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Potential Antibacterial Agents: Part VI- Synthesis and Structure Elucidation of Schiff Bases Derived from Hydralazine

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Abstract. Synthesis of hydrazones (Schiff bases) of an antihypertensive drug, hydralazine was carried out. The study afforded the hitherto unreported 1-[4-chlorobenzylidene]-hydrazinophthalazine (**III-a**), 1-[benzylidene]-hydrazinophthalazine (**III-b**), 1-[4-nitrobenzylidene]-hydrazinophthalazine (**III-c**), 1-[2-nitrobenzylidene]-hydrazinophthalazine (**III-d**), 3-[4-chlorophenyl]-*s*-triazolo [3-4-a] phthalazine (**IV-a**) and 3-phenyl-*s*-triazolo [3-4-a] phthalazine (**IV-b**). The structures of these compounds were established using spectroscopic techniques i.e. IR, UV, ^{1}HNMR, EIMS, FD and HRMS. Antibacterial activity of the drugs was evaluated.

Keywords: hydrazone, triazole, antibacterial activity, hydralazine, spectroscopic techniques

Introduction

Hydralazine **(1)** (1-hydrazinophthalazine) is an established antihypertensive agent, and is one of the most commonly prescribed drug in hypertension therapy today, although it was introduced forty five years ago. The effectiveness of hydralazine as antihypertensive agent was first reported by Gross *et al.* (1950) and currently it is sold under the trade name Apresoline (Rudy and Reece, 1988; Reece, 1981).

Hydralazine may be used in the treatment of early hypertension emergencies in conjunction with other antihypertensive drugs. It increases renal blood flow and it is also used to treat 'toxemia' of pregnancy. Hydralazine is particularly effective in combination with β-adrenergic antagonist. Its role in the treatment of hypertension has extended during recent years (Reece *et al.*, 1980; Orzech *et al.*, 1979; Druey and Tripod, 1967).

The hydrazino group of hydralazine is highly reactive towards carbonyl groups to give hydrazones. The products thus obtained may not be stable and further transformation may occur. For example, reaction of hydralazine with pyruvic acid at pH<7, gives 3-methyl-4-keto-*s*-triazino [3-4-a]phthalazine. However, when the hydrazones obtained from different conditions, were heated above their melting points, the same cyclized triazole derivatives were obtained. Reaction of hydralazine with different biogenic carbonyl compounds including carbohydrates and pyridoxol gave benzyledene (Schiff bases) derivatives. These Schiff bases were also employed in the assay of the drug in biological fluids (Mandour *et al.*, 1995; Shinozaki *et al.*, 1987; Reece, 1981).

Keeping in view the structure-activity relationship of hydralazine and its effectiveness in combination with β-adrenergic antagonist, the technical know-how for the 'basic manufacture' of hydralazine in the laboratory was developed. To prepare new biologically active compounds the following methods were adopted. Hydralazine was allowed to react with corresponding aromatic aldehydes (**IIa-d**) to introduce an additional heterocyclic ring adjacent to phthalazine moiety as a component of biological importance. The present study deals with the synthesis, characterization and evaluation of antimicrobial activity of the new Schiff bases and their corresponding cyclized analogues.

Materials and Methods

Melting points were taken on Buchi-510 melting point apparatus and are uncorrected. The IR spectra were measured in KBr and $CHCl_3$ on a JASCO-A-302 spectrophotometer. The ^{1}HNMR spectra were recorded in $CDCl_3$ unless otherwise stated at 500 MHz on Bruker AM-300 ASPECT 3000 spectrophotometer. Mass spectra (MS) were determined using a Finnigan MAT-112 and MAT-312 spectrometer connected to PAD-11/34 and MAT-188 computer. The UV spectra were measured in MeOH on a JASCO Model 7800 UV/Vis spectrophotometer. Field desorption (FD) measurements were also performed on MAT-312 spectrometer.

Synthesis of hydralazine. Several methods have been reported in literature, for the manufacture of hydralazine. How-

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ever, we have synthesized it in the laboratory, starting from naphthalene to yield 1-hydroxyphthlazine via phthaldehydic acid. It was then transformed into hydralazine by condensation with hydralazine hydrate.

General procedure for the preparation of arylidenes (Schiff bases) (IIIa-d) from 1-hydrazinophthalazine (I). 1-Hydrazinophthalazine (**I**, 0.01 mol) was refluxed on water bath with different aryl aldehydes (**IIa-d**, 0.01 mol) in the presence of acetic acid/acetic anhydride (anhydrous) in absolute ethanol (40 ml) for 4-6 h to afford crude **IIIa-d**, which on recrystallization from appropriate solvent yielded the corresponding arylidenes (**IIIa-d**, scheme-1).

1-[4-chlorobenzylidene]hydrazinophthalazine(III-a). Yellow crystalline solid obtained from EtOH/pet ether yielded 1.75 g; mp. 250-5 °C (decomp); IR: ν_{max} 3300 (NH), 1620, 1580 (N=C) cm^{-1}; UV: λ_{max} (ε) 204.6 (4.40), 262.2 (4.49), 370.8 (3.44) nm; Mass: (m/z, rel.int.), 282 (M^+,49), 280(18), 171(100 base peak), 172(11), 129(5), 115(10), 103(10), 89(19), 88(6); Peak matching: m/z 282.0333 ($C_{15}H_{12}ClN_4$, calc. 282.074); ^{1}HNMR (Table 1).

1-[benzylidene]hydrazinophthalazine(III-b). Yellow shinning crystals obtained yield 1.22 g; mp. 243-45 °C; IR: ν_{max} 3300 (NH), 1615, 1582 (N=C) cm^{-1}; UV: λ_{max} (ε) 208 (4.42), 260 (4.50); 366.4 (3.52) nm; Mass: (m/z, rel.int.), 248 (M^+44%), 247(30), 172(10), 171(100), 103(16), 51(9); F.D: m/z 248. Peak matching: m/z 248.1027 ($C_{15}H_{12}N_4$ calc. 248.1027); ^{1}HNMR (Table 1).

1-[4-nitrobenzylidene]hydrazinophthalazine(III-c). Orange crystalline solid crystallized from acetic acid yield 1.02 g; mp. 265-8 °C; IR: ν_{max} 3300 (NH)1610, 1585(N=C) cm^{-1}; UV: λ_{max} (ε) 212 (4.22), 282 (4.07), 408.8 nm; Mass: (m/z, rel.int.), 293 (M^+, 27), 290(19), 246(5), 171(100), 172(12), 129(11), 103(39), 89(26), 76(15), 63(13); F.D: m/z 293$^+$. Peak matching m/z 293.1012 ($C_{15}H_{11}N_5O_2$ calc. 293.091); ^{1}HNMR (Table 1).

1-[2-nitrobenzylidene]hydrazinophthalazine(III-d). Orange crystals from EtOH yield 1.7 g; mp. 230-35 °C (decomp); IR: ν_{max} 3300 (NH),1618 (aromatic), 1580 (N=C), cm^{-1}; UV: λ_{max} (ε) 216 (4.23), 280 (4.16), nm; Mass: (m/z, rel.int.), 293 (M^+35), 245(17), 219(10), 161(30), 130(37), 103(100), 89(23), 176(27), 63(14), 51(12); F.D: 293 m/z; Peak matching m/z 293.1012 ($C_{15}H_{11}N_5O_2$ calc. 293.041); ^{1}HNMR (Table 1).

3-[4-chlorophenyl]-s-triazolo[3-4-a]phthalazine(IV-a). White crystals from EtOH yield 1.55 g; IR: ν_{max} 1680 (aromatic), 1560 (N = C); UV: λ_{max} (ε) 204 (4.42), 260 (4.50), nm; Mass: (m/z, rel.int.), 280 (M^+100), 152(5), 140(6), 137(5), 116(5), 115(51), 111(27), 102(11), 89(14.5), 88(45), 87(11), 76(7), 75(13), 65(6), 64(9), 63(17), 62(13), 51(13); Peak matching: m/z 280.1015 ($C_{15}H_9ClN_4$ calc. 280.711); ^{1}HNMR (Table 1).

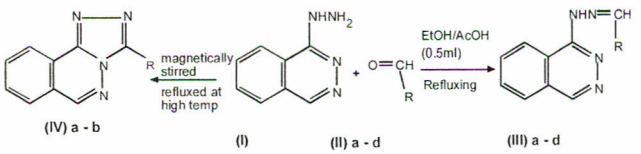
3-phenyl-s-triazolo[3-4-a]phthalazine (IV-b). White shinning needles from EtOH yield 1.22 g; mp. 215-217 °C; IR: ν_{max} 1620 (aromatic), 1560 (N = C) cm^{-1}; UV: λ_{max} (ε) 204 (4.40), 262 (4.49), Mass; (m/z, rel.int.), 246 (M^+100), 245(71), 218(2), 189(4), 128.7(5), 114.7(51), 102(17), 90(2), 76(12), 61.8(29). Peak matching: m/z 246.1016 ($C_{15}H_{10}N_4$ calc. 246.266); ^{1}HNMR (Table 1).

Evaluation of antibacterial activities. Compounds (**IIIa-d**) were tested for their antibacterial activity against gram–ve and gram +ve bacteria namely *Escherichia coli, Salmonella typhi, Salmonella para A, Shigella flexneri, Sh.sonnei, Sh.dysenteriae, Pseudomonas* sp., *Aeromonas* sp., *Klebsiella pneumoniae, Bordetella branchiseptica* (gram-ve); *Lactobacillus plantarum, Bacillus pumilus, Staphylococcus aureus* (gram+ve). The cultures of bacteria grown overnight at 37 °C were used for testing antibacterial activity. The assay was carried out by overlay agar method (Mandour *et al.*, 1995).

The compounds were dissolved in DMF and blank experiments with pure DMF were also performed. Two antibiotics Ampiclox and Oxytetracycline were used for comparison. Results are reported as zone of inhibition after 24 to 48 h of growth at 37 °C (Table 2).

Results and Discussion

The reaction of 1-hydrazinophthalazine (**I**) with carbonyl compounds has been reported in literature for the formation of open chain hydrazones (Schiff bases) alongwith *s*-triazoles, but the reaction conditions favouring the formation of one over the other were not well documented. In the present work we have studied the formation of benzylidenes (Schiff bases) under different experimental conditions. The reaction when performed under mild heating and in presence of some activated reagents such as acetic acid and anhydrous acetic anhy-



Scheme 1

dride, Schiff bases were formed as major products alongwith some *s*-triazoles. Under these conditions (**I**) with *p*-chlorobenzaldehyde (**IIa**) and benzaldehyde (**IIb**) yielded Schiff bases (**IIIa-b**) alongwith the corresponding *s*-triazoles (**IVa-b**) while O-8*p*-benzaldehyde (**IIc-d**) resulted only in (**IIIc**) and (**IIId**). The compounds have been characterized by IR, UV, EIMS, FD, HREIMS and ^{1}HNMR spectroscopy.

These Schiff bases (**IIIa-d**) showed the characteristic –NH band between 3200-3300 cm^{-1} in IR spectra, absorption at λ_{max} 204.6-292 nm and λ_{max} 366-408.8 nm in UV spectra. The former were atributable to a hydrazone moiety while the latter to a phthalazine ring.

The EIMS spectra of (**IIIa-d**) showed the corresponding molecular ion peaks at m/z 282, 248 and 293, respectively

Table 1. ^{1}HNMR data of (**IIIa-d**) and (**IVa-b**)

Compound	NH-protons (δ)	Azomethine protons (δ)	Methine protons of ring B (δ)	Aromatic protons of ring A (δ)	Aromatic protons of ring C (δ)
III-a	10.59 (s,1H)	8.48 (s,1H)	7.87 (s, 1H)	7.67-7.53 (m, 4H)	7.73 (dd, 2H, *J* = 8.5, 1.95 Hz) 7.39 (d, 2H, *J* = 8.5 Hz)
III-b	10.81 (s,1H)	8.72 (bs,1H)	8.72 (bs,1H)	7.58-7.41 (m, 4H)	7.82-7.72 (m, 5H)
III-c	10.20 (s,1H)	8.78 (s,1H)	8.76 (s, 1H)	8.00-7.7 (m, 4H)	8.78 (d, 2H, *J* = 9.1Hz) 8.41 (d, 2H, *J* = 9.1Hz)
III-d	10.61 (s,1H)	8.99 (bs,1H)	8.5 (s, 1H)	7.75-7.50 (m, 4H)	8.27 (dd, 1H, *J* = 8.2, 1.25Hz) 8.20 (d, 1H, *J* = 7.6Hz) 7.95 (dd, 1H, *J* = 8.2, 1.2Hz) 7.84 (d, 1H, *J* = 7.70, Hz)
IV-a			9.17 (s, 1H)	7.6 (dd, 1H, *J* = 8.7, 2.05) 7.57 (dd, 2H, *J* = 8.55, 1.95) 8.07 (t, 1H, *J* = 7.85, 1.70Hz) 7.94 (t, 1H, *J* = 7.85, 1.70Hz)	7.57 (dd, 2H, *J* = 8.6, 1.95) 7.60 (dd, 2H, *J* = 8.7, 1.90)
IV-b			9.08	8.30 (dd, 1H, *J* = 8.2, 2.50 Hz) 8.21 (d, 1H, *J* = 7.9Hz) 8.05 (dd, 1H, *J* = 7.9, 1.10Hz) 7.55 (dd, 1H, *J* = 7.55, 1.15Hz)	7.59-7.55, 1m, 5H

Table 2. Antibacterial activity (zone of inhibition in mm)

Name of organism	**III-a**	**III-b**	**III-c**	**III-d**	Oxytetracycline	Ampiclox
Gram –ve						
Salmonella typhi	22	19	20	23	23.3	18.3
Salmonella para A	21	24	22	21	21.7	18.6
Shigella flexneri	23	21	23	25		
Shigella sonnei	24	22	20	22		
Shigella dysenteriae	23	20	20	21		
Pseudomonas sp.	22	21	17	20	13.6	20.8
Aeromonas sp.	23	22	20	22	13.3	18.0
Klebsiella pnemoniae	20	20	20	23	24.8	17.4
Escherichia coli	21	19	20	21	12.7	18.5
Bordetella branchiseptica	23	23	23	25		
Gram +ve						
Lactobacillus plantarum	-ve	15	13	11		
Bacillus punilus	-ve	13	-ve	-ve	22.6	18.1
Staphylococcus aureus	16	16	15	15	22.1	22.2

concentration = 10 mg/ml DMF; diameter of the well = 8.2 mm; -ve= no activity

for (**IIIa-d**) which were confirmed by field desorption (FD) and high resolution electron impact mass spectroscopic (HREIMS) studies. The ^{1}HNMR spectra of all these Schiff bases revealed signal for an azomethine proton at δ 8.48-8.99 (Table 1), while peak of NH was obtained for (**IIIa-d**) as singlet at δ 10.20-10.81 which disappeared when shaken with D_2O.

The closed ring *s*-triazolo[3,4-a] phthalazines (**IVa-b**) in IR spectrum did not show absorption for NH group and in UV spectrum no absorption due to hydrazone group was observed. The ^{1}HNMR spectra of (**IVa-b**), also did not show signals for azomethine and NH protons. The EIMS spectra of both the compounds showed molecular ion peak M^+ at m/z 280 and 246, respectively, two mass units less than their open chain hydrazones, which was further confirmed by field desorption (FD) studies.

Antibacterial screening. The antibacterial activity test was performed by overlay agar method in dimethyl formamide and compared with known antibiotics (Oxytetracycline and Ampiclox). The compounds were tested against ten gram negative and three gram positive organisms (Table 2). The compounds did not show any remarkable activity against gram positive bacteria but showed more activity than the standard antibiotics against gram negative bacteria. The compound (**IIId**) was more active against *Shigella flexneri* and *Bordetella branchiseptica* as compared to (**IIIa-c**). All the four Schiff bases (**IIIa-d**) had less activity than the known antibiotics against gram + ve bacteria. Even for some gram +ve cultures no activity was observed.

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