubscript{2} release in the coronary circulation during ischemic attacks has also been reported in many studies although the role of TXA\textsubscript{2} in angina remains controversial. TXA\textsubscript{2} is also implicated in thrombosis, circulatory shock, coronary vasospasm, myocardial infraction, sudden cardiopulmonary death and atherosclerosis.

3. Asthma

It has been reported that TXA\textsubscript{2} may contribute to asthmatic symptoms since TXA\textsubscript{2} has potent effects on bronchial smooth muscles contraction.\textsuperscript{77}

1.3.3 THROMBOXANE A\textsubscript{2} RECEPTOR ANTAGONISTS

The stable endoperoxide analogs U 46619 (29) and U44069 (30), Figure 25, mimic the effects of TXA\textsubscript{2}. These endoperoxides are often used for the study of potential physiological and pathophysiological roles of TXA\textsubscript{2}.\textsuperscript{78} Thromboxane A\textsubscript{2} receptors are usually referred to as TXA\textsubscript{2}/PGH\textsubscript{2} receptors\textsuperscript{78}
because both TXA₂ and PGH₂ produce the same biological effects although TXA₂ is slightly more active than PGH₂.²³ It has been indicated that TXA₂/PGH₂ receptor in the platelet and blood vessel may be different.⁷⁹-⁸⁰ Mais et al.⁸⁰ have classified the TXA₂ receptor subtypes as α (alpha) for aggregation in platelets and τ (tau) for tone on vascular smooth muscle.

The antagonism of TXA₂ at the receptor level is an attractive approach because this can inhibit the effect of endoperoxide which also has action at this receptor. A number of TXA₂ antagonists have been reported and are shown in Figure 26. Most of them are structurally related to the prostaglandins such as 13-azaprostanoic acid (31), pinane thromboxane A₂ (PTA₂, 32), SQ 29,548 (33), ONO 11120 (34), ONO 3708 (35), EP 045 (36), ICI 180080 (37) and ICI 192605 (38). Only a very few compounds with non-prostanoid structures are shown to be TXA₂ antagonists; these include: BM 13.177 (sulotroban, 39), BM 13.505 (daltroban, 40), and trimetoquinol (21).⁸⁹

![Figure 25: Compounds 29-30](image-url)
Figure 26: TXA₂ Antagonists
CHAPTER II

STATEMENT OF PROBLEMS AND OBJECTIVES

As discussed in chapter I, the nature of adrenoceptors has been intensively studied in order to gain the knowledge of the physiological roles of these adrenoceptors. A number of compounds have been synthesized, and the structure-activity relationship requirements for each adrenoceptor have been investigated. The major objective of these studies is to understand the causes of some pathophysiology in which these adrenoceptors might be involved and to find selective drugs which are highly potent and low in toxicity for the treatment of these pathological conditions.

2.1 NOREPINEPHRINE DERIVATIVES

Norepinephrine is a potent agonist at \( \alpha \)-adrenoceptors (somewhat less active than epinephrine), and has little action on \( \beta_2 \)-adrenoceptor. It is approximately equipotent to epinephrine in stimulating \( \beta_1 \) (cardiac) receptors. Kirk et al.\textsuperscript{61,63,90} found that the fluorine substitution at the 2, 5, or 6 position of norepinephrine affects the activity on adrenergic receptors: 2-fluoronorepinephrine (2F-NE, 41) is nearly a pure \( \beta \)-adrenergic agonist, whereas 6-fluoronorepinephrine (6F-NE, 43) is an \( \alpha_2 \)-adrenergic agonist (greater activity as an \( \alpha_2 \)-adrenergic agonist relative to \( \alpha_1 \) activity), and 5-fluoronorepinephrine (5F-NE, 42) maintains both
α- and β-adrenergic agonist properties, similar in specificity to norepinephrine. The specificity of 2F-NE and 6F-NE for β-and α-adrenoceptors has been postulated to arise from a conformational bias induced by electrostatic repulsion between the aromatic fluorine at either the 2- or 6-position and the side-chain β-hydroxyl group. Because of these distinct effects on adrenergic activity of the fluoronorepinephrinines, an investigation into the biological effects of other halogens was launched. Changing the substitution from a fluorine to an iodine atom should provide a less electronegative, more lipophilic and a large steric effect as shown on Table 5. Although there are few reports on the effect of iodine, it was found by Klepping et al. that iodometanephrine and iodonormetanephrine can enhance hypertensive activity by about 12- and 10-fold in comparison to metanephrine and normetanephrine, respectively, indicating that the iodo analogs can enhance the biological activity of various parent compounds. In an attempt to find potent and selective adrenergic stimulants, the 2-iodonorepinephrine (2I-NE, 44), 5-iodonorepinephrine (5I-NE, 45), and 6-iodonorepinephrine (6I-NE, 46), shown in Figure 27, were synthesized in order to examine their pharmacological profile of activities on α- and β-adrenoceptors. The synthesis of 5I-NE was previously described by Gordon et al. No biological investigation of this compound has been reported. In order to examine the biological properties, this compound was resynthesized (by a different method which will be discussed in the next chapter).

Another objective for the synthesis of iodonorepinephrine derivatives is to test whether these compounds can be used to enhance
myocardial hemodynamics during cardiopulmonary resuscitation (CPR) in patients suffering cardiac arrest. A number of adrenergic agonists including selective $\alpha_1$- and $\alpha_2$-agonists have been examined for their use in CPR.\textsuperscript{96} It has been demonstrated that the $\beta_2$ adrenergic agonist effect is not required for improving myocardial hemodynamics. Beta$_1$ adrenergic agonist effect may not be required during CPR; this is due to its positive inotropic and chronotropic effects which cause an increase in myocardial oxygen consumption and the development of post-defibrillation ventricular dysrhythmias. Epinephrine is a drug of choice for use in CPR;\textsuperscript{97} however, both epinephrine and norepinephrine can improve the conditions during CPR. The lack of $\beta_2$ adrenergic properties in norepinephrine might make this compound more attractive as an adrenergic drug used in CPR. Because of the lack of information on the effects of iodine on catecholamines, the effects of these iodonorepinephrine derivatives will be evaluated.

Table 5

Properties of hydrogen, fluorine and iodine atoms.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>H</th>
<th>F</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomic weight</td>
<td>1.008</td>
<td>19.00</td>
<td>126.9</td>
</tr>
<tr>
<td>Electronegativity</td>
<td>2.1</td>
<td>4.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Covalent radius</td>
<td>0.30</td>
<td>0.72</td>
<td>1.33</td>
</tr>
<tr>
<td>Hydrophobic constant $\pi$</td>
<td>0.00</td>
<td>0.19</td>
<td>1.22</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The $\pi$ values for aromatic substituents were obtained from substituted phenylacetic acid.
As previously mentioned in chapter I, imidazolines have effects on α-adrenoceptors and generally lack activity at β-adrenoceptors. Some imidazolines are selective for α₁-adrenoceptors, whereas others are selective for α₂-adrenoceptor. It has been reported recently that catecholimidazoline (3,4-dihydroxytolazoline, 47) not only possesses agonist activity at α₁- and α₂-adrenoceptors but also exhibits very weak agonist activity at β₁- and is even weaker at β₂-adrenoceptors. The fluoro analogs of catecholimidazolines have been synthesized and it was found that aromatic fluorine substitution produces significant changes in agonist activity at both α- and β-adrenoceptors. 2-Fluoro-3,4-dihydroxytolazoline (48) and the 5-fluoro analog (49) show potent full
agonist activities on $\alpha_1$-adrenoceptor, whereas 6-fluoro analog (50) is a partial agonist. The rank order of potency for $\alpha_1$-adrenoceptor is 48 = 49 > 47 > phenylephrine > 50. The parent compound (47) is 10 fold less potent at presynaptic $\alpha_2$-adrenoceptor than at $\alpha_1$-adrenoceptor. The 6-fluoro analog is equipotent at both receptor subtypes, whereas the 2- and 5-fluoro analogs are 30-fold more potent at $\alpha_1$-adrenoceptor. The 2- and 5-fluoro analogs produce an increase in activity at the $\beta_1$-adrenoceptor, whereas the 6-fluoro analog abolishes activity at both $\beta_1$- and $\beta_2$-adrenoceptors. Due to the marked effects at the adrenoceptors especially at the $\alpha_1$-adrenoceptors by the substitution of fluorine on the aromatic ring, compounds 51-53 were synthesized to study the effects of iodine substitution. Compounds 47-53 are shown in Figure 28.

Figure 28: Structure of compounds 47-53
From the previous discussion, the classification of α-adrenoceptors is controversial. Some receptor subtypes have been classified based on the stimulation by imidazolines and/or phenethylamines. Recently, Reis et al.\textsuperscript{102} have described imidazoline/imidazole receptors in the brain. They suggest that the antihypertensive actions of clonidine are not attributed to its properties as an α\textsubscript{2}-adrenergic agonist but may be due to the action of an imidazoline receptor. Parani et al.\textsuperscript{103} have characterized an imidazoline/guanidinium binding site which is distinct from the α\textsubscript{2}-adrenoceptor. The imidazoline/guanidinium receptor is rauwolscine-insensitive but can be identified with the $[^3H]$idazoxan (16, an α\textsubscript{2} antagonist which contains an imidazoline ring in the molecule). Compounds 51-53 may be used as probes for this biological investigation and/or studies of the action of this imidazoline receptor. Possessing an imidazoline moiety, compounds 51-53 can be recognized by the imidazoline binding site and the iodine moiety may serve as a potential photoaffinity labeling reagent. It has been reported\textsuperscript{104} that iodo derivatives are used in the photoaffinity labeling of β-adrenoceptors. Because the C-I bond is a weak bond with the bond dissociation energy about 52 kcal/mol,\textsuperscript{105} it can be easily cleaved by ultraviolet light, generating a phenyl radical which can interact or form a covalent bond with the receptor.

2.3 \textbf{TRIMETOQUINOL ANALOGS}

Trimetoquinol (TMQ, 21) is a potent β-adrenoceptor agonist\textsuperscript{106,107} The S (-)-isomer of TMQ is more potent than the R (+)-isomer in β-adrenergic systems.\textsuperscript{108} The S (-)-isomer is used in Japan for treatment of bronchial asthma. TMQ is found to be 7 times more selective than isoproterenol as
a bronchial relaxant versus a cardiotonistimulant in guinea pig tracheal and atrial preparations respectively.109 In 1976, Shtacher et al.110 reported the antiaggregatory activity of TMQ. It has been reported that TMQ can inhibit collagen, secondary-wave of ADP- and epinephrine-induced human platelet aggregation. The mechanism of inhibition is mediated by a different isomer than that for adrenergic activity since the stereoselectivity [R(+)-TMQ > S(-)-TMQ] of TMQ for inhibition of platelet aggregation is reversed compared to the stereoselectivity [S(-)-TMQ > R(+)-TMQ] for β-adrenergic activity.111 Mayo et al. found that R(+)-TMQ is more active than S(-)-TMQ as an inhibitor of aggregation induced by arachidonic acid, collagen, U44069 (30), U46619 (29), and thromboxane A₂. Due to the competitive inhibition of U46619, it is proposed that TMQ is a TXA₂/PGH₂ receptor antagonist.112 Armstrong et al.113 have disagreed with this mechanism based on their finding that TMQ cannot displace [³H]U44069 from high-affinity specific binding sites in platelets. They suggested that TMQ may mediate its action by blockade of a specific transduction process that links receptor occupancy to cellular function. Recently, TMQ isomers have been shown to block U46619 receptor-mediated degradation of phosphoinositides114 and to displace [³H] U46619 with the expected stereoselectivity [R(+)-TMQ > S(-)-TMQ].115 Mukhopadhyay et al.116 have shown that TMQ acts functionally as a TXA₂ antagonist in vascular smooth muscle and human platelets. Extensive studies of TMQ for β-adrenergic and antithromboxane A₂ activities have been recently reviewed.99,117

Recently, it has been found that fluorine substitution at the 5- or 8-position of TMQ provides molecules that are more β₂-selective than TMQ in guinea pig tracheal (β₂) versus atrial (β₁) systems. This is because
the 5-fluoro-TMQ (5F-TMQ, 54) and 8-fluoro-TMQ (8F-TMQ, 55) can maintain their potent \( \beta_2 \)-adrenergic activity but reduce \( \beta_1 \)-adrenergic agonist activity.\textsuperscript{118} The fluoro analogs of TMQ also enhance the selectivity of the parent compound for inhibition of U46619 action on thoracic aorta versus human platelets. Of particular interest is the observation that 8F-TMQ (55) is more active than TMQ in the aorta (\( \tau \) receptor).\textsuperscript{119}

The addition of fluorine on the aromatic ring can contribute to tissue selectivity and greater potency for TMQ. To study the effect of changing the substitution from a fluorine to an iodine atom on the biological activities in \( \beta \)-adrenergic (\( \beta_1 \)– and \( \beta_2 \)-adrenoceptors) as well as \( \text{TXA}_2 \) (\( \tau \)- and \( \alpha \)-receptors) responsive systems, compounds 56 and 57 were synthesized. The structure of compounds 54-57 are shown in Figure 29.

2.4 SUMMARY

The ring fluorinated catecholamines produce distinct effects by increasing activities and tissue selectivities. It was our objective to study the effects that might be generated by iodine substitution. Changing a fluorine to an iodine atom will give differences in electronegativity, size, and lipophilicity as mentioned above and this might provide insight into our understanding of the factors which might be necessary for enhancing activity and selectivity of each of the receptor systems studied. Iodonorepinephrine derivatives will also be examined for their effects of enhancing the myocardial hemodynamics during cardiopulmonary resuscitation. The iodo derivatives of imidazolines (44-45) might also be used as photoaffinity agents for study the nature of the receptors for imidazoline-like substances if the activities of the
compounds are retained. Because the intermediates in the synthesis of the iodo-catecholamines can be used for the synthesis of other analogs, this makes it convenient to synthesize and study a variety of iodo analogs in different biological systems.

\[
\begin{array}{c}
\text{Figure 29: Structure of compounds 54-57}
\end{array}
\]
CHAPTER III
RESULTS AND DISCUSSION

3.1 CHEMISTRY

3.1.1 SYNTHESIS OF 5- AND 8-IDO-TRIMETOQUINOL

The synthesis of 5-iodo-trimetoquinol (5I-TMQ, 56) and 8-iodo-
trimetoquinol (8I-TMQ, 57) utilized 2-iodo-3,4-dimethoxyphenyl-
acetonitrile (58) and 3-iodo-4,5-dimethoxyphenylacetonitrile (59),
respectively. The synthesis of 58 has been previously reported from our
laboratory.\textsuperscript{117,120} The synthesis of 59 requires the protected catechol of
2-iodobenzaldehyde as the starting material. 2-Iodovanillin, one of the
possible starting materials, was previously synthesized in a 6-step
reaction by Freudenberg et al.\textsuperscript{121} The alternative material is 2-
iodopiperonal which was synthesized from piperonal (60) in a 3-step
reaction with an overall yield of only 25%.\textsuperscript{122} Due to the need of a large
amount of this starting material, a new synthesis is desired. A survey
of the literature reveals the most common iodinating agents are: iodine
(I\textsubscript{2}), iodine monochloride (ICl), chloramine T/sodium iodide.\textsuperscript{123} Iodine is
an unreactive agent for the aromatic electrophilic substitution; an
oxidizing agent is usually needed to oxidize I\textsubscript{2} to a better electrophile.
Chloramine T/sodium iodide combination produced p-toluenesulfonamide as
a by product which required chromatographic separation. This reagent is
normally used for the small-scale iodination. ICl is reported to be a
better iodinating reagent than I₂. The iodine-chlorine bond in ICl is polarized with a partial positive charge on the iodine. The electrophilic species has been suggested to be HOI or H₂OIT at low pH and ICl at higher pH. Since ICl has been previously used for the synthesis of 5-iodovanillin (61) in a one step reaction from vanillin in the presence of dilute HCl, the same procedure was adopted for preparing the required material. Isovanillin (62) was used instead of vanillin in order that the presence of hydroxy group at the 3 position can induce the iodination to occur at the 2 position and give 2-iodoisovanillin (63) as the product. Unfortunately, the yield obtained by this procedure was only 6%. Changing the condition from diluted HCl to glacial acetic acid, glacial acetic acid with heat or using dioxane, with the increase of reaction time to 3 days did not give a satisfactory yield. Dioxane and pyridine have been reported as another solvent system for iodination with ICl. The use of this solvent system provided a 75% yield with the reaction time of 6 days at room temperature (Scheme I). The proton NMR spectrum of the product shows a pair of doublets in the aromatic region with a coupling constant (J) value of 8.4 Hz indicating an ortho coupling (Figure 30).

With sufficient quantities of 2-iodoisovanillin (63), the synthesis of 2-Iodo-3,4-dimethoxyphenylacetonitrile (58) was performed by routine reactions as outlined in Scheme I. 2-Iodoisovanillin (63) was methylated with Me₂SO₄ to afford the dimethoxybenzaldehyde 64. The aldehyde was then reduced with NaBH₄ to give the benzyl alcohol 65. The alcohol was converted to the benzylbromide 66 with PBr₃. Displacement of the benzyl bromide with NaCN gave phenylacetonitrile 58.
Figure 30: 250 MHz proton NMR spectrum of 2-iodoisovanillin (63) at aromatic region.

Once 58 was obtained, the same chemistry was used for the synthesis of both 56 and 57 as shown in Scheme II. The protecting group of 58 and 59 was changed from dimethoxy groups to dibenzyloxy groups by demethylation of 58 or 59 with BBr₃ and the resulted catechols 67 and 68 were dibenzylated with benzyl bromide to afford dibenzyloxy phenylacetonitrile 69 and 70, respectively. Both phenylacetonitriles were converted into the phenethylamines by reduction with diborane. Without isolation, the phenethylamines were condensed with trimethoxyphenylacetic acid to give the phenylacetamides 71 and 72. Bischler-Napieralski cyclization¹²⁸,¹²⁹ of the amides with the use of POC₁₃ in acetonitrile gave the intermediate dihydroisoquinolines. These products are not necessary to be purified but can be directly reduced with NaBH₄ to give the tetrahydroisoquinolines 73 and 74. It is interesting to note that with
**Scheme I**

*Synthesis of 2-iodo-3,4-dimethoxyphenylacetonitrile (58)*

\[
\begin{align*}
62 & \xrightarrow{ICl, \text{pyridine, dioxane}} 63 \\
64 & \xrightarrow{NaBH_4, \text{THF}} 65 \\
66 & \xrightarrow{NaCN, \text{DMF}} 58
\end{align*}
\]
the aqueous workup dihydroisoquinoline intermediate 75 was obtained as almost the only product from the cyclization of 71. The proton NMR spectrum at the aromatic region of the crude product (a yellow solid) shows a disappearance of a pair of doublets of two ortho protons at 6.83 and 6.68 ppm (of starting material, 71) and gives a singlet at 7.15 ppm instead suggesting that the product is 75 and this indicates that Bischler-Napieralski cyclization is a clean method for the cyclization giving rise primarily to one compound. The proton NMR spectra of 71 and 75 are shown in Figure 31. There is no question that the cyclization of 71 gives 73 as the product, whereas it is possible that the cyclization of 72 might give either 74 or 76. Spectroscopy techniques cannot differentiate between these two compounds. An attempt to elucidate the structure of 74 was achieved as shown in Scheme III by catalytic hydrogenation (5% Pd/C in ethanol) of 74 resulting in removal of the iodo moiety to give 77. The structure of 77 is assigned by comparison of the proton NMR spectrum of 77 with that of authentic compound previously synthesized.³⁰ The proton NMR spectrum of the deiodinated product shows two singlets for the aromatic protons at 6.89 and 6.62 ppm. This confirms that 72 undergoes cyclization to 74 and not to 76 which upon removal of iodine should show an AB quartet for the two ortho protons.

Catalytic hydrogenation is not a method for deprotecting the benzyl groups of compounds containing iodide moiety on the aromatic ring but its dehalogenation effect sometimes proves to be a useful technique for structure elucidation. An alternative method, using a 50:50 mixture of refluxing methanol and concentrated HCl accomplished the cleavage of the protecting groups of 73 and 74 and gives the target materials 56 and 57.
Scheme II

Synthesis of 5I-TMO (56) and 8I-TMO (57)

58 \( X_1 = I, X_2 = H \)

59 \( X_1 = H, X_2 = I \)

67 \( X_1 = I, X_2 = H \)

68 \( X_1 = H, X_2 = I \)

69 \( X_1 = I, X_2 = H \)

70 \( X_1 = H, X_2 = I \)

71 \( X_1 = I, X_2 = H \)

72 \( X_1 = H, X_2 = I \)

75 \( X_1 = I, X_2 = H \)
Scheme II (continued)

\[
\begin{align*}
73 & \quad X_1 = I, \quad X_2 = H \\
74 & \quad X_1 = H, \quad X_2 = I
\end{align*}
\]
Figure 31: Proton NMR spectra of 71 (a) and 75 (b) in CDCl₃
Scheme III

Structure elucidation of 74 by catalytic hydrogenation
3.1.2 SYNTHESIS OF IODONOREPINEPHRINE ANNLOGS

An attempt to synthesize 44-46 is shown in Scheme IV. 2-Iodoisovanillin (63), 5-iodovanillin (61), and 2-iodo-4,5-dimethoxybenzaldehyde (78) were used as starting materials. 2-Iodo-4,5-dimethoxybenzaldehyde (79) was prepared using the procedure of Piatak et al. Treatment of these benzaldehydes with trimethylsilyl cyanide in the presence of a small amount (10 mg) of anhydrous zinc iodide produced cyanohydrin trimethylsilyl ethers 79-81. The reaction was monitored by the disappearance of aldehydic proton at about 9.7 ppm. Lithium aluminum hydride is usually used for reducing cyanohydrin trimethylsilyl ether to amino alcohol. Due to the fact that lithium aluminum hydride can displace an iodine of an iodine-arene bond, diborane was utilized for this reduction. Trimethylsilyl ethers were hydrolyzed with dilute HCl to give cyanohydrins 82-84 which upon reduction with diborane gives amino alcohols 85-87. Boron tribromide can demethylate 85-87 (observed by proton NMR) but the products cannot be purified. Attempts at crystallization of the products led to extensive decomposition. This problem was previously reported when Kirk et al. used BBr₃ to demethylate at the last step in the synthesis of fluoronorepinephrines. They solved the problem by changing protecting groups from dimethoxy derivatives to dibenzyl ether groups; deprotection at the last step by hydrogenolysis in the presence of 1 equivalent of oxalic acid produced crystalline 2F-NE (41). Because of a difficulty in changing the protecting groups, 5F-NE (42) and 6F-NE (43) were purified after demethylation by ion exchange chromatography as HCl salts. Both 42 and 43 were obtained as hygroscopic air-sensitive off-white solids but the purity and identity were confirmed.
by a combination of HPLC, TLC, mass spectroscopy, UV, and NMR. Gordon et al.\textsuperscript{95} used a similar technique in the synthesis 5I-NE (45), and this compound was also obtained as a light sensitive, hygroscopic, off-white solid.

Although catalytic hydrogenation cannot be used to remove the protecting groups (since it causes deiodination), the dibenzyl ether protecting groups can be removed by refluxing with 50:50 mixture of methanol and concentrated HCl. Compound 88 was prepared to test this process. Benzaldehyde 89 was synthesized using the same method of Piatak et al\textsuperscript{131} for the preparation of 78, by the reaction of 3,4-dibenzyloxybenzaldehyde (90) with I\textsubscript{2} and CF\textsubscript{3}CO0Ag in CCl\textsubscript{4}. Using the same chemistry as mentioned earlier, the cyanohydrin 91 was obtained and 91 was converted to amino alcohol 92 by diborane reduction. Deprotection of the benzyl groups by refluxing 88 in 50:50 mixture of methanol and concentrated HCl was accomplished but further purification by crystallization of the product was not successful.

The attempt to change the deprotecting group for the last reaction step is still being explored, since a cleaner final product is desired. A methylenedioxy protecting group might be an alternative. The starting material, 6-iodopiperonal (92) was prepared using the adopted method of Patiak et al.\textsuperscript{131} instead of the method reported by Ziegler et al.\textsuperscript{122} which involved a 3-step reaction sequence. The yield obtained of 92 from piperonal (60) was only 10\%. 6-Iodopiperonal was converted to cyanohydrin 93 by trimethylsilyl cyanide. Reduction with diborane gave amino alcohol 94. Using BCl\textsubscript{3} to deprotect the methylenedioxy group gave a complex mixture. A complicated mixture is also observed when BCl\textsubscript{3} was used for
Scheme IV

Unsuccessful approach to synthesize 44-46

\[ \text{RO} \quad \text{H} \quad \text{I}_2 \quad \text{CF}_3\text{COOAg} \quad \text{CCl}_4 \quad \text{RO} \quad \text{H} \]

63 \[ R_1 = \text{I}, R_3 = \text{CH}_3, R_2 = R_4 = R_5 = \text{H} \]

61 \[ R_4 = \text{I}, R_2 = \text{CH}_3, R_1 = R_3 = R_5 = \text{H} \]

78 \[ R_5 = \text{I}, R_2 = R_3 = \text{CH}_3, R_1 = R_4 = \text{H} \]

89 \[ R_5 = \text{I}, R_2 = R_3 = \text{Bn}, R_1 = R_4 = \text{H} \]

92 \[ R_5 = \text{I}, R_2 = R_3 = \text{CH}_2, R_1 = R_4 = \text{H} \]

\[ \text{TMSCN} \quad \text{ZnI}_2 \quad \text{OTMS} \quad \text{CN} \quad \text{aq HCl} \quad \text{OTMS} \quad \text{CN} \]

79 \[ R_1 = \text{I}, R_3 = \text{CH}_3, R_2 = R_4 = R_5 = \text{H} \]

80 \[ R_4 = \text{I}, R_2 = \text{CH}_3, R_1 = R_3 = R_5 = \text{H} \]

81 \[ R_5 = \text{I}, R_2 = R_3 = \text{CH}_3, R_1 = R_4 = \text{H} \]
Scheme IV (continued)

82 \( R_1 = I, R_3 = CH_3, R_2 = R_4 = R_5 = H \)

83 \( R_4 = I, R_2 = CH_3, R_1 = R_3 = R_5 = H \)

84 \( R_5 = I, R_2 = R_3 = CH_3, R_1 = R_4 = H \)

91 \( R_5 = I, R_2 = R_3 = Bn, R_1 = R_4 = H \)

93 \( R_5 = I, R_2 = R_3 = CH_2, R_1 = R_4 = H \)

85 \( R_1 = I, R_3 = CH_3, R_2 = R_4 = R_5 = H \)

86 \( R_4 = I, R_2 = CH_3, R_1 = R_3 = R_5 = H \)

87 \( R_5 = I, R_2 = R_3 = CH_3, R_1 = R_4 = H \)

88 \( R_5 = I, R_2 = R_3 = Bn, R_1 = R_4 = H \)

94 \( R_5 = I, R_2 = R_3 = CH_2, R_1 = R_4 = H \)
deprotection 88.

Another model study was undertaken using 3,4-dihydroxybenzaldehyde (95), a commercial available material as the starting material (Scheme V). Since a cyanohydrin could not be produced when the catechol 95 was treated with trimethylsilyl cyanide, other labile groups for the protection of the catechol were investigated. Trimethylsilyl ether, a labile protecting group which can be removed by methanolysis was experimentally explored. The reaction of trimethylsilyl chloride with 95 in triethylamine using THF as the solvent gave 96 after the reaction mixture was diluted with hexane, filtered and the excess reagents and solvent were evaporated in vacuo. Aqueous workup can sometimes detach these protecting groups. Without further purification, 96 was converted to cyanohydrin trimethylsilyl ether 97 using trimethylsilyl cyanide and a catalytic amount of anhydrous ZnI₂. Usually, the yield of cyanohydrins obtained (after acid hydrolysis and aqueous workup) are low, but the results from proton NMR spectra indicated clean products formed in the reaction. In general, it is not necessary to workup the reaction or isolate the product if lithium aluminum hydride is used as the reducing agent, so the trimethylsilyl ether 97 was divided into two portions to experiment with different methods of workup. The first attempt was to directly reduced with diborane. The other was to undergo aqueous workup and then reduction. Methanol was added to quench the reaction and it also caused methanolysis of the protecting groups. Interestingly, the proton NMR spectrum from the first portion indicated that the product was dopamine instead of norepinephrine. Dopamine was purified as an oxalate salt and its identity was reconfirmed by mass spectrum and proton NMR comparison of authentic dopamine. Using the
Scheme V

Model synthesis of norepinephrine

1. TMSCl, Et$_3$N, THF
2. TMSCN, ZnI$_2$
3. aq. workup
4. BH$_3$·THF
5. MeOH

NOREPINEPHRINE

DOPAMINE
alternate procedure with the aqueous workup, norepinephrine was obtained. The aqueous workup approach was employed for the synthesis of 44-46. The starting materials are catechols 98-100. The catechol 99 was synthesized from 5-iodovanillin (61) using the procedure of Anhoury et al.\textsuperscript{133} Boron tribromide demethylation of 63 and 81 gave catechols 98 and 100, respectively. Using this chemistry, from catechols 98-100 the iodo derivatives 44-46 were obtained as oxalate salts (Scheme VI).

3.1.3 SYNTHESIS OF IODOIMIDAZOLINE DERIVATIVES

The retrosynthesis of iodoimidazoline derivatives is shown in Scheme VII. Deprotection at the last step by refluxing 50:50 mixture of methanol and concentrated HCl was of concern due to a possible breakdown of the imidazoline ring. To verify this approach (Figure 32), 101 was debenzylated using refluxing 50:50 mixture of HCl and methanol. The product obtained (47) by this method was the same as that afforded by hydrogenation indicating that this set of condition can be used for debenzylolation of imidazoline-containing compound. The synthesis of 51-53 is shown in Scheme VIII. Compounds 69 and 70 are intermediates in the synthesis of 51-TMQ (56) and 81-TMQ (57) and their syntheses are shown in Scheme II. The synthesis of 102 is shown in Scheme IX using 89 as the starting material. Reduction with sodium borohydride afforded benzyl alcohol 103 which was converted to benzyl bromide 104 with PBr\textsubscript{3}. Displacement of the benzyl bromide 104 with NaCN gave phenylacetonitrile 102. The reaction of cyanide 69, 70, and 102 with HCl and ethanol gave imidates 105-107. The imidates were allowed to react with ethylenediamine to give imidazolines 108-110. Deprotection of the dibenzyl analogs
Scheme VI

Synthesis of 44-46

63 \( \text{R}_1 - \text{I}, \text{R}_3 = \text{CH}_3, \text{R}_2 = \text{R}_4 = \text{R}_5 = \text{H} \)

61 \( \text{R}_4 = \text{I}, \text{R}_2 = \text{CH}_3, \text{R}_1 = \text{R}_3 = \text{R}_5 = \text{H} \)

81 \( \text{R}_5 = \text{I}, \text{R}_2 = \text{R}_3 = \text{CH}_3, \text{R}_1 = \text{R}_4 = \text{H} \)

"1. TMSCl, Et\(_3\)N"

"2. TMSCN, ZnI\(_2\)"

"3. aq. workup"

"4. BH\(_3\).THF"

"5. MeOH"

98 \( \text{R}_1 - \text{I}, \text{R}_2 = \text{R}_3 = \text{H} \)

99 \( \text{R}_2 = \text{I}, \text{R}_1 = \text{R}_3 = \text{H} \)

100 \( \text{R}_3 = \text{I}, \text{R}_1 = \text{R}_2 = \text{H} \)

44 \( \text{R}_1 - \text{I}, \text{R}_2 = \text{R}_3 = \text{H} \)

45 \( \text{R}_1 = \text{I}, \text{R}_2 = \text{R}_3 = \text{H} \)

46 \( \text{R}_1 = \text{I}, \text{R}_2 = \text{R}_3 = \text{H} \)
Figure 32: Deprotection of 101

Scheme VII

Retrosynthesis of imidazoline derivative
Scheme VIII

Synthesis of 51-53

69 $R_1 - I, R_2 - R_3 - H$

70 $R_2 - I, R_1 - R_3 - H$

102 $R_3 - I, R_1 - R_2 - H$

105 $R_1 - I, R_2 - R_3 - H$

106 $R_2 - I, R_1 - R_3 - H$

107 $R_3 - I, R_1 - R_2 - H$

108 $R_1 - I, R_2 - R_3 - H$

109 $R_2 - I, R_1 - R_3 - H$

110 $R_3 - I, R_1 - R_2 - H$

51 $R_1 - I, R_2 - R_3 - H$

52 $R_2 - I, R_1 - R_3 - H$

53 $R_3 - I, R_1 - R_2 - H$
Scheme IX

Synthesis of 102

89 + NaBH₄ → THF → 103

108-110 yielded the target compounds 51-53.

3.2 BIOLOGY

Trimetoquinol (TMQ, 21), 5-iodo-trimetoquinol (5I-TMQ, 56), 8-iodo-trimetoquinol (8I-TMQ, 57) have been evaluated for their ability to inhibit U46619-mediated platelet aggregation and contraction of rat thoracic aorta. Complete experimental details are reported elsewhere.¹²⁰ Each compound blocked the response to U46619-mediated contraction of rat thoracic aorta in a competitive manner giving Kᵦ values of 3.47 µM, 48.98 µM and 14.45 µM for TMQ, 56, and 57, respectively (Table 6).
Table 6
Comparison of the contractile activities for TMQ (21), 5I-TMQ (56), and 8I-TMQ (57) against U46619 in rat thoracic aorta in the presence of indomethacin.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>n</th>
<th>Control, 10^{-4}M</th>
<th>+ Drug, 10^{-4}M</th>
<th>pK_b ± SEM^b</th>
<th>K_b (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMQ (21)</td>
<td>4</td>
<td>8.48 ± 0.07</td>
<td>7.00 ± 0.09</td>
<td>5.46 ± 0.17</td>
<td>3.47</td>
</tr>
<tr>
<td>5I-TMQ (56)</td>
<td>4</td>
<td>8.57 ± 0.15</td>
<td>8.05 ± 0.05</td>
<td>4.31 ± 0.19^c</td>
<td>48.98</td>
</tr>
<tr>
<td>8I-TMQ (57)</td>
<td>4</td>
<td>8.58 ± 0.20</td>
<td>7.65 ± 0.03</td>
<td>4.84 ± 0.21</td>
<td>14.45</td>
</tr>
</tbody>
</table>

^a pD_2 = -log EC_{50} where EC_{50} is the drug concentration which produce a response equal to 50% of its maximum.

^b pK_b = -log [I] where [I] is molar propranolol concentration and CR is CR-1 concentration ratio.

^c p < 0.01 compared to TMQ in unpaired t-test.

The inhibition of human platelets were shown as superimposed light transmittance tracings in the presence of varying concentration of each analog. In the absence of an inhibitor, 0.5 µM U46619 caused maximal irreversible aggregation which resulted in a "settling out" of the platelets and other debris causing a clearing of the originally turbid solution and an increasing in light transmittance. Addition of TMQ and iodo analogs caused inhibition of platelet aggregation and resulted in a decrease in light transmittance (Figure 33). It was demonstrated that both iodo analogs inhibit aggregation and serotonin secretion of human
Figure 33: Superimposed aggregation responses showing the effects of TMQ and iodo analogs on U46619-induced human platelet activation.
platelets induced by U46619 with IC$_{50}$ values of 119 µM and 3.10 µM for 56 and 57 respectively as compared to TMQ which has an IC$_{50}$ of 0.28 µM (Table 7). Similar inhibitory potencies were observed for these compounds against U46619-induced serotonin secretion (Table 7). In addition, TMQ and iodo analogs blocked the specific binding of $[^3$H]-SQ 29548 to receptor sites with an identical rank order and concentration range as observed for their inhibition of U46619-mediated aggregatory and secretory (Table 7).

**Table 7**

Comparison inhibitory potencies of TMQ (21), 5I-TMQ (56), and 8I-TMQ (57) in human platelets aggregation and serotonin secretion by U46619 (0.5-0.7 µM)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Platelet Function</th>
<th>IC$_{50}$ (µM)$^a$</th>
<th>$[^3$H] SQ 29548 inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aggregation</td>
<td>Secretion</td>
</tr>
<tr>
<td>TMQ (21)</td>
<td></td>
<td>0.28 ± 0.03</td>
<td>0.24 ± 0.06</td>
</tr>
<tr>
<td>5I-TMQ (56)</td>
<td></td>
<td>119.27 ± 8.35$^c$</td>
<td>92.00 ± 6.37$^c$</td>
</tr>
<tr>
<td>8I-TMQ (57)</td>
<td></td>
<td>3.10 ± 0.35$^c,d$</td>
<td>1.78 ± 0.26$^d,e$</td>
</tr>
</tbody>
</table>

$^a$ Data were express as the mean ± SEM of n = 3-4 donors

$^b$ All samples contained 1 mM aspirin

$^c$ p < 0.001 compared to 21 in unpaired t-test

$^d$ p < 0.001 compared to 56 in unpaired t-test

$^e$ p < 0.01 compared to 21 in unpaired t-test
Iodine substitution at either the 5- or 8-position of TMQ (21) caused a reduction in TXA$_2$ receptor antagonist properties. However, 56 and 57 were about 30- and 3-fold more selective than TMQ (21) as antagonist of TXA$_2$ responses in rat thoracic aorta versus human platelets. In the similar manner as fluorine substitution at the 5- and 8-position of TMQ, these ring substitution analogs of TMQ are more tissue selective for inhibiting TXA$_2$ responses in rat aorta. Most interesting in this study is the large difference in the reduction in activity between the substitution at the 5- and 8-positions. The TXA$_2$ blocking activity of TMQ is shown to be sensitive to iodine substitution at the 5-position whereas it is more tolerant to the presence of this substituent at the 8-position.

The effects of TMQ (21) and iodo analogs were also evaluated on guinea pig right atria and tracheal strips as representative $\beta_1$- and $\beta_2$-adrenoceptor systems, respectively (Figure 34). Both 56 and 57 were less active than TMQ as agonist in both atria and trachea, and the rank order of potency was 21 $>$ 57 $>$ 56. Whereas TMQ (21) gave a maximal response nearly equal to that of $10^{-5}$ M isoproterenol in these tissues, both 57 and 56 gave reduced maximal effects. Analog 56 gave a maximal effect equal to only 39% and 58% of isoproterenol stimulation in guinea pig atria and trachea, respectively. Analogs 56 and 57 were active in a similar concentration range in both guinea pig atria and trachea, and gave $\beta_2/\beta_1$-selectivity ratios similar to that of TMQ (21).

Additional experiments were conducted in the presence of 30 nM propranolol to determine if the stimulatory effects were mediated through the activation of $\beta$-adrenoceptors in atria and trachea. As shown in Table 8, the experimentally determined $pK_a$ values of propranolol against each
Figure 34: Tracheal relaxant (right panel) and chronotropic (left panel). Effects of TMQ (21), 5I-TMQ (56), and 8I-TMQ (57). Key: 21, O; 56, △; 57, O. Data were expressed as the mean ± SEM (n = 4-5).
### Table 8

**Comparison of Trimetoquinol (TMQ) and Iodinated Analogs on B₂- and B₁-adrenoceptors; Effect of Propranolol**

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Treaches (B₂)</th>
<th>Atria (B₁)</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pD₂ ± SEM</td>
<td>IAR ± SEM</td>
<td>pK₆ ± SEM</td>
</tr>
<tr>
<td>TMQ (21)</td>
<td>7.58 ± 0.05</td>
<td>0.99 ± 0.01</td>
<td>-</td>
</tr>
<tr>
<td>51-TMQ (56)</td>
<td>5.65 ± 0.16</td>
<td>0.58 ± 0.04</td>
<td>-</td>
</tr>
<tr>
<td>51-TMQ plus 3 x 10⁻⁶M propranolol</td>
<td>4.78 ± 0.13</td>
<td>0.57 ± 0.02</td>
<td>8.31 ± 0.16</td>
</tr>
<tr>
<td>81-TMQ (57)</td>
<td>5.93 ± 0.10</td>
<td>0.89 ± 0.02</td>
<td>-</td>
</tr>
<tr>
<td>81-TMQ plus 3 x 10⁻⁶M propranolol</td>
<td>4.85 ± 0.03</td>
<td>0.71 ± 0.02</td>
<td>8.57 ± 0.04</td>
</tr>
</tbody>
</table>

- **a** Drug concentrations used varied from 10⁻⁹ to 3 x 10⁻⁵M. Concentration of propranolol was 3 x 10⁻⁶M.
- **b** Values are the mean ± SEM of N = 3-6 (numbers given in parentheses).
- **c** IAR = intrinsic activity ratio = ratio of maximal drug effect to maximal response of TMQ.
- **d** pK₆ = -log[I] where [I] = molar propranolol concentration and CR = concentration ratio.
- **e** Potency ratio = EC₅₀ (trimetoquinol)/EC₅₀ (drug).
- **f** Selectivity ratio = potency ratio (B₂)/potency ratio (B₁) for each drug.
- **g** nd = not determined.
- **h** Value is given as pEC₃₀ ± SEM. Corresponding control pEC₃₀ ± SEM value for 81-TMQ was 5.77 ± 0.28 (n = 3).
compound were nearly identical in atria and trachea, and are similar to that previously reported for the antagonism of TMQ responses by propranolol in these tissues.\textsuperscript{109}

Iodo imidazoline analogs (51-53) were evaluated for $\alpha_2$-adrenergic activity on human platelets. 3,4-Dihydroxytolazoline and iodo analogs did not stimulate platelet aggregation but antagonized epinephrine-induced primary wave aggregation in aspirin-treated platelets giving the IC$_{50}$ value of 26 $\mu$M, 4.9 $\mu$M, 52 $\mu$M and 20 $\mu$M for 47, 51, 52, and 53, respectively (Table 9).

3,4-Dihydroxytolazoline (47) and iodo analogs (51-53) produced vasoconstriction of rat aorta with the rank order of potency (ED$_{50}$ values): $47$ (8.7 nM) > $52$ (40 nM) > $51$ (82 nM) > $53$ (120 nM) as shown in Table 10. Contractile responses to these compounds were sensitive to blockade by prazosin with $K_b$ values ranging from 0.36 to $1.5 \times 10^{-10}$ M.

Iodonorepinephrine derivatives (44-46) are in the process of being tested for their adrenoceptor activities.

### 3.2 SUMMARY

1. Iodine substitution at the 5- and 8-position of TMQ were synthesized.
2. 5I-TMQ and 8I-TMQ can inhibit the response of U46619-mediated contraction of rat thoracic aorta in a competitive manner.
3. Both iodo analogs of TMQ can also inhibit aggregation and serotonin secretion of human platelets induced by U46619 in the same rank order of potency.
4. Iodine substitution at either 5- or 8-position of TMQ caused a
Table 9

Comparative inhibitory potency of 3,4-dihydroxytolazoline (47) and iodo analogs against primary wave aggregation by epinephrinea

<table>
<thead>
<tr>
<th>compound</th>
<th>n</th>
<th>pIC50 ± SEM</th>
<th>IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>47</td>
<td>4</td>
<td>4.58 ± 0.23</td>
<td>26.3</td>
</tr>
<tr>
<td>51</td>
<td>3</td>
<td>5.31 ± 0.13</td>
<td>4.9</td>
</tr>
<tr>
<td>52</td>
<td>4</td>
<td>4.29 ± 0.26</td>
<td>51.2</td>
</tr>
<tr>
<td>53</td>
<td>4</td>
<td>4.69 ± 0.06</td>
<td>20.4</td>
</tr>
</tbody>
</table>

a platelet-rich plasma samples were incubated in the presence of 1 mM aspirin. (-)-Epinephrine (1-30 μM) was monitored for 6 min in the presence of varying compound concentration.

Table 10

Activities of 3,4-dihydroxytolazoline (47) and iodo analogs on rat aorta (α1)

<table>
<thead>
<tr>
<th>compound</th>
<th>ED50 (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>47</td>
<td>2.6 x 10^{-9}</td>
</tr>
<tr>
<td>51</td>
<td>8.2 x 10^{-8}</td>
</tr>
<tr>
<td>52</td>
<td>1.2 x 10^{-7}</td>
</tr>
<tr>
<td>53</td>
<td>4.0 x 10^{-8}</td>
</tr>
</tbody>
</table>
reduction in TXA₂ antagonist activities with the same rank order of potency \([\text{TMQ} (21) > \text{8I-TMQ} (57) >> \text{5I-TMQ} (56)]\) as blockade of U46619-mediated serotonin release and platelets aggregation.

5. Compare to TMQ, both 5I-TMQ and 8I-TMQ show about 30-fold and 3-fold more selective for blockade of U46619 responses in rat aorta versus human platelets.

6. The reduction in TXA₂ antagonist activity of 5- and 8- iodo TMQ suggests that the receptor cannot tolerate the steric bulk near the catechol. TMQ is shown to be sensitive to iodine substitution at the 5-position but it is more tolerant to the presence of this substituent at the 8-position.

7. TMQ and iodo analogs also inhibit the specific binding of \[^3H\]-SQ 29548 to TXA₂ receptor sites in platelets with the rank order potency of TMQ (21) > 8I-TMQ (57) >> 5I-TMQ (56). This suggests that TMQ and iodo TMQ analogs are TXA₂ receptor antagonists and bind to the receptor at the same binding site.

8. 5I-TMQ and 8I-TMQ are less active than TMQ at both \(\beta_1\)- and \(\beta_2\)-adrenoceptor tissues. This reduced potency resulting from the substitution of an iodine atom suggests that the \(\beta\)-adrenoceptor cannot easily accommodate the presence of a large lipophilic atom such as iodine in the binding area near the catechol group to the receptor sites.

9. Norepinephrine and 3,4-dihydroxytolazoline analogs with substitution of iodine at the 2-, 5- and 6-positions were synthesized.

10. The 2-iodoimidazoline analog is about 5-fold more potent than the
parent compound (3,4-dihydroxytolazoline) and the 6-iodo analog and about 10-fold more potent than the 5-iodo analog as an inhibitor of epinephrine-induced platelet aggregation.

11. The iodoimidazoline analogs are less active than the parent compound (3,4-dihydroxytolazoline) at $\alpha_1$-adrenoceptors.

12. Thus, overall iodine produces a quantitative difference in activity of both $\alpha$- and $\beta$-adrenergic agonists and the $\alpha_2$-adrenergic receptor system is the only one that can accommodate the iodine atom at the 2-position of 3,4-dihydroxytolazoline with an enhanced activity.
CHAPTER IV
EXPERIMENTAL

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared data were collected on a Beckman 4230 spectrophotometer or an Analect RFX-40 FTIR spectrometer. The NMR spectra were recorded on an IBM AF-250 spectrometer, and reported in parts per million. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration, interpretation, coupling constant (Hz). The mass spectra were obtained with a Kratos MS-25 RFA mass spectrometer or at the Ohio State University Chemical Instrument Center, by use of a VG 70-250S or a Kratos MS-30 mass spectrometer. Elemental analysis were performed by Galbraith Laboratories, Inc., Knoxville, TN and were within ± 0.4% of the theoretical values for the elements indicated. Anhydrous THF was produced by refluxing with, and distillation from, CaH₂. THF was kept in refluxing with sodium using benzophenone as an indicator for dryness. Acetone was dried over K₂CO₃ for at least 18 h prior to use. Acetonitrile was dried by refluxing over P₂O₅ for 3 h followed by distillation. Chloroform and CH₂Cl₂ were dried overnight over anhydrous CaCl₂ and fractionally distilled. Anhydrous benzene was produced by refluxing with and distillation from sodium.
2-Iodo-3-hydroxy-4-methoxybenzaldehyde (63)

To a stirred solution of 62 (12.76 g, 83.95 mmol) in pyridine (48 mL) at 0°C was added a solution of ICl (13.61 g, 84.01 mmol) in dioxane (82 mL). The reaction mixture was stirred at room temperature for 6 days. The solvents were evaporated under reduced pressure to give an oil which was added to H₂O (200 mL) and acidified with 6 N HCl. The solution was extracted with EtOAc (2 x 150 mL), and the EtOAc extracts were washed successively with 5% sodium bisulfite solution, H₂O (2 x 200 mL), and brine (200 mL), dried (MgSO₄), and concentrated under reduced pressure to give a solid. The solid was crystallized from EtOAc/Et₂O to give 17.10 g (73%) of 63 as light yellow needles: mp 169-171.5°C; IR (KBr, cm⁻¹) 3300 (OH), 1655 (C=O); ¹H NMR (CDCl₃) δ 10.03 (s, 1 H, ArCHO), 7.56 (d, 1 H, ArH, J = 8.4 Hz), 6.93 (d, 1 H, ArH, J = 8.4 Hz), 6.31 (s, 1 H, ArOH), 3.99 (s, 3 H, ArOCH₃); MS m/z 278 (M⁺, base). Anal. Calcd for C₈H₇IO₃: C, 34.56; H, 2.54. Found: C, 34.87; H, 2.59.
2-Iodo-3,4-dimethoxybenzaldehyde (64)

To a solution of 63 (25.12 g, 90.36 mmol) in acetone (250 mL) was added K$_2$CO$_3$ (25 g) and Me$_2$SO$_4$ (22.77 g, 180.72 mmol). The mixture was refluxed for 6 h, cooled, and concentrated under reduced pressure. Water (100 mL) was added and the solution was extracted with Et$_2$O (2 x 100 mL). The organic extracts were combined, washed with H$_2$O (100 mL), dried (MgSO$_4$) and concentrated to give an oil which was crystallized from CH$_2$Cl$_2$/petroleum ether to give 22.3 g (85%) of 64 as a white crystal: mp 77-78°C; IR (KBr, cm$^{-1}$) 1660 (C=O); $^1$H NMR (CDCl$_3$) $\delta$ 10.00 (s, 1 H, CHO), 7.70 (d, 1 H, ArH, $J$ = 8.5 Hz), 6.90 (d, 1 H, ArH, $J$ = 8.5 Hz), 3.94 (s, 3 H, ArOCH$_3$), 3.84 (s, 3 H, ArOCH$_3$); MS m/z 292 (M$^+$, base). Anal. Calcd for C$_9$H$_9$I0$_3$: C, 37.01; H, 3.11. Found: C, 37.04; H, 3.22.
2-Iodo-3,4-dimethoxybenzyl alcohol (65)

To a solution of 64 (6.65 g, 22.77 mmol) in dry THF (50 mL) was added NaBH₄ (2.58 g, 68.25 mmol). The mixture was stirred at 40°C for 3 h. The excess NaBH₄ was destroyed by the addition of H₂O. THF and H₂O were evaporated under reduced pressure and the residue was added H₂O (100 mL) and extracted with Et₂O (3 x 100 mL). The organic layer was dried (MgSO₄) and concentrated under reduced pressure to give an oil. The oil was crystallized from Et₂O/hexane to give 5.5 g (83%) of 65 as a white solid: mp 90-92°C; IR (KBr, cm⁻¹) 3300 (OH); ¹H NMR (CDCl₃) δ 7.13 (d, 1 H, ArH, J = 8.4 Hz), 6.89 (d, 1 H, ArH, J = 8.4 Hz), 4.66 (s, 2 H, CH₂OH), 3.87 (s, 3 H, ArOCH₃), 3.84 (s, 3 H, ArOCH₃); MS m/z 294 (M⁺, base). Anal. Calcd for C₉H₁₁IO₃: C, 36.76; H, 3.77. Found: C, 36.98; H, 3.64.
2-Iodo-3,4-dimethoxybenzyl bromide (66)

![Chemical Structure Image]

To a solution of 65 (15.9 g, 54.08 mmol) in dry CHCl₃ (30 mL) at 0°C, PBr₃ (14.63 g, 54.03 mmol) was added dropwise. The solution was stirred for 2 h at 0°C, H₂O (50 mL) was added to the reaction mixture and the organic layer was separated. The aqueous layer was reextracted with CHCl₃ (2 x 50 mL). The organic layers were combined, washed with H₂O (2 x 50 mL), dried (MgSO₄), and concentrated to give an oil. The oil was crystallized from EtOH to give 14.92 g (77%) of 66 as white crystals: mp 47-49°C; 'H NMR (CDCl₃) δ 7.23 (d, 1 H, ArH, J = 8.4 Hz), 6.83 (d, 1 H, ArH, J = 8.4 Hz), 4.65 (s, 2 H, CH₂Br), 3.86 (s, 3 H, ArOCH₃), 3.83 (s, 3 H, ArOCH₃); MS m/z 358 (M⁺, ³⁷Br), 356 (M⁺, ⁷⁹Br), 277 (base).
To the solution of 66 (12.33 g, 34.54 mmol) in DMF (50 mL) was added NaCN (8.46 g, 172.65 mmol) and the mixture was stirred at room temperature for 30 min. The reaction mixture was then poured into H₂O (50 mL) and extracted with CH₂Cl₂ (3 x 50 mL). The organic extracts were combined, washed with H₂O (100 mL), dried (MgSO₄), concentrated to give an oil which was crystallized from MeOH/acetone to give 7.61 g (73%) of 58 as white crystals: mp 77-78°C; IR (KBr, cm⁻¹) 2250 (C≡N); ¹H NMR (CDCl₃) δ 7.24 (d, 1 H, ArH, J = 8.4 Hz), 6.92 (d, 1 H, ArH, J = 8.4 Hz), 3.88 (s, 3 H, ArOCH₃), 3.84 (s, 3 H, ArOCH₃), 3.80 (s, 2 H, CH₂CN); MS m/z 302.97 (M⁺, base). Anal. Calcd for C₁₀H₁₀INO₂: C, 39.63; H, 3.33; N, 4.62. Found: C, 39.69; H, 3.40; N, 4.52.
2-Iodo-3,4-dihydroxyphenylacetonitrile (67)

To a solution of 58 (3.03 g, 10 mmol) in CH₂Cl₂ (10 mL) at -78°C, 1M BBr₃ in CH₂Cl₂ (30 mL, 30 mmol) was added dropwise. The reaction mixture was allowed to attain room temperature and stirred at that temperature overnight. The mixture was concentrated under reduced pressure to give a solid. The solid was dissolved in EtOAc (50 mL), washed with brine (3 x 50 mL), and H₂O (3 x 50 mL), dried (MgSO₄), and concentrated under reduced pressure to give a solid which was crystallized from EtOAc/Et₂O to give 1.88 g (68%) of 67 as white crystals: mp 135-137°C; IR (KBr, cm⁻¹) 3480-3200 (OH), 2230 (C≡N); ¹H NMR (CDCl₃) δ 6.91 (d, 1 H, ArH, J = 8.3 Hz), 6.88 (d, 1 H, ArH, J = 8.3 Hz), 5.57 (s, 2 H, 2 x ArOH), 3.72 (s, 2 H, CH₂CN); MS m/z 275 (M⁺), 273 (base). Anal. Calcd for C₈H₆INO₂: C, 34.94; H, 2.20; N, 5.09. Found: C, 35.00; H, 2.16; N, 4.99.
3-Iodo-4,5-dihydroxyphenylacetonitrile (68)

Prepared by the procedure as described for compound 67, from 59 (3.03 g, 10 mmol) with 1 M BBr\textsubscript{3} in CH\textsubscript{2}Cl\textsubscript{2} (30 mL, 30 mmol) was obtained 1.89 g (69%) of 68 as white crystals: mp 153-154°C; IR (KBr, cm\textsuperscript{-1}) 2250 (C=\textbar N); \textsuperscript{1}H NMR (MeOH-d\textsubscript{4}) \textdelta 7.11 (d, 1 H, ArH, J = 1 Hz), 6.76 (d, 1 H, ArH, J = 1 Hz), 3.30 (s, 2 H, CH\textsubscript{2}CN); MS m/z 275 (M\textsuperscript{+}), 127 (base). Anal. Calcd for C\textsubscript{8}H\textsubscript{6}INO\textsubscript{2}: C, 34.94; H, 2.20; N, 5.09. Found: C, 34.90; H, 2.17; N, 4.96.
2-iodo-3,4-dibenzoxypheynylacetonitrile (69)

To a solution of 67 (1.50 g, 5.45 mmol) in acetone (50 mL) was added K$_2$CO$_3$ (1.5 g, 10.85 mmol), KI (1 g, 6.02 mmol) and benzyl bromide (1.86 g, 10.90 mmol). The mixture was refluxed overnight, cooled, and concentrated under reduced pressure. The residue was dissolved in EtOAc (100 mL) and washed with H$_2$O (3 x 50 mL), dried (MgSO$_4$), and concentrated under reduced pressure to give a solid which was crystallized from EtOAc/Et$_2$O to give 2.27 g (92%) of 69 as white crystals: mp 105.5-106.5°C; IR (KBr, cm$^{-1}$) 3060-3025 (OH), 2230 (C=N); $^1$H NMR (CDCl$_3$) δ 7.50-7.32 (m, 10 H, ArH), 7.23 (d, 1 H, ArH, J = 8.4 Hz), 7.01 (d, 1 H, ArH, J = 8.4 Hz), 5.15 (s, 2 H, ArCH$_2$O), 5.04 (s, 2 H, ArCH$_2$O), 3.81 (s, 2 H, CH$_2$CN); MS m/z 455 (M$^+$), 91 (base). Anal. Calcd for C$_{22}$H$_{18}$INO$_2$: C, 58.04; H, 3.98; N, 3.08. Found: C, 58.06; H, 3.86; N, 2.93.
3-Iodo-4,5-dibenzylxyphenylacetonitrile (70)

Prepared by the same procedure as described for compound 69, from 68 (3.11 g, 11.30 mmol) with K$_2$CO$_3$ (3.12 g), KI (1 g), and benzyl bromide (3.87 g, 22.61 mmol) was obtained 3.74 g (73%) of 70 as white crystals:

mp 99.5-101°C; IR (KBr, cm$^{-1}$) 2250 (C≡N); $^1$H NMR (CDCl$_3$) δ 7.46-7.29 (m, 11 H, ArH), 6.92 (d, 1 H, ArH, J = 1 Hz), 5.10 (s, 2 H, ArCH$_2$O), 4.99 (s, 2 H, ArCH$_2$O), 3.63 (s, 2 H, CH$_2$CN); MS m/z 455 (M$^+$), 91 (base). Anal. Calcd for C$_{22}$H$_{18}$INO$_2$: C, 58.04; H, 3.98; N, 3.08. Found: C, 58.02; H, 3.98; N, 2.95.
To a cool solution (0°C) of 69 (3.72 g, 8.2 mmol) in THF (20 mL) was added dropwise a 1 M BH₃·THF solution (24.5 mL, 24.5 mmol). The mixture was refluxed under an argon atmosphere for 18 h, cooled to 0°C, and MeOH was added cautiously to quench the reaction. The mixture was concentrated under reduced pressure to an oil. The oil was dissolved in MeOH (50 mL) and reconcentrated under reduced pressure (this was repeated two more times) to give the oily phenethylamine. The oil was dissolved in toluene (100 mL) and 3,4,5-trimethoxyphenylacetic acid (1.85 g, 8.2 mmol) was added. The mixture was refluxed for 72 h with removal of H₂O via a Dean-Stark trap. The mixture was cooled and concentrated under reduced pressure to give a solid. The solid was dissolved in CH₂Cl₂ (150 mL), washed with H₂O (100 mL), 10% HCl (2 x 100 mL), H₂O (2 x 100 mL), 10% NaHCO₃ (2 x 100 mL), and H₂O (2 x 100 mL), dried (MgSO₄), and concentrated under reduced pressure to give a white solid. The solid was crystallized from EtOAc to give 3.05 (56%) of 71 as a white solid: mp 126-127°C; IR (KBr, cm⁻¹) 1640 (C=O); ¹H NMR (CDCl₃) δ 7.50-7.31 (m, 10 H, ArH), 6.83 (d, 1 H,
ArH, J = 8.35 Hz), 6.68 (d, 1 H, ArH, J = 8.35 Hz), 6.4 (s, 2 H, 2 x ArH), 5.46 (br, 1 H, NH), 5.11 (s, 2 H, ArCH₂O), 4.99 (s, 2 H, ArCH₂O), 3.84 (s, 3 H, ArOCH₃), 3.80 (s, 6 H, 2 x ArOCH₃), 3.47 (s, 2 H, COCH₂Ar), 3.45 (t, 2 H, ArCH₂C), 2.90 (t, 2 H, CH₂N); MS m/z 667 (M⁺). Anal. Calcd for C₃₃H₃₄INO₆: C, 59.38; H, 5.13; N, 2.10. Found: C, 59.39; H, 5.27; N, 2.21.

N-(3-Iodo-4,5-dibenzylxoxyphenylethyl)-3,4,5-trimethoxyphenylacetamide (72)

Phenylacetamide 72 was synthesized by the procedure previously described for phenylacetamide 71, from phenylacetonitrile 70 (2.74 g, 6.02 mmol), with 1 M BH₃·THF solution (18.06 mL, 18.06 mmol), and 3,4,5-trimethoxyphenylacetic acid (1.36 g, 6.02 mmol) was obtained 2.66 g (66%) of 72 as a white solid: mp 123-124°C; IR (KBr, cm⁻¹) 1640 (C=O); ¹H NMR (CDCl₃) δ 7.50-7.31 (m, 10 H, ArH), 7.17 (d, 1 H, ArH, J = 1.8 Hz), 6.77 (d, 1 H, ArH, J = 1.8 Hz), 6.40 (s, 2 H, 2 x ArH), 5.50 (br, 1 H, NH), 5.07 (s, 2 H, ArCH₂O), 4.99 (s, 2 H, ArCH₂O), 3.83 (s, 3 H, ArOCH₃), 3.82 (s, 6 H, 2 x ArOCH₃), 3.47 (s, 2 H, COCH₂Ar), 3.42 (t, 2 H, ArCH₂C), 2.69 (t, 2 H, CH₂N); MS m/z 667 (M⁺). Anal. Calcd for C₃₃H₃₄INO₆: C, 59.38; H,
To a solution of the phenylacetamide 71 (1 g, 1.50 mmol) in CH$_2$CN was added POCl$_3$ (1.64 g, 10.75 mmol). The mixture was refluxed for 5 h under an argon atmosphere. The mixture was then concentrated under reduced pressure to give an oil. The oil was dissolved in MeOH (25 mL) and reconcentrated under reduced pressure (this was repeated two more times). The oil was dissolved in MeOH (50 mL) and cooled to 0°C whereupon excess NaBH$_4$ (2.3 g, 60.81 mmol) was added. The mixture was stirred overnight at room temperature and concentrated under reduced pressure. The residue was dissolved in H$_2$O (25 mL) and 10% NaOH (25 mL) was added. The mixture was extracted with Et$_2$O (2 x 50 mL). The organic extracts were washed with H$_2$O (2 x 50 mL), brine (50 mL), dried (MgSO$_4$), and concentrated to give an oil. The oil was dissolved in MeOH and a solution
of C₂H₂O₄·2H₂O (189 mg, 1.5 mmol) in MeOH was added. The solvent was evaporated and the residue was crystallized from MeOH/Et₂O to give 750 mg (68%) of 73 as a white solid: mp 199.5-200.5°C; 'H NMR (DMSO-d₆) δ 7.44-7.32 (m, 10 H, ArH), 6.90 (s, 1 H, ArH), 6.65 (s, 2 H, 2 x ArH), 5.03 (d, 1 H, ArCH₂O, J = 11.6 Hz), 4.95 (d, 1 H, ArCH₂O, J = 11.6 Hz), 4.91 (s, 2 H, ArCH₂O), 4.65 (br, 1 H, ArCHNH), 3.76 (s, 6 H, 2 x ArOCH₃), 3.41 (s, 3 H, ArOCH₃), 3.3-2.7 (m, 6 H, 3 x CH₂); MS (FAB) m/z 652 (MH⁺·C₂H₂O₄), 91 (base). Anal. Calcd for C₃₃H₃₄INO₅·C₂H₂O₄: C, 56.69; H, 4.89; N, 1.89. Found: C, 56.77; H, 4.77; N, 1.79.

1-(3,4,5-Trimethoxybenzyl)-6,7-dibenzyl oxy-8-iodo-1,2,3,4-tetrahydroisoquinoline (74)

Prepared by the same procedure as described for compound 73, from phenylacetamide 72 (1.2 g, 1.80 mmol) with POCl₃ (2.47 g, 16.14 mmol), NaBH₄ (2.5 g, 66.14 mmol) was obtained 790 mg (67%) of 74 as a white solid: mp 140.5-142°C dec; 'H NMR (CDCl₃) δ 7.53-7.31 (m, 10 H, ArH), 6.78 (s, 1 H, ArH), 6.63 (s, 2 H, 2 x ArH), 5.11 (s, 2 H, ArCH₂O), 5.05 (d, 1 H, ArCH₂O, J = 10.2 Hz), 5.00 (d, 1 H, ArCH₂O, J = 10.2 Hz), 4.24 (dd, 1 H,
ArCHNH, J = 2.7 Hz and 10.9 Hz), 3.88 (s, 6 H, 2 x ArOCH₃), 3.85 (s, 3 H, ArOCH₃), 3.34-2.60 (m, 6 H, 3 x CH₂); MS m/z 470 (M⁺-trimethoxybenzyl), 91 (base). Anal. Calcd for C₃₃H₃₄INO₅·0.5H₂O: C, 60.00; H, 5.30; N, 2.12. Found: C, 59.90; H, 5.09; N, 2.12.

1-(3,4,5-Trimethoxybenzyl)-5-iodo-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (56)

An equivolume mixture of MeOH/concentrated HCl (6 mL) was added to 73 (200 mg, 0.27 mmol). The mixture was refluxed for 10 min under an argon atmosphere. The solvent and excess HCl were evaporated. The residue was dissolved in EtOH, and the solution was evaporated to dryness. This was repeated three times. The residue was crystallized from MeOH to give 62.29 mg (46%) of 56 as white needles: mp 217-218°C dec; ¹H NMR (MeOH-d₄) δ 6.59 (s, 1 H, ArH), 6.53 (s, 2 H, 2 x ArH), 4.64 (dd, 1 H, ArCHNH, J = 6.1 and 8.2 Hz), 3.79 (s, 6 H, 2 x ArOCH₃), 3.70 (s, 3 H, ArOCH₃), 3.55-3.30, 3.00-2.80 (m, 6 H, 3 x CH₂); MS (FAB) m/z 472 (MH⁺-HCl), 85
(base). Anal. Calcd for \( \text{C}_{19}\text{H}_{22}\text{INO}_5 \cdot \text{HCl} \): C, 44.94; H, 4.56; N, 2.75. Found: C, 44.62; H, 4.50; N, 2.66.

1-(3,4,5-Trimethoxybenzyl)-6,7-dihydroxy-8-iodo-1,2,3,4-tetrahydroisoquinoline Hydrochloride (57)

According to the same procedure as described for compound 56, from 74 (361.2 mg, 0.55 mmol) with an equivolume mixture of MeOH/concentrated HCl (6 mL) was obtained 188 mg (67%) of 57 as white needles: mp 180-181.5°C dec; \(^1\)H NMR (pyridine-\(d_5\)) \( \delta \) 7.17 (s, 2 H, 2 x ArH), 7.07 (s, 1 H, ArH), 5.39 (dd, 1 H, ArCHNH, J = 2.9 and 9.6 Hz), 3.92-3.77 (m, 2 H, CH\(_2\)), 3.80 (s, 3 H, ArOCH\(_3\)), 3.77 (s, 6 H, 2 x ArOCH\(_3\)), 3.55-3.41 (m, 2 H, CH\(_2\)), 3.81-3.16 (m, 2 H, CH\(_2\)NH); MS (FAB) \( m/z \) 472 (MH\(^+\) -HCl), 290 (base). Anal. Calcd for \( \text{C}_{19}\text{H}_{22}\text{INO}_5 \cdot \text{HCl} \cdot 0.5\text{H}_2\text{O} \): C, 44.18; H, 4.65; N, 2.17. Found: C, 43.86; H, 4.55; N, 2.67.
Benzeneacetronitrile, \( \alpha \)-hydroxy-2-iodo-4,5-dimethoxy (84)

\[
\begin{align*}
&\text{CH}_3\text{O} &\text{CN} \\
&\text{OH} &\text{I} \\
&\text{CH}_3\text{O} &
\end{align*}
\]

To aldehyde 78 (675 mg, 2.31 mmol) in 50 mL dried flask under argon atmosphere was added (1.12 g, 11.25 mmol) of trimethylsilyl cyanide and approximately 10 mg of anhydrous ZnI\(_2\). The mixture was stirred at room temperature for 1 h. To reaction mixture was then added dilute HCl (10 mL) and the mixture was stirred further for 1 h. The product was extracted with Et\(_2\)O (50 mL), washed with H\(_2\)O (10 mL), brine (25 mL), dried (MgSO\(_4\)) and evaporated to give an oil which was crystallized from EtOAc/petroleum ether to give 658 mg (89%) of 84 as a white solid: mp 124-125°C; IR (KBr, cm\(^{-1}\)) 3447 (OH); \({^1}\)H NMR (CDCl\(_3\)) \& 7.22 (s, 1 H, ArH), 7.19 (s, 1 H, ArH), 5.68 (d, 1 H, CH, J = 5.6 Hz), 3.90 (s, 3 H, OCH\(_3\)), 3.87 (s, 3 H, OCH\(_3\)), 2.97 (d, 1 H, OH, J = 5.6 Hz); MS m/z 319 (M\(^+\)), 292 (base). Anal. Calcd for C\(_{10}\)H\(_{10}\)IN\(_3\): C, 37.64; H, 3.16; N, 4.39. Found: C, 37.66; H, 3.15; N, 4.31.
2-Amino-1-(2-iodo-4,5-dimethoxyphenyl)ethanol hydrochloride (87)

To a cool solution (0°C) of 84 (390 mg, 1.22 mmol) in anhydrous THF (10 mL) was added a 1 M BH$_3$.THF solution (4 mL, 4.00 mmol). The mixture was refluxed under an argon atmosphere for 1.30 h, cooled to 0°C, and MeOH was added cautiously to quench the reaction. The mixture was concentrated under reduced pressure to give an oil. The oil was dissolved in MeOH (50 mL) and reconcentrated under reduced pressure (this was repeated two more times). The oil was dissolved in methanol and a stream of hydrogen chloride was passed through the mixture. Recrystallization from MeOH/Et$_2$O provided 383 mg (90%) of 87 as white solids: mp 225-226.5°C (dec); IR (KBr, cm$^{-1}$) 3500-3200 (br, OH); $^1$H NMR (D$_2$O) δ 7.31 (s, 1 H, ArH), 7.00 (s, 1 H, ArH), 4.97 (dd, 1 H, CH, J = 9.1 and 3.3 Hz), 3.72 (s, 3 H, ArOCH$_3$), 3.68 (s, 3 H, ArOCH$_3$), 3.15 (dd, 1 H, CH$_2$, J = 13.2 and 3.3 Hz), 2.95 (dd, 1 H, CH$_2$, J = 13.2 and 9.1 Hz); MS m/z 323 (M$^+$-HCl), 293 (base).
2-Amino-1-(2-iodo-3-hydroxy-4-methoxyphenyl)ethanol hydrobromide (85)

By the procedure described for the synthesis of 84 and 87 from benzaldehyde 63 (1.5 g, 5.39 mmol), trimethylsilyl cyanide (1.12 g, 11.25 mmol), anhydrous ZnI₂ approximately 10 mg, yielded the crude cyanohydrin 82 1.47 g (89%). ¹H NMR (acetone-d₆) δ 7.25 (d, 1 H, ArH, J = 8.4 Hz), 7.09 (d, 1 H, ArH, J = 8.4 Hz), 5.79 (s, 1 H, CH), 3.89 (s, 3 H, ArOCH₃), 2.85 (s, 1 H, OH). This cyanohydrin (1 g, 3.28 mmol) was reduced by 1 M BH₃·THF solution (16 mL, 16 mmol). After acidification with HBr and crystallization from MeOH/Et₂O gave 1.03 g (81%) of 85 as white crystals: mp 193.5-195°C (dec); IR (KBr, cm⁻¹) 3600-3200 (br, OH); ¹H NMR (methanol-d₄) δ 7.10 (d, 1 H, ArH, J = 8.4 Hz), 6.99 (d, 1 H, ArH, J = 8.4 Hz), 5.12 (dd, 1 H, CH, J = 9.5 and 2.9 Hz), 3.87 (s, 3 H, ArOCH₃), 3.19 (dd, 1 H, CH₂, J = 12.7 and 2.9 Hz), 2.80 (dd, 1 H, CH₂, J = 12.7 and 9.5 Hz); MS (FAB) m/z 310 (MH⁺ - HBr), 292. Anal. Calcd for C₉H₁₂INO₃·HBr: C, 27.72; H, 3.36; N, 3.59 Found: C, 27.65; H, 3.20; N, 3.47.
Benzeneacetronitrile, 3-iodo-α,4-dihydroxy-5-methoxy (83)

By the procedure described for the synthesis of 84 from benzaldehyde 61 (1.13 g, 4.06 mmol), trimethylsilyl cyanide (803 mg, 8.10 mmol), anhydrous ZnI₂ approximately 10 mg, yielded 1 g (81%) of 83 as white solids: mp 137-139°C (lit. 95 mp 133-134.5°C); IR (KBr, cm⁻¹) 2250 (C=N); ¹H NMR (CDCl₃) δ 7.42 (d, 1 H, ArH, J = 1.9 Hz), 6.98 (d, 1 H, ArH, J = 1.9 Hz), 6.25 (s, 1 H, ArOH), 5.42 (d, 1 H, CH, J = 6.8 Hz), 3.92 (s, 3 H, ArOCH₃), 2.71 (d, 1 H, OH, J = 6.8 Hz).
2-Iodo-4,5-dibenzyl oxy benzaldehyde (89)

A solution of iodine (16.28 g, 51.13 mmol) in CCl₄ (500 mL) was added to benzaldehyde 90 (16.28, 51.14 mmol) and CF₃CO₂Ag (11.31 g, 51.20 mmol) with stirring. The reaction mixture was stirred overnight. The yellow AgI was removed by filtration. The filtrate was washed with 5% NaHSO₃ (2 x 200 mL), H₂O (2 x 200 mL), brine (200 mL), dried (MgSO₄), concentrated under reduced pressure to give a solid. The solid was crystallized from CH₂Cl₂/Et₂O to give 14.98 g (66%) of 89 as white crystals: mp 122.5-123.5°C; IR (KBr, cm⁻¹) 2854 (CHO), 1670 (C=O); ¹H NMR (CDCl₃) δ 9.81 (s, 1 H, ArCHO), 7.48-7.29 (m, 12 H, ArH), 5.19 (s, 2 H, ArCH₂O), 5.16 (s, 2 H, ArCH₂O); MS m/z 444 (M⁺), 91 (base). Anal. Calcd for C₂₁H₁₇I'O₃: C, 56.78; H, 3.86. Found: C, 56.54; H, 3.82.
2-Amino-1-(2-iodo-4,5-dibenzoxophenyl)ethanol hydrochloride (88)

The procedure was similar to that for compounds 84 and 87. Treatment of 89 (1.122 g, 2.53 mmol) with trimethylsilyl cyanide (744 mg, 7.5 mmol) and approximately 10 mg ZnI₂ gave the crude cyanohydrin 91 which was reduced with 1 M BH₃·THF (7.58 mL, 7.58 mmol) to give 860 mg (68%) of 88 as white crystals: mp 223-224°C (dec); IR (KBr, cm⁻¹) 3600-3200 (br, OH); ¹H NMR (free base, CDCl₃) δ 7.44-7.29 (m, 11 H, ArH), 7.13 (s, 1 H, ArH), 5.18 (d, 1 H, CH₂O, J = 13.2 Hz), 5.12 (d, 1 H, CH₂O, J = 13.2 Hz), 5.09 (s, 2 H, CH₂O), 4.67 (dd, 1 H, CH, J = 7.6 and 3.6 Hz), 3.00 (dd, 1 H, CH₂, J = 12.8 and 3.6 Hz), 2.55 (dd, 1 H, CH₂, J = 12.8 and 7.6 Hz); MS (FAB) m/z 310 (MH⁺ - HCl), 292. Anal. Calcd for C₂₂H₂₂INO₃·HCl: C, 51.63; H, 4.53; N, 2.74 Found: C, 51.90; H, 4.58; N, 2.67.
2-Iodo-4,5-methylenedioxybenzaldehyde (92)

Prepared by the procedure as described for compound 89, from piperonal 60 (5 g, 33.30 mmol) with CF₃COOAg (8.09 g, 36.62 mmol), I₂ (8.45 g, 33.26 mmol) and CCl₄ (325 mL) was obtained 900 mg (10%) of 92 as yellow crystals: mp 110-112°C (lit. 122 mp 108.5-110.5, lit. 135 mp 111°C); IR (KBr, cm⁻¹) 2859, 2775 (CHO), 1663 (C=O); ¹H NMR (CDCl₃) δ 9.86 (s, 1 H, ArCHO), 7.35 (s, 1 H, ArH), 7.31 (s, 1 H, ArH), 6.06 (s, 2 H, CH₂).
2-Amino-1-(2-iodo-4,5-methylenedioxyphenyl)ethanol (94)

According to the same procedure as described for compound 84 and 87, from 92 (900 mg, 3.26 mmol) with trimethylsilyl cyanide (967 mg, 9.75 mmol) and approximately 10 mg ZnI₂ gave 910 mg (92%) of crude cyanohydrin 93. This cyanohydrin was reduced with 1 M BH₃·THF (13 mL, 13 mmol) to give 679 mg (68%) of 94 as white crystals: mp 134.5-136°C; IR (KBr, cm⁻¹) 3355 (OH); ¹H NMR (MeOH-d₄) δ 7.22 (s, 1 H, ArH), 7.02 (s, 1 H, ArH), 5.97 (d, 1 H, CH₂, J = 1.1 Hz), 5.94 (d, 1 H, CH₂, J = 1.1 Hz), 4.75 (dd, 1 H, CH, J = 8.4 and 3.3 Hz), 2.79 (dd, 1 H, CH₂, J = 13.2 and 3.3 Hz), 2.54 (dd, 1 H, CH₂, J = 13.2 and 8.4 Hz); MS m/z 307 (MH⁺), 277 (base). Anal. Calcd for C₉H₁₀INO₃: C, 35.20; H, 3.28; N, 4.56 Found: C, 35.31; H, 3.29; N, 4.45.
2-Iodo-3,4-dihydroxynzentaldehyde (98)

To a solution of 63 (1 g, 3.5 mmol) in dry CH$_2$Cl$_2$ at -78°C, 1 M BBr$_3$ in CH$_2$Cl$_2$ (10.79 mL, 10.79 mmol) was added dropwise. The reaction mixture was allowed to stir at room temperature for 24 h, then cooled to 0°C, and treated with H$_2$O (20 mL). The mixture was stirred further at room temperature for 24 h and extracted with EtOAc (50 mL). The organic extract was washed with H$_2$O (2 x 20 mL), brine (50 mL), dried (MgSO$_4$), and concentrated under reduced pressure to give a yellow solid. The solid was crystallized from CH$_2$Cl$_2$ to give 800 mg (84%) of 98 as yellow crystals: mp 169-170°C; IR (KBr, cm$^{-1}$) 1657 (C=O); $^1$H NMR (acetone-d$_6$) δ 9.96 (d, 1 H, ArCHO, J = 0.6 Hz), 7.35 (d, 1 H, ArH, J = 8.2 Hz), 6.99 (dd, 1 H, ArH, J = 8.2 and 0.6 Hz); MS m/z 264 (M$^+$, base), 263.
2-amino-1-(2-iodo-3,4-dihydroxyphenyl)ethanol oxalate (44)

To a solution of 98 (800 mg, 3.03 mmol) in dry THF (10 mL) at 0°C under argon atmosphere was added triethylamine (922 mg, 9.11 mmol). A solution of trimethylsilyl chloride (1g, 9.20 mmol) in dry THF (10 mL) was added dropwise. The mixture was stirred at 0°C for 2 h, then diluted with n-hexane (50 mL) and filtered through filter paper. The filtrate was concentrated under reduced pressure to dryness. The residue was redissolved with Et2O (20 mL), filtered and concentrated to give a crude 3,4-trimethylsilylether benzaldehyde as a clear oil which was dried under high vacuum and used in the next step without further purification. Following a similar procedure as outline for preparing 84 (except adding of dilute HCl) and 87, by the treatment of trimethylsilyl cyanide (595 mg), approximately 10 mg ZnI2 and aqueous workup, follow by the reduction with 1 M BH3·THF (10 mL, 10 mmol) and addition of C2H2O4·2H2O (382 mg, 3.03 mmol) and crystallization from MeOH/CH3CN compound 44 was prepared in 46% yield as white solids: dp 170°C; IR (KBr, cm⁻¹) 3600-3200 (br, OH); 1H NMR (DMSO-d6) δ 6.80 (d, 1 H, ArH, J = 8.3 Hz), 6.76 (d, 1 H, ArH, J = 8.3 Hz), 4.79 (dd, 1 H, CH, J = 9.3 and 2.8 Hz), 2.86 (dd, 1 H, CH2, J = 12.9 and 2.8 Hz), 2.54 (dd, 1 H, CH2, J = 9.3 peaks obscured under solvent peaks);
MS (FAB) $m/z$ 296 ($M^+ - C_2H_2O_4$). Anal. Calcd for $C_8H_{10}INO_3\cdot C_2H_2O_4\cdot 0.25CH_3CN$: C, 31.87; H, 3.22; N, 4.42. Found: C, 32.00; H, 3.39; N, 4.15.

2-Amino-1-(3-iodo-4,5-dihydroxyphenyl)ethanol oxalate (45)

![Chemical Structure]

Prepared by the procedure as described for compound 44, from 99 (1.1 g, 4.16 mmol) with trimethylsilyl chloride (1.35 g, 12.5 mmol), triethylamine (1.26 g, 12.48 mmol); trimethylsilyl cyanide (617 mg, 6.22 mmol), approximately 10 mg ZnI$_2$; 1 M BH$_3$.THF (12.5 mL, 12.5 mmol); C$_2$H$_2$O$_4\cdot 2H_2O$ (525 mg, 4.16 mmol) yielded 402 mg (25%) of 45 as white solids after crystallization from H$_2$O/CH$_3$CN and recrystallization from MeOH/CH$_2$Cl$_2$: mp 155-156°C (dec); IR (KBr, cm$^{-1}$) 3600-3200 (br, OH); $^1$H NMR (MeOH-d$_4$) $\delta$ 7.21 (d, 1 H, ArH, J = 1.9 Hz), 6.83 (d, 1 H, ArH, J = 1.9 Hz), 4.68 (dd, 1 H, CH, J = 9.4 Hz and 3.5 Hz), 3.07 (dd, 1 H, CH$_2$, J = 12.7 and 3.5 Hz), 2.92 (dd, 1 H, CH$_2$, J = 12.7 and 9.4 Hz); MS (FAB) $m/z$ 296 ($M^+ - C_2H_2O_4$). Anal. Calcd for $C_8H_{10}INO_3\cdot C_2H_2O_4$: C, 31.19; H, 3.14; N, 3.64 Found: C, 31.39; H, 3.25; N, 3.60.
2-Iodo-4,5-dihydroxybenzaldehyde (100)

Prepared by the procedure as described for compound 98, from 78 (2 g, 6.85 mmol) with 1 M BBr₃ in CH₂Cl₂ (20.54 mL, 20.54 mmol) was obtained 1.65 g (91%) of 100 as yellow crystals: mp 202-204°C (dec); IR (KBr, cm⁻¹) 1649 (C=O); ¹H NMR (acetone-d₆) δ 9.78 (s, 1 H, ArCHO), 7.42 (s, 1 H, ArH), 7.36 (s, 1 H, ArH); MS m/z 264 (M⁺, base). Anal. Calcd for C₇H₅IO₃: C, 31.85; H, 1.91 Found: C, 32.14; H, 1.88.
2-Amino-1-(2-iodo-4,5-dihydroxyphenyl)ethanol oxalate (46)

Prepared by the procedure as described for compound 44, from 100 (680 mg, 2.57 mmol) with trimethylsilyl chloride (838 mg, 7.71 mmol), triethylamine (784 mg, 7.75 mmol); trimethylsilyl cyanide (766 mg, 7.72 mmol), approximately 10 mg ZnI₂; 1 M BH₃·THF (7.72 mL, 7.72 mmol); C₂H₂O₄·2H₂O (324 mg, 2.57 mmol) yielded 250 mg (25%) of 46 as white solids after crystallization from MeOH/CH₃CN: mp 170-171°C (dec); IR (KBr, cm⁻¹) 3600-3200 (br, OH); ¹H NMR (D₂O) δ 7.28 (s, 1 H, ArH), 6.94 (s, 1 H, ArH), 4.95 (dd, 1 H, CH, J = 8.9 Hz and 3.4 Hz), 3.16 (dd, 1 H, CH₂, J = 13.2 and 3.4 Hz), 2.97 (dd, 1 H, CH₂, J = 13.2 and 8.9 Hz); MS (FAB) m/z 295 (MH⁻ - C₂H₂O₄). Anal. Calcd for C₈H₁₀INO₃·C₂H₂O₄·0.25CH₃CN: C, 31.87; H, 3.22; N, 4.42. Found: C, 31.95; H, 3.34; N, 4.13.
Ethyl-2-(2-iodo-3,4-dibenzoxysteraphenyl)iminoacetate hydrochloride (105)

To the suspension of 69 (1.5 g, 3.29 mmol) in dry benzene (5 mL) and dry EtOH (0.22 mL, 3.75 mmol), cooled at 0°C with stirring, was bubbled HCl gas until no absorption of HCl gas by the mixture (indicated by the change of moist pH paper to red). The mixture was allowed to stir at room temperature for 45 min and then kept in the refrigerator for 40 h. The solid material was poured into Et<sub>2</sub>O (50 mL) and a white solid was filtered and dried to yield 1.05 (89%) of 105: mp 164-164.5°C (dec); IR (KBr, cm<sup>-1</sup>) 1652 (C-NH); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.47-7.29 (m, 10 H, ArH), 7.18 (d, 1 H, ArH, J = 8.4 Hz), 6.96 (d, 1 H, ArH, J = 8.4 Hz), 5.12 (s, 2 H, ArCH<sub>2</sub>O), 5.03 (s, 2 H, ArCH<sub>2</sub>O), 4.62 (q, 2 H, OCH<sub>2</sub>, J = 7.0 Hz), 4.25 (s, 2 H, CH<sub>2</sub>), 1.38 (t, 3 H, CH<sub>3</sub>, J = 7.0 Hz); MS (FAB) m/z 502 (MH<sup>+</sup> - HCl). Anal. Calcd for C<sub>24</sub>H<sub>25</sub>CINO<sub>3</sub>: C, 53.60; H, 4.69; N, 2.60. Found: C, 53.33; H, 4.59; N, 2.51.
2-(2-Iodo-3,4-dibenzylxoybenzyl)imidazoline hydrochloride (108)

To the solution of iminoacetate 105 (1.69 g, 3.15 mmol) in dry CH₂Cl₂ (20 mL), cooled at 0°C with stirring, was added ethylenediamine (472 mg, 7.85 mmol) dropwise. The mixture was allowed to warm up to room temperature and stirring continued for another 12 h. The mixture was poured into CH₂Cl₂ (50 mL) and H₂O (50 mL). The organic layer was separated and washed with H₂O (50 mL), brine (50 mL), dried (MgSO₄), and concentrated under reduced pressure to give an oily residue which was crystallized from acetone to give 1.20 g (71%) of 108 as white crystals: mp 102-104°C; ¹H NMR (CDCl₃) δ 7.47-7.30 (m, 11 H, ArH), 7.00 (d, 1 H, ArH, J = 8.4 Hz), 5.12 (s, 2 H, ArCH₂O), 5.03 (s, 2 H, ArCH₂O), 4.28 (s, 2 H, CH₂), 3.87 (s, 4 H, CH₂CH₂); MS (FAB) m/z 499 (MH⁺ - HCl). Anal. Calcd for C₂₄H₂₃IN₂O₂·HCl·1.5H₂O: C, 51.34; H, 4.81; N, 4.99. Found: C, 51.25; H, 4.72; N, 5.13.
2-(2-Iodo-3,4-dihydroxybenzyl)imidazoline hydrochloride (51)

According to the same procedure as described for compound 56, from 108 (400 mg, 0.749 mmol) with an equimolecular mixture of MeOH/concentrated HCl (10 mL) was obtained 230 mg (87%) of 51 as white crystals after crystallization from MeOH/Et₂O and recrystallization from MeOH: mp 196.5-197.5°C (dec); IR (KBr, cm⁻¹) 3600-3100 (br, OH); ¹H NMR (MeOH-d₄) δ 6.81 (d, 1 H, ArH, J = 8.1 Hz), 6.77 (d, 1 H, ArH, J = 8.1 Hz), 3.94 (s, 2 H, CH₂), 3.89 (s, 4 H, CH₂CH₂); MS (FAB) m/z 319 (MH⁺ - HCl). Anal. Calcd for C₁₀H₁₁IN₂O₂.HCl: C, 33.88; H, 3.41; N, 7.90. Found: C, 34.21; H, 3.44; N, 7.90.
Ethyl-2-(3-iodo-4,5-dibenzoyloxyphenyl)iminoacetate hydrochloride (106)

According to the same procedure as described for compound 105, from 70 (2.05 g, 4.51 mmol) with EtOH (0.3 mL, 5.11 mmol) and HCl gas was obtained 2.18 g (90%) of 106 as white crystals: mp 133-134.5°C; IR (KBr, cm⁻¹) 1651 (C=NH); ¹H NMR (CDCl₃) δ 7.45-7.28 (m, 12 H, ArH), 5.14 (s, 2 H, ArCH₂O), 4.98 (s, 2 H, ArCH₂O), 4.59 (q, 2 H, OCH₂, J = 7.0 Hz), 3.90 (s, 2 H, CH₂), 1.41 (t, 3 H, CH₃, J = 7.0 Hz); MS (FAB) m/z 502 (MH⁺ - HCl). Anal. Calcd for C₂₄H₂₃ClINO₃: C, 53.60; H, 4.69; N, 2.60. Found: C, 53.49; H, 4.74; N, 2.58.
2-(3-Iodo-4,5-dibenzylxoybenzyl)imidazoline hydrochloride (109)

According to the same procedure as described for compound 108, from 106 (2.10 g, 3.91 mmol) with ethylenediamine (589 mg, 9.72 mmol) was obtained 1.71 g (82%) of 109 as white crystals after crystallization from MeOH/Et₂O: mp 210.5-212°C (dec); IR (KBr, cm⁻¹) 1652 (C-N); ¹H NMR (MeOH-d₄) δ 7.49-7.27 (m, 11 H, ArH), 7.11 (d, 1 H, ArH, J = 1.9 Hz), 5.18 (s, 2 H, ArCH₂O), 4.98 (s, 2 H, ArCHO), 3.90 (s, 4 H, CH₂CH₂), 3.81 (s, 2 H, CH₂); MS m/z 498 (M⁺ - HCl), 91 (base). Anal. Calcd for C₂₄H₂₃IN₂O₂·HCl: C, 53.90; H, 4.52; N, 5.24. Found: C, 53.98; H, 4.53; N, 5.15.
2-(3-Iodo-4,5-dihydroxybenzylimidazoline hydrochloride (52)

Prepared by the procedure as described for compound 56, from 109 (600 mg, 1.13 mmol) with an equivolume mixture of MeOH/concentrated HCl (10 mL) was obtained 360 mg (91%) of 52 as white crystals after crystallization from MeOH/aceton: dp 184°C; $^1$H NMR (MeOH-d$_4$) $\delta$ 7.13 (d, 1 H, ArH, J = 2 Hz), 6.74 (d, 1 H, ArH, J = 2 Hz), 3.89 (s, 4 H, CH$_2$CH$_2$), 3.68 (s, 2 H, CH$_2$); MS (FAB) m/z 319 (MH$^+$ - HCl). Anal. Calcd for C$_{10}$H$_{11}$IN$_2$O$_2$.HCl: C, 33.88; H, 3.41; N, 7.90. Found: C, 34.14; H, 3.40; N, 7.94.
2-Iodo-4,5-dibenzylxybenzyl alcohol (103)

Prepared by the procedure as described for compound 65, from 89 (4 g, 9.01 mmol) with NaBH₄ (1.02 g, 26.98 mmol) was obtained 3.82 g (95%) of 103 as white crystals after crystallization from CH₂Cl₂/n-hexane: mp 106.5-107.5°C; IR (KBr, cm⁻¹) 3600-3200 (br, OH); ¹H NMR (CDCl₃) δ 7.43-7.26 (m, 11 H, ArH), 7.07 (s, 1 H, ArH), 5.13 (s, 2 H, ArCH₂O), 5.09 (s, 2 H, ArCH₂O), 4.54 (d, 2 H, CH₂, J = 6.3 Hz), 1.87 (t, 1 H, OH, J = 6.3 Hz); MS m/z 446 (M⁺), 91 (base). Anal. Calcd for C₂₁H₁₉I0₃: C, 56.52; H, 4.29. Found: C, 56.89; H, 4.24.
2-Iodo-4,5-dibenzylxoybenzyl bromide (104)

Prepared by the same procedure as described for compound 66, from 103 (1.5 g, 3.63 mmol) with PBr₃ (910 mg, 3.36 mmol) was obtained 1.6 g (93%) of 66 as white crystals after crystallization from CH₂Cl₂/n-hexane: mp 118-119°C; ¹H NMR (CDCl₃) δ 7.39-7.726 (m, 11 H, ArH), 7.00 (s, 1 H, ArH), 5.08 (s, 2 H, ArCH₂O), 5.06 (s, 2 H, ArCH₂O), 4.47 (s, 2 H, CH₂Br); MS m/z 510 (M⁺, ³¹Br), 508 (M⁺, ⁷⁹Br), 91 (base). Anal. Calcd for C₂₁H₁₈IBrO₂: C, 49.54; H, 3.56. Found: C, 49.24; H, 3.50.
2-Iodo-4,5-dibenzylxyphenylacetonitrile (102)

Prepared by the same procedure as described for compound 58, from 104 (3.07 g, 6.03 mmol) with NaCN (1.48 g, 30.20 mmol) was obtained 2.42 (88%) of 102 as white crystals after crystallization from CH₂Cl₂/n-hexane: mp 107.5-108°C; IR (KBr, cm⁻¹) 2254 (C≡N); ¹H NMR (CDCl₃) δ 7.44-7.28 (m, 11 H, ArH), 7.09 (s, 1 H, ArH), 5.15 (s, 2 H, ArCH₂O), 5.11 (s, 2 H, ArCH₂O), 3.70 (s, 2 H, CH₂CN); MS m/z 455 (M⁺), 91 (base). Anal. Calcd for C₂₂H₁₈INO₂: C, 58.04; H, 3.99; N, 3.08. Found: C, 58.05; H, 3.87; N, 2.94.
Ethyl-2-(2-iodo-4,5-dibenzoxypyphenyl)iminoacetate hydrochloride (107)

According to the same procedure as described for compound 105, from 102 (710 mg, 1.6 mmol) with EtOH (0.11 mL, 1.87 mmol) and HCl gas was obtained 760 mg (91%) of 107 as white crystals: mp 159-160.5°C; $^1$H NMR (CDCl$_3$) $\delta$ 7.46-7.26 (m, 11 H, ArH), 7.08 (s, 1 H, ArH), 5.18 (s, 2 H, ArCH$_2$O), 5.10 (s, 2 H, ArCH$_2$O), 4.58 (q, 2 H, OCH$_2$, J = 6.6 Hz), 4.14 (s, 2 H, CH$_2$), 1.37 (t, 3 H, CH$_3$, J = 6.6 Hz); MS (FAB) m/z 502 (MH$^+$ - HCl). Anal. Calcd for C$_{24}$H$_{25}$ClNO$_3$: C, 53.60; H, 4.69; N, 2.60. Found: C, 53.31; H, 4.58; N, 2.54.
According to the same procedure as described for compound 108, from 107 (716 mg, 1.33 mmol) with ethylenediamine (198 mg, 3.29 mmol) was obtained 510 mg (77%) of 110 as white crystals after crystallization from CH₂Cl₂/acetone: mp 150.5-152°C; IR (KBr, cm⁻¹) 1625 (C=N); 'H NMR (CDCl₃) δ 7.43-7.27 (m, 11 H, ArH), 6.91 (s, 1 H, ArH), 5.11 (s, 2 H, ArCH₂O), 5.08 (s, 2 H, ArCH₂O), 3.61 (s, 2 H, CH₂), 3.7-3.3 (br s, 4 H, CH₂CH₂); MS m/z 499 (M⁺). Anal. Calcd for C₂₄H₂₃IN₂O₂·0.25H₂O: C, 57.31; H, 4.67; N, 5.57. Found: C, 57.39; H, 4.35; N, 5.49.
2-(2-Iodo-4,5-dihydroxybenzyl)imidazoline hydrochloride (53)

Prepared by the procedure as described for compound 56, from 110 (500 mg, 1.00 mmol) with an equimolar mixture of MeOH/concentrated HCl (10 mL) was obtained 261 mg (73%) of 53 as white crystals after crystallization from MeOH/Et₂O: mp 204.5-205°C (dec); IR (KBr, cm⁻¹) 1621 (C=N); "H NMR (D₂O) δ 7.40 (s, 1 H, ArH), 7.28 (s, 1 H, ArH), 3.78 (s, 6 H, CH₂CH₂ and CH₂); MS (FAB) m/z 319 (MH⁺ - HCl). Anal. Calcd for C₁₁H₁₁N₂O₂·HCl: C, 33.88; H, 3.41; N, 7.90. Found: C, 34.22; H, 3.46; N, 7.87.
PART 2
SYNTHESIS OF IRREVERSIBLE INHIBITORS OF
ALDOSE REDUCTASE
CHAPTER V
SYNTHESIS OF IRREVERSIBLE INHIBITORS OF
ALDOSE REDUCTASE

5.1 DIABETES MELLITUS

Diabetes mellitus is the disease involving a series of complex and chronic metabolic disorders characterized by symptomatic glucose intolerance. Currently there are more than 10 million diabetics in the United States and about 60 million diabetics worldwide. Until recently diabetes was classified primarily by age of onset. The National Diabetes Data Group has reclassified diabetes, and this new classification has been adopted by the American Diabetes Association. The two major types of diabetes include insulin-dependent diabetes mellitus (IDDM or type I) and non-insulin-dependent diabetes mellitus (NIDDM or type II). Type I of diabetes is a severe form. It usually occurs in childhood or early adulthood and accounts for only 5% to 10% of all diabetics. These patients are often thin and have an absolute lack of insulin, and are prone to develop ketosis if insulin is withheld. Heredity, viruses, and autoimmune syndromes have been linked to the development of this type of diabetes. Type II diabetes manifests itself in adulthood around the age of 40 or later and the symptoms develop more slowly. It is more genetically linked than type I diabetes. About 90% of patients are obese. The type II diabetic can synthesize and secrete insulin; however,
the insulin does not appear to be useful in the transport of glucose into cells because of hidden receptors, desensitized receptors, or low quality of insulin. These patients do not usually progress to ketosis.

The treatment of diabetes varies considerably between type I and type II diabetes. Type I diabetics have an absolute lack of insulin, so diet, exercise and insulin are necessary. The therapy for type II diabetes also consists of diet and exercise; however, oral hypoglycemic agents or insulin may or may not be required. Hypoglycemic agents have been classified into two major groups: the first-generation agents such as tolbutamide, acetohexamide, tolazamide and chlorpropamide, and the second-generation agents such as glyburide and glipizide. Second-generation agents are more potent than the older agents, have a quicker onset of action, exhibit a longer duration of action and produce fewer side effects than do the older drugs.

Currently available treatments of diabetes can correct acutely life-threatening symptoms but do not prevent diabetic complications which are largely responsible for the large degree of morbidity and mortality present in the diabetic population. Several different types of diabetic complications can occur. Macroangiopathy occurs more in type II than type I diabetics. Larger blood vessels such as coronary, cerebral, and peripheral vessels may be more prone to occlusion. These complications can lead to coronary heart disease, stroke, or peripheral vascular disease. The mechanism of this complication is unclear but relates to an altered metabolism of low-density lipoprotein (LDL), cholesterol or to a direct effect of diabetes on arterial wall cells. Other diabetic complications: diabetic nephropathy, cataract, retinopathy, and neuropathy
occur almost exclusively in type I diabetes. Approximately one of three people with type I diabetes eventually develops kidney failure.\textsuperscript{141} Diabetic retinopathy occurs in 80\% to 90\% of people who have had type I diabetes for at least 20 years.\textsuperscript{142} In young and middle-aged adults in the United States, diabetes is both the leading cause of kidney failure requiring dialysis and the leading cause of blindness.\textsuperscript{143} Neuropathy can lead to a high incidence of motor, sensory and autonomic dysfunction. Although the mechanism of these diabetic complications has not been fully elucidated, these complications have been linked to increased sorbitol levels resulting from glucose metabolism via a polyol pathway.\textsuperscript{144} It has been demonstrated that the use of an aldose reductase inhibitor such as sorbinil can reduce the sorbitol concentration and can prevent or improve these complications.\textsuperscript{145,146}

5.2 ALDOSE REDUCTASE AND THE POLYOL PATHWAY

The polyol pathway (Figure 35) consists of two enzymes, aldose reductase (aldol: NADP\textsuperscript{+}oxidoreductase, EC 1.1.1.21) and sorbitol dehydrogenase(l-iditol dehydrogenase, ED 1.1.1.14). Aldose reductase utilizes NADPH to reduce the aldehyde form of glucose to its corresponding sugar alcohol, sorbitol. In the second step, sorbitol dehydrogenase utilizes NAD\textsuperscript{+} to oxidize sorbitol to fructose. In addition to reducing glucose, aldose reductase can also reduce a variety of aromatic and aliphatic aldehydes, including galactose (Figure 35), xylolose and arabinose.\textsuperscript{144} The enzyme has a greater affinity for galactose than for glucose but the product (galactitol) cannot be further oxidized by sorbitol dehydrogenase. The accumulating galactitol can develop a
cellular pathology similar to that observed in diabetic tissues. Aldose reductase in most tissues competes with hexokinase for the utilization of glucose. Since the affinity of hexokinase for glucose is greater than that of aldose reductase, glucose is preferentially phosphorylated by hexokinase under normal conditions. However, at high glucose levels, such as in diabetes, hexokinase is saturated and there is an increased flux through the polyol pathway. Under these conditions, sorbitol is formed more rapidly than it is converted to fructose, resulting in the accumulation of sorbitol. This accumulation of sorbitol is enhanced by its polarity which hinders its facile penetration through membranes and subsequent removal from tissues through diffusion. The intracellular accumulation of sorbitol can produce hyperosmotic effects which result in an influx of fluid and membrane permeability changes that lead to the onset of cellular pathology. It has been suggested that polyol induced pathology may also result from the rapid depletion of NADPH and from an inverse relationship between the cellular concentration of myoinositol and aldose reductase activity.$^{148}$

![Polyol Pathway](image)

**Figure 35:** Polyol Pathway.$^{147}$
5.3 ALDOSE REDUCTASE INHIBITORS

A variety of structurally diverse compounds have been reported to be inhibitors of aldose reductase. Almost all inhibitors are acidic and can be grouped into certain functionality classes. Major structural classes are the acidic cyclic amides and the carboxylic acids. Nonsteroidal antiinflammatory agents (sulindac, indomethacin, and oxyphenbutazone, etc.) and antiallergic agents also inhibit aldose reductase in vitro, although both classes lack significant in vivo inhibitory activity. Some weakly acidic phenols and natural product extracts have also been reported to possess aldose reductase inhibitory activity in vitro. Table 11 shows the clinically tested aldose reductase inhibitors containing acidic cyclic amide: sorbinil (111), M 79175 (112), CT 112 (113), alconil (114), AL 1576 (115), and ADN 138 (116). Table 12 lists the aldose reductase inhibitors containing carboxylic acid: alrestatin (117), tolrestat (118), epalrestat (119), ponalrestat (statil, 120), FR 74366 (121), and AD 5467 (122). The structure activity relationships of these inhibitors have been summarized in many review articles.

These inhibitors are either uncompetitive or noncompetitive inhibitors. The inhibition is reversible and has been proposed to result from a direct interaction between the enzyme and its inhibitors. This interaction may result in either a reversible conformational perturbation, before or after the binding of substrate and nucleotide, or in steric interference of the catalytic site produced by a partial overlapping of bound inhibitors.
Clinically tested aldose reductase inhibitors containing acidic cyclic amides.\textsuperscript{150}

![Chemical structures](image)
Table 12

Clinically tested aldose reductase inhibitors containing carboxylic acids.\textsuperscript{150}

\begin{align*}
\text{117} & \quad \text{[Chemical structure]} \\
\text{118} & \quad \text{[Chemical structure]} \\
\text{119} & \quad \text{[Chemical structure]} \\
\text{120} & \quad \text{[Chemical structure]} \\
\text{121} & \quad \text{[Chemical structure]} \\
\text{122} & \quad \text{[Chemical structure]} \\
\end{align*}
5.4 IRREVERSIBLE INHIBITORS

Kinetic and competition studies indicate that there are three distinct binding sites on the enzyme: a NADPH cofactor site, a substrate site, and an inhibitor binding site (the common region that both acidic cyclic amide and carboxylic acid inhibitors react with on the enzyme\textsuperscript{153}). Protein modification and protection studies reveal the presence of a sulfhydryl group on the enzyme,\textsuperscript{155} the basic amino acids (lysine, arginine, and histidine) at the cofactor site,\textsuperscript{156} and tyrosine at the substrate and inhibitor binding sites.\textsuperscript{155}

To gain the information about the nature of the inhibition, the location of amino acids at the inhibitor site, and the structural and spatial limitations of the binding site, affinity and photoaffinity analogs of known aldose reductase inhibitors are being used as specific probes. This is possible because a nucleophilic residue which is present at the inhibitor site will be able to irreversibly react with appropriately electrophilic functional groups on the inhibitor molecules. These irreversible inhibitors \textit{in vivo} can be used as tools for the study of aldose reductase biosynthesis and activation. In addition, irreversible inhibitors can give a prolonged inactivation of the enzyme so the daily administration of the agents is not necessary.\textsuperscript{157} Ares et al.\textsuperscript{158-160} and Smar et al.\textsuperscript{150,161} have synthesized and biologically evaluated several affinity and photoaffinity irreversible inhibitors. Isothiocyanate, haloacetamides, and sulfonyl fluoride have been used as affinity labels and azides have been examined as potential photoaffinity labels. By using various inhibitors (alrestatin, sorbinil, alconil etc.), they believed, when superimposed, the corresponding affinity labels will
each cover a different portion of the inhibitor binding site and the information of the various portions of the inhibitor site can be obtained.

The results of affinity and photoaffinity labels of alrestatin (117), racemic sorbinil (111), alconil (114) and an alconil analog (compound without fluorine at the 2 position of alconil, 123) are shown in Table 13, 150,160 Table 14, 162 and Table 15, 150 respectively.

Alrestatin azide 125, sorbinil azide 132, and alconil azide 139 upon appropriate photolysis irreversibly inhibit aldose reductase.

The isothiocyanate and haloacetamide substitution at the 5-position of alrestatin gives irreversible inhibition of the enzyme (Table 13). The iodoacetamide is the most active irreversible inhibitor whereas the chloroacetamide is the least active in the series of haloacetamide substitutions. The IC₅₀ values for reversible inhibition of the halo-substituted analogs and the 5-acetamido parent compound are not very different indicating that the increased activity of the iodo-analog is not due to the higher affinity of the compound to the inhibitor site but due to the higher chemical reactivity or leaving group ability of iodine. The trend of activity is shown to follow I > Br > Cl.

Affinity labels with a chloroacetamide or an isothiocyanate group at the 8-position of racemic sorbinil are inactive as irreversible inhibitors (Table 14). However, both compounds reversibly inhibit the enzyme at a level not very different from that of sorbinil, 111. This indicates the lack of a nucleophilic residue on the region of the inhibitor binding site of aldose reductase for interaction with the electrophile on the inhibitor molecules. Isothiocyanate and sulfonyl fluoride substitution at the 2 position of alconil, compound 137 and 138,
Table 13

Reversible and irreversible inhibition of aldose reductase by
photoaffinity and affinity labeled alrestatin.\textsuperscript{150,158,162}

<table>
<thead>
<tr>
<th>Inhibition</th>
<th>Irreversible % at 10^-4 M</th>
<th>Reversible IC\textsubscript{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>117 [R = H]</td>
<td>0</td>
<td>0.11</td>
</tr>
<tr>
<td>124 [R = NCS]</td>
<td>34</td>
<td>-</td>
</tr>
<tr>
<td>125 [R = N\textsubscript{3}]</td>
<td>34 ± 4.9</td>
<td>-</td>
</tr>
<tr>
<td>126 [R = NHCOCH\textsubscript{3}]</td>
<td>0</td>
<td>0.63 ± 0.02</td>
</tr>
<tr>
<td>127 [R = NHCOCH\textsubscript{2}Cl]</td>
<td>18</td>
<td>0.55 ± 0.04</td>
</tr>
<tr>
<td>128 [R = NHCOCH\textsubscript{2}Br]</td>
<td>46 ± 3.8</td>
<td>0.60 ± 0.04</td>
</tr>
<tr>
<td>129 [R = NHCOCH\textsubscript{2}I]</td>
<td>89 ± 0.8</td>
<td>0.40 ± 0.02</td>
</tr>
</tbody>
</table>
Table 14

Reversible and irreversible inhibition of aldose reductase by photoaffinity and affinity labeled sorbinil.¹⁶⁰,¹⁶²

<table>
<thead>
<tr>
<th>R</th>
<th>Inhibition</th>
<th>Irreversible % at 10⁻⁴ M</th>
<th>Reversible IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>0</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>NCS</td>
<td>-</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>NHCOCH₂Cl</td>
<td>-</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>N₃</td>
<td>48 ± 3.0</td>
<td>0.19 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>
Table 15

Reversible and irreversible inhibition of aldose reductase by photoaffinity and affinity labeled alconil and alconil analog.\textsuperscript{150,162}

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Inhibition</th>
<th>Irreversible % at 10^{-4} M</th>
<th>Reversible IC\textsubscript{50} (\mu M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>133</td>
<td>1 = NCS</td>
<td>2 ± 3.5</td>
</tr>
<tr>
<td>134</td>
<td>2 = NCS</td>
<td>56 ± 10.7</td>
</tr>
<tr>
<td>135</td>
<td>3 = NCS</td>
<td>54 ± 13.0</td>
</tr>
<tr>
<td>136</td>
<td>4 = NCS</td>
<td>21 ± 4.0</td>
</tr>
<tr>
<td>115</td>
<td>2' = F, 2 = F</td>
<td>0</td>
</tr>
<tr>
<td>137</td>
<td>2' = F, 2 = NCS</td>
<td>56 ± 10.8</td>
</tr>
<tr>
<td>138</td>
<td>2' = F, 2 = SO\textsubscript{2}F</td>
<td>12 ± 6.7</td>
</tr>
<tr>
<td>139</td>
<td>2' = F, 2 = N\textsubscript{3}</td>
<td>84 ± 6.2</td>
</tr>
</tbody>
</table>
also give the irreversible inactivation of the enzyme. The isothiocyanate \textit{137} is more active as an irreversible inhibitor than the sulfonyl fluoride \textit{138}.

Irreversible inhibition is also observed with the isothiocyanate substitution at the 1, 2, 3, and 4 position of \textit{123}, compounds \textit{133}, \textit{134}, \textit{135}, and \textit{136}, respectively. The order of irreversible inactivation depends on the substitution position on the molecule. Similar irreversible inhibition is observed with either the 2 or 3 substituted analogs, \textit{134} or \textit{135}, which are more active than the 4 substituted analog, \textit{136}, and much more active than the 1 substituted analog, \textit{133}. The IC$_{50}$ of \textit{133} is much larger that the others indicating that the inhibitor binding site is poorly tolerant to the steric bulk at the 1-position of this fluorene ring. A comparison of % irreversible inhibition and IC$_{50}$ values of compound \textit{134} and \textit{137} shows that in this series, fluorine exerts little affect on either the reversible or irreversible inhibitory activity.

\textbf{5.5 STATEMENT OF PROBLEMS AND OBJECTIVES}

The preceding results indicate the presence of a nucleophilic residue at a specific region of the inhibitor binding site and the potency of each analog depends on the reactivity of each functional group substituted on the inhibitor molecule, as well as the close proximity of the electrophile to the nucleophilic residue. Interestingly, using various reversible inhibitors, the enzyme can be protected against the irreversible inhibition caused by \textit{124} and \textit{137},\textsuperscript{158} this suggests that the compounds bind at the common aldose reductase binding site.
To continue studies with the irreversible inhibitors of aldose reductase, haloacetamide of alconil analog, compounds 140-145, were synthesized and evaluated for their irreversible inhibitory activity. Due to lack of difference in the irreversible activities between 2-isothiocyanato derivative of alconil, 137, and that of alconil analog, 134, the alconil analog is used for studies instead of alconil. As shown in Table 13, the isothiocyanate and haloacetamides of alrestatin gave the differences in the irreversible inhibitory activity. The chloro-, bromo-, and iodo-acetamides of alconil analog were prepared to see if these functional groups could increase the irreversible activity as that compared to the isothiocyanate. As shown in Table 15, the use of an isothiocyanato group at the 2 or 3 positions of 123 provides more active compounds than that at 4 position. Because of the differences in size and occupied space between the isothiocyanate and the haloacetamide, the possibility that the nucleophile on the enzyme molecule can reach and react with the functional groups might be different. In order to verify this possibility the haloacetamides were placed at the 2 and the 4 positions as illustrated in compounds 140-145 (Figure 36).

5.6 RESULTS AND DISCUSSION

5.6.1 CHEMISTRY

The synthesis of compounds 140-142 and 143-145 involved the use of the known intermediates, 146 and 147, respectively. The attempt to prepare the spirohydantoin 146 using the reaction condition and time as reported in the literature gave some of the unreacted starting material so the reaction time was increased from 1 day to 4 days to attain a
The synthesis of the haloacetamides of alrestatin were previously reported,\textsuperscript{161} using 5 equivalents of the appropriate haloacetic anhydride and 4-dimethylaminopyridine (DMAP) in THF at 0°C for 1 hour. This procedure was adopted to synthesize the bromoacetamide of 123 but the proton NMR spectrum indicated undesired product with the quartet of two protons at 4.69 and 4.61 ppm and a singlet of two protons at 4.03 ppm plus the aromatic and the amide protons. Mass spectrum of the product (Figure 32) showed prominent molecular ions at masses 504.93 (51.13% rel.abund.), 506.93 (100% rel.abund.), and 508.92 (51.03% rel.abund.). It is known that the mass spectrum of Br\textsubscript{2} shows the molecular ions at masses 158, 160, and
162, of relative abundances 1:2:1, due to the ions $^{79}\text{Br}_2$, $^{79}\text{Br}^{81}\text{Br}$ and $^{81}\text{Br}^{79}\text{Br}$, and $^{81}\text{Br}_2$, respectively, so the mass spectrum of this compound indicated the presence of two bromine atoms in the molecule. These informative spectra suggested that the product might be 148 (Figure 38).

![Mass spectrum of 148](image)

**Figure 37:** Mass spectrum of 148

![Compound 148](image)

**Figure 38:** Compound 148

To prevent the excessive reaction of the amine, only 1 equivalent of the appropriate haloacetic anhydride should be used or the reaction should be performed without using DMAP. It was found that by using acetonitrile as the solvent and one to two equivalents of haloacetic anhydride the reaction could be complete in 1-3 hours without the use of DMAP. The synthesis of these compounds 140-145 is shown in scheme X.
It should be noted that the proton NMR spectra of the products showed good correlation between the inductive effect of each halogen atom, which can give a different deshielding effects, and the chemical shift of the attached methylene protons. Compound 140-142 and 143-145, using DMSO-d$_6$ as the solvent, showed the chemical shift of -CH$_2$-$X$ at 4.25, 4.04, 3.81 ppm and 4.43, 4.20, 3.97 ppm when $X$ is Cl, Br, and I, respectively.

\textit{Scheme X}

\textit{Synthesis of (140-145)}

\begin{center}
\begin{align*}
\text{146} & \xrightarrow{\text{CH$_3$CN}} \text{140} & \ x = \text{Cl} \\
\text{141} & \ x = \text{Br} \\
\text{142} & \ x = \text{I} \\
\text{147} & \xrightarrow{\text{CH$_3$CN}} \text{143} & \ x = \text{Cl} \\
\text{144} & \ x = \text{Br} \\
\text{145} & \ x = \text{I}
\end{align*}
\end{center}
5.6.2 BIOLOGICAL RESULTS

The method for determining irreversible activities of aldose reductase inhibitors has been described in the literature.\textsuperscript{150,162}

The irreversible activity of compound 140-145 is shown in Table 16. Surprisingly, the substitution of haloacetamides at the 4 position of the alconil analog (123) gave the most active irreversible inhibitor, the results obtained were different than those obtained with isothiocyanato group. As expected, the iodoacetamides were more active than the bromo- and the chloroacetamides, respectively.

Table 16

<table>
<thead>
<tr>
<th>Compound</th>
<th>Irreversible Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Inhibition (10^{-14} M)</td>
</tr>
<tr>
<td>140</td>
<td>0</td>
</tr>
<tr>
<td>141</td>
<td>0</td>
</tr>
<tr>
<td>142</td>
<td>25.6 ± 6.6</td>
</tr>
<tr>
<td>143</td>
<td>4.3 ± 1.3</td>
</tr>
<tr>
<td>144</td>
<td>27.9 ± 18.4</td>
</tr>
<tr>
<td>145</td>
<td>41.2 ± 11.4</td>
</tr>
</tbody>
</table>

5.6.2 SUMMARY

1. Chloro-, bromo- and iodoacetamide substitution at the 2- and 4- positions of an alconil analog were synthesized as affinity labels for aldose reductase.
2. Substitution of chloro- and bromoacetamide at the 2-position of the alconil analog does not provide irreversible inactivation of enzyme.

3. The data from Table 14 indicate that a nucleophile exists at the inhibitor binding site of aldose reductase and may be localized in an area accessible to the haloacetamide substitution at the 4-position.

3. Iodoacetamides are more active as irreversible aldose reductase inhibitors than bromo- and chloroacetamide. This is due to the leaving group ability of iodine.

5.7 EXPERIMENTAL

The general information concerning instrumentation, solvent purification and storage and elemental analysis can be found in the experimental portion in chapter IV.
To a magnetically stirred 0°C suspension of 146 (100 mg, 0.37 mmol) in dry acetonitrile (5 ml), in a flask equipped with a drying tube, was added chloroacetic anhydride (129 mg, 0.75 mmol). After stirring for 1 h, water (10 ml) was added and the mixture was allowed to stir at room temperature for 20 min. The reaction mixture was concentrated under reduced pressure and the resulting solid residue was crystallized from acetone to give 122 mg (94%) of 140 as a slightly yellow solid: mp > 285°C; IR (KBr, cm⁻¹) 1778 (C=O), 1716 (C=O), 1674 (C=O); ¹H NMR (DMSO-d₆) δ 11.26 (s, 1 H, NH), 10.48 (s, 1 H, NH), 8.62 (s, 1 H, NH), 7.84-7.77 (m, 3 H, ArH), 7.61 (dd, 1 H, ArH, J = 8.3 and J = 1.9 Hz, ), 7.49-7.43 (m, 2 H, ArH), 7.34-7.28 (m, 1 H, ArH), 4.25 (s, 1 H, CH₂); MS m/z 343 (M⁺, 37Cl), 341 (M⁺, 35Cl, base). Anal. Calcd for C₁₇H₁₂ClN₃O₃·0.25H₂O: C, 59.04; H, 3.47; N, 12.15. Found: C, 59.08; H, 3.55; N, 12.02.
Spirohydantoin 141 was synthesized by the same procedure previously described for spirohydantoin 140. Thus, 146 (100 mg, 0.37mmol) was allowed to react with bromoacetic anhydride (195 mg, 0.75mmol) for 1 h at 0°C, then water was added and the mixture was stirred for about 20 min. The reaction mixture was concentrated under reduced pressure and the resulting solid residue was crystallized from acetone to give 132 mg (90%) of 141 as a pale yellow solid: mp > 285°C; IR (KBr, cm⁻¹) 1772 (C=O), 1725 (C=O), 1652 (C=O); ¹H NMR (DMSO-d₆) δ 11.26 (s, 1 H, NH), 10.56 (s, 1 H, NH), 8.62 (s, 1 H, NH), 7.84-7.77 (m, 3 H, ArH), 7.59 (dd, 1 H, ArH, J = 8.2 and 1.9 Hz), 7.49-7.43 (m, 2 H, ArH), 7.34-7.28 (m, 1 H, ArH), 4.04 (s, 1 H, CH₂); MS m/z 387 (M⁺, 81Br), 385 (M⁺, 79Br), 194 (base). Anal. Calcd for C₁₇H₁₂BrN₃O₃·0.25H₂O: C, 52.24; H, 3.07; N, 10.75. Found: C, 52.41; H, 3.24; N, 10.75.
2-Iodoacetamidospiro[9H-fluoren-9,4'-imidazoline]-2',5'-dione (142)

Spirohydantoin 142 was synthesized by the same procedure previously described for spirohydantoin 140. Thus, 146 (106 mg, 0.40 mmol) was allowed to react with iodoacetic anhydride (160 mg, 0.45 mmol) for 1 h at 0°C, then water was added and the mixture was stirred for about 20 min. The reaction mixture was concentrated under reduced pressure and the resulting solid residue was crystallized from acetone to give 170 mg (98%) of 142 as a pale yellow solid; mp 267-268°C; IR (KBr, cm⁻¹) 1771 (C=O), 1728 (C=O), 1669 (C=O); ¹H NMR (DMSO-d⁶) δ 11.27 (s, 1 H, NH), 10.52 (s, 1 H, NH), 8.62 (s, 1 H, NH), 7.84-7.76 (m, 3 H, ArH), 7.56 (dd, 1 H, ArH, J = 8.3 and 1.9 Hz), 7.48-7.42 (m, 2 H, ArH), 7.33-7.27 (m, 1 H, ArH), 3.81 (s, 1 H, CH₂); MS m/z 433 (M⁺), 69 (base). Anal. Calcd for C₁₇H₁₂IN₃O₃: C, 47.13; H, 2.79; N, 9.70. Found: C, 47.09; H, 2.81; N, 9.43.
4-Chloracetamidospiro[9H-fluoren-9,4'-imidazoline]-2',5'-dione (143)

Spirohydantoin 143 was synthesized by the same procedure previously described for spirohydantoin 140. Thus, 147 (150 mg, 0.57 mmol) was allowed to react with chloroacetic anhydride (193 mg, 1.13 mmol) for 1.5 h at 0°C, then water was added and the mixture was stirred for about 30 min. The reaction mixture was concentrated under reduced pressure and the resulting solid residue was crystallized from methanol/petroleum ether to give 182 mg (94%) of 143 as a white solid; mp 270-271°C (dec); IR (KBr, cm⁻¹) 1765 (C=O), 1722 (C=O); ^1H NMR (DMSO-d₆) δ 11.22 (s, 1 H, NH), 10.33 (s, 1 H, NH), 8.61 (s, 1 H, NH), 7.83 (apparent d, 1 H, ArH, J = 8 Hz), 7.46-7.36 (m, 6 H, ArH), 4.43 (s, 1 H, CH₂); MS m/z 343 (M⁺, 37Cl), 341 (M⁺, 35Cl), 270 (base). Anal. Calcd for C₁₇H₁₂ClN₃O₃·0.5H₂O: C, 58.28; H, 3.71; N, 12.00. Found: C, 58.04; H, 3.81; N, 11.88.
4-Bromoacetamidospiro[9H-fluoren-9.4’-imidazoline]-2’5’-dione (144)

Spirohydantoin 144 was synthesized by the same procedure previously described for spirohydantoin 140. Thus, 147 (150 mg, 0.57 mmol) was allowed to react with bromoacetic anhydride (292 mg, 1.13 mmol) for 1.5 h at 0°C, then water was added and the mixture was stirred for about 30 min. The reaction mixture was concentrated under reduce pressure and the residue was crystallized from acetone/H₂O to give 205 mg (94%) of 144 as a white solid; mp 176-178°C (dec); IR (KBr, cm⁻¹) 1764 (C=O), 1719 (C=O), 1651 (C=O); ¹H NMR (DMSO-d₆) δ 11.22 (s, 1 H, NH), 10.39 (s, 1 H, NH), 8.62 (s, 1 H, NH), 7.88 (apparent d, 1 H, ArH, J = 7.7 Hz), 7.48-7.36 (m, 6 H, ArH), 4.20 (s, 1 H, CH₂); MS m/z 387 (M⁺, 61Br), 385 (M⁺, 79Br), 69 (base). Anal. Calcd for C₁₇H₁₂BrN₃O₃·H₂O: C, 50.49; H, 3.46; N, 10.39. Found: C, 50.76; H, 3.29; N, 10.34.
4-Iodoacetamidospiron[9H-fluoren-9.4'-imidazoline]-2',5'-dione (145)

Spirohydantoin 145 was synthesized by the same procedure previously described for spirohydantoin 140. Thus, 147 (128 mg, 0.48 mmol) was allowed to react with iodoacetic anhydride (342 mg, 0.97 mmol) for 3 h at 0°C, then water was added and the mixture was stirred for about 30 min. The reaction mixture was concentrated under reduced pressure and the residue was crystallized from methanol/petroleum ether to give 198 mg (95%) of 145 as a white solid; mp 260-261°C; IR (KBr, cm⁻¹) 1775 (C=O), 1722 (C=O), 1671 (C=O); ¹H NMR (DMSO-d₆) δ 11.22 (s, 1 H, NH), 10.32 (s, 1 H, NH), 8.62 (s, 1 H, NH), 7.92 (apparent d, 1 H, ArH, J = 7.7 Hz), 7.49-7.35 (m, 6 H, ArH), 3.97 (s, 1 H, CH₂); MS m/z 433 (M⁺), 69 (base).

Anal. Calcd for C₁₇H₁₂IN₃O₃•0.25H₂O: C, 46.62; H, 2.85; N, 9.6. Found: C, 46.84; H, 2.76; N, 9.58.


1985, 28, 1398.


Obshchei, Khim. 1954, 24, 1265; Chem. Abstr. 1958, 49, 12327d. year?


