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Synthesis, antibacterial, antifungal and anti-HIV activities of Schiff and Mannich bases derived from isatin derivatives and *N*-[4-(4′-chlorophenyl)thiazol-2-yl] thiosemicarbazide

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Abstract

Isatin, its 5-chloro and 5-bromo derivatives have been reacted with *N*-[4-(4′-chlorophenyl)thiazol-2-yl] thiosemicarbazide to form Schiff bases and the *N*-Mannich bases of these compounds were synthesized by reacting them with formaldehyde and three secondary amines. Their chemical structures have been confirmed by means of IR, ^{1}H-NMR data and by elemental analysis. Investigation of antimicrobial activity of compounds was done by agar dilution method against 28 pathogenic bacteria, 8 pathogenic fungi and anti-HIV activity against replication of HIV-1 (IIIB) in MT-4 cells. Among the compounds tested 1-[*N*,*N*-dimethylaminomethyl]-5-bromo isatin-3-{1′-[4″-(p-chlorophenyl) thiazol-2″-yl] thio semicarbazone} **10** showed the most favourable antimicrobial activity.

Keywords: Isatin; Thiazole; Thiosemicarbazone; Schiff bases; Mannich bases; Antimicrobial

1. Introduction

Isatin (Indolin-2,3-dione) derivatives are reported to show variety of biological activities like antibacterial (Daisley and Shah, 1984) antifungal (Piscopo et al., 1987) and anti-HIV (Pandeya et al., 1998) activities. Thiazoles have been reported to possess antibacterial (Agarwal et al., 1997), antifungal (Sup et al., 1995) and anti-HIV (Maass et al., 1993) activities. It is also reported that isatin-β-thiosemicarbazones have shown antimicrobial activity (Teitz et al., 1994). Further amino derivative of pyridine 2-carboxaldehyde thiosemicarbazone have been found to be shown antitumor activity (Liu et al., 1992). Recently cytotoxic activities of Mannich bases of chalcones (Dimmock et al., 1998) have been reported. In view of the antimicrobial property of the above pharmacophores, it was envisaged that the combined effect of all the entities will result in increased antimicrobial activity. Thus the present work to synthesize Schiff and Mannich bases of isatin derivatives and *N*-[4-(4′-chlorophenyl) thiazol-2-yl] thiosemicarbazide and screen for their antibacterial, antifungal activity by agar dilution method and anti-HIV activity against HIV-1 (III B) in MT-4 cells were accomplished.

2. Experimental

2.1. Chemistry

The melting points were determined by using Thomas-Hoover melting point apparatus and are uncorrected. Spectroscopic data were recorded on the following instruments IR, Jasco infrared spectrometer, Jeol FX 90Q FT-NMR spectrometer (90 MHz). Microanalyses were performed by the microanalytical unit Central Drug Research Institute, India.

2.1.1. Synthesis of 2-amino-4-(4′-chlorophenyl) thiazole

A mixture of 4-chloro acetophenone (0.1 mol), thiourea (0.2 mol) and iodine (0.1 mol) was heated on a steam bath

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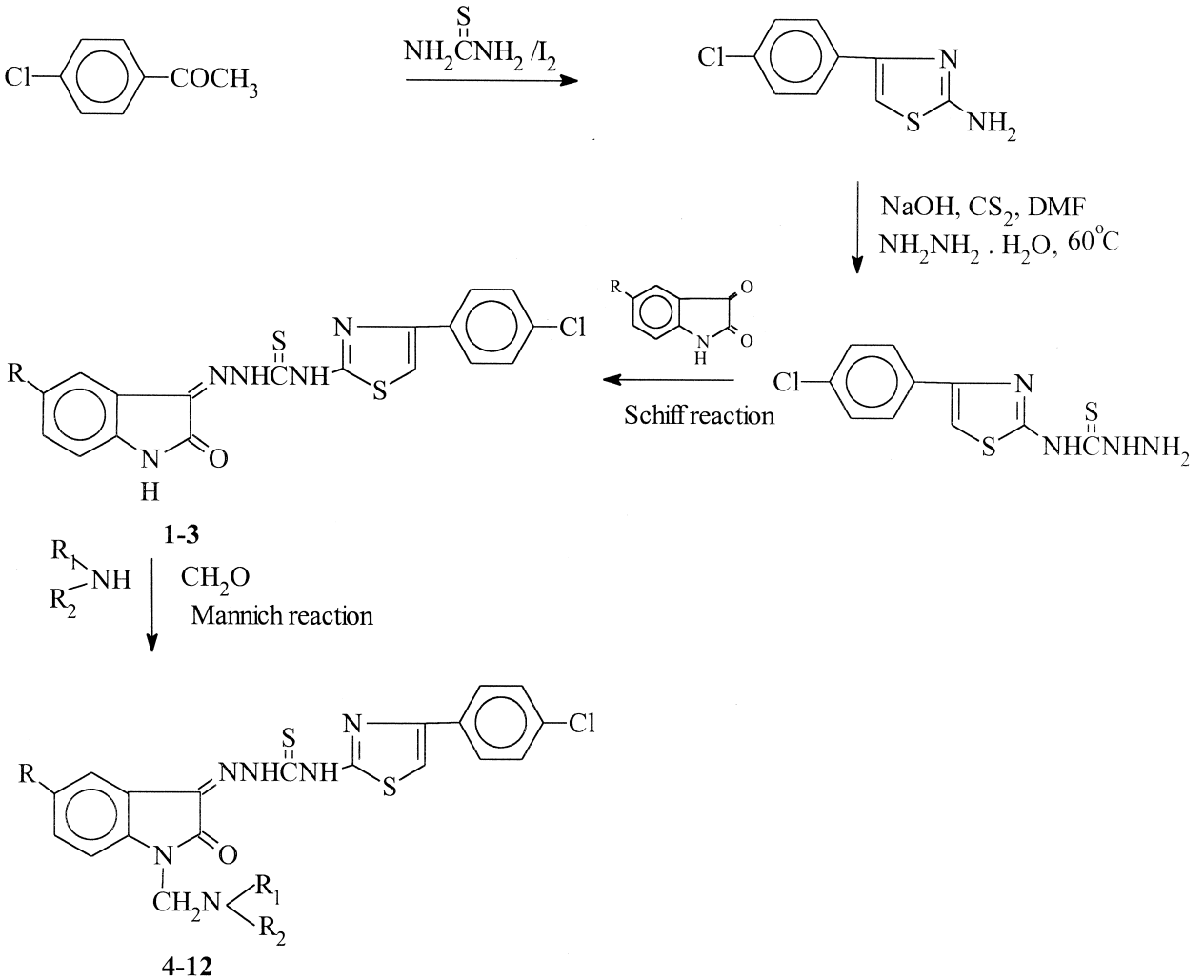
for 4 h. The hydroiodide, thus separated, was filtered, washed with ether and dried. It was dissolved in hot water, filtered while hot and the clear solution neutralized with a strong solution of ammonia. The solid separated was filtered, washed with water and recrystallized from benzene. Yield: 96%; m.p. 145°C; IR (KBr): 3320 cm^{-1} (NH_2), 1510, 1460, 1045 cm^{-1} (characteristic of thiazole nucleus); ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.35 (s, 2H, NH_2, D_2O exchangeable), 6.75 (s, 1H, H-5), 7.21–7.56 (m, 4H, Ar-H). Anal. ($C_9H_7N_2SCl$) C, H, N.

2.1.2. Synthesis of N-[4-(4′-chlorophenyl thiazol-2-yl] thiosemicarbazide

To a solution of 2-amino-4-(4′-chlorophenyl) thiazole (0.01 mol) in DMF (10 ml) was added sodium hydroxide (0.01 mol) and carbon disulphide (0.75 ml). The mixture was stirred at 15–20°C for 1 h, to the stirred mixture was added hydrazine hydrate (0.01 mol) and stirring continued at 60°C for 1 h more. On adding water, a pale yellow solid separated out which is recrystallized from DMF-ethanol afforded pale yellow crystals. Yield: 90%; m.p. 175°C; IR (KBr): 1020 cm^{-1} (C=S), 3100 cm^{-1} (NH), 3250 cm^{-1} (NH_2); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.5 (s, 2H, NH_2, D_2O exchangeable), 6.30 (s, 1H, H-5), 6.92–7.75 (m, 4H, Ar-H), 9.70 (s, 2H, 2×NH, D_2O exchangeable); Anal. ($C_{10}H_9N_45_2C1$) C, H, N.

*2.1.3. Synthesis of isatin-3-{1′-[4″-(p-chlorophenyl) thiazol-2″-yl] thiosemi carbazone} **(1)***

Equimolar quantities (0.02 mol) of isatin and *N*-[4-(4′-chlorophenyl) thiazol-2-yl] thiosemicarbazide were dissolved in warm ethanol containing 1 ml of glacial acetic acid. The reaction mixture was refluxed for 15 h and set aside. The resultant solid was washed with dilute ethanol dried and recrystallized from ethanol–chloroform mixture. Yield 94.6%; m.p. 125°C, IR (KBr): 1620 cm^{-1} (C=N), 1015 cm^{-1} (C=S); ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.2 (s, 1H, H-5′), 7.0–7.75 (m, 8H, Ar-H), 9.6 (s, 2H, 2×-NH-D_2O exchangeable), 10.4 (s, 1H, NH of isatin, D_2O exchangeable) Anal. ($C_{18}H_{12}ON_5S_2C1$) C, H, N.

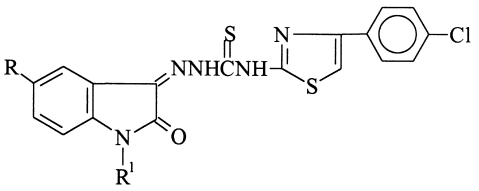


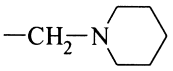
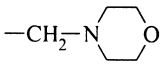
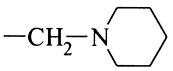
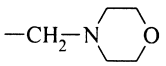
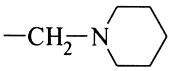
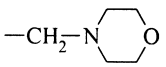
Scheme 1. Syntheses of the studied compounds.

*2.1.4. Synthesis of 1-(morpholino methyl) isatin-3-{l′-[4″-(p-chlorophenyl) thiazol-2″-yl] thiosemicarbazone} **(6)***

A slurry consisting of the S-1 (0.005 mol), tetrahydrofuran (5 ml) and 37% formalin (2 ml) was made. To this morpholine (0.005 mol) was added dropwise, with cooling and shaking. The reaction mixture was allowed to stand at room temperature for 1 h with occasional shaking after which it was warmed on a steam bath for 15 min. At the end of the period the contents were cooled and the product obtained was recrystallized from chloroform–petroleum ether. Yield 90.4%; m.p. 131°C; IR (KBr): 1615 cm^{-1} (C=N), 2850 cm^{-1} (CH_2); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.6 (t, 4H, $-CH_2NCH_2-$), 3.7 (t, 4H, $-CH_2OCH_2-$) 4.45 (s, 2H, CH_2), 6.3 (s, 1H, H-5), 6.9–7.8 (m, 7H, Ar-H), 9.45 (s, 2H, 2×-NH-, D_2O exchangeable); Anal. ($C_{23}H_{21}O_2N_6S_2C1$) C, H, N.

Table 1
Physical constants of the synthesized compounds



Code	R	R^1	Yield (%)	M.P. °C	Mol. formula	Elemental analysis calculated/found		
						% C	% H	% N
1	H	H	94.6	125	$C_{18}H_{12}ON_5S_2C1$	52.1	2.8	16.9
						52.3	2.9	17.0
2	Cl	H	86.7	208	$C_{18}H_{11}ON_5S_2C1_2$	48.1	2.4	15.6
						48.0	2.5	15.9
3	Br	H	91.2	128	$C_{18}H_{11}ON_5S_2ClBr$	43.8	2.2	14.2
						43.9	2.2	14.2
4	H-	CH_2-N(CH_3)$_2$	82.8	186	$C_{21}H_{19}ON_6S_2C1$	53.5	4.0	17.8
						53.7	4.2	17.6
5	H		91.3	122	$C_{24}H_{23}ON_6S_2C1$	56.3	4.5	16.4
						56.4	4.6	16.4
6	H		90.4	131	$C_{23}H_{21}O_2N_6S_2C1$	53.7	4.0	16.3
						53.8	4.1	16.4
7	Cl	-CH_2-N(CH_3)$_2$	89.0	268	$C_{21}H_{18}ON_6S_2C1_2$	49.8	3.5	16.6
						49.1	3.5	16.8
8	Cl		90.6	224	$C_{24}H_{22}ON_6S_2C1$	52.7	4.0	15.3
						52.8	4.1	15.4
9	Cl		90.4	220	$C_{23}H_{20}0_2N_6S_2C1_2$	50.4	3.6	15.3
						50.3	3.7	15.2
10	Br	-CH_2-N(CH_3)$_2$	80.0	197	$C_{21}H_{18}ON_6S_2ClBr$	45.8	3.2	15.2
						45.8	3.3	15.3
11	Br		72.0	207	$C_{24}H_{22}ON_6S_2ClBr$	48.8	3.7	14.2
						48.5	3.7	14.2
12	Br		89.5	191	$C_{23}H_{20}O_2N_6S_2C1Br$	46.6	3.3	14.2
						46.6	3.4	14.0

2.2. Biological evaluation

2.2.1. In vitro antibacterial activity

Compounds were evaluated for their in vitro antibacterial activity against 26 pathogenic bacteria procured from Dept. of Microbiology, Institute of Medical Sciences, Banaras Hindu University. The agar dilution method (Barry, 1991) was performed using Mueller–Hinton agar (Hi-Media) medium. Suspensions of each microorganisms were prepared to contain approximately 10^6 colony forming units (cfu)/mL and applied to plates with serially diluted compounds to be tested and incubated at 37°C for overnight (approx. 18–20 h). The minimum inhibitory concentration (MIC) was considered to be the lowest concentration that completely inhibited growth on agar plates, disregarding a single colony or a faint haze caused by the inoculum.

2.2.2. In vitro antifungal activity

The compounds were evaluated for their in vitro antifungal activity against *Cryptococus neoformans*, *Microsporum audouinii*, *Trichophyton mentagrophytes*, *Microsporum gypsum*, *Histoplasma capsulatum*, *Candida albicans* and *Aspergillus niger* using agar dilution method with Saburoud's dextrose agar (Hi-Media). Suspensions of each microorganisms were prepared to contain 10^5 cfu/mL and applied to agar plates which have been serially diluted with compounds to be tested. The plates were incubated at 26°C during 48–72 h and MIC's were determined.

2.2.3. Anti-HIV activity

The procedure to measure anti-HIV activity in MT-4 cells has been described previously (Pandeya et al., 1998). Either mock-infected or HIV-1 infected MT-4 cells were incubated in the presence of various concentrations of test compounds and the number of viable cells was determined by the MTT [3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide] method on day five after virus infection.

3. Result and discussion

In the present study *N*-[4-(4′-Chlorophenyl)thiazol-2-yl] thiosemicarbazide has been synthesized from 4-chloro acetophenone. This has been condensed with isatin and its 5-chloro and 5-bromo derivatives to form Schiff bases. The *N*-Mannich bases of the above Schiff bases were synthesized by condensing acidic imino group of isatin with formaldehyde and secondary amines (Scheme 1). All

Table 2
Antibacterial activity of the compounds MIC's in μg/mL[a]

Microorganisms/Drugs	1	2	3	4	5	6	7
1. *Salmonella typhimurium*	625	625	312.5	156.2	312.5	312.5	312.5
2. *Vibrio parahaemolyticus*	78.1	156.2	78.1	19.5	39.1	78.1	39.1
3. *Salmonella paratyphi* B	625	312.5	312.5	78.1	156.2	312.5	78.1
4. *Edwardsiella tarda*	625	312.5	312.5	156.2	156.2	312.5	156.2
5. *Vibrio cholerae* 0139	312.5	312.5	78.1	39.1	78.1	156.2	312.5
6. *Staphylococcus aureus* (G+ve)	1250	1250	1250	625	1250	1250	312.5
7. *Escherchia coli* NCTC 10418	625	312.5	312.5	312.5	156.2	312.5	78.1
8. *Vibrio cholerae non*-01	39.1	39.1	39.1	39.1	19.5	39.1	19.5
9. *Enterococcus faecalis* (G+ve)	4.9	9.8	9.8	2.4	2.4	4.9	2.4
10. *Salmonella typhi*	312.5	156.2	156.2	156.2	156.2	78.1	78.1
11. *Pseudomonas aeruginosa*	2500	1250	1250	1250	1250	1250	625
12. *Klebsiella pneumoniae*	625	312.5	625	312.5	312.5	625	156.2
13. *Staphylococcus albus* (G+ve)	625	312.5	312.5	625	625	625	312.5
14. *Salmonella enteritidis*	312.5	312.5	156.2	156.2	156.2	312.5	78.1
15. *Aeromonas hydrophile*	156.2	156.2	156.2	39.1	78.1	78.1	156.2
16. *Vibrio cholerae*-01	312.5	156.2	78.1	78.1	78.1	156.2	39.1
17. *Bacillus subtilis* (G+ve)	312.5	156.2	156.2	156.2	156.2	78.1	156.2
18. *Shigella sonnei*	156.2	78.1	156.2	78.1	156.2	156.2	78.1
19. *Shigella boydii*	312.5	156.2	156.2	156.2	156.2	312.5	78.1
20. *Plesiomonas shigelloides*	312.5	78.1	156.2	78.1	312.5	156.2	78.1
21. *Proteus rettgeri*	156.2	156.2	156.2	39.1	39.1	78.1	39.1
22. *Shigella flexnari*	312.5	312.5	78.1	156.2	156.2	312.5	78.1
23. *Proteus vulgaris*	312.5	78.12	156.2	156.2	78.1	156.2	78.1
24. *Enterobacter*	156.2	156.2	156.2	156.2	156.2	156.2	39.1
25. *Morgonella morgonii*	156.2	312.5	78.1	78.1	156.2	156.2	78.1
26. *Citrobacter ferundii*	156.2	156.2	78.1	39.1	39.1	78.1	156.2
27. *Proteus morgonii*	312.5	312.5	156.2	156.2	312.5	312.5	78.1
28. *Salmonella paratyphi* B	156.2	78.1	19.5	39.1	156.2	156.2	78.1

[a] MIC – Minimum inhibitory concentration.

Table 3
Antibacterial activity of the compounds MIC's in μg/mL[a]

Microorganisms/Drugs	8	9	10	11	12	Suiphamethoxazole	Trimethoprim
1. *Salmonella typhimurium*	312.5	312.5	78.1	156.2	156.2	5000	5000
2. *Vibrio parahaemolyticus*	78.1	156.2	39.1	78.1	39.1	1250	2.4
3. *Salmonella paratyphi* B	312.5	156.2	39.1	156.2	156.2	5000	9.8
4. *Edwardsiella tarda*	156.2	156.2	78.1	312.5	156.2	5000	312.5
5. *Vibrio cholerae* 0139	78.1	156.2	78.1	39.1	156.2	>5000	39.1
6. *Staphylococcus aureus* (G+ve)	625	625	312.5	625	625	5000	>5000
7. *Escherchia coli* NCTC 10418	156.2	312.5	312.5	312.5	156.2	2500	19.5
8. *Vibrio cholerae non*-01	39.1	39.1	9.8	39.1	39.1	312.5	1.2
9. *Enterococcus faecalis* (G+ve)	9.8	9.8	1.2	4.9	4.9	5000	78.1
10. *Salmonella typhi*	156.2	156.2	156.2	78.1	156.2	2500	4.9
11. *Pseudomonas aeruginosa*	625	625	625	625	1250	78.12	5000
12. *Klebsiella pneumoniae*	156.2	312.5	625	312.5	625	2500	5000
13. *Staphylococcus albus* (G+ve)	156.2	156.2	78.1	156.2	156.2	2500	>5000
14. *Salmonella enteritidis*	156.2	312.5	78.1	156.2	156.2	2500	4.9
15. *Aeromonas hydrophile*	78.1	156.2	78.1	78.1	78.1	2500	1250
16. *Vibrio cholerae*-01	39.1	78.1	39.1	78.1	156.2	5000	5000
17. *Bacillus subtilis* (G+ve)	156.2	78.1	78.1	156.2	78.1	5000	5000
18. *Shigella sonnei*	39.1	78.1	19.5	78.1	156.2	2500	9.8
19. *Shigella boydii*	78.1	156.2	78.1	156.2	156.2	2500	9.8
20. *Plesiomonas shigelloides*	39.1	156.2	156.2	156.2	156.2	5000	4.9
21. *Proteus rettgeri*	39.1	39.1	156.2	78.1	156.2	2500	2500
22. *Shigella flexnari*	156.2	312.5	9.8	39.1	78.1	2500	156.2
23. *Proteus vulgaris*	78.1	156.2	39.1	78.1	39.1	2500	156.2
24. *Enterobacter*	156.2	78.1	39.1	39.1	156.2	1250	156.2
25. *Morgonella morgonii*	312.5	156.2	19.5	78.1	19.5	2500	156.2
26. *Citrobacter ferundii*	78.1	78.1	39.1	78.1	78.1	5000	19.5
27. *Proteus morgonii*	156.2	156.2	78.1	78.1	78.1	5000	156.2
28. *Salmonella paratyphi* B	78.1	156.2	19.5	78.1	39.1	2500	156.2

[a] MIC – Minimum inhibitory concentration.

compounds (Table 1) gave satisfactory elemental analysis. IR and ^{1}H-NMR spectra were consistent with the assigned structures. All the synthesized compounds were tested for in vitro antibacterial activity by agar dilution method. The MIC's of the compounds against 28 pathogenic bacteria are presented in Tables 2 and 3. Also included is the activity of reference compounds sulphamethoxazole and trimethoprim. It has been observed that all the compounds tested showed mild to moderate activity against tested bacteria. All the compounds showed more activity (less MIC) than sulphamethoxazole except *Pseudomonas aeruginosa*. When compared to trimethoprim all the compounds are more active against *Salmonella typhimurium*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus albus*, *Aeromonas hydrophile*, *Vibrio cholerae*-01, *Bacillus subtilis*, *Proteus rettgeri*, seven compounds are more active against *Edwardsiella tarda*, *Proteus vulgaris* and *Salmonella paratyphi* A, four compounds are more active against *Shigella flexnari*, *Enterobacter* and *Proteus morgonii* and six compounds are more active against *Morgonella morgonii*. In general the antibacterial activity of the substituents at the 5th position is Br>Cl>H. In case of substitution at the 1st position in Mannich bases the dimethylaminomethyl derivative showed better activity as compared to other Mannich bases. The site of action of thiosemicarbazones is the enzyme ribonucleotide reductase (RR) (Liu et al., 1992). Deoxyribonucleotides the blocks of DNA, which are derived from corresponding ribonucleotide, by reaction in which the 2-carbon atom of the D-ribose portion of the ribonucleotide is directly reduced to form the 2-deoxy derivative. The substrate for this reaction is catalysed by RR. Antimicrobial activity of thiosemicarbazones synthesized in the present study could be based on the inactivation of RR. Inactivation of RR in general leads to the reduction of intracelluar pools of deoxynucleotides and this will affect the biosynthesis of DNA. Further it has been observed that Mannich bases derived from α,β-unsaturated ketones inhibit DNA and protein synthesis marketly (Dimmock and Kumar, 1997) and such compounds do not inhibit dihydrofolate reductase. Thus taking into the account of the both Mannich bases and thiosemicarbazones, the possible mechanism of present compounds could be inhibition of DNA synthesis by controlling the inhibition of RR.

The antifungal activity of the compounds was studied with eight pathogenic fungi. The results are summarized in Table 4. Clotrimazole has been used as reference for inhibitory activity against fungi. All the compounds showed good antifungal activity. When compared to clotrimazole, 10 compounds are more active (MIC: 1.2 μg/

Table 4
Antifungal activity of the compounds MIC's in μg/mL

Drugs/ Microorganism	*Cryptococcus neoformans*	*Microsporum audouinii*	*Trichophyton mentagrophytes*	*Epidermophyton floccosum*	*Microsporum gypsum*	*Histoplasma capsulatum*	*Candida albicans*	*Aspergillus niger*
1	4.9	4.9	9.8	1.2	2.4	39.l	156.2	39.1
2	2.4	2.4	4.9	1.2	1.2	19.5	78.1	19.5
3	4.9	2.4	1.2	2.4	1.2	19.5	39.1	9.8
4	4.9	4.9	4.9	1.2	1.2	78.1	78.1	39.1
5	4.9	2.4	4.9	2.4	1.2	39.1	156.5	39.1
6	2.4	1.2	2.4	2.4	1.2	39.1	156.5	9.8
7	2.4	2.4	2.4	1.2	1.2	19.5	78.2	19.5
8	4.9	4.9	4.9	4.9	1.2	19.5	78.1	9.8
9	4.9	2.4	2.4	2.4	2.4	19.5	78.1	9.8
10	2.4	2.4	2.4	2.4	1.2	19.5	78.1	9.8
11	4.9	9.8	2.4	1.2	1.2	39.1	78.1	9.8
12	9.8	4.9	9.8	1.2	1.2	78.1	78.1	9.8
Clotrimazole	2.4	4.9	2.4	2.4	2.4	19.5	0.3	2.4

mL) and two compounds are equipotent (2.4 μg/mL) against *Microsporum gypsum*, seven compounds are more active (MIC: <4.8 μg/mL against *Microsporum audouinii*, six compounds are more active (MIC: 1.2 μg/mL) against *Epidermophyton floccosum*. All the compounds inhibit *Cryptococcus neoformans*, *Microsporum audouinii*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum* and *Microsporum gypsum* with MIC of less than 10 μg/mL.

Compound 1-[*N*,*N*-dimethyl amino methyl]-5-bromo isatin 3-{1′-[4″-(p-chlorophenyl) thiazol-2″-yl] thiosemicarbazone} **10** showed the most favourable antimicrobial activity.

The synthesized compounds were evaluated for their inhibitory effect of the replication of HIV-1 in human MT-4 cells. None of the compounds showed marked anti-HIV at a concentration significantly below their toxicity threshold (Table 5). This may be due to the fact that these drugs may not be inhibiting the HIV-1 reverse transcriptase (RT). It appears that for this RT inhibitory effect a small hydrophobic group is necessary (Teitz et al., 1994) whereas in our case, we have a big p-chlorophenyl thiazolyl group at thiosemicarbazide end.

Table 5
Anti-HIV activity of the compounds

Compounds	EC_{50}[a], μg/mL	CC_{50}[b], μg/mL	SI[c]
1	>12	12.1	<1
2	>10	10.1	<1
3	>10	10.4	<1
4	>54	53.8	<1
5	>57	57.2	<1
6	>19	19.4	<1
7	>10	10.3	<1
8	>12	11.8	<1
9	>12	11.7	<1
10	>29	29.2	<1
11	>21	20.7	<1
12	>14	13.7	<1

[a] Effective concentration of compound, achieving 50% protection of MT-4 cells against the cytopathic effect of HIV.

[b] Cytotoxic concentration of compound, required to reduce the viability of mock infected MT-4 cells by 50%.

[c] Selectivity index or ratio of CC_{50} to EC_{50}.

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