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Synthesis and *in vitro* antimicrobial studies of some new 3-[phenyldiazenyl] benzaldehyde *N*-phenyl thiosemicarbazones

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Abstract
The increasing clinical importance of drug resistant microbial pathogens has lent additional urgency to microbiological research and new antimicrobial compound development. For this purpose, a new series of 3-[phenyldiazenyl] benzaldehyde *N*-phenylthiosemicarbazones were synthesized and evaluated for antifungal and antibacterial activity. The reaction of 2-hydroxy-5-[phenyldiazenyl] benzaldehyde **(I)** with *N*-phenylhydrazinecarbothioamide **(II)** were carried out in DMF. The antimicrobial activity of the synthesized target compounds **(III)** were evaluated by screening on different human pathogens using the disc diffusion assay. All the compounds exhibited considerable inhibition against the bacteria and fungi tested.

Keywords: *Thiosemicarbazones, antimicrobial activity*

Introduction

Infections caused by microorganisms pose a serious challenge to the medical community and there is a need for a reliably effective therapy for such infectious diseases that had previously caused extensive mortality, morbidity and fear. The resurgence of infections in industrialized countries as well as the appearance of multidrug resistant (MDR) strains of bacterial and fungal pathogens have prompted the quest for new drugs acting both as antibacterial and antifungal agents, without cross- resistance with known antimicrobials. Development of MDR [1,2] to existing drugs is a constant growing phenomenon that has concerned researchers world-wide, and now has reached an alarming level for certain infectious diseases lacking ready treatment regimens [3–5].

There are two basic approaches to develop a new drug for microbial infections: (i) Synthesis of analogues, modifications or derivatives of existing compounds for improving microbial treatment, (ii) Searching for novel structures, that the concerned organisms has never encountered, for the treatment of MDR microbial infections, which may act either by the same or a new mechanism [6].

To pursue this goal, our research efforts are directed to finding new chemical classes of antimicrobial agents. The methods of investigation enabled us to find some new pharmacophores of the above mentioned activities. Many studies have been conducted on compounds bearing —N═N-, -N-C═S, -CH═N- and electron withdrawing moieties as a pharmacophore [7–10]. Various substituted aromatic rings were utilised as a basis to constitute a large series of N-and S heteroatomic compounds. Special attention was paid to correlate their biological activity with their structure which proved that, activity is enhanced by electron withdrawing groups attached to aryl ring [11–16]. Furthermore, interest in the chemistry, synthesis and biology of these pharmacophores continues to be fuelled by their wide range of biological properties viz. antifungal, anticancer, antibacterial, antiviral, antiamoebic, antiproliferative, antitubercular, antitumor, anticonvulsant, antimalarial and trypanocidal activities [17–37].

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In view of conclusions drawn from our previous work [38–40]and considering the antimicrobial efficacy of —OCH_3, Cl and F groups attached to an aryl ring it seems logical and attractive to combine all these moieties together in a molecular framework. In the present work we have selected substituted amines containing these substituents for the synthesis of azosalicylaldehydes(**I**) and thiosemicarbazides(**II**) and finally synthesized some new 3-[phenyldiazenyl] benzaldehyde *N*-phenylthiosemicarbazones(**III**) by condensing (**I**) & (**II**). which were evaluated for their antimicrobial efficacy.

Experimental

Chemistry

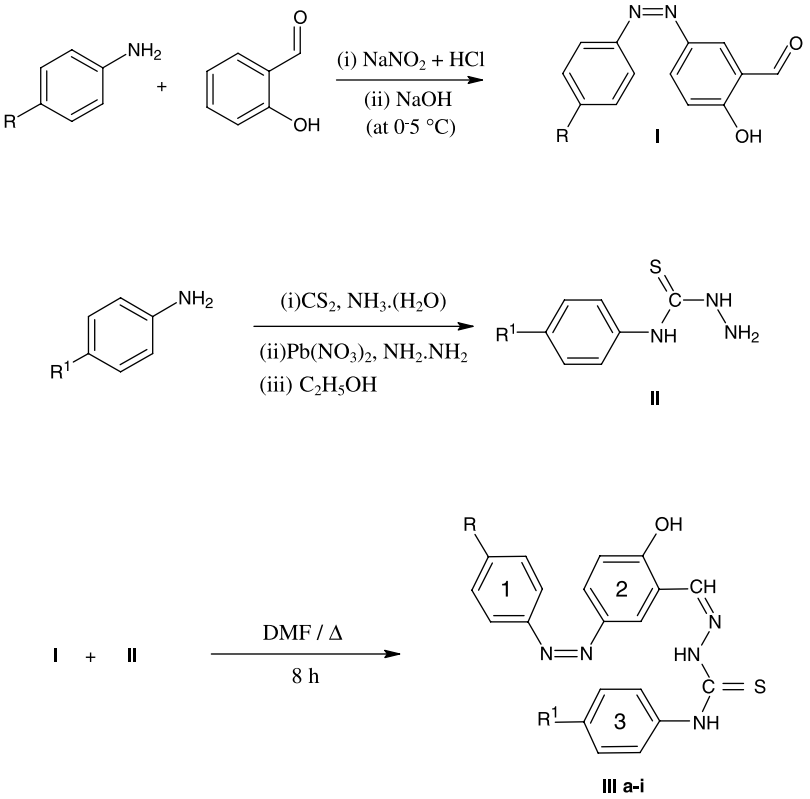
All melting points were determined in open capillaries and are uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel 60G (Merck) with acetone/-hexane (1:3). Spectroscopic data were recorded using the following instruments. IR: Perkin Elmer RxI (FT IR) spectrophotometer;^{1}NMR: Bruker DRX 300 (300MHz, FT NMR); MS –FAB: Jeol –SX 102 Mass spectrometer.

The synthetic route to the required compounds is outlined in Scheme 1. For the synthesis of the titled compounds, 2-hydroxy-5-[phenyldiazenyl] benzaldehyde **(I)** required as a starting material was prepared by the diazotisation and coupling method. *N*-phenylhydrazinecarbothioamide **(II)** was prepared by reacting *p*-substituted phenyl isothiocyanate with hydrazine hydrate in the presence of ethanol. The reaction of equimolar quantities of **(I)** with **(II)** in the presence of DMF resulted in the formation of the titled compounds **(III a-i)** [Table I].

General procedure for synthesis of compounds

2-hydroxy-5-[phenyldiazenyl] benzaldehyde (**I**). Aniline (3.72mL) was dissolved in aqueous hydrochloric acid (28mL,6N) and mechanically stirred at 0–5°C. A cold solution of sodium nitrite (5gm/10 mL water) was added dropwise to the constantly stirred reaction mixture. The diazotized solution was immediately added in small portions to salicylaldehyde (5mL dissolved in 40mL 6N NaOH), with constant stirring at 0–5°C. The stirring was continued for 4h. The solid obtained was filtered under suction washed with cold water and recrystallised from glacial acetic acid.

N-*phenylhydrazinecarbothioamide* (**II**). Carbon disulphide(12mL) was added dropwise to a mixture of an ethanolic(20mL) solution of aniline(10 mL) and liquor ammonia (15 mL) and stirred at 10–15°C for 2h. The reaction mixture was transferred to another flask containing lead nitrate (75gm in 200mL distilled water) and stirred further until the precipitation of lead sulphide was complete. The reaction mixture was



Scheme 1. The general synthesis reactions.

Table I. Some characteristics of the compounds.

Comp.	R	R′	M.P.(°C)	Yield (%)	Mol. Formula	Mol. Wt.
IIIa	F	H	121	58	$C_{20}H_{16}N_5OSF$	463
IIIb	F	F	210	55	$C_{20}H_{15}N_5OSF_2$	481
IIIc	F	Cl	171	56	$C_{20}H_{15}N_5OSClF$	497.5
IIId	Cl	H	185	59	$C_{20}H_{16}N_5OSCl$	479.5
IIIe	Cl	F	135	51	$C_{20}H_{15}N_5OSClF$	497.5
IIIf	Cl	Cl	131	45	$C_{20}H_{15}N_5OSCl_2$	514
IIIg	OCH_3	H	145	60	$C_{21}H_{19}N_5O_2S$	475
IIIh	OCH_3	F	149	52	$C_{21}H_{18}N_5O_2SF$	493
IIIi	OCH_3	Cl	137	58	$C_{21}H_{18}N_5O_2SCl$	509.5

steam distilled and isothiocyanatobenzene was collected, dissolved in ethanol (10mL) and refluxed with an ethanolic solution of hydrazine hydrate(25%, 0.6mL in 10 mL ethanol) for 5h. The product obtained was collected by filtration, washed with cold water and recrystallised from ethanol.

5-[(4- chlorophenyl) diazenyl]- 2- hydroxybenzaldehyde N *-(4- methylphenyl) thiosemicarbazones* (**III a-i**). A mixture of the appropriate, 2-hydroxy-5-[phenyldiazenyl] benzaldehyde **(I)**(2.5 gm) and the *N*-phenylhydrazinecarbothioamide **(II)** (1.5 gm) were refluxed for 8h in DMF (30 mL). The mixture was then cooled in an ice bath and the product separated was repeatedly washed with water followed by ethanol and recrystallised from diethyl ether.

III a-i. IR (KBr)ν max (cm^{-1}): 3460-3215(-OH & -NH), 1670-1655 (-CH═N), 1610-1595(-N═N-), 1270-1240(-C═S)

III a. ^{1}H NMR (DMSO-d_6) δ (ppm): 6.59(1H,s,-OH), 9.2(1H,s,-CH═N),9.60 (1H,s,═N-NH), 7.05-7.50 (12H,m,ArH), 8.41(1H,s,-NH-Ar). MS (FAB): *m/z* 389.

IIId. ^{1}HNMR (DMSO-d_6) δ (ppm):6.54 (1H,s,-OH), 8.9 (1H,s,-CH═N), 9.58(1H,s,═N-NH), 7.45-7.66 (12H,m,ArH), 8.20(1H,s,-NH-Ar).MS(FAB): *m/z* 305,[M + 2]:m/z 307.

III g. ^{1}H NMR (DMSO-d_6) δ (ppm):6.49(1H,s,-OH)9.02(1H,s,-CH═N), 9.54(1H, s,═N-NH), 6.95-7.40 (12H,m,Ar H),8.3(1H,s,-NH-Ar) 3.5(3H,s,-OCH_3).MS(FAB): *m/z* 301.

Microbiology

All the compounds were screened for their *in vitro* antimicrobial activity at the Birla institute of Medical Research and College of Life Sciences, Gwalior, against 24h old cultures of bacterial and fungal pathogens. Antimicrobial activity was determined against *Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Shigella flexinerri and Klebisiella pneumoniae* bacterial strains and *Candida albicans, Aspergillus fumigatus, Candida neoformans, Aspergillus niger and Penicillium ittalecum* fungal strains using the Disc Diffusion assay. For this, a sterile filter paper disc (6mm) impregnated with fixed doses (1000 μg/mL) and (800 μg/mL) of the synthesized compounds under investigation were placed upon the seeded petri dishes. Similar discs were prepared for the standard drugs, chloramphenicol, fluconazole and the solvent control, dimethyl formamide. The plates were incubated for 24h at 37°C for the bacterial strains and 48 h at 37°C for the fungal strains. The zone of inhibition, observed around the disc after incubation was measured. The results are presented in Table II.

Results and discussion

The structure of the nine new compounds synthesized were elucidated by spectral data. In the IR spectra, some significant stretching bands due to —OH and -NH, -N═N- and —C═S were observed at 3460-3215, 1610-1595and 1270-1240 cm^{-1} respectively. The specific band for thiosemicarbazone (-CH═N-) was observed at 1670-1655 cm^{-1}. In the ^{1}NMR spectra, the signal due to —CH═N protons, present in all compounds, appeared at 8.9-9.2 ppm as a singlet. The, ═N-NH and NH-Ar, —OH protons were observed at 9.54-9.60, 8.20-8.41 and 6.49-6.59 ppm as a singlet, respectively. All the aromatic protons were observed in the expected regions. Mass spectra[MS(FAB)] of compounds showed a (M + 1) and (M + 2) peaks, in agreement with their molecular formula.

The antimicrobial activities of the synthesized compounds were screened *in vitro* using the Disc Diffusion technique against different human pathogens at 1000 μg/mL and 800 μg/mL. All of the compounds tested, showed moderate activity (Table II). The major findings gathered from the antimicrobial study of the compounds are highlighted as follows:

i) a *p*-Chloro group of ring 1 and/or 3, in the new thiosemicarbazones enhanced the antimicrobial profile.
ii) a *p*- methoxy substituent in aryl ring 1 showed poor antimicrobial activity.

Table II. *In vitro* antimicrobial activity of compounds **III a-i**.

Comp.	Inhibition zone diameter [a] (mm)					Inhibition zone diameter [b] (mm)				
	S.aureus	*E. coli*	*S. typhimurium*	*S. flaxinerri*	*K. pneumoniae*	*C. albicanes*	*A.fumigatus*	*C. neoformans*	*A. niger*	*P. ittalecium*
IIIa	05	02	08	09	07	02	08	08	05	09
IIIb	06	08	11	11	12	04	06	10	06	09
IIIc	25	26	33	30	32	18	22	20	18	20
IIId	22	20	24	25	26	09	10	15	05	08
IIIe	24	24	31	32	33	12	20	19	19	21
IIIf	22	28	38	32	34	23	29	24	19	23
IIIg	05	09	10	12	11	03	07	10	05	10
IIIh	20	19	22	22	23	10	12	16	08	07
IIIi	21	27	37	30	34	20	27	23	20	19
Chloramphenicol	52	42	44	52	50	–	–	–	–	–
Fluconazole	–	–	–	–	–	25	30	26	32	24

[a]1000 μg/mL; [b]800 μg/mL

iii) Substitution of *p*-chloro and *p*-methoxy moieties of aryl rings 1 & 3 by a *p*-fluoro group markedly reduced antimicrobial activity.
iv) The incorporation of halogen moieties in an unsubstituted ring gradually increased activity.

We concluded from our investigation that **IIIf** and **IIIi** may be considered promising for the development of new antimicrobial agents.

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