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Short communication

Imidazole derivatives as possible microbicides with dual protection☆

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ABSTRACT

Twenty seven derivatives (**2**–**28**) of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethanol were synthesized and evaluated for anti-*trichomonas*, spermicidal and antifungal activities. Twenty six compounds were active against *Trichomonas vaginalis* at MIC ranging from 1–42 μM and seven compounds (**9**,**18**,**19**,**22**,**24**,**26**,**28**) immobilized 100% human spermatozoa at 1% concentration (w/v). Twenty three compounds (**2**,**3**,**5**,**8**–**26**,**28**) exhibited antifungal activity at 25–50 μg/mL concentration. Seven compounds (**9**,**18**,**19**,**22**,**24**,**26**,**28**) showed significant anti-*trichomonas* and spermicidal activities and also exhibited mild antifungal activity. All the compounds were highly safe towards human cervical cell line (HeLa) as shown by the cell-viability assay of HeLa cells at 200 μg/mL concentration, whereas nonoxynol-9 (N-9, the marketed spermicidal microbicide) was highly cytotoxic. Therefore, it may be concluded that introduction of the pharmacophore responsible for spermicidal activity into a proven anti-*trichomonas* structure may lead to a potent dual function microbicide better and safer than N-9.

1. Introduction

The epidemics of AIDS and unwanted pregnancies appear to thrive in the presence of overpopulation [1], poverty and other sexually transmitted diseases (STDs) [2–4]. It is now well established that trichomoniasis [5] (the most prevalent, non-viral STD) significantly increases the vulnerability to HIV [6,7] and therefore controlling trichomoniasis alone could significantly reduce the incidence of new HIV infections. On the other hand, high number of unintended pregnancies [8] may also indicate the need for newer, cost-effective, safe and convenient contraceptives. A recent survey [9] has shown that most women would prefer a product capable of preventing HIV, pregnancy and STIs and almost half of respondents would use contraceptive microbicides as a dual protection method. Therefore, efforts are being undertaken to develop novel, dually-active microbicidal agents [10–17]. Also since microbicides lacking contraceptive activity may not be used with the required compliance, especially during the "most vulnerable" sexual contacts where the immediate worry of an unwanted pregnancy often overwhelms the otherwise bigger fret of STD and/or HIV acquisition [11], it would be worthwhile to develop dually active agents. The healthy human vagina is firmly resistant to HIV infection [18], still ~5 million new patients are added annually to ~40 million living with HIV, half of which are women [18]. This indicates, (a) high prevalence of HIV in heterosexual contacts, and (b), increase in number of women with compromised vaginal resistance caused by vaginally applied chemical products and/or STD pathogens. Currently all commercially available spermicidal microbicides [19] have detergent ingredients that disrupt cell membranes. The most widely used vaginal spermicide, nonoxynol-9 (N-9) has been shown to damage the cervicovaginal epithelium because of its membrane-disruptive properties, causing an acute inflammatory tissue response, altered vaginal microflora, and enhanced risk of opportunistic infections in the genitourinary tract. Such opportunistic infections are known to enhance the susceptibility of the ectocervical epithelium and the endocervical mucosa to HIV infection. Hence, despite its ability to inactivate HIV *in vitro*, the reported failure [20] of N-9 to prevent heterosexual vaginal transmission of HIV in clinical settings has prompted the search for new female-controlled, non-detergent, topical vaginal spermicidal microbicides that are more effective as well as safer than N-9 [17]. Thus, a challenge is thrown open to chemists to design molecules with spermicidal and anti-HIV/STI activities. Consequently, it was thus thought worthwhile to introduce the pharmacophore responsible for spermicidal activity into a proven structure with anti-STI potential (and vice versa).

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Previously, some attempts have been made to modify metronidazole structure with a view to improve microbicidal activities [21–23]. Accordingly, the authors were prompted to carry out modifications in the structure of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethanol (Metronidazole, 1, Scheme 1, the currently used drug for vaginal trichomoniasis [24]) with a view to introduce spermicidal activity. The pharmacophores imparting spermicidal activity have been reported to be dithiocarbamates [25], disulfide esters [14], benzenepropanamine [26], selective serotonin reuptake inhibitor (SSRI) antidepressants [27], thiourea derivatives [13], acrylophenones [28] etc. It was planned to derivatize at hydroxyl group of the side chain (Gen. Str I, Fig. 1), to introduce alkylamino ester (Gen. Str II, Fig. 1) and dithiocarbamaoyl group (Gen. Str III, Fig. 1) at position-2 of side chain. The compounds (**2**–**28**) synthesised were screened for spermicidal, anti-*trichomonas* and antifungal activities and also their safety was evaluated against human cervical (*HeLa*) cell line.

2. Results and discussion

2.1. Chemistry

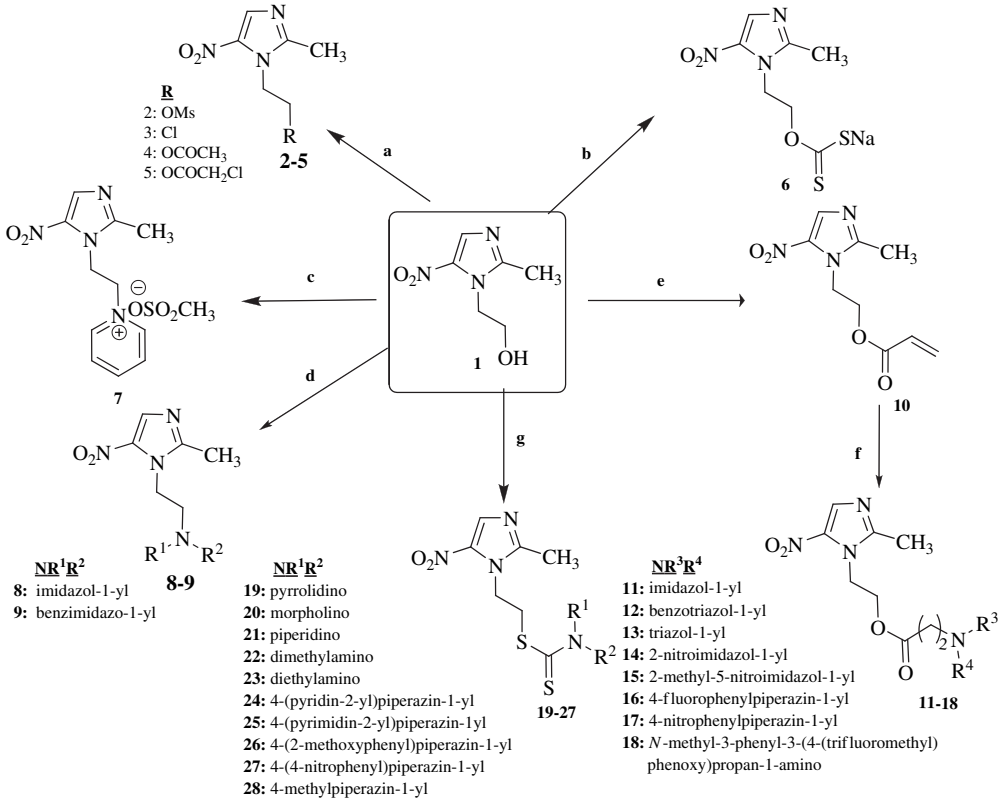
Compounds (**2**–**10**, Scheme 1) were synthesized by the reactions of appropriate reagents (**a**–**e**, Scheme 1) on hydroxyl group of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethanol (**1**, Scheme 1). Compounds (**11**–**18**, Scheme 1) were prepared from 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl acrylate (**10**, Scheme 1) on addition with substituted azoles, substituted piperazines or *N*-methyl-3-phenyl-3-(4-(trifluoromethyl)phenoxy)propan-1-amine in the presence of triethylamine. Compounds (**19**–**28**, Scheme 1) were synthesized by the reaction of compound **2** with sodium salt of substituted carbodithioic acid in acetonitrile under reflux.

2.2. Biology

The compounds synthesized (**2**–**28**) were evaluated for spermicidal, anti-*trichomonas* (Table 1) and antifungal activities (Table 2). All the compounds were active against *Trichomonas vaginalis* at MIC ranging from 1.0–111.0 μM (Table 1) whereas the standard drug Metronidazole was active at 11.7 μM. Seven compounds (**9**,**18**,**19**,**22**,**24**,**26**,**28**) immobilized 100% human spermatozoa at 1% concentration (w/v) (Table 1). Twenty three compounds (**2**,**3**,**5**,**8**–**26**,**28**) exhibited antifungal activity at 50 μg/mL concentration (Table 2). Metronidazole, and all of the compounds (except 5) were highly safe towards human cervical cell line (HeLa) as shown by the cell-viability assay of HeLa cells at 200 μg/mL concentration, whereas N-9 was highly cytotoxic (Fig. 2).

The results indicated that on functionalization (**2**–**10**) at hydroxyl group in 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethanol (**1**) the anti-*trichomonas* activity was retained, and high spermicidal activity (100% immobilization) in compound **9** suggested that we have been able to introduce the dual activity as metronidazole (**1**) showed no effect on sperm motility (Table 1). On the other hand, compounds **2**–**8** exhibited moderate to appreciable anti-trichomonas activity but their spermicidal activity was negligible. However, 5-nitroimidazoles bearing an arylsulfonylmethyl group [21] and N-acetamide(sulfonamide)-2-methyl-4-nitro-1*H*-imidazoles [22] have been reported to exhibit substantial activity against *T. vaginalis*.

With a view to further enhance the spermicidal action in 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethanol framework, alkylamino side chain (**11**–**18**) and dithiocarbamoyl moieties (**19**–**28**) were introduced at hydroxyl function. The compounds with alkylamino side chain showed high anti-*trichomonas* activity. Compounds (**16**,**17**) were seven to eight times more active than metronidazole



Scheme 1. Reagents and conditions; a: CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0–5 °C/ $SOCl_2$, $CHCl_3$(dry), reflux/ $(CH_3CO)_2O$, Et_3N, CH_2Cl_2, DMAP, 0–5 °C/ $ClCH_2COCl$, Et_3N, CH_2Cl_2, 0–5 °C; b: CS_2, NaOH, MeOH, rt; c:(i) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0–5 °C; (ii) Py, 110-115°C; d: (i) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0–5 °C; (ii) NR^1R^2, Et_3N, toluene, reflux; e: $ClCOCH_2CH_2Cl$, Et_3N, CH_2Cl_2, 0–5 °C; f: NR^3R^4, Et_3N, CH_2Cl_2/$ClCH_2CH_2Cl$; g:(i) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0–5 °C; (ii) $R^1R^2NC{=}SSNa$, CH_3CN, reflux.

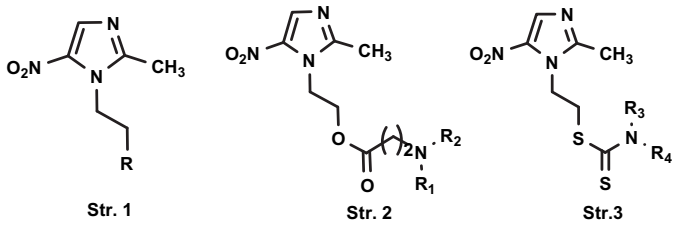
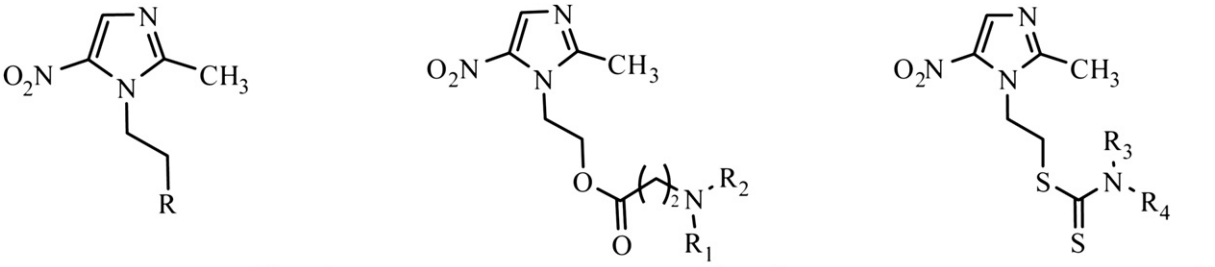


Fig. 1. General Structures of the compounds synthesized.

and compound **18** immobilized 100% sperms at 1% concentration. The addition of dithiocarbamoyl moiety further enhanced both the activities. Three compounds **21**–**23** were ten to eleven times, compound **20** six times and compound **27** was about four times as active as metronidazole and four compounds (**19**,**24**,**26**,**28**) had MIC significantly better than metronidazole. Moreover, five compounds (**19**,**22**,**24**,**26**,**28**) also immobilized 100% human spermatozoa and therefore exhibited dual activity. Compound **22** showed remarkable anti-*trichomonas* and spermicidal activities and also exhibited mild antifungal activity. The notable activity in seven compounds (**9**,**18**,**19**,**22**,**24**,**26**,**28**) may possibly be attributed to their high sulfhydryl affinity [14] resulting in interactions between sulfhydryl group present on sperm membrane with benzimidazolyl side-chain, benzenepropanamine [26] and dithiocarbamoyl group [16], respectively.

Therefore, it may be concluded that introduction of the pharmacophore responsible for spermicidal activity into a proven anti-*trichomonas* structure may lead to a potent dual function spermicide, which can be better and safer than N-9.

Table 1
In-vitro Spermicidal activity and Anti-*Trichomonas* activity of the compounds (**2**–**28**) against human spermatozoa and *Trichomonas vaginalis*.



Compounds (str. 1)	R	Spermicidal activity (percent sperm motility of control at 1.0% concentration)	Anti-*Trichomonas* activity (μM) (MIC ± SE)
2	OMs	94.12	5.02 ± 0.89
3	Cl	58.82	6.60 ± 0.44
4	OAc	82.35	23.47 ± 4.14
5	$OCOCH_2Cl$	98.37	10.10 ± 1.78
6	OC = SSNa	90.24	111.52 ± 4.96
7	Pyridinium methanesulfonate	85.09	9.53 ± 1.02
8	imidazol-1-yl	92.36	14.14 ± 1.51
9	benzimidazol-1-yl	0	4.61 ± 0.81
10	acrylate	70.58824	42.22 ± 2.96
Compounds (str. 2)	NR_1R_2		
11	Imidazol-1-yl	100	10.67 ± 3.01
12	Benzotrizol-1-yl	95.734	27.62 ± 0.32
13	Triazol-1-yl	100	21.26 ± 0.57
14	2-nitroimidazol-1-yl	100	9.25 ± 0.99
15	2-methyl-5-nitroimidazol-1-yl	88.23529	20.60 ± 1.71
16	4-fluorophenylpiperazin-1-yl	88.23529	1.54 ± 0.82
17	4-nitrophenylpiperazin-1-yl	82.35294	1.45 ± 0.77
18	N-methyl-3-phenyl-3-(4-(trifluoromethyl) phenoxy)propan-1-amino	0	11.70 ± 0.21
Compounds (str. 3)	NR_3R_4		
19[a]	pyrrolidino	0	8.33 ± 1.47
20	morpholino	70.58824	1.98 ± 0.35
21	piperidino	70.58824	1.00 ± 0.18
22[a]	dimethylamino	0	1.14 ± 0.20
23	diethylamino	100	1.03 ± 0.18
24[a]	4-(pyridin-2-yl)piperazin-1-yl	0	6.38 ± 0.43
25	4-(pyrimidin-2-yl)piperazin-1yl	94.11765	25.45 ± 2.94
26[a]	4-(2-methoxyphenyl)piperazin-1-yl	0	5.94 ± 0.69
27	4-(4-nitrophenyl)piperazin-1-yl	41.17647	2.87 ± 0.51
28	4-methylpiperazin-1-yl	0	7.60 ± 0.33
N-9	–	0	60.78 ± 4.05
Metronidazole	–	100	11.70 ± 1.46
Vehicle (**Control**)	–	100	Inactive

[a] These compounds were tested as D-(-)tartrate salt for spermicidal activity.

Table 2
Antifungal activity against six different strains of fungi.

Compound	Antifungal activity (MIC in μg/mL)					
	1	2	3	4	5	6
2	>50	>50	50	>50	>50	>50
3	>50	50	50	>50	>50	>50
4	>50	>50	>50	>50	>50	>50
5	>50	>50	>50	25	>50	>50
6	>50	>50	>50	>50	>50	>50
7	>50	>50	>50	>50	>50	>50
8	25	>50	50	>50	>50	>50
9	>50	>50	50	>50	>50	>50
10	>50	50	50	50	>50	>50
11	>50	>50	>50	50	>50	>50
12	>50	>50	>50	50	>50	>50
13	>50	>50	>50	50	>50	>50
14	>50	>50	>50	50	>50	>50
15	>50	>50	>50	50	>50	>50
16	>50	>50	>50	50	>50	>50
17	>50	>50	>50	50	>50	>50
18	>50	>50	>50	50	>50	>50
19	50	50	25	50	>50	>50
20	>50	>50	50	50	>50	>50
21	>50	>50	25	50	>50	>50
22	>50	>50	>50	50	>50	>50
23	50	50	25	25	>50	>50
24	>50	>50	50	50	>50	>50
25	>50	>50	>50	50	>50	>50
26	>50	>50	50	25	>50	>50
27	>50	>50	>50	>50	>50	>50
28	>50	>50	50	50	>50	>50
N-9	>50	–	–	–	–	50
Fluconazole	0.5	1.0	2.0	1.0	2.0	1.0

1: *Candida albicans*; 2: *Cryptococcus neoformans*; 3: *Sporothrix schenckii*; 4: *Trichophyton mentagrophytes*; 5: *Aspergillus fumigatus*; 6: *Candida parapsilosis*.

3. Experimental section

3.1. Chemistry

Melting points were determined in open capillary tubes on an electrically heated block and are uncorrected. IR spectra (ν_{max} in cm^{-1}) of the compounds were recorded on Perkin Elmer's FT-IR RX1 PC spectrophotometer. ^1H NMR & ^{13}C NMR spectra were recorded on Bruker Supercon Magnet Avance DPX-200/DRX-300 spectrometers (operating at 200 and 300 MHz respectively for ^1H; 50 and 75 MHz respectively for ^{13}C) in deuterated solvents with TMS as internal reference (chemical shifts in δ ppm, J in Hz.). Electronspray Ionisation Mass spectra (ESI-MS) were recorded on a Micromass Quattro II triple quadruple mass spectrometer. Elemental analyses were performed on Carlo Erba EA-1108 micro analyzer/Vario EL-III C H N S analyzer. All compounds were analyzed of C, H, N and the results obtained were within ±0.4% of calculated values. The reaction progress was routinely monitored by thin layer chromatography (TLC) on precoated alumina/silica gel plates (Aldrich). Column chromatography was performed over Merck silica gel (60–120 mesh). All chemicals and solvents were procured from Sigma-Aldrich/Merck India Ltd. 1-dialkylamino carbodithioic acid sodium salts [25] were prepared by known procedures.

3.1.1. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl methanesulfonate (**2**)

Into a ice-cooled solution of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethanol (**1**, 0.011 mol) and triethylamine (0.017 mol) in dry dichloromethane was added methane sulfonyl chloride (0.017 mol) drop wise under stirring. The reaction mixture was further stirred at 0–5 °C for 2 h. The separated thick material was filtered and washed with water (15 mL × 3) gave a cream coloured solid. The solid was crystalized from acetonitrile/ethyl acetate (1:1, v/v). Yield 80%; Mp 139–140 °C; IR (KBr): 3024, 2934, 1526, 1260, 745 cm^{-1}; ESI-MS: m/z 250 ($M^+ + 1$); ^1H NMR (300 MHz, DMSO-d_6, δ ppm): δ 8.05 (s, 1H), 4.65 (t, 2H, $j = 4.7$ Hz), 4.55 (t, 2H, $j = 4.6$ Hz), 3.14 (s, 3H), 2.46 (s, 3H); Anal. Calcd. for $C_7H_{11}N_3O_5S$ (249): C, 33.73; H, 4.45; N, 16.86; Found: C, 33.51; H, 4.25; N, 16.53.

3.1.2. 1-(2-chloroethyl)-2-methyl-5-nitro-1H-imidazole (**3**)

Into a ice-cooled solution of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethanol (**1**, 0.003 mol) in dry chloroform (20 mL) was added thionyl chloride (0.004 mol) dropwise under stirring. A sticky solid separated out which was dissolved on heating the mixture to 65 °C in an oil bath. The reaction mixture was further heated at 65–70 °C for 3 h. Excess thionyl chloride was removed azeotropically. The residue was taken into ethyl acetate (30 mL) and washed with water (10 mL × 3). Organic layer was dried over sodium sulfate and concentrated under reduce pressure to provide a brown solid. Yield 85%; Mp 78–79 °C; IR (KBr): 3132, 2976, 1531, 1365, 1263, 756 cm^{-1}; ESI-MS: m/z 190 ($M^+ + 1$), 192 ($M^+ + 3$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 7.91 (s, 1H), 4.61 (t, 2H, $j = 5.6$ Hz), 3.87 (t, 2H, $j = 5.6$ Hz), 2.54 (s, 3H); Anal. Calcd.: for $C_6H_8ClN_3O_2$ (189): C, 38.01; H, 4.25; N, 22.16; Found: C, 37.93; H, 4.16; N, 22.01.

3.1.3. Acetic acid 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethyl ester (**4**)

In to an ice-cooled solution of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethanol (**1**, 0.015 mol) in dry dichloromethane (10 mL) was added triethylamine (0.022 mol) and dimethylaminopyridine

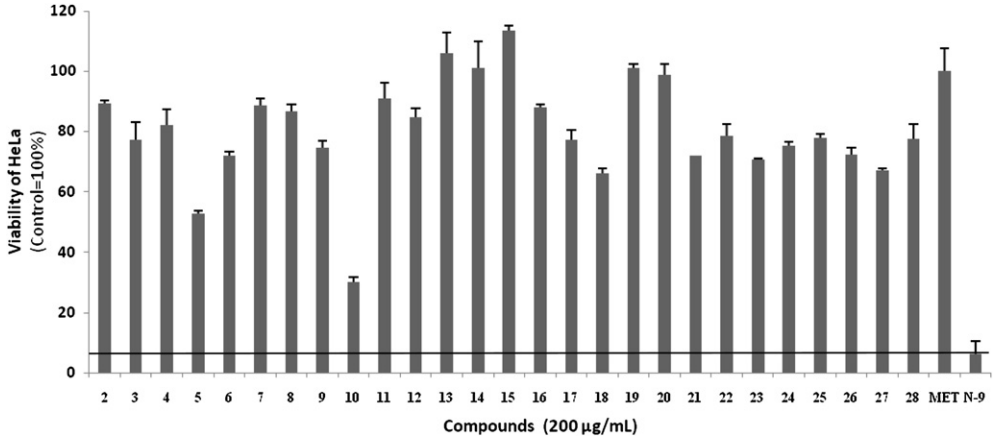


Fig. 2. The viability of HeLa cells after 24 h incubation in 200 μg/mL of test compounds (Mean ± SE of 3 estimations). MET (metronidazole); N-9 (nonoxynol-9).

(10 mol%) under stirring. Acetic anhydride (0.003 mol) was added dropwise and the reaction mixture was further stirred for 1 h (0–5 °C). The reaction mixture was concentrated under reduced pressure, diluted with water (15 mL) and extracted with ethyl acetate (15 mL × 3). The combined organic phase was washed with water (10 mL × 3) and dried over sodium sulfate. Sodium sulfate was filtered off and washed with ethyl acetate (5 mL × 2). The combined ethyl acetate layer was concentrated under reduce pressure to give a light brown solid. Yield 88%; Mp 61–62 °C; IR (KBr): 3124, 2963, 1741, 1528, 1263, 744 cm^{-1}; ESI-MS: *m/z* 213 (M^+), 214 ($M^+ + 1$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 7.92 (s, 1H); 4.55 (t, 2H, $j = 5.2$ Hz), 4.37 (t, 2H, $j = 5.1$ Hz), 2.47 (s, 3H), 1.99 (s, 3H); Anal. Calcd. for $C_8H_{11}N_3O_4$ (213): C, 45.07; H, 5.20; N, 19.71; Found: C, 45.12; H, 5.39; N, 19.61.

3.1.4. Chloro-acetic acid 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethyl ester (**5**)

Into a ice-cooled solution of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethanol (**1**, 0.0011 mol) and triethylamine (0.0022 mol) in dichloromethane (10 mL) was added chloroacetyl chloride (0.0022 mol) dropwise under stirring. The reaction mixture was further stirred at 0–5 °C for 30 min. Dichloromethane was distilled off and the residue was taken in ethyl acetate (20 mL). The ethyl acetate layer was washed with water (10 mL × 3) and dried over sodium sulfate. Sodium sulfate was filtered off and washed with ethyl acetate (5 mL × 2). The filtrate was concentrated to give a brown semisolid. Yield 71%; IR (KBr): 3021, 1747, 1534, 1364, 1262, 756 cm^{-1}; ESI-MS: *m/z* 248 ($M^+ + 1$), 250 ($M^+ + 3$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 7.95 (s, 1H), 4.62 (t, 2H, $j = 4.9$ Hz), 4.52 (t, 2H, $j = 4.9$ Hz), 4.01 (s, 2H), 2.53 (s, 3H); Anal. Calcd. for $C_8H_{10}ClN_3O_4$ (247): C, 38.80; H, 4.07; N, 16.97; Found: C, 38.52; H, 4.15; N, 17.13.

3.1.5. Sodium O-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl carbonodithioate (**6**)

The title compound was prepared by the known procedure [29]. To a ice-cooled solution of sodium hydroxide (0.0028 mol, 0.287 g) in methanol (10 mL) was added 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethanol (**1**, 0.0018 mol) in small portions under stirring. Carbon disulfide was added dropwise at 0–5 °C and the reaction mixture was further stirred for 24 h at room temperature, during the course of reaction pH maintained basic (10–12). Methanol was distilled off and crude product was taken in acetone (10 mL) to give a precipitate. Yellow solid was filtered off and dried in vacuum desiccator. Yield 77%; Mp 250 °d; IR (KBr): 1640, 1384, 1272, 674 cm^{-1}; ^1H NMR (300 MHz, D_2O, δ ppm): δ 7.31 (s, 1H), 3.97 (t, 2H, $j = 5.2$ Hz), 3.75 (t, 2H, $j = 5.1$ Hz), 2.26 (s, 3H); Anal. Calcd. for $C_7H_8N_3NaO_3S_2$ (269): C, 31.22; H, 2.99; N, 15.60; Found: C, 31.39; H, 3.17; N, 15.91.

3.1.6. 1-[2-(2-methyl-5-nitro-1H-imidazol-1-yl)-ethyl]pyridinium methanesulfonate (**7**)

2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl methanesulfonate (**2**, 0.0006 mol) was added into excess of pyridine (2 mL) and the reaction mixture was heated at reflux under stirring for 24 h. Pyridine was distilled off under reduced pressure. The residual brown solid was washed with ethyl acetate/hexane mixture (1:1; 10 mL × 2). Yield 82%; Mp 145–146 °C; IR (KBr): 3444, 2929, 1742, 1650, 1187, 778 cm^{-1}; ^1H NMR (300 MHz, D_2O, δ ppm): δ 8.48 (*d*, 2H, $j = 5.9$ Hz), 8.36–8.31 (m, 1H), 7.82 (s, 1H), 7.80–7.77 (m, 2H), 4.82 (t, 2H, $j = 5.5$ Hz), 4.69 (t, 2H, $j = 5.4$ Hz), 2.48 (s, 3H), 1.88 (s, 3H); Anal. Calcd. for $C_{12}H_{16}N_4O_5S$ (328): C, 43.90; H, 4.91; N, 17.06; Found: C, 43.53; H, 5.21; N, 17.31.

3.1.7. Synthesis of 1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl) 1H-substituted azoles (**8**,**9**; Scheme 1)

To a solution of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl methanesulfonate (1 eq) and triethylamine (1.5 eq) in toluene (10 mL) was added imidazole/benzimidazole (1 eq) portionwise at room temperature. The reaction mixture was heated at 110–120 °C for 6 h under stirring in an oil bath. Toluene was distilled off under reduced pressure and the crude material was taken in chloroform (20 mL). The chloroform layer was washed with water (10 mL × 3) and dried over sodium sulfate. Sodium sulfate was filtered off and washed with chloroform (5 mL × 2). The filtrate was concentrated under reduced pressure. The crude material was purified by column chromatography over silica gel (60–120 mesh) with chloroform/hexane as an eluent.

3.1.7.1. 1-(2-(1H-imidazol-1-yl)ethyl) 2-methyl-5-nitro-1H-imidazole (**8**).

Light red solid; Yield 63%; Mp 158–159 °C; IR (KBr): 3032, 2964, 1531, 1259, 736 cm^{-1}; ESI-MS: *m/z* 222 ($M^+ + 1$), 154; ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 7.97 (s, 1H), 7.26 (s, 1H), 7.03 (s, 1H), 6.60 (s, 1H), 4.54 (t, 2H, $j = 5.4$ Hz), 4.36 (t, 2H, $j = 5.4$ Hz), 1.85 (s, 3H); Anal. Calcd. for $C_9H_{11}N_5O_2$ (221): C, 48.86; H, 5.01; N, 31.66; Found: C, 48.58; H, 5.34; N, 31.82.

3.1.7.2. 1-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl) 1H-benzimidazole (**9**).

Light yellow solid; Yield 67%; Mp 210–211 °C; I.R.(KBr): 2922, 1519, 1257, 760 cm^{-1}; ESI-MS: *m/z* 272 ($M^+ + 1$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 8.00 (s, 1H), 7.83–7.79 (m, 1H), 7.64 (s, 1H), 7.33–7.30 (m, 2H), 7.23–7.20 (m, 1H), 4.69–4.61 (m, 4H), 1.70 (s, 3H); Anal. Calcd. for $C_{13}H_{13}N_5O_2$ (271): C, 57.56; H, 4.83; N, 25.82; Found: C, 57.87; H, 4.96; N, 25.49.

3.1.8. Acrylic acid 2-(2-methyl-5-nitro-1H-imidazol-1-yl)e thyl ester (**10**)

To a ice-cooled solution of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethanol (**1**, 0.023 mol) and triethylamine (0.035 mol) in dry dichloromethane (70 mL) was added a solution of 1,3-dichloropropanone (0.046 mol) in chloroform (10 mL) under stirring in 2 h. The reaction mixture was allowed to attain room temperature in 1 h under stirring. The reaction mixture was further stirred for 3 h at room temperature. The reaction mixture was concentrated and then diluted with chloroform (50 mL) and water (20 mL). The two layers were stirred for 30 min and then separated. Organic layer was washed with water (15 mL × 3) and dried over sodium sulfate. Sodium sulfate was filtered off and washed with chloroform (5 mL × 2) and the filtrate was concentrated under reduce pressure. A yellowish solid was formed. Yield 79%; Mp 42–43 °C; IR (KBr): 3123, 2957, 1721, 1529, 1369, 1264, 742 cm^{-1}; ESI-MS: *m/z* 226 ($M^+ + 1$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 7.92 (s, 1H), 6.34 (d, 1H, $j = 15.8$ Hz), 6.07–5.97 (m, 1H), 5.85 (d, 1H, $j = 9.0$ Hz), 4.61–4.58 (m, 2H), 4.49–4.48 (m, 2H), 2.46 (s, 3H); Anal. Calcd. for $C_9H_{11}N_3O_4$ (225): C, 48.00; H, 4.92; N, 18.66; Found: C, 47.68; H, 5.24; N, 18.33.

3.1.9. Synthesis of 3-substituted-propionic acid 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl ester (**11**–**18**, Scheme 1)

To a solution of acrylic acid 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-ethyl ester (**10**, 0.003 mol) in dichloromethane/dichloroethane (10 mL) was added substituted diazoles, substituted piperazines or *N*-methyl-3-phenyl-3-(4-(trifluoromethyl)phenoxy)propan-1-amine at room temperature under stirring. Reaction mixture was heated at reflux under stirring for 5–8 h in an oil bath. Solvent was distilled off and crude product was taken into ethyl acetate (20 mL). Organic layer was washed with water (10 mL × 3) and dried over sodium sulfate. Sodium sulfate was filtered off and washed with ethyl acetate (5 mL × 2) and the filtrate was concentrated under reduce pressure. Crude material was purified by column chromatography using chloroform/hexane or ethyl acetate/hexane as eluent to give the product.

*3.1.9.1. 3-Imidazol-1-yl-propionic acid 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl ester (**11**).* Light yellow solid; Yield 77%; Mp 81–82 °C; IR (KBr): 3019, 1744, 1534, 1428, 1263, 758 cm^{-1}; ESI-MS: *m/z* 294 ($M^+ + 1$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 7.93 (s, 1H), 7.47 (s, 1H), 7.03 (s, 1H), 6.88 (s, 1H), 4.54 (t, 2H, *j* = 5.2 Hz), 4.40 (t, 2H, *j* = 5.1 Hz), 4.22 (t, 2H, *j* = 6.4 Hz), 2.71 (t, 2H, *j* = 6.4 Hz), 2.41 (s, 3H); Anal. Calcd. for $C_{12}H_{15}N_5O_4$ (293): C, 49.14; H, 5.16; N, 23.88; Found: C, 49.42; H, 5.29; N, 23.68.

*3.1.9.2. 3-Benzotriazol-1-yl-propionic acid 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl ester (**12**).* Light yellow solid; Yield 51%; Mp 94–95 °C; IR (KBr): 2969, 1742, 1530, 1364, 1263, 757 cm^{-1}; ESI-MS: *m/z* 345 ($M^+ + 1$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 7.99 (d, 1H, *j* = 8.3 Hz), 7.81 (s, 1H), 7.54–7.43 (m, 2H), 7.35–7.30 (m, 1H), 4.81 (t, 2H, *j* = 6.5 Hz), 4.46 (t, 2H, *j* = 5.0 Hz), 4.34 (t, 2H, *j* = 4.9 Hz), 3.04 (t, 2H, *j* = 6.5 Hz), 2.34 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): δ 170.0, 150.7, 145.9, 138.5, 133.2, 127.6, 124.0, 120.0, 109.4, 63.2, 44.9, 43.1, 34.0, 14.3; Anal. Calcd. for $C_{15}H_{16}N_6O_4$ (344): C, 52.32; H, 4.68; N, 24.41; Found: C, 52.09; H, 4.99; N, 24.25.

*3.1.9.3. 3-[1,2,4]Triazol-1-yl-propionic acid-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl ester (**13**).* Yellow solid; Yield 55%; Mp 57–58 °C; IR (KBr): 3018, 1743, 1533, 1365, 1265, 760 cm^{-1}; ESI-MS: *m/z* 295 ($M^+ + 1$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 8.07 (s, 1H), 7.89 (s, 1H), 7.86 (s, 1H), 4.54 (t, 2H, *j* = 5.1 Hz), 4.41 (t, 4H, *j* = 5.6 Hz), 2.87 (t, 2H, *j* = 6.1 Hz), 2.42 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): δ 169.8, 151.9, 150.3, 143.5, 138.0, 132.8, 62.8, 44.6, 44.3, 33.5, 14.0; Anal. Calcd. for $C_{11}H_{14}N_6O_4$ (294): C, 44.90; H, 4.80; N, 28.56; Found: C, 44.61; H, 4.98; N, 28.38.

*3.1.9.4. 3-(4-Nitro-1H-imidazol-1-yl)-propionic acid-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl ester (**14**).* Light yellow solid; Yield 68%; Mp 108–109 °C; IR (KBr): 3083, 2965, 1730, 1522, 1267, 744 cm^{-1}; ESI-MS: *m/z* 339 ($M^+ + 1$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 8.02 (s, 1H), 7.92 (s, 1H), 7.58 (s, 1H), 4.61 (t, 2H, *j* = 4.9 Hz), 4.45 (t, 2H, *j* = 5.0 Hz), 4.33 (t, 2H, *j* = 6.0 Hz), 2.84 (t, 2H, *j* = 6.1 Hz), 2.48 (s, 3H); Anal. Calcd. for $C_{12}H_{14}N_6O_6$ (338): C, 42.61; H, 4.17; N, 24.84; Found: C, 42.38; H, 4.28; N, 24.69.

*3.1.9.5. 3-(2-Methyl-5-nitro-1H-imidazol-1-yl)-propionic acid 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl ester (**15**).* White solid; Yield 81%; Mp 207–208 °C; IR (KBr): 3055, 2899, 1742, 1506, 1260, 750 cm^{-1}; ESI-MS: *m/z* 353 ($M^+ + 1$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 7.94 (s, 1H), 7.91 (s, 1H), 4.61 (t, 2H, *j* = 4.9 Hz), 4.45 (t, 2H, *j* = 5.0 Hz), 4.22 (t, 2H, *j* = 6.3 Hz), 2.83 (t, 2H, *j* = 6.3 Hz), 2.49 (s, 3H), 2.46 (s, 3H); Anal. Calcd. for $C_{13}H_{16}N_6O_6$ (352): C, 44.32; H, 4.58; N, 23.85; Found: C, 44.02; H, 4.68; N, 23.65.

*3.1.9.6. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl-3-(4-(4–fluorophenyl)piperazin-1-yl)propanoate (**16**).* Yellow solid; Yield 81%; Mp 89–90 °C; IR (KBr): 2955, 1730, 1514, 1268, 744 cm^{-1}; ESI-MS: *m/z* 406 ($M^+ + 1$); ^1H NMR (200 MHz, $CDCl_3$, δ ppm): δ 7.89 (s, 1H), 6.95–6.77 (m, 4H), 4.57 (t, 2H, *j* = 5.2 Hz), 4.39 (t, 2H, *j* = 5.2 Hz), 3.06–3.01 (m, 4H), 2.69–2.61 (m, 2H), 2.58–2.53 (m, 4H), 2.49 (s, 3H), 2.46–2.42 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): δ 171.3, 158.7, 155.5, 150.3, 147.8, 138.6, 132.8, 117.8, 117.7, 115.6, 115.3, 62.4, 53.2, 50.0, 44.8, 32.0, 14.2; Anal. Calcd. for $C_{19}H_{24}FN_5O_4$ (405): C, 56.29; H, 5.97; N, 17.27; Found: C, 56.60; H, 6.21; N, 17.22.

*3.1.9.7. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 3-(4-(4-nitrophenyl)piperazin-1-yl)propanoate (**17**).* Dark yellow solid; Yield 66%; Mp 115–116 °C; IR (KBr): 2926, 2823, 1738, 1596, 1526, 1248, 750 cm^{-1}; ESI-MS: *m/z* 433 ($M^+ + 1$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 8.11 (d, 2H, *j* = 9.2 Hz), 7.92 (s, 1H), 6.81 (d, 2H, *j* = 9.3 Hz), 4.63 (t, 2H, *j* = 5.2 Hz), 4.43 (t, 2H, *j* = 5.2 Hz), 3.40–3.37 (m, 4H), 2.69 (t, 2H, *j* = 6.8 Hz), 2.59 (d, 4H, *j* = 4.9 Hz), 2.57 (s, 3H), 2.53–2.47 (m, 2H); Anal. Calcd. for $C_{19}H_{24}N_6O_6$ (432): C, 52.77; H, 5.59; N, 19.43; Found: C, 52.51; H, 5.81; N, 19.79.

*3.1.9.8. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 3-(methyl(3-phenyl-3-(4-(trifluoromethyl)phenoxy)propyl) amino)propanoate (**18**).* Light red semisolid; Yield 77%; IR (neat): 3020, 1740, 1616, 1529, 1326, 1216, 762 cm^{-1}; ESI-MS: *m/z* 535 ($M^+ + 1$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 7.85 (s, 1H), 7.39 (d, 2H, *j* = 8.5 Hz), 7.30–7.22 (m, 5H), 6.84 (d, 2H, *j* = 8.5 Hz), 5.32–5.28 (m, 1H), 4.52–4.49 (m, 2H), 4.34–4.30 (m, 2H), 2.77–2.53 (m, 6H), 2.43 (s, 3H), 2.34 (s, 3H), 2.19–2.12 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): δ 171.3, 160.6, 150.3, 141.0, 138.6, 132.8, 128.8, 127.8, 126.8, 126.75, 126.71, 126.1, 125.7, 123.1, 122.7, 122.5, 115.7, 78.0, 62.3, 53.4, 52.4, 44.8, 41.8, 36.1, 32.1, 14.1; Anal. Calcd. for $C_{26}H_{29}F_3N_4O_5$ (534): C, 58.42; H, 5.47; N, 10.48; Found: C, 58.73; H, 5.18; N, 10.76.

*3.1.10. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl dialkylamino-1-carbodithioate (**19**–**28**, Scheme 1)*

To a solution of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl methanesulfonate (**2**, 0.002 mol) in acetonitrile (20 mL) was added dialkylamino carbodithioic acid sodium salt (0.002 mol) at room temperature. The reaction mixture was heated at reflux in an oil bath under stirring for 6–9 h. The reaction mixture was concentrated and diluted with ethyl acetate (25 mL). The ethyl acetate layer was washed with water (10 mL × 3) and dried over sodium sulfate. Sodium sulfate was filtered off and washed with ethyl acetate (5 mL × 2). The filtrate was concentrated under reduce pressure to provide the crude product which was purified by column chromatography over silica gel with ethylacetate/hexane as eluent. The free base (1 eq) was dissolved in methanol (20 mL) and a solution of D-(-) tartaric acid (1 eq) in methanol (10 mL) was added with stirring at room temperature. The reaction mixture was further stirred overnight at room temperature. Adding dry ethyl ether precipitated the tartrate salts (**19**,**22**,**24**,**26**).

*3.1.10.1. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl pyrrolidine-1-carbodithioate (**19**).* Brown solid; Yield 86%; Mp 138–139 °C, IR (KBr): 3020, 1529, 1436, 1216, 760, cm^{-1}; ESI-MS: *m/z* 300 (M^+), 301 ($M^+ + 1$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 7.95 (s, 1H), 4.67 (t, 2H, *j* = 6.9 Hz), 3.93 (t, 2H, *j* = 6.9 Hz), 3.70–3.62 (m, 4H), 2.59 (s, 3H), 2.15–1.96 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 190.8, 151.4, 138.5, 133.4, 55.5, 50.9, 45.3, 34.9, 26.2, 24.4, 14.9; Anal. Calcd. for $C_{11}H_{16}N_4O_2S_2$ (300): C, 43.94; H, 5.32; N, 18.64; Found: C, 43.69; H, 5.54; N, 18.46; Tartrate salt mp 92–93 °C.

*3.1.10.2. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl morpholine-4-carbodithioate (**20**).* Off white solid; Yield 76%; Mp 127–128 °C; IR (KBr): 3018, 1530, 1264, 762 cm^{-1}; ESI-MS: *m/z* 316 (M^+), 317 ($M^+ + 1$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 7.94 (s, 1H), 4.60 (t, 2H, *j* = 6.9 Hz), 4.43–4.23 (m, 2H), 4.01–3.89 (m, 2H), 3.75–3.68 (m, 6H), 2.57 (s, 3H); Anal. Calcd. for $C_{11}H_{16}N_4O_3S_2$ (316): C, 41.76; H, 5.10; N, 17.71; Found: C, 41.68; H, 5.33, N, 17.63.

*3.1.10.3. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl piperidine-1-carbodithioate (**21**).* Light yellow solid; Yield 76%; Mp 109–110 °C; IR (KBr): 3020, 1529, 1363, 1216, 761, cm^{-1}; ESI-MS: *m/z* 314 (M^+), 315 ($M^+ + 1$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 7.96 (s, 1H), 4.62 (t, 2H, *j* = 6.8 Hz), 4.33–4.25 (m, 2H), 3.91–3.87 (m, 2H), 3.71 (t, 2H, *j* = 6.9 Hz), 2.58 (s, 3H), 1.82–1.60 (m, 6H); Anal. Calcd. for $C_{12}H_{18}N_4O_2S_2$ (314): C, 45.84; H, 5.77; N, 17.82; Found: C, 46.17; H, 6.04; N, 17.42.

*3.1.10.4. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl dimethylcarbamodithioate (**22**).* Yellow solid; Yield 79%; Mp 109–110 °C; IR (KBr):

3020, 1530, 1261, 786, cm^{-1}; ESI-MS: *m/z* 274 (M^+), 275 ($M^+ + 1$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 7.93 (s, 1H), 4.58 (t, 2H, $j = 7.0$ Hz), 3.66 (t, 2H, $j = 6.9$ Hz), 3.55 (s, 3H), 3.36 (s, 3H), 2.56 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): δ 195.3, 151.5, 138.6, 133.5, 45.8, 45.2, 41.8, 36.0, 14.9; Anal. Calcd. for $C_9H_{14}N_4O_2S_2$ (274): C, 39.40; H, 5.14; N, 20.42; Found: C, 39.66; H, 5.36; N, 20.66; Tartrate salt mp 88–89 °C.

*3.1.10.5. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl diethylcarbamodithioate (**23**).* Light yellow solid; Yield 70%; Mp 65–66 °C; IR (KBr): 2926, 1526, 1267, 739 cm^{-1}; ESI-MS: *m/z* 303 ($M^+ + 1$); ^1H NMR ($CDCl_3$, 300 MHz, δ ppm): δ 7.99 (s, 1H), 4.63 (t, 2H, $j = 6.9$ Hz), 4.07–4.00 (m, 2H), 3.76–3.74 (m, 2H), 3.68 (t, 2H, $j = 6.9$ Hz), 2.57 (s, 3H), 1.28 (t, 6H, $j = 7.9$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 193.7, 151.4, 138.5, 133.4, 50.15, 47.18, 45.2, 35.6, 14.9, 12.8, 11.8; Anal. Calcd. for $C_{11}H_{18}N_4O_2S_2$ (302): C, 43.69; H, 6.00; N, 18.53; Found: C, 43.71; H, 6.22, 18.66.

*3.1.10.6. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-(pyridin-2-yl)piperazine-1-carbodithioate (**24**).* Yellow solid; Yield 65%; Mp 144–145 °C; IR (KBr): 3021, 1597, 1522, 1216, 764 cm^{-1}; ESI-MS: *m/z* 393 ($M^+ + 1$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 8.18–8.16 (m, 1H), 7.93 (s, 1H), 7.54–7.48 (m, 1H), 6.69–6.60 (m, 2H), 4.59 (t, 2H, $j = 6.9$ Hz), 4.54–4.32 (m, 2H), 4.13–3.93 (m, 2H), 3.73–3.68 (m, 6H), 2.55 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): δ 194.8, 158.0, 151.0, 147.6, 138.2, 137.6, 133.1, 113.9, 106.8, 44.7, 44.1, 35.0, 14.5; Anal. Calcd. for $C_{16}H_{20}N_6O_2S_2$ (392): C, 48.96; H, 5.14; N, 21.41; Found; C, 48.77; H, 5.38; N, 21.19; Tartrate salt hygroscopic.

*3.1.10.7. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-(pyrimidin-2-yl) piperazine-1-carbodithioate (**25**).* Yellow solid; Yield 78%; Mp 179–180 °C; IR (KBr): 3020, 1586, 1529, 1261, 761 cm^{-1}; ESI-MS: *m/z* 394 ($M^+ + 1$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 8.33–8.31 (m, 2H), 7.93 (s, 1H), 6.58–6.54 (m, 1H), 4.60 (t, 2H, $j = 6.9$ Hz), 4.50–4.31 (m, 2H), 4.19–3.94 (m, 6H), 3.72 (t, 2H, $j = 6.9$ Hz), 2.56 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): δ 195.3, 161.3, 158.0, 151.4, 138.6, 133.5, 111.0, 45.2, 43.1, 35.4, 14.9; Anal. Calcd. for $C_{15}H_{19}N_7O_2S_2$ (393): C, 45.79; H, 4.87; N, 24.92; Found: C, 45.64; H, 4.84; N, 25.13.

*3.1.10.8. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-(2-methoxyphenyl)piperazine-1-carbodithioate (**26**).* Yellow solid; Yield 70%; Mp 131–132 °C; IR (KBr): 3020, 1594, 1530, 1216, 760 cm^{-1}; ESI-MS: *m/z* 422 ($M^+ + 1$); ^1H NMR (200 MHz, $CDCl_3$, δ ppm): δ 7.93 (s, 1H), 7.09–7.00 (m, 1H), 6.93–6.85 (m, 3H), 4.60 (t, 2H, $j = 6.9$ Hz), 4.53–4.42 (m, 2H), 4.19–3.95 (m, 2H), 3.86 (s, 3H), 3.70 (t, 2H, $j = 6.9$ Hz), 325–3.04 (m, 4H), 2.56 (s, 3H); Anal. Calcd. for $C_{18}H_{23}N_5O_3S_2$ (421): C, 51.29; H, 5.50; N, 16.61; Found: C, 51.50; H, 5.33; N, 16.49; Tartrate salt mp 99–100 °C.

*3.1.10.9. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-(4-nitrophenyl)piperazine-1-carbodithioate (**27**).* Orange solid; Yield 75%; Mp 199–200 °C; IR (KBr): 3114, 2919, 1595, 1318, 1254, 745 cm^{-1}; ESI-MS: *m/z* 437 ($M^+ + 1$); ^1H NMR (300 MHz, TFA-d, δ ppm): δ 8.04 (d, 2H, $j = 8.6$ Hz), 7.82 (s, 1H), 7.48 (d, 2H, $j = 8.7$ Hz), 4.54 (t, 2H, $j = 6.2$ Hz), 4.40–4.21 (m, 4H), 3.55–3.48 (m, 6H), 2.52 (s, 3H); Anal. Calcd. for $C_{17}H_{20}N_6O_4S_2$ (436): C, 46.78; H, 4.62; N, 19.25; Found: C, 46.61; H, 4.75; N, 19.06.

*3.1.10.10. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl 4-methylpiperazine-1-carbodithioate (**28**).* Brown solid; Yield 65%; Mp 105–106 °C; IR (KBr): 3020, 1531, 1217, 761 cm^{-1}; ESI-MS: *m/z* 330 ($M^+ + 1$); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): δ 7.89 (s, 1H), 4.61–4.49 (m, 2H), 4.45–4.24 (m, 2H), 4.04–3.82 (m, 2H), 3.64 (t, 2H, 6.9 Hz), 2.53–246 (m, 7H), 2.34 (s, 3H). Anal. Calcd. for $C_{12}H_{19}N_5O_2S_2$ (328): C, 43.75; H, 5.81; N, 21.26; Found: C, 43.66; H, 5.65; N, 21.32.

3.2. Biology

3.2.1. Spermicidal activity

Spermicidal test was adapted from the standard procedure [30]. Briefly, the test compounds were dissolved in a minimum volume of DMSO and diluted with physiological saline (0.85% NaCl in distilled water) to make a 1.0% solution. A spermicidal test was performed with each compound solution and for this purpose 0.05 mL of liquefied human semen was added to 0.25 mL of test solution and vortexed for 10 s at low speed. A drop of the mixture was then placed on a microscope slide, covered with a cover glass and examined under a phase contrast microscope in five fields of vision. The percentage of motile spermatozoa was determined by visual scoring in the next 30–40 s and recorded (Table 1). N-9 was used as reference control.

3.2.2. Anti-Trichomonas activity

T. vaginalis parasites to be used in drug susceptibility assays were grown in TYM medium [31] supplemented with 10% Fetal Calf Serum (FCS), vitamin mixture and 100 U/mL penicillin/streptomycin mixtures, at 37 °C in 15 mL tubes for one day following regular subculturing, and were in the log phase of growth. The cultures routinely attained a concentration of 2×10^7 cells/mL in 48 h. An inoculum of 1×10^4 cells per tube was used for maintenance of the culture. *In vitro* drug susceptibility assays were carried out using the standard procedure [32]. Stock solutions (100 μg/μL) of test compounds were prepared in DMSO. These stock solutions were serially diluted with TYM medium to obtain concentrations up to 0.1 μg/mL in 48-well plates. DMSO/TYM was used as vehicle in control wells. Parasites (5×10^4 trophozoites/L) were added to these wells and incubated anaerobically at 37 °C. Cells were checked for viability at different time intervals from 3 to 48 h under the microscope at 200× magnification. Viability of the cells was determined by trypan blue exclusion assay. Minimum concentration of the test agent at which all cells were found dead in 48 h was considered as its minimum inhibitory concentration (MIC). Metronidazole (Sigma-Aldrich), the anti-*trichomonas* drug presently available in the market, was used as reference standard. The experiment was repeated three times to confirm the MIC (Table 1).

3.2.3. Antifungal activity

The MIC of compounds were determined [33] by broth microdilution technique as per guide lines of National Committee for Clinical Laboratory Standards using RPMI 1640 media buffered with MOPS [3-(N-Morpholino)propanesulfonic acid]. Starting inoculums of test culture was $1–5 \times 10^3$ CFU/mL. Micro titer plates were incubated at 35 °C. MICs were recorded after 48 h of incubation (Table 2).

3.2.4. Cyto-toxicity assay using human cervical (HeLa) cell line

The cyto-toxicity of compounds were assessed [12] against human cervical (*HeLa*) cell line using the MTT (3-[4,5-dimethyl thiazol-2-yl]-2,5-diphenyltetrazolium bromide) based colorimetric assay. *HeLa* cells (procured from National Centre for Cell Science, Pune, India) seeded at a density of 5.0×10^4 per well in 96 well plates were incubated in culture medium (DMEM with 10% FCS) for 24 h at 37 °C in 5%CO_2/95% air atmosphere. After 24 h, culture medium was replaced with fresh medium containing dilutions of test compounds (0–200 μg/mL). Vehicle (10% DMSO) was added to 0 μg/mL (control) wells. After incubation for another 24 h, 10 μL of MTT solution (5 mg/mL in PBS, pH 7.4) was added to each well. The formazan crystals formed inside the viable cells were solubilized in DMSO and the OD was recorded at 540 nm in a microplate reader (Microquant, Bio-Tek, USA). Cyto-toxicity at the highest concentration tested (200 μg/mL) has been displayed in Fig. 2.

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