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Research Article

Design, synthesis and *in- vitro* antimicrobial screening of some biorelevant thiosemicarbazones

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ABSTRACT

Thiosemicarbazones exhibit various biological activities and have attracted considerable pharmaceutical interest. They have been evaluated over the last 50 years as antibacterial, antifungal, antiviral, antitubercular, antitumor and anticancer therapeutics. Studies have been conducted on compounds bearing –N=N-, -N-C=S, and -CH=N- as pharmacophore. 5-[(3-Substitutedphenyl)diazenyl]-2-hydroxybenzaldehydeN-(3-Substitutedphenyl)thiosemicarbazones(**3a-l**) has been Synthesized by the condensation of 2-hydroxy 5-(substituted phenyl azo)benzaldehydes (**1**) and thiosemicarbazides (**2**). Purity of compounds were ascertained by Column chromatography and the structures were assigned on the basis of elemental analysis and spectral analysis. The synthesized compounds were screened for antimicrobial activities using Kirby–Bauer disc diffusion technique against two bacterial pathogens viz *Bacillus anthresis* (gram positive), *Escherichia coli* (gram negative) & two fungal pathogens *Candida albicanes*, *Aspergillus niger*. Chloramphenicol and fluconazole were used as standard drug respectively. The compounds exhibit moderate activity.

Key words: Antibacterial activity, Antifungal activity Thiosemicarbazide, Thiosemicarbazones

INTRODUCTION

Thiosemicarbazone is emerging moiety with wide spectrum of biological activity and having sound scope in research and developing process in pharmaceutical and medicinal chemistry. Since 1946, thiosemicarbazone derivatives have been studied for their biological properties[1]. Presently, the areas in which thiosemicarbazones are receiving more attention can be broadly classified according to their activity as antiparasitic[2-4], antibacterial[5,6] and antitumoral agents[7]. In 1950, Hamre D et al.[8] found that thiosemicarbazone derived from several benzaldehydes were active against neurovaccinial infection in mice. As anticancer agents, it is believed that their mechanism of action is through the inhibition of ribonucleotide reductase[9]. Thiosemicarbazones were reported to be associated with antimicrobial activity[10]. Several semicarbazones, as well as their sulfur analogues and its derivatives, have proved their efficiency and efficacy in combating various diseases[11]. Thiosemicarbazones are of considerable interest because of their chemistry and potentially beneficial biological activities, such as antitumor[12], antibacterial[13], antiviral[14] and antimalarial activities[15]. For many years, thiosemicarbazones and their metal complexes have been the subject of most structural and medicinal studies due to their potential biological value[16]. Thiosemicarbazones have drawn great interests especially for their antitumor activities[17,18]. Thiosemicarbazones possess a wide range of biological activity depending on the parent aldehyde or ketone[19]. Heterocyclic thiosemicarbazones are important because of their possible beneficial biological activity[20]. Thiosemicarbazones exhibit various biological activities such as antituberculosis[21], antimicrobial[22], anti-inflammatory[23], anticonvulsant[24], antihypertensive[25], local anesthetic[26], anticancer[27,28], hypoglycemic[29], and cytotoxic activities and also antioxidant agents[30,31]. In view of these facts and as a part of our extensive research program to rapidly assemble novel bioactive compounds under mild conditions[32-34], the present work was intended for the synthesis of a novel series of thiosemicarbazone derivatives bearing a azo moiety and a semicarbazide moiety in this compound. Some of the synthesized derivatives were screened to probe their potential antimicrobial activity against some selected bacteria and fungi species.

MATERIALS AND METHODS

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}H-NMR and ^{13}C-NMR: Bruker DRX 300 (300MHz, FT-NMR) and Varian (300MHz); MS – FAB: Jeol –SX 102 Mass spectrometer. The synthetic route is shown in **Scheme 1**. For the synthesis of the titled compounds, 2-hydroxy-5-[phenyldiazenyl] benzaldehyde (**1**) required as a starting material was prepared by the diazotisation and coupling reaction. N-phenyl hydrazine carbothioamide (**2**) was prepared by reacting phenyl isothiocyanate with hydrazine hydrate in the presence of ethyl alchohal. The reaction of equimolar quantities of (**1**) with (**2**) were refluxed in the dimethylformamide for 8hrs.

General procedure for synthesis of compounds

(1) 2-hydroxy-5-[phenyldiazenyll benzaldehyde: Aniline (3.72mL) was dissolved in aqueous hydrochloric acid (28mL, 6N) and stirred at 0–5^{o}C. A cold solution of sodium nitrite (5gm/10mL water) was added drop wise into the 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^{o}C. The stirring was continued for 4h. The solid obtained was filtered under suction and washed with cold water and recrystallised from glacial acetic acid.

(2) N-phenylhydrazine carbothioamide: Carbon disulphide(12mL) was added drop wise into the mixture of ethanolic (20mL) solution of aniline(10 mL) and liquid ammonia (25mL) stirred at 10–15^{0}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 steam distilled and isothiocyanatobenzene was collected dissolved in ethanol (10mL) and refluxed with an ethanolic solution of hydrazine hydrate(25%, 0.6mL in 10mL ethanol) for 5h. The product obtained was collected by filtration, washed with cold water.

5-[(3-Substitutedphenyl) diazenyl]- 2-hydroxybenzaldehyde N-(3- Substitutedphenyl) thiosemicarbazones (3a-l). A mixture of the appropriate, 2-hydroxy-5-[phenyldiazenyl] benzaldehyde (**1**) (2.5 gm) and the N-phenyl hydrazine carbothioamide (**2**) (1.5 gm) were refluxed for 8h in DMF (20 mL). The mixture was cooled and poured in ice water and the product was obtained and recrystallised with diethyl ether.

(2*E*)-2-{2-hydroxy-5-[(*Z*)-phenyldiazenyl]benzylidene}-*N* phenyl -hydrazinecarbothioamide (3a) Yield-71%, M.P.-187^{o}C, IR(KBr, ν_{max} cm^{-1}): 1655 (-CH=N), 1595 (-N=N-), 1240 (-C=S); ^{1}HNMR=(300MHz, DMSO) δ =2.5(1H,s,NH), 4.4(1H,s,NH), 5.5(1H,s,OH), 6.56-7.4(5H,m,Ar-H), 7.5-8.0(5H,m,Ar-H), 7.8-8.2(3H,m,Ar-H), 8.5(1H,s,CH) ppm; ^{13}C NMR: δ 186.5, 160.5, 154.9, 152.9, 145.6, 139.9, 131.0, 129.2, 126.9, 125.9, 124.9, 123.2, 119.2, 116.9; MS(EI) m/z=375.12[M]$^{+}$, Anal. calcd. For $C_{20}H_{17}N_5OS$: C,63.98%; H, 4.56%; N, 18.65%; Found: C, 63.91%; H, 4.52%; N, 18.56%.

(2*E*)-2-{2-hydroxy-5-[(*Z*)-phenyldiazenyl]benzylidene}-*N*-(3-methylphenyl)hydrazinecarbothioamide (3b)
Yield-62%, M.P.-216^{o}C, IR(KBr, ν_{max} cm^{-1}): 1660 (-CH=N), 1599 (-N=N-), 1256 (-C=S); ^{1}HNMR=(300MHz,DMSO) δ = 2.3(1H,s,NH), 2.45(3H,s,CH_3), 4.2(1H,s,NH), 5.3(1H,s,OH), 6.29-6.91(4H,m,Ar-H), 7.56-7.93(5H,m,Ar-H), 7.7-8.4(3H,m,Ar-H), 8.43(1H,s,CH)ppm; ^{13}C NMR: δ 186.3, 161.0, 155.0, 152.8, 146.2, 139.4, 138.3, 130.8, 128.9, 126.6, 126.0, 125.7, 124.3, 123.2, 118.9, 116.7, 21.2; MS(EI) m/z=389.13[M]$^{+}$, Anal. calcd. for $C_{21}H_{19}N_5OS$: C, 64.76%; H, 4.92%; N, 17.98%; Found: C, 64.52%; H, 4.62%; N, 18.06%.

(2*E*)-2-{2-hydroxy-5-[(*Z*)-phenyldiazenyl]benzylidene}-*N*-(3-methoxyphenyl)hydrazinecarbothioamide (3c)
Yield-65%, M.P.-239^{o}C, IR(KBr, ν_{max} cm^{-1}): 1659 (-CH=N), 1610 (-N=N-), 1249 (-C=S); ^{1}HNMR=(300MHz,DMSO) δ = 2.6(1H,s,NH), 3.8(3H,s,OCH_3), 4.30(1H,s,NH), 5.45(1H,s,OH), 6.01-7.0(4H,m,Ar-H), 7.57-8.06(5H,m,Ar-H), 8.3-8.25(3H,m,Ar-H), 8.65(1H,s,CH)ppm; ^{13}C NMR: δ 186.1, 162.5, 161.2, 155.2, 153.1, 145.8, 141.0, 131.3, 129.9, 128.9, 127.2, 125.2, 123.4, 119.2, 117.6, 111.9, 111.2, 57.0; MS(EI) m/z= 405.13[M]$^{+}$, Anal. calcd. For $C_{21}H_{19}N_5O_2S$: C,62.21%; H, 4.72%; N, 17.27%; Found: C, 62.51%; H, 4.42%; N, 17.36%.

(2*E*)-*N*-(3-chlorophenyl)-2-{2-hydroxy-5-[(*Z*)-phenyl -diazenyl]benzylidene}hydrazinecarbothioamide (3d)
Yield-58%, M.P.-215^{o}C, IR(KBr, ν_{max} cm^{-1}): 1662 (-CH=N), 1615 (-N=N-), 1252 (-C=S); ^{1}HNMR=(300MHz,DMSO) δ = 2.23(1H,s,NH), 4.54(1H,s,NH), 5.6(1H,s,OH), 6.36-6.99(4H,m,Ar-H), 7.0-8.2(3H,m,Ar-H), 8.33-8.5(5H,m,Ar-H), 8.4(1H,s,CH)ppm; ^{13}C NMR: δ 187.1, 165.0, 155.0, 152.3, 146.3, 141.6, 135.2, 131.7, 131.2, 130.1, 127.1, 125.9, 124.9, 126.7, 123.7, 120.7, 117.8; MS(EI) m/z= 409.08[M]$^{+}$, Anal. calcd. For $C_{20}H_{16}N_5OSCl$: C,58.60%; H, 3.93%; N, 17.09%; Found: C, 58.87%; H, 4.02%; N, 17.15%.

(2*E*)-2-{2-hydroxy-5-[(*Z*)-(2-nitrophenyl) diazenyl] benzylidene}-*N*-phenylhydrazine carbothioamide (3e)
Yield-62%, M.P.-185^{o}C, IR(KBr, ν_{max} cm^{-1}): 1656 (-CH=N), 1621 (-N=N-), 1254 (-C=S); ^{1}HNMR=(300MHz,DMSO) δ = 2.56(1H,s,NH), 4.67(1H,s,NH), 5.2(1H,s,OH), 6.53-7.05(5H,m,Ar-H), 7.2-8.03(3H,m,Ar-H), 7.8-8.49(4H,m,Ar-NH), 8.5(1H,s,CH)ppm; ^{13}C NMR: δ 187.3, 167.0, 157.2, 147.6, 145.2, 142.9, 140.3, 135.9, 132.6, 129.3, 127.2, 126.2, 125.9, 123.6, 119.2, 116.6; MS(EI) m/z= 420.10[M]$^{+}$, Anal. calcd. For $C_{20}H_{16}N_6O_3S$: C, 57.13%; H, 3.84%; N, 19.99%; Found: C, 57.20%; H, 4.02%; N, 20.06%.

(2*E*)-2-{2-hydroxy-5-[(*Z*)-(2-nitrophenyl)diazenyl] benzylidene}-*N*-(3-methylphenyl)hydrazine carbothioamide (3f)

Yield-64%, M.P.-195^{o}C, IR(KBr, ν_{max} cm^{-1}): 1673 (-CH=N), 1617 (-N=N-), 1262 (-C=S); ^{1}HNMR=(300MHz,DMSO)δ= 2.42(1H,s,NH), 2.43(3H,s,CH_3), 4.70(1H,s,NH), 5.25(1H,s,OH), 6.37-6.95(4H,m,Ar-H), 7.3-8.09(3H,m,Ar-H),7.8-8.39(4H,m,Ar-H), 8.5(1H,s,CH)ppm; ^{13}C NMR: δ 186.4, 170.3, 154.8, 148.0, 145.6,142.4, 141.3, 139.0,135.9, 132.5, 129.4, 126.5, 126.1, 125.9, 124.6, 123.9, 123.7, 122.6, 118.9, 117.1, 21.2; MS(EI) m/z=434.12[M]$^{+}$, Anal. calcd. For $C_{21}H_{18}N_6O_3S$: C, 58.05%; H, 4.18%; N, 19.34%; Found: C, 58.20%; H, 4.22%; N, 9.76%.

(2*E*)-2-{2-hydroxy-5-[(*Z*)-(2-nitrophenyl)diazenyl] benzylidene}-*N*-(3-methoxyphenyl)hydrazine carbothioamide (3g)

Yield-67%, M.P.-218^{o}C, IR(KBr, ν_{max} cm^{-1}): 1664 (-CH=N), 1609 (-N=N-), 1250 (-C=S); ^{1}HNMR=(300MHz,DMSO)δ= 2.49(1H,s,NH), 3.85(3H,s,OCH_3), 4.53(1H,s,NH), 5.19(1H,s,OH), 6.01-7.01(4H,m,Ar-H), 7.06-8.2(3H,m,Ar-H), 7.87-8.59(4H,m,Ar-H), 8.2(1H,s,CH)ppm; ^{13}C NMR: δ 186.3, 163.4, 161.2 155.4, 147.7, 145.3, 142.7, 141.2, 135.0, 132.6, 130.1, 127.3, 124.3, 123.9, 118.3, 117.8, 116.7, 111.2, 110.5, 57.2; MS(EI) m/z= 450.11[M]$^{+}$, Anal. calcd. For $C_{21}H_{18}N_6O_4S$: C, 55.99%; H, 4.03%; N, 18.66%; Found: C, 56.05%; H, 4.12%; N, 19.06%.

(2*E*)-*N*-(3-chlorophenyl)-2-{2-hydroxy-5-[(*Z*)-(2-nitrophenyl)diazenyl]benzylidene}hydrazinecarbothioamide (3h)

Yield-60%, M.P.-175^{o}C, IR(KBr, ν_{max} cm^{-1}): 1661 (-CH=N), 1601 (-N=N-), 1258 (-C=S); ^{1}HNMR=(300MHz,DMSO)δ= 2.69(1H,s,NH), 4.59(1H,s,NH), 5.23(1H,s,OH), 6.43-6.97(4H,m,Ar-H), 7.1-8.05(3H,m,Ar-H), 7.87-8.39(4H,m,Ar-H), 8.23(1H,s,CH)ppm; ^{13}C NMR: δ 187.3, 161.2, 155.7, 148.3, 146.2, 143.2, 141.2, 140.9, 135.5, 134.1, 132.6, 131.2, 127.3, 126.4, 125.2, 125.1, 124.0, 123.9, 118.8, 117.5; MS(EI) m/z= 454.06[M]$^{+}$, Anal. calcd. For $C_{20}H_{15}N_6O_3SCl$: C, 52.81%; H, 3.32%; N, 18.47%; Found: C, 53.05%; H, 3.52%; N, 18.76%.

(2*E*)-2-{2-hydroxy-5-[(*Z*)-(4-nitrophenyl)diazenyl] benzylidene}-*N*-phenylhydrazinecarbothioamide (3i)

Yield-72%, M.P.-170^{o}C, IR(KBr, ν_{max} cm^{-1}): 1666 (-CH=N), 1602 (-N=N-), 1247 (-C=S); ^{1}HNMR=(300MHz,DMSO)δ= 2.66(1H,s,NH), 4.67(1H,s,NH), 5.20(1H,s,OH), 6.50-7.09(5H,m,Ar-H), 7.22-8.24(3H,m,Ar-H), 8.26-8.38(4H,m,Ar-H), 8.17(1H,s,CH)ppm; ^{13}C NMR: δ 186.2, 161.1, 159.6, 155.3, 151.3, 145.3, 140.4, 130.5, 127.3, 126.3, 125.6, 125.1, 124.5, 124.0, 123.6; MS(EI) m/z= 420.10[M]$^{+}$, Anal. calcd. For $C_{20}H_{16}N_6O_3S$: C, 57.13%; H, 3.84%; N, 19.99%; Found: C, 56.05%; H, 3.62%; N, 19.46%.

(2*E*)-2-{2-hydroxy-5-[(*Z*)-(4-nitrophenyl)diazenyl] benzylidene}-*N*-(3-methylphenyl)hydrazine carbothioamide (3j)

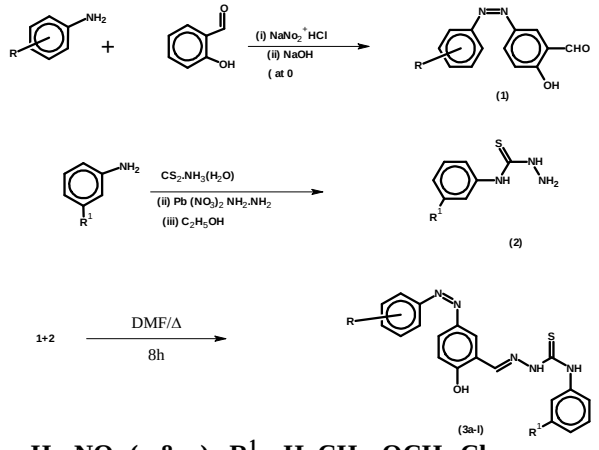
Yield-68%, M.P.-200^{o}C, IR(KBr, ν_{max} cm^{-1}): 1667 (-CH=N), 1599 (-N=N-), 1248 (-C=S); ^{1}HNMR=(300MHz,DMSO)δ= 2.36(1H,s,NH), 2.38(3H,s,CH_3), 4.37(1H,s,NH), 5.10(1H,s,OH), 6.36-6.92(4H,m,Ar-H), 7.05-8.3(3H,m,Ar-H), 8.28-8.64(4H,M,Ar-H), 8.06(1H,s,CH)ppm; ^{13}C NMR: δ 187.0, 161.3, 159.3, 155.3, 152.2, 147.3, 140.1, 138.3, 129.3, 127.3, 126.0, 125.9, 124.9, 124.1, 123.7, 123.2, 119.1, 117.2, 22.0; MS(EI) m/z= 434.12[M]$^{+}$, Anal. calcd. For $C_{21}H_{18}N_6O_3S$: C, 58.05%; H, 4.18%; N, 19.34%; Found: C, 57.85%; H, 4.02%; N, 19.16%.

(2*E*)-2-{2-hydroxy-5-[(*Z*)-(4-nitrophenyl)diazenyl] benzylidene}-*N*-(3-methoxyphenyl)hydrazine carbothioamide (3k)

Yield-70%, M.P.-230^{o}C, IR(KBr, ν_{max} cm^{-1}): 1663 (-CH=N), 1607 (-N=N-), 1265 (-C=S); ^{1}HNMR=(300MHz,DMSO)δ= 2.38(1H,s,NH), 3.87(3H,s,OCH_3), 4.57(1H,s,NH), 5.03(1H,s,OH), 6.03-7.05(4H,m,Ar-H), 7.03-8.3(3H,m,Ar-H), 8.32-8.55(4H,m,Ar-H), 8.16(1H,s,CH)ppm; ^{13}C NMR: δ 187.9, 163.1, 161.3, 159.0, 156.2, 152.2, 147.3, 141.4, 131.5, 127.0, 125.9, 124.3, 123.9, 119.5, 118.3, 116.9, 111.8, 110.9, 57.3; MS(EI) m/z= 450.11[M]$^{+}$, Anal. calcd. For $C_{21}H_{18}N_6O_4S$: C, 55.99%; H, 4.03%; N, 18.66%; Found: C, 56.15%; H, 4.13%; N, 18.86%.

(2*E*)-*N*-(3-chlorophenyl)-2-{2-hydroxy-5-[(*Z*)-(4-nitrophenyl)diazenyl]benzylidene}hydrazinecarbothioamide (3l)

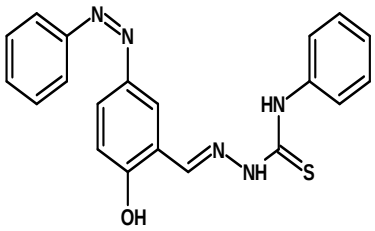
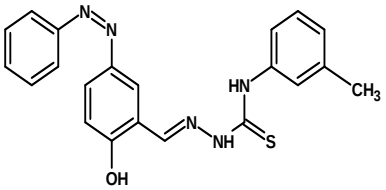
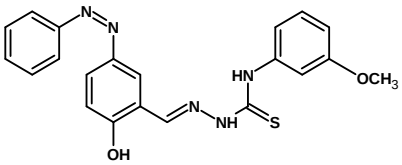
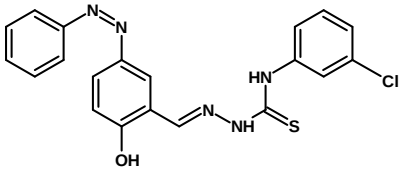
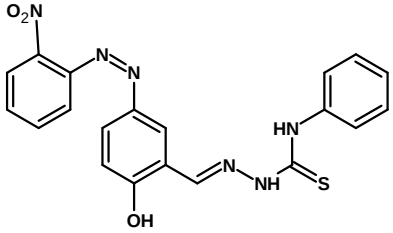
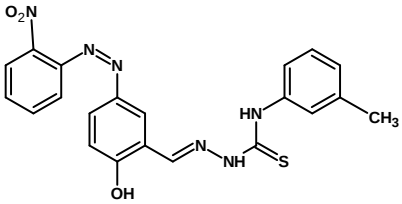
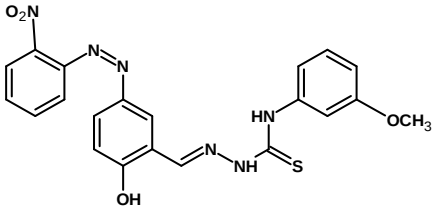
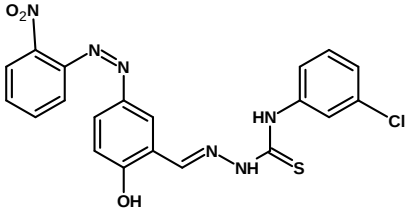
Yield-65%, M.P.-180^{o}C, IR(KBr, ν_{max} cm^{-1}): 1664 (-CH=N), 1593 (-N=N-), 1245 (-C=S); ^{1}HNMR=(300MHz,DMSO)δ= 2.18(1H,s,NH), 4.37(1H,s,NH), 5.09(1H,s,OH), 6.38-7.05(4H,m,Ar-H), 7.03-8.30(3H,m,Ar-H), 8.2-8.65(4H,m,Ar-H), 8.09(1H,s,CH)ppm; ^{13}C NMR: δ 187.3, 163.0, 159.6, 156.2, 152.1, 146.2, 142.0, 135.1, 132.1, 127.4, 126.0, 125.6, 125.1, 124.0, 123.6, 119.9, 118.3; MS(EI) m/z= 454.06[M]$^{+}$, Anal. calcd. For $C_{20}H_{15}N_6O_3SCl$: C, 52.81%; H, 3.32%; N, 18.47%; Found: C, 52.05%; H, 3.12%; N, 18.06%.



R = H, -NO_2 (*o* & *p*) , R^1 = H, CH_3 ,OCH_3, Cl

Scheme-1 synthesis of thiosemicarbazone derivatives

Table - I Physical characterization data of the compounds (3a-l)

Comp	Structure	M.P. ^{o}C	Yield (%)	Mole. Formula	Mole. Wt.
3a		187	71	$C_{20}H_{17}N_5OS$	375
3b		216	62	$C_{21}H_{19}N_5OS$	389
3c		239	65	$C_{21}H_{19}N_5O_2S$	405
3d		215	58	$C_{20}H_{16}N_5OSCl$	391
3e		185	62	$C_{20}H_{16}N_6O_3S$	418
3f		195	64	$C_{21}H_{18}N_6O_3S$	434
3g		218	67	$C_{21}H_{18}N_6O_4S$	440
3h		175	60	$C_{20}H_{15}N_6O_3SCl$	434

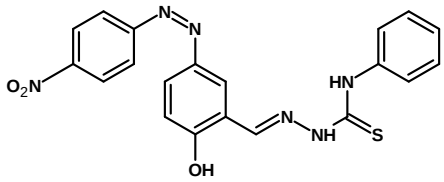
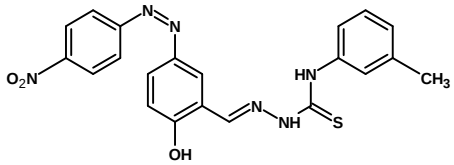
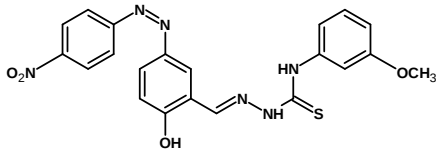
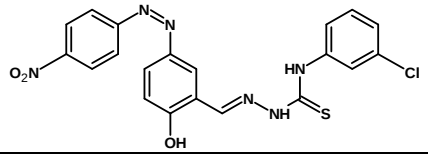
3i		170	72	$C_{20}H_{16}N_6O_3S$	418
3j		200	68	$C_{21}H_{18}N_6O_3S$	434
3k		230	70	$C_{21}H_{18}N_6O_4S$	440
3l		180	65	$C_{20}H_{15}N_6O_3SCl$	434

Table - II *in-vitro* antimicrobial activity of compounds

S.No.	Compound	Inhibition zone in diameter (mm)			
		Bacterial strains		Fungal Strains	
		E.coli	*B. anthrecis*	*C. albicanes*	*A. niger*
1	3a	12	14	14	17
2	3b	11	13	13	13
3	3c	18	16	15	14
4	3d	12	15	14	13
5	3e	13	16	12	17
6	3f	14	14	15	14
7	3g	14	21	17	18
8	3h	16	19	14	16
9	3i	13	15	16	14
10	3j	13	12	12	15
11	3k	15	16	19	17
12	3l	14	16	16	18
	Chloramphenicol	24	22	-	-
	Fluconazole	-	-	20	25

Antimicrobial studies

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, *Escherichia coli, Bacillus anthrecis* bacterial strains and *Candida albicans, Aspergillus niger* fungal strains using the disc diffusion assay[35]. For this, a sterile filter paper disc (6mm) impregnated with fixed doses 600µg/ml of the synthesized compounds under investigation were placed upon the seeded petridishes. Similar discs were prepared for the standard drugs, chloramphenicol, fluconazole and the solvent control, dimethyl formamide. The plates were incubated for 24h at 37.8^{o}C for the bacterial strains and 48 h at 37.8^{o}C for the fungal strains. The zone of inhibition, observed around the disc after incubation was measured. The results are presented in **Table II**.

Preparation of anti-microbial Suspension (1mg/mL)

The antimicrobial activities of the synthesized compounds were screened *in-vitro* using the disc diffusion technique against different human pathogens at 600µg/mL. All the compounds tested, showed moderate activity. (Dissolved

10 mg of each compound in 10 mL of dimethyl formamide to get 1 mg/mL concentration).

Preparation of Media

The media employed in microbiological studies were prepared by dissolving the required amount of individual components of the subjective media in distilled water and then autoclaved at 121^{o}C for 20 minutes. For solid medium, agar was added to the broth @1.6% prior to autoclaving. The fresh media plates were prepared by pouring lukewarm (40^{o}C) autoclaved agar medium in sterile petri-plates inside a laminar flow cabinet[36].

Interpretation

After incubation, the disc showing no visible growth were considered to be representing the MIC. The details of results are furnished in **Table-II**.

RESULTS AND DISCUSSION

The targeted thiosemicarbazone derivatives (**3a-l**) were obtained in excellent yields by refluxing 2-hydroxy-5-[phenyldiazenyl] benzaldehyde (**1**) with various substituted N-phenylhydrazine carbothioamide (**2**) in DMF for 8 h. The reaction pathway has been summarized in **Scheme-1**. Newly synthesized compounds (**3a-l**) were characterized by IR, NMR, Mass and C, H, N elemental analysis. Formation of 5-[(3-Substitutedphenyl) diazenyl]- 2-hydroxybenzaldehyde N-(3- Substituted phenyl) thiosemicarbazones (**3a-l**) were confirmed by recording their IR and ^{1}H NMR and ^{13}C NMR spectra. In the IR spectra, some significant stretching bands due to CH=N, N=N- and C=S were observed at 1670-1655, 1610-1595 and 1270-1240cm^{-1} respectively. The specific band for thiosemicarbazones (CH=N) was observed at 1670-1655 cm^{-1}. In the NMR spectra, the signal due to –CH=N protons, present in all compounds, appeared at 8.1- 8.7 ppm as a singlet. The, =N-NH and NH-Ar, -OH protons were observed at 2.2-27, 4.0-4.8 and 5.0-5.5 ppm as a singlet, respectively. All the aromatic protons were observed in the expected regions. ^{13}C NMR spectra recorded signals corresponding to azo and semicarbazide moiety and other aromatic corbons. The mass spectrum of **3a** showed molecular ion peak at m/z = 375.12(M)$^{+}$, which is in agreement with the molecular formula $C_{20}H_{17}N_5OS$. Similarly the spectral values for all the compounds and C, H, N analyses are given in the experimental part and characterization is provided in **Table-I.**

CONCLUTION

Novel substituted thiosemicarbazone derivatives were synthesized in reasonably good yields. They were characterized by Mass, ^{1}H-NMR, ^{13}C NMR, IR studies and elemental analyses. All the newly synthesized compounds were screened for their antimicrobial activity by MIC method. Among the screened samples, **3c** and **3h** have showed excellent antibacterial activity against *Escherichia coli* bacteria as compared to the standard drug Chloramphenicol. They also showed similar activity as that of standard drug against selected bacteria and fungi. Compounds **3g** and **3h** were shown to inhibit the growth of *Bacillus antresis* bacteria respectively. Although, a definite structure activity relationship could not be established with the limited experimental data and available compounds, it appears that with the incorporation of N=N and –NNH_2SCN in the resulting products **3** might have a positive influence, enhancing the antimicrobial activity of the designed compounds. The antifungal activities of compounds **3a**–**l** against *Candida albicans* and *Aspergillus niger* fungal strain. The results of the *in vivo* bioassay against are given in **Table-II**. Fluconazole was used as a reference antifungal drug. Compounds **3g** and **3k** were shown to inhibit the growth of *Candida albicans*, respectively; compounds **3a, 3e, 3g** and **3l** exhibited good activities on *Aspergillus niger* respectively. On the other hand, the remaining compounds showed moderately good activity against all of the tested bacterial and fungal strains compared to standard drugs, Chloramphenicol and Fluconazole. From the obtained results, here it is clear that the thiosemicarbazones play a major role for antimicrobial activity due to the aromatic rings which are bonded to N-C=S moiety.

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