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Synthesis and Pharmacological Evaluation of Certain Schiff Bases and Thiazoldine Derivatives as AT1 Angiotension-II(AII) Receptor Antagonists

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Abstract:

Many Schiff bases were prepared by condensation reaction of nitro compound containing biphenyl ether amines with aromatic aldehydes and ketone derivatives and thiazolidines wer prepared by Schiff base with thioglycolic acid. The synthesized compounds were screened for AT1 Angiotension (A II) Receptor Antagonist activity. The nitro compound containing biphenyl ether Schiff bases and thiazolidines shows good activity compared with losartan.

Keywords: Schiff base, Biphenyl ether Thiazolidines, Angitotensin II and Losartan

Introduction

The renin-angiotensin system (RAS) plays an important role in the regulation of blood pressure through the actions of angiotensin II (AII) (vasoconstriction, aldosterone secretion, renal sodium reabsorption, and nor epinephrine release) and thus is an appropriate target for therapeutic intervention in hypertension.[1] Inhibition and antagonism of the various components of the RAS have been the subject of extensive research, culminating in such drugs as angiotensin converting enzyme (ACE) inhibitors,[2] renin inhibitors,[3] and AII antagonists.[4] ACE inhibitors effectively reduce hypertension but can produce undesirable side effects such as cough and angioedema. ACE inhibitors may interact with enzymes that process bradykinin and substance P to produce such side effects.[5] Angiotensin II antagonists selectively block AII at the receptor level and should be devoid of the adverse effects associated with ACE inhibitors

Presently the attention has been on the synthesis and evaluation of non-peptide antagonists Angiotension II. The great breakthrough has been made particularly with the synthesis of Losartan.[6]

Numerous patents and publications on AII antagonists are reported in the last several years. Most of the published work on variations of the heterocyclic system in Losartan molecule (e.g. ICI D8731 (2), L-159,093 (3)) (Chart-1) with retaining tetrazole biphenyl moiety and the side chain has been linked directly through a heteroatom.

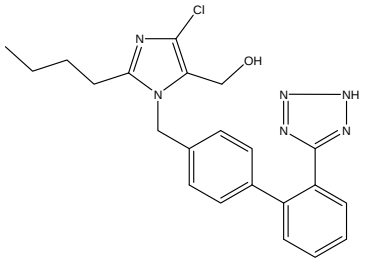
The objective of the work is to establish the essentiality of biphenyl tetrazole and heterocyclic ring in exhibiting the biological activity. In this context Schiff bases of 2-phenoxynitroaniline (IV), Isonicotinic acid and also thiazolidine (VIII) derivatives are prepared.

Schiff bases derived from aromatic amines and aromatic aldehydes have a wide variety of applications in many fields, *e.g.*, biological, inorganic and analytical chemistry.[7-9] They are known to exhibit potent antibacterial, anticonvulsant, anti-inflammatory activities.[10] In addition some Schiff bases show pharmacologically useful activities like anticancer,[11] anti-hypertensive and hypnotic[12] activities.

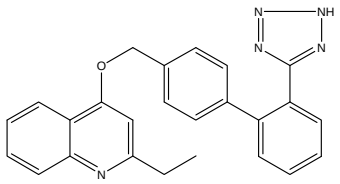
Thiazolidinones are to an important group of heterocyclic compounds containing sulfur and nitrogen in a five member ring, which have fungicidal, local anesthetic, anti-tubercular and anti-inflammatory activities.[13-14]

In view of above facts, certain Schiff bases containing 2-Phenoxynitroaniline (IV) and Isonicotinic acid containing thiazolidine moiety has been synthesized in the hope of getting better bioactive agents. The 2-Phenoxynitroaniline was prepared by condensation of o-Chloro nitrobenzene with phenol followed by acetylation, nitration and Schiff bases are prepared by condensing with aromatic aldehyde and ketone

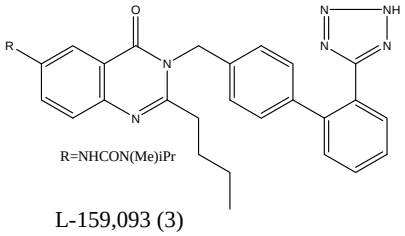
Chart-1:



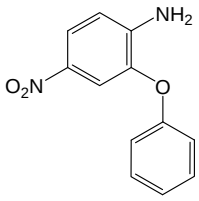
Losartan(1)



ICI D8731(2)



L-159,093 (3)



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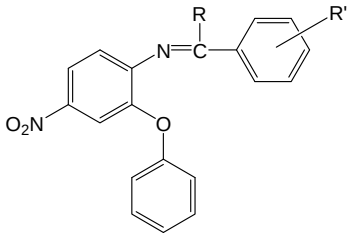
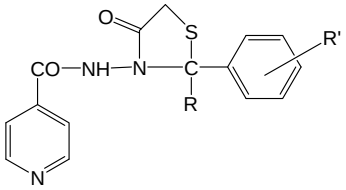


(5) R=H/CH_3



(7) R=H/CH_3

Numerous patents and publications on AII antagonists are reported in the last several years. Most of the published work on variations of the heterocyclic system in Losartan molecule (e.g. ICI D8731 (2), L-159,093 (3)) (Chart-1) with retaining tetrazole biphenyl moiety and the side chain has been linked directly through a heteroatom.

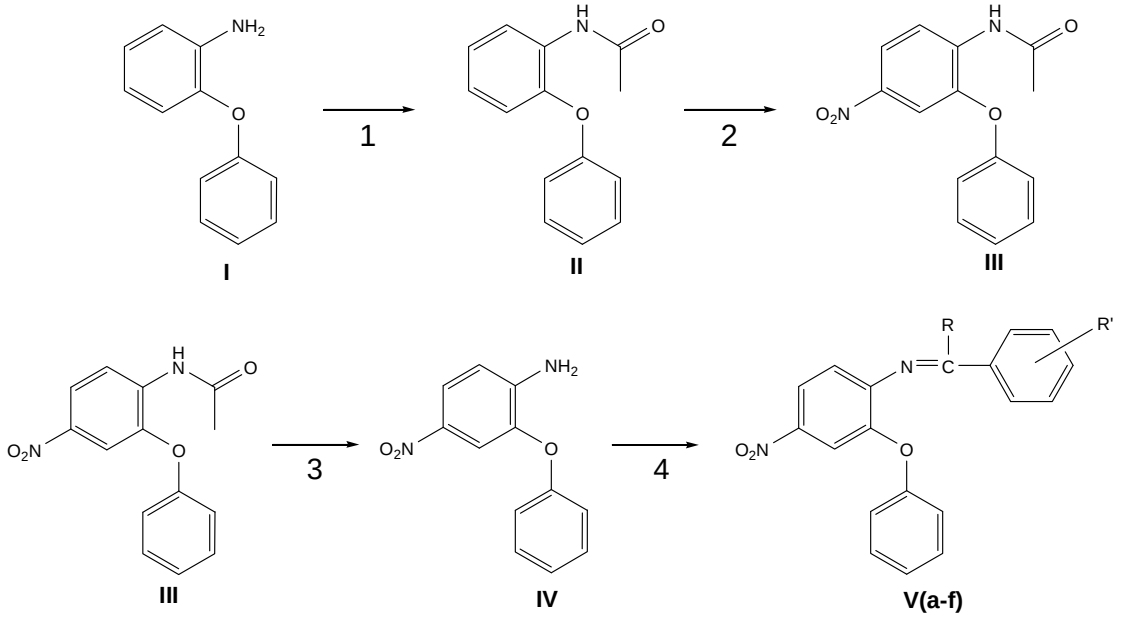
(***Scheme-1***). Thiazolidine derivative are prepared by condensing Schiff base of isnicotinic acid with thioglycolic acid (***Scheme-2***). The structures of synthesized compounds were suitably characterized on the basis of chemical, analytical and spectral data and selected compounds were evaluated for AngiotensionII receptor antagonistic activity.

Materials and Methods:

The 2-Phenoxyaniline was either commercially available (purchased from Aldrich Chemical Company, USA) or prepared according to standard procedures reported earlier.[15] All the reagents and solvents were purchased from SISCO Research Laboratories Pvt. Ltd., Bombay (India). All of the solvents used were of analytical grade or were purified according to standard procedures. Thin layer chromatography was carried out on silica gel plates obtained from Whatman Inc. The melting points were determined by using a Thomas–Hoover melting point apparatus and are uncorrected. IR spectra were recorded on a Infrared spectra (KBr) were recorded on Perkin-Elmer 599-B spectrophotometer and NMR spectra ($CDCl_3$

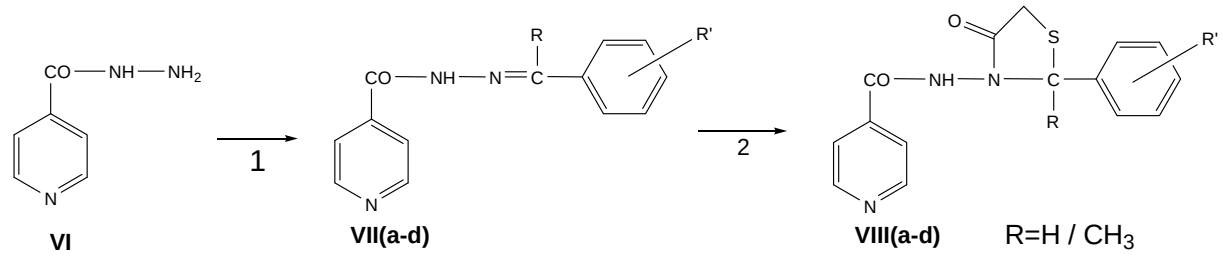
and DMSO-d_6) on a Varian EM 390 spectrophotometer.

Scheme-1



Reagents: (1) AcOH, Pyridine, Ac_2O, Reflux,1hr, (2) 69% HNO_3, RT, 15min, ice, (3) Ethanolic HCl, Reflux, 5hr, (4) Ar-CHO / Ar-CO-CH_3, Zinc(II)Chloride, Ethanol, Reflux, 4hr.

Scheme-2



Reagents: (1) Ar-CHO / AR-CO-CH_3, Zinc(II)Chloride, Ethanol, Reflux, 4hr, (2) Thioglycolic acid, Benzene, Reflux

The purification was carried out by using 60-120 mesh silica gel for column chromatography (obtained from Spectrochem) with a suitable eluting system. The chemicals used for pharmacological screening were procured from Sigma Chemicals and Himedia, Bombay. The animals like albino rats were obtained from the Venkateshwara enterprises, Bangalore.

Experimental

Preparation of 2-Acetoaminodiphenyl ether:

50.0 gm of 2-Phenoxy aniline (0.2699moles) was dissolved in 125.0 ml of Acetic acid and 100.0 ml of pyridine in a 1000 ml two neck round bottom flask. The reaction mixture was heated to reflux with stirring and added acetic anhydride solution drop wise to reaction mixture and continued at same temperature for 1hr. After completion of the reaction was poured into beaker containing crushed ice to get buff colored solid, filtered and dried under vacuum. Yield-49.0 gm (79.88%), m.p.: 81-83^{0}C

Preparation of N-(4-nitro-2-phenoxyphenyl)acetamide:

250.0 g f Con.Nitric acid was suspended in 500 ml two neck round bottom flask. The flask was cooled to 0-5^{0}C and added 49.0 g (0.2156moles) 2-Acetamidodiphenyl ether in a portion wise under stirring during 10min and continued at same temperature for 15 min. and poured into beaker containing crushed ice, the light yellow colored solid formed. The solid was filtered under suction and crystallized from methanol. Yield-29.0 gm (49%), m.p.: 178-182^{0}C.

Preparation of 4-Nitro-2-Phenoxyaniline (IV):

N-(4-nitro-2-phenoxyphenyl)acetamide 29.0 g (0.1065 moles) and Ethanolic HCl (300 ml) in a 1000 ml two neck round bottom flask was heated to reflux for 5hrs. After completion of the reaction (monitored by TLC), the reaction was poured into beaker containing crushed ice and the product was precipitated and basified with aqueous ammonia. The yellow colored precipitate of 4-Nitro-2-phenoxyaniline was filtered by suction and dried in vacuum. Yield-19.6 g (80%), m.p.: 116-118^{0}C, IR (KBr) cm^{-1}: 1079, 1103(-C-O), 3500(-NH), 1505 (-NO_2), 1326(C-N), 1585(C=N).^{1}H NMR (DMSO-d^6): δ 6.5-6.70(2H, s, -NH2) δ 6.80-7.90(8H, Ar-H).

General Procedure for Synthesis of Schiff Bases: (Va-f) and (VIIa-d):[16-18]

The mixture of compound IV (0.01 M) and aryl aldehyde / ketone (0.01M) in 25 ml of ethanol and a pinch of anhydrous zinc chloride was refluxed for a period of 4 hr. and cooled. The solid separated was filtered and crystallized from ethanol. The characterization data of synthesized compounds are given in the (***Table-1***).

General Procedure for Synthesis of 4-Thiazolidinones (VIIIa-d):[19]

A mixture of equimolar quantity of Schiff bases and thioglycolic acid was taken in dry benzene. The reaction mixture was refluxed for a period of 24 hr. using a Dean Stack apparatus till no more water separated. The residue obtained after removal of benzene was washed successively with saturated sodium bicarbonate solution (10%) and then with water. The obtained compounds were purified by column chromatography.

Pharmacological Evaluation

Screening Methods for Anti-hypertensive Activity:

a) Angiotensin II induced Hypertension: [20]

1) Invasive method (Direct method).

2) Non-invasive Tail cuff method (Indirect method).

b) In-vitro determination of vasodilator activity by aortic rings.

Experimental Techniques:

1) Invasive Method (Direct Method):[20]

Male albino wistar (300-400 gm) rats were used and housed at 22±2^{0}C room temperature. The rats were anaesthetized with urethane hydrochloride 80 mg/kg i.p. The Jugular vein and carotid artery were surgically cannulated for drug administration for recording the blood pressure respectively by using P-50 tubing. By means of three way stop cock and a stainless steel needle at the end of P.E. tubing was attached to arterial cannula for B.P., Transducers and the venus cannula to a syringe.

Table-1

Compound No.	R R	[1] M.P.	°C	Yield (%)	Mol. Form	Mol. Wt
V a	H	Phenyl	148	70	$C_{19}H_{14}N_20_3$	256
V b	H	p-Cl	152	80	$C_{19}H_{13}N_20_3C1$	290.5
V c	H	p-OH	150	73	$C_{19}H_{14}N_20_4$	272
V d	H	p-OCH_3	142	60	$C_{19}H_{16}N_20_4$	281
V e	H	o-OH, p-OCH_3	137-140	75	$C_{19}H_{16}N_20_5$	302
V f	H	o-Cl	145	65	$C_{19}H_{13}N_20_3C1$	290
V g	CH_3	p-Cl	135	80	$C_{20}H_{16}N_2O_3C1$	304.5
VII a	H	Phenyl	195	83	$C_{14}H_{12}N_3O$	225
VII b	H	p-Cl	210	80	$C_{14}H_{11}N_3OC1$	259.5
VII c	H	p-OCH_3	180	75	$C_{14}H_{14}N_2O_3$	255
VII d	CH_3	Phenyl	160	70	$C_{15}H_{14}N_3O$	239
VIII a	H	Phenyl	240	70	$C_{16}H_{14}N_2SO_2$	300
VIII b	H	p-Cl	220-225	89	$C_{16}H_{13}N_2SO_2C1$	334.5
VIII c	H	p-OCH_3	295	65	$C_{16}H_{16}N_2SO_3$	330
VIII d	CH_3	Phenyl	>300	60	$C_{17}H_{16}N_3SO_2$	326

Then both the cannulas were filled by heparinized saline before the administration. Arterial cannula was connected via transducer to physiograph recorder. Several baseline readings of systolic and diastolic pressure were recorded. The physiograph shows the reduction of the blood pressure with compare to losratan.

Synthesized compounds were screened in presence of Angiotensin II induced hypertension (0.5 µg/kg i.v.). (Physiograph-1)

2) Non-invasive Tail cuff Method (Indirect Method):[21-22]

Albino rats weighing 300-400 gm were used to produce 2-kidney -1-clip rats. Under sodium pentobarbital anesthesia 10mg/kg i.p.), the left renal artery was constricted with a silver clip (internal slit of 0.20 mm). Three weeks after the operation, blood pressure was measured by the Tail-cuff method and rats with mean blood pressure above 150mm Hg or with a systolic blood pressure above 170 mm Hg were used for the experiment. Observations are given in the table 2.

Results and Discussion

The synthesized compounds were characterized on the basis of chemical and spectral data. The present work was mainly intended to establish the moieties which are responsible for Angiotension-II inhibition. It was interesting to note that, the 4-Nitro-2-phenoxyaniline (IV) was able to reduce Angiotensin-II induced hypertension and also phenyl epinephrine induced vasoconstriction, compared with Losartan (1). The similar results were noticed with synthesized Schiff (V) bases and thiazolidinones. From the preliminary screening the synthesized molecules shows good Angiotensin II induced anti hypertension activity and above molecules can be taken as a lead molecule for Angiotensin II induced hypertension.

Therefore we conclude in our preliminary screening that it may not to be necessary to go for a synthesis of big molecule. The above four moieties themselves can be taken as a lead molecules for Angiotensin II induced hypertension. Further study of these molecules and its analogues under progress in our laboratory.

Table-2:

Compound. No.	Dose* (mg/kg i.v)	Systolic Blood Pressure		
		Control	Ang II (i.v)	After Test Compound
Losartan[5]	0.3	115	150	118
V	0.3	115	145	142
	0.6	115	145	130
	1.0	115	145	122
V a	0.3	110	135	135
	0.6	110	135	135
	1.0	110	135	132
V b	0.3	118	143	141
	0.6	118	143	132
	1.0	118	143	125
V d	0.3	110	140	135
	0.6	110	140	130
	1.0	110	140	120
VII a	0.3	112	138	138
	0.6	112	138	138
	1.0	112	138	134
VIII a	0.3	116	140	140
	0.6	116	140	138
	1.0	116	140	132
VIII c	0.3	114	140	140
	0.6	114	140	138
	1.0	114	140	136

- Doses were selected on Trial and Error Basis.
- Standard Angiotension II & Test compounds were administered through Jugular vein.
- Reduction in systolic blood pressure 20-40 mm Hg was considered as an index of anti-hypertensive activity.

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