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A unique dithiocarbamate chemistry during design & synthesis of novel sperm-immobilizing agents†‡

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1-Substituted piperazinecarbodithioates were obtained by an unusual removal of CS_2 in benzyl substituted dithiocarbamate derivatives under acid and basic conditions during design and synthesis of 1,4-(di-substituted)piperazinedicarbodithioates as double edged spermicides. A plausible mechanism for CS_2 removal has been proposed. All synthesized compounds were subjected to spermicidal, antitrichomonal and antifungal activities. Twenty-one compounds irreversibly immobilized 100% sperm (MEC, 0.06–31.6 mM) while seven compounds exhibited multiple activities. Benzyl 4-(2-(piperidin-1-yl)ethyl) piperazine-1-(carbodithioate) (**18**) and 1-benzyl 4-(2-(piperidin-1-yl)ethyl)piperazine-1,4-bis(carbodithioate) (**24**) exhibited appreciable spermicidal (MEC, 0.07 and 0.06 mM), antifungal (MIC, 0.069–0.14 and >0.11 mM) and antitrichomonal (MIC, 1.38 and 0.14 mM) activities. The probable mode of action of these compounds seems to be through sulfhydryl binding which was confirmed by fluorescence labeling of sperm thiols.

Introduction

The current world population is expected to rise to 9.1 billion by year 2050[1] accompanied by an equally challenging rise in number of sexually transmitted diseases (STD)[2] and HIV infections.[3] Hence there is a global need to control the human population and spread of sexually transmitted infections (STI), through the development of dually active agents capable of preventing conception and disease, and anti-STD vaginal spermicides could be one of best choice. Furthermore the spermicide currently on the market, nonoxynol-9 (N-9), increases the risk of transmission of these infections owing to its surfactant action.[4–7] Therefore, urgent efforts are warranted to develop antimicrobial, non-detergent spermicidal agents, preferably in a single chemical entity. The free sulfhydryls modulate sperm membrane conformations and regulate energy metabolism, which are vital for motility and viability.[8] Moreover, sulfhydryl groups also play an important role in survival of anaerobic microbes such as the STI, *Trichomonas vaginalis*.[9] Thus, sulfhydryl binding scaffolds have been utilized as viable option for the development of dually active sperm immobilizing agents.[10]

In our ongoing efforts[10–14] to design non-surfactant dual action vaginal spermicides (I–V, Fig. 1), the most viable pharmacophore was found to be the dithiocarbamate (DTC) group as it interacts with sulfhydryl groups present on spermatozoa and *T. vaginalis*.[10] Moreover, the DTC group being a versatile pharmacophore exhibits various biological activities *i.e.*, microbicidal–spermicidal,[15] fungicidal,[16] antabuse,[17] anti HIV[18] activities, and being a very fascinating chemical appendage,

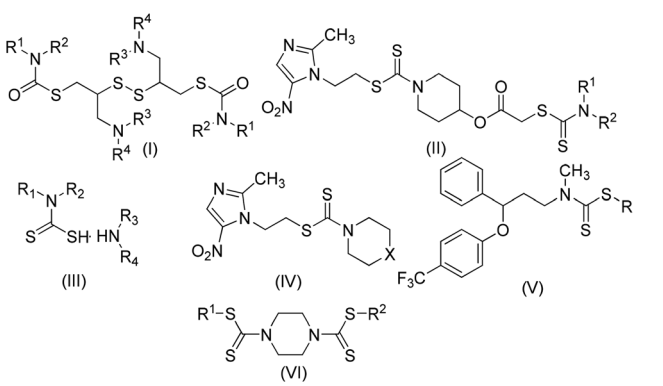

Fig. 1 General structures of compounds studied (I–V) and envisaged scaffold (VI).

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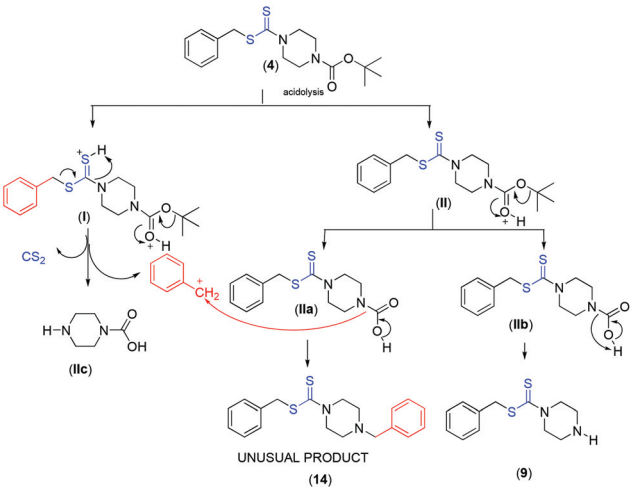
it generates unique and interesting chemistry *e.g.*, O–S exchange,[19,20] *N*-trifluoromethyl amine formation,[21] elimination to give alkene,[22] unusual epithio product.[19]

Consequently, it was thought worthwhile to design and synthesize chemical entities with more than one dithiocarbamate groups in a single framework. Since 4-(*N*-methyl)piperazine carbothioic acid sodium salt[23] has mild spermicidal activity and piperazine itself has been designated as a privileged scaffold,[24] attempts were carried out to incorporate a dithiocarbamate group on both the nitrogen atoms of piperazine moiety. Accordingly, 1,4-(disubstituted)piperazinedicarbodithioate derivatives (VI, Fig. 1) were synthesized and evaluated for spermicidal, antitrichomonal and antifungal activities. The chemical synthesis, biological evaluation and structure–activity relationship (SAR) are being discussed in this communication.

Chemistry

1,4-Piperazinedicarbodithioic acid esters were synthesized by di-*tert*-butyl dicarbonate (DIBOC) protection and incorporation of dithiocarbamate on the other nitrogen, followed by esterification and deprotection, and incorporation of dithiocarbamate on this free amine. Piperazine (**1**) was protected selectively at one nitrogen (**2**) which on being reacted with carbon disulfide under alkaline conditions gave dithiocarbamate sodium salt (**3**). Compound (**3**) was reacted with alkyl halides to yield carbodithioic esters (**4–8**), which were deprotected with TFA to provide desired compounds (**9–13**), however, in the case of benzyl chloride an unusual *N*-benzyl product (**14**) was isolated in 20–30% yield (Scheme 1).

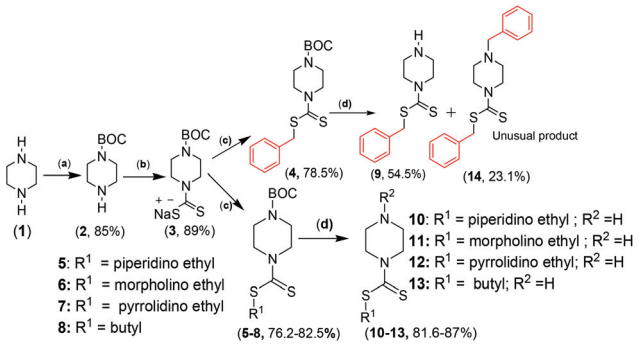
The formation of an unusual *N*-benzyl product (**14**) can be explained (Scheme 2) on the basis of acid catalyzed N-deprotection of the *tert*-butyloxy carbonyl group.[25,26] Under acidic conditions N-deprotection occurred *via* intermediates **II** and **IIb** to give the required product **9**. The unusual product (**14**) might have been formed when both the carbonyl and thiocarbonyl groups get protonated (**I**) and carbon disulfide is lost to provide a benzyl cation,[22,27] which in turn attacked the free amine of **IIa**. The presence of a piperazine-1-carboxylic acid fragment (**IIc**) in the mass spectrum further suggested this mechanism.



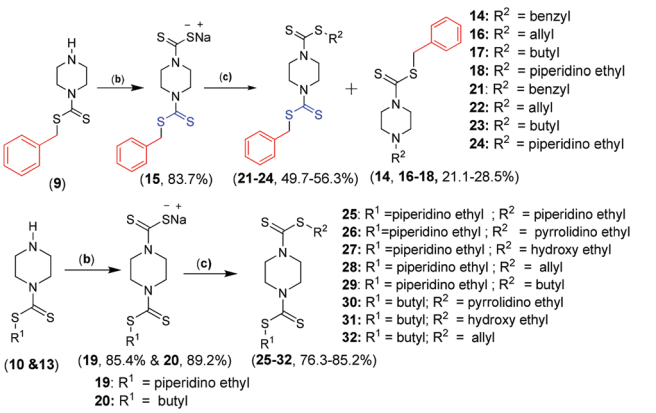
Scheme 2 Possible mechanism for synthesis of unusual product (**14**) under acidic condition.

The N-deprotected compounds (**9**, **10**, **13**) were subjected to the incorporation of another dithiocarbamate group (Scheme 3) to get the desired scaffold (VI, Fig. 1). The reaction of these compounds with carbon disulfide under alkaline conditions gave dithiocarbamate sodium salts (**15**, **19**, **20**), which on being alkylated with alkyl halide in presence of triethyl amine yielded desired 1,4-bisdithiocarbamate compounds (**21–32**). Surprisingly, with *S*-benzyl compound (**15**) again unusual products, benzyl 4-alkylpiperazine-1-carbodithioates (**14**, **16–18**) were isolated in 20–30% yields.

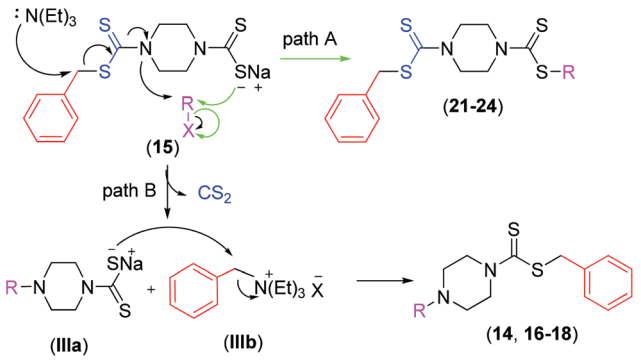
The formation of these unusual *N*-benzyl products (**14**, **16–18**) can be explained (Scheme 4) on the basis of the formation of a triethylbenzyl ammonium salt (**IIIb**) in the presence of triethyl amine followed by loss of carbon disulfide.



Scheme 1 Reaction conditions for synthesis of alkyl piperazine-1-carbodithioate; (a) DIBOC, $CHCl_3$, TEA, 0–5 °C, 4 h; (b) CS_2, NaOH, EtOAc, 0–5 °C, 10 h; (c) alkyl halide, MeOH, TEA, rt, 3 h; (d) (i) TFA, DCM, 0–5 °C, 6 h; (ii) $NaHCO_3$, H_2O, 0–5 °C, 4 h.



Scheme 3 Reaction conditions for synthesis of 1-substituted and 1,4-(disubstituted) piperazinedicarbodithioate derivatives; (b) CS_2, NaOH, EtOAc, 0–5 °C, 10 h; (c) alkyl halide, MeOH, TEA, rt, 3 h.



Scheme 4 Possible mechanism for synthesis of unusual product (**14**, **16–18**) under basic conditions.

S-Debenzylation is known to occur under basic conditions[28–30] and the benzyl group is known to dance from one anionic centre to other within the same molecule.[31] The free amine formed after the loss of carbon disulfide reacts with alkyl halide and the carbodithioate anion of **IIIa** attacks the electron-deficient benzyl carbon to give unusual products (**14**, **16–18**). Thus, loss of carbon disulfide and migration of the benzyl group occurred simultaneously.

Biological evaluation

Spermicidal activity

All compounds (except **11**, **14** and **17**) exhibited 100% spermicidal activity (Table 1) at a minimum effective concentration (MEC) ranging from 0.06–31.6 mM while reference compound (**33**) and standard N-9 exhibited spermicidal activity at MEC, 50.4 and 0.8 mM respectively. Twenty one compounds (**9**, **10**, **12**, **13**, **15**, **16** and **18–32**) were more potent than reference compound **33**. Additionally two compounds (**18** and **24**) demonstrated extremely potent sperm immobilizing potential and showed spermicidal activity at (MEC, 0.07 and 0.06 mM), which were more active than commercially available spermicide N-9.

Antifungal activity

Seven compounds (**9**, **13**, **15**, **18**, **20**, **26** and **30**) showed antifungal activity (Table 1) against one or more fungal strains *viz.*, *Candida albicans. Cryptococcus neoformans*, *Sporothrix schenckii*, *Trichophyton mentagrophytes*, *Aspergillus fumigates*, *Candida parapsilosis* with minimum inhibitory concentration (MIC) 0.002–0.23 mM. Compounds **13**, **15** and **18** inhibited all fungal strains while compounds **26** and **30** inhibited five and compounds **9** and **20** inhibited four strains. Compound **15** was found to be the most potent antifungal compound, which inhibits all fungal strains at MIC, 0.002–0.14 mM. The standard Fluconazole showed antifungal activity at MIC, 0.003 to >0.104 mM.

Anti-*Trichomonas* activity

Twenty compounds (**9–13**, **15**, **16**, **18–24** and **26–31**) showed antitrichomonal activity (Table 1) at MEC, 0.08–1.81 mM. Eleven compounds (**9**, **10**, **12**, **13**, **15**, **19**, **20**, **24**, **26**, **27**, and **31**) exhibited moderate antitrichomonal activity (MEC, 0.14–0.96 mM) while one compound (**30**) showed remarkable activity at MEC, 0.08 mM. The standard Drug Metronidazole demonstrated antitrichomonal activity at 0.018 mM.

Cytotoxicity to cervical epithelium (HeLa) and lactobacilli

Compounds **18** and **24** exhibited an IC_{50} of >1500 μM against HeLa cells and lactobacilli (normal vaginal flora), *in vitro*. In contrast N-9 displayed much lower IC_{50} against these cells (82.3 and 35.0 μM, respectively).

Structure–activity relationship (SAR)

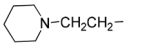
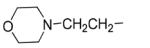
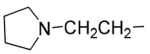
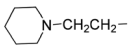
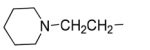
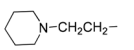
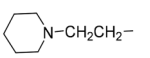
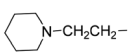
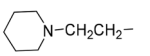
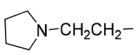
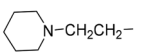
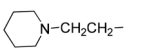
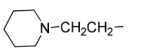
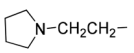
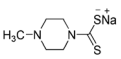
A structure–activity relationship (SAR) study revealed that N-demethylation and S-esterification of the reference compound (**33**) enhanced the spermicidal activity by two and a half fold (**9**, **10**, **13**) and thirteen-fold (**12**) among alkyl piperazine-1-carbodithioates (**9–13**) whereas the activity was lost with *S*-morpholinoethyl group (**11**). Two compounds (**9**, **13**) exhibited mild antifungal (MIC, 0.11–0.23 mM) and antitrichomonal activity (MIC 0.50 and 0.57 mM). The N-alkylation of benzyl 4-piperazine-1-carbodithioate (**9**) with piperidinoethyl group (**18**) gave highly potent spermicidal compound, which was 283 and 720 times more active than compound **9** and the reference compound, **33** respectively. Whereas an *N*-allyl group (**16**), *N*-benzyl (**14**) and *N*-butyl (**17**) groups were less desirable. The incorporation of an additional carbodithioic acid group as sodium salt in compound **9** and **13** retained the spermicidal and antitrichomonal activity while the antifungal activity was highly enhanced (**15**, **20**).

The study also demonstrated that two alkyl variants at R^1 and R^2 played a significant role in sperm immobilization in 1,4-(disubstituted) piperazinedicarbodithioate derivatives (**21–32**). When R^1 was benzyl and R^2 was varied from benzyl (**21**), allyl (**22**), butyl (**23**) to piperidinoethyl (**24**), the spermicidal activity increased **24** > **23** > **21** > **22** and compound **24** was 840 times more active than the reference compound, (**33**) and also exhibited antitrichomonal activity (MIC 0.14 mM), but the antifungal activity was lost. Whereas with R^1 as piperidinoethyl and R^2 as different alkyl groups (**25–29**), spermicidal, antitrichomonal and antifungal activities became moderate. Further, with R^1 as butyl and R^2 with pyrrolidinoethyl (**30**), hydroxyethyl (**31**) or allyl (**32**), the spermicidal activity was enhanced significantly (19 times) for **30** and marginally (1.7 fold) for **31** and **32** with respect to **33** while antifungal and antitrichomonal activity increased considerably (**30**).

Fluorescence labeling of sperm thiols

To study the mode of action of the most active compounds (**18** and **24**), free –SH groups were localized by fluorescence detec-

Table 1 Biological activity of compounds (9–33)

Compd	(9-20) –R^1	(21-32) –R^2	Antifungal activity[a] (MIC[d], mM) 1	2	3	4	5	6	Spermicidal activity (MEC, mM)	Anti-*Trichomonas* activity (MEC ± SE in mM)
9	$PhCH_2$–	H–	0.20	0.20	>0.11	0.20	>0.11	0.20	19.8	0.50 ± 0.055
10		H–	>0.11	>0.11	>0.11	>0.11	>0.11	>0.11	18.3	0.91 ± 0.009
11		H–	>0.11	>0.11	>0.11	>0.11	>0.11	>0.11	—	1.81 ± 0.15
12		H–	>0.11	>0.11	>0.11	>0.11	>0.11	>0.11	3.9	0.96 ± 0.05
13	$CH_3CH_2CH_2CH_2$–	H–	0.11	0.11	0.23	0.11	0.23	0.23	22.9	0.57 ± 0.37
14	$PhCH_2$–	$PhCH_2$–	>0.11	>0.11	>0.11	>0.11	>0.11	>0.11	—	—
15	$PhCH_2$–		0.009	0.002	0.14	0.14	0.14	0.018	14.3	0.71 ± 0.006
16	$PhCH_2$–	CH_2=$CHCH_2$–	>0.11	>0.11	>0.11	>0.11	>0.11	>0.11	3.4	1.71 ± 0.07
17	$PhCH_2$–	$CH_3CH_2CH_2CH_2$–	>0.11	>0.11	>0.11	>0.11	>0.11	>0.11	—	—
18	$PhCH_2$–		0.069	0.069	0.14	0.069	0.14	0.14	0.07	1.38 ± 0.087
19			>0.11	>0.11	>0.11	>0.11	>0.11	>0.11	26.9	0.67 ± 0.020
20	$CH_3CH_2CH_2CH_2$–		0.039	0.005	>0.11	>0.11	0.15	0.039	31.6	0.79 ± 0.067
21	$PhCH_2$–	$PhCH_2$–	>0.11	>0.11	>0.11	>0.11	>0.11	>0.11	11.9	1.19 ± 0.075
22	$PhCH_2$–	CH_2=$CHCH_2$–	>0.11	>0.11	>0.11	>0.11	>0.11	>0.11	13.6	1.36 ± 0.12
23	$PhCH_2$–	$CH_3CH_2CH_2CH_2$–	>0.11	>0.11	>0.11	>0.11	>0.11	>0.11	2.6	1.30 ± 0.095
24	$PhCH_2$–		>0.11	>0.11	>0.11	>0.11	>0.11	>0.11	0.06	0.14 ± 0.03
25			>0.11	>0.11	>0.11	>0.11	>0.11	>0.11	21.7	—
26			0.11	0.056	0.11	0.11	>0.11	0.11	22.4	0.56 ± 0.068
27		$OHCH_2CH_2$–	>0.11	>0.11	>0.11	>0.11	>0.11	>0.11	12.7	0.64 ± 0.038
28		CH_2=$CHCH_2$–	>0.11	>0.11	>0.11	>0.11	>0.11	>0.11	12.8	1.28 ± 0.10
29		$CH_3CH_2CH_2CH_2$–	>0.11	>0.11	>0.11	>0.11	>0.11	>0.11	12.3	1.23 ± 0.061
30	$CH_3CH_2CH_2CH_2$–		0.032	0.032	0.032	0.016	>0.11	0.032	2.6	0.08 ± 0.006
31	$CH_3CH_2CH_2CH_2$–	$OHCH_2CH_2$–	>0.11	>0.11	>0.11	>0.11	>0.11	>0.11	29.5	0.74 ± 0.026
32	$CH_3CH_2CH_2CH_2$–	CH_2=$CHCH_2$–	>0.11	>0.11	>0.11	>0.11	>0.11	>0.11	29.9	—
33[b]									50.4	—
N-9[c]									0.8	—
Metronidazole										0.018 ± 0.006
Fluconazole			0.003	0.006	0.006	>0.104	>0.104	0.006	—	—

[a] 1. *Candida albicans*; 2. *Cryptococcus neoformans*; 3. *Sporothrix schenckii*; 4. *Trichophyton mentagrophytes*; 5. *Aspergillus fumigates*; 6. *Candida parapsilosis* (ATCC-22019). [b] Prepared by known procedure.[23] [c] Spectrum Chemical Manufacturing Corp. (New Brunswick, N. J.). [d] Mean of three replicates, ±SE value ranged from 0.00 to 0.03 mM.

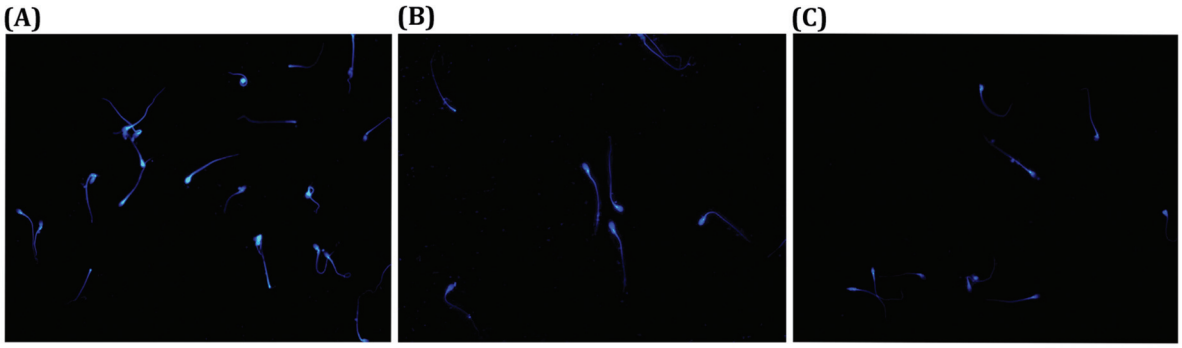


Fig. 2 Fluorescence due to free thiols on human sperm treated with (A) control, (B) compound **18**, (C) compound **24**.

tion (after labeling with the thiols capturing dye mBBr) of human sperm that were either motile (control) or immobilized by compounds (**18** and **24**) treatment, and digitally imaged for qualitative assessment. It became clearly evident by visual assessment of fluorescence intensities that control sperm (Fig. 2A) had remarkably higher number of free thiols as compared with sperm immobilized by compounds **18** and **24** (Fig. 2B and 2C). Even though the difference was marked throughout the structure of sperm, it was prominently noticeable in the tail region (principal piece). The diminished fluorescence of compounds (**18** and **24**) suggested the interaction with free thiol might be the mechanism of spermicidal action.

Conclusions

It may be inferred that a benzyl dithiocarbamate at a nitrogen of piperazine scaffold and a piperidinoethyl group (**18**) or an additional DTC group having piperidinoethyl group (**24**) at the other nitrogen were essential for sperm immobilization. The sodium dithiocarbamate group (**15**, **20**) seems to be desirable for high antifungal activity. The high activity of these compounds may be attributed to the interaction[13] of a dithiocarbamate group with free sulfhydryl groups present over sperm membrane[10] and *Trichomonas*.[10] The remarkable antifungal activity might be due to the interaction of the DTC group with Lanosterol 14α-demethylase (CYP-51), a prospective target in *Candida albicans*.[32–34] Compounds **18** and **24** of this series were found to be the most potent double-edged spermicides as these were 11–13 times and 720–840 times more potent than N-9 and **33**. These were also found to be much safer than N-9 in cytotoxicity assays. The mode of action of these compounds was imaged (Fig. 2) by their interaction with free thiols on human sperm using a fluorescent thiol probe and a fluorescence microscope. A diminished fluorescence as compared to control sperm suggested the sulfhydryl binding mechanism. Thus, novel scaffolds, 1-substituted piperazinecarbodithioate (**9–20**) and 1,4-(disubstituted) piperazinedicarbodithioate (**21–32**) have evolved along with unique dithiocarbamate chemistry. Further lead optimization is being carried out to arrive a better dually active spermicidal agents.

Experimental

Chemistry

In general, all reagents and solvents were commercial quality and were used without further purification. 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 and ^{13}C NMR spectra were recorded on Bruker Supercon Magnet Avance/DPX-300 spectrometers (300 MHz for ^1H; 50, 75, 100 MHz ^{13}C) in deuterated solvents with TMS as internal reference (chemical shifts δ in ppm, J in Hz.). Electrospray Ionisation Mass spectra (ESI-MS) were recorded on Thermo Lcq Advantage Max-IT and HR-DART MS were recorded on JEOL, JMS T100LC Accu TOF. 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.

Synthesis of *tert*-butyl 4-(benzylthiocarbonothioyl)piperazine-1-carboxylate (4). To the mixture of sodium 4-(*tert*-butoxycarbonyl)piperazine-1-carbodithioate (**3**, 10.0 g, 35.2 mmol), methanol (70.0 mL) and triethyl amine (7.33 mL, 52.1 mmol) was added benzyl chloride (4.0 g, 35.2 mmol) and stirred at room temperature for 3 h. The methanol from the reaction mixture obtained was evaporated under reduced pressure. EtOAc (60.0 mL) was added to the reaction mixture, and the resulting solid salt was filtered off. The filtrate was washed with water (2 × 20 mL). The organic layer was collected, dried over sodium sulfate and concentrated under reduced pressure to give the title compound (9.7 g, 78.5%) white solid; mp: 81–82 °C; IR (KBr) ν (cm^{-1}): 2977, 2928, 1689, 1557, 1460, 1223; ^1H NMR (300 MHz, $CDCl_3$): δ 7.40–7.24 (m, 5H), 4.57 (s, 2H), 4.47–3.79 (m, 4H), 3.54 (t, J = 5.2 Hz, 4H), 1.47 (s, 9H); ^{13}C (50 MHz, $CDCl_3$): δ 197.1 (C═S), 154.5 (C═O), 135.7, 129.4, 128.7, 127.7, 80.6, 50.1, 42.9, 42.2, 28.4; ESI-MS: m/z 353

(MH^+); Anal. calcd for $C_{17}H_{24}N_2O_2S_2$: C, 57.92; H, 6.86; N, 7.95; found, C, 58.14; H, 6.98; N, 8.17.

The compounds (**5–8**) were prepared using a procedure similar to that described for compound **4**.

***tert*-Butyl 4-((2-(piperidin-1-yl)ethylthio)carbonothioyl)-piperazine-1-carboxylate (5).** The title compound was synthesized from sodium 4-(*tert*-butoxycarbonyl)piperazine-1-carbodithioate (**3**) and 1-(2-chloroethyl)piperidine in 81.7% yield as off-white solid; mp: 92–94 °C; IR (KBr) ν (cm^{-1}): 2974, 2859, 1690, 1222; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.10–4.05 (m, 4H), 3.56–3.52 (m, 4H), 3.40–3.37 (m, 1H), 2.82–2.79 (m, 1H), 2.68–2.63 (m, 2H), 2.48 (bs, 4H), 1.59 (bs, 4H), 1.48 (s, 11H); ^{13}C (50 MHz, $CDCl_3$): δ 197.9 (C═S), 154.6 (C═O), 80.7, 57.6, 54.4, 42.8, 34.2, 28.5, 25.8, 24.3; ESI-MS: *m*/*z* 374 (MH^+); Anal. calcd for $C_{17}H_{31}N_3O_2S_2$: C, 54.66; H, 8.36; N, 11.25; found, C, 54.58; H, 8.49; N, 11.36.

***tert*-Butyl 4-((2-morpholinoethylthio)carbonothioyl)piperazine-1-carboxylate (6).** The title compound was synthesized from sodium 4-(*tert*-butoxycarbonyl)piperazine-1-carbodithioate (**3**) and 4-(2-chloroethyl)morpholine in 76.2% yield as white solid; mp: 118–119 °C; IR (KBr) ν (cm^{-1}): 2968, 2858, 1690, 1223; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.32–3.98 (m, 4H), 3.73–3.70 (m, 4H), 3.61–3.47 (m, 6H), 2.74–2.66 (m, 2H), 2.55–2.50 (m, 4H), 1.48 (s, 9H); ESI-MS: *m*/*z* 376 (MH^+); Anal. calcd for $C_{16}H_{29}N_3O_3S_2$: C, 51.17; H, 7.78; N, 11.19; found, C, 51.35; H, 7.61; N, 11.07.

***tert*-Butyl 4-((2-(pyrrolidin-1-yl)ethylthio)carbonothioyl)-piperazine-1-carboxylate (7).** The title compound was synthesized from sodium 4-(*tert*-butoxycarbonyl)piperazine-1-carbodithioate (**3**) and 1-(2-chloroethyl)pyrrolidine in 82.5% yield as light yellow solid; mp: 75–76 °C; IR (KBr) ν (cm^{-1}): 2973, 2802, 1689, 1221; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.13 (bs, 4H), 3.56–3.52 (m, 6H), 2.80 (t, J = 7.0 Hz, 2H), 2.60 (bs, 4H), 1.81–1.80 (m, 4H), 1.48 (s, 9H); ^{13}C (100 MHz, $CDCl_3$): δ 197.8 (C═S), 154.5 (C═O), 80.6, 54.8, 54.0, 50.1, 42.9, 36.0, 28.4, 23.5; ESI-MS: *m*/*z* 360 (MH^+); Anal. calcd for $C_{16}H_{29}N_3O_2S_2$: C, 53.45; H, 8.13; N, 11.69; found, C, 53.61; H, 8.27; N, 11.75.

***tert*-Butyl 4-(butylthiocarbonothioyl)piperazine-1-carboxylate (8).** The title compound was synthesized from sodium 4-(*tert*-butoxycarbonyl)piperazine-1-carbodithioate (**3**) and 1-bromobutane in 79.5% yield as white solid; mp: 78–79 °C; IR (KBr) ν (cm^{-1}): 2937, 2855, 1643, 1219; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.13 (bs, 4H), 3.56–3.53 (m, 4H), 3.32 (t, J = 7.4 Hz, 2H), 1.74–1.64 (m, 2H), 1.48 (s, 11H), 0.95 (t, J = 7.3 Hz, 3H); ^{13}C (100 MHz, $CDCl_3$): δ 198.1 (C═S), 154.5 (C═O), 80.5, 49.9, 42.9, 37.0, 30.6, 28.4, 22.1, 13.7; ESI-MS: *m*/*z* 319 (MH^+); Anal. calcd for $C_{14}H_{26}N_2O_2S_2$: C, 52.79; H, 8.23; N, 8.80; found, C, 52.57; H, 8.14; N, 8.73.

Synthesis of benzyl piperazine-1-carbodithioate (9). To the mixture of *tert*-butyl 4-(benzylthiocarbonothioyl)piperazine-1-carboxylate (**4**, 9.2 g, 26.2 mmol) and dichloromethane (60.0 mL) was added 16% TFA in dichloromethane at (0–5 °C) and stirred at room temperature for 6 h. A saturated solution of sodium bicarbonate was added to the reaction mixture at (0–5 °C), and stirred at room temperature for 4 h. The dichloromethane layer was separated and washed with water (2 × 10 mL). The organic layer was collected, dried over sodium sulfate, concentrated under reduced pressure and purified by column chromatography using silica (60–120 mesh) to give the title compound (**9**, 3.6 g, 54.5%) light yellow oil; IR (neat) ν (cm^{-1}): 3436, 2920, 2852, 1639, 1557, 1462, 1422, 1227; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39–7.23 (m, 5H), 4.57 (s, 2H), 4.33–3.91 (m, 4H), 2.92 (bs, 4H); ^{13}C (75 MHz, $CDCl_3$): δ 196.2 (C═S), 135.8, 129.3, 128.5, 127.4, 52.3, 45.6, 41.9; ESI-MS: *m*/*z* 253 (MH^+); Anal. calcd for $C_{12}H_{16}N_2S_2$: C, 57.10; H, 6.39; N, 11.10; found, C, 57.26; H, 6.45; N, 10.98.

An unusual side product benzyl 4-benzylpiperazine-1-carbodithioate (**14**, 1.5 g, 23.1%) was also isolated as semisolid by using column chromatography.

Benzyl 4-benzylpiperazine-1-carbodithioate (14). White semisolid; IR (KBr) ν (cm^{-1}): 2923, 2855, 1646, 1538, 1462, 1421, 1224; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39–7.25 (m, 10H), 4.56 (s, 2H), 4.32–3.92 (m, 4H), 3.53 (s, 2H), 2.94 (bs, 1H), 2.52 (bs, 3H); ^{13}C (50 MHz, $CDCl_3$): δ 196.4 (C═S), 137.3, 135.9, 129.4, 129.2, 128.6, 128.4, 127.6, 127.5, 62.5, 52.4, 45.6, 42.2; HRMS *m*/*z* calcd for $C_{19}H_{22}N_2S_2$ (MH^+): 343.1224; found 343.1258; Anal. calcd for $C_{19}H_{22}N_2S_2$: C, 66.62; H, 6.47; N, 8.18; found, C, 66.46; H, 6.52; N, 8.31.

Compounds **10–13** were prepared using the procedure similar to that described for compound **9**.

2-(Piperidin-1-yl)ethyl piperazine-1-carbodithioate (10). The title compound was synthesized from *tert*-butyl 4-((2-(piperidin-1-yl)ethylthio)carbonothioyl)piperazine-1-carboxylate (**5**), TFA and sodium bicarbonate in 85.7% yield as light brown solid; mp: 66–67 °C; IR (KBr) ν (cm^{-1}): 3441, 2970, 2836, 1688, 1220; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.49–3.94 (m, 4H), 3.50 (t, J = 7.5 Hz, 2H), 2.96–2.93 (m, 4H), 2.67 (t, J = 7.5 Hz, 2H), 2.51 (bs, 4H), 1.62–1.59 (m, 4H), 1.46–1.44 (m, 2H); ^{13}C (100 MHz, $CDCl_3$): δ 196.8 (C═S), 57.6, 54.3, 52.4, 51.6, 45.7, 33.8, 25.7, 24.2; ESI-MS: *m*/*z* 274 (MH^+); Anal. calcd for $C_{12}H_{23}N_3S_2$: C, 52.71; H, 8.48; N, 15.37; found, C, 52.59; H, 8.32; N, 15.19.

2-Morpholinoethyl piperazine-1-carbodithioate (11). The title compound was synthesized from *tert*-butyl 4-((2-morpholinoethylthio)carbonothioyl)piperazine-1-carboxylate (**6**), TFA and sodium bicarbonate in 84.2% yield as white solid; mp: 54–55 °C; IR (KBr) ν (cm^{-1}): 3402, 2963, 2846, 1638, 1224; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.31–3.87 (m, 4H), 3.74–3.71 (m, 4H), 3.52–3.43 (m, 3H), 3.03–3.00 (m, 3H), 2.71–2.66 (m, 2H), 2.54 (bs, 4H); ESI-MS: *m*/*z* 276 (MH^+); Anal. calcd for $C_{11}H_{21}N_3OS_2$: C, 47.97; H, 7.68; N, 15.26; found, C, 47.76; H, 7.57; N, 15.19.

2-(Pyrrolidin-1-yl)ethyl piperazine-1-carbodithioate (12). The title compound was synthesized from *tert*-butyl 4-((2-(pyrrolidin-1-yl)ethylthio)carbonothioyl)piperazine-1-carboxylate (**7**), TFA and sodium bicarbonate in 81.6% yield as light yellow oil; IR (neat) ν (cm^{-1}): 3437, 2923, 2851, 1639, 1219; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.31–4.00 (m, 4H), 3.52 (t, J = 7.1 Hz, 2H), 2.96–2.94 (m, 4H), 2.80 (t, J = 7.1 Hz, 2H), 2.60 (bs, 4H), 1.91–1.80 (m, 4H); ESI-MS: *m*/*z* 260 (MH^+); Anal. calcd for $C_{11}H_{21}N_3S_2$: C, 50.93; H, 8.16; N, 16.20; found, C, 50.82; H, 8.05; N, 16.32.

Butyl piperazine-1-carbodithioate (13). The title compound was synthesized from *tert*-butyl 4-(butylthiocarbonothioyl) piperazine-1-carboxylate (**8**), TFA and sodium bicarbonate in 87.0% yield as colourless oil; IR (neat) ν (cm^{-1}): 3436, 2927, 2863, 1641, 1227; ^1H NMR (300 MHz, $CDCl_3$): δ 4.28–3.98 (m, 4H), 3.32 (t, J = 7.4 Hz, 2H), 2.96–2.93 (m, 4H), 1.74–1.64 (m, 2H), 1.51–1.39 (m, 2H), 0.95 (t, J = 7.3 Hz, 3H); ^{13}C (100 MHz, $CDCl_3$): δ 197.4 (C═S), 51.8, 45.7, 36.9, 30.7, 22.2, 13.7; ESI-MS: m/z 219 (MH^+); Anal. calcd for $C_9H_{18}N_2S_2$: C, 49.50; H, 8.31; N, 12.83; found, C, 49.68; H, 8.45; N, 12.92.

Synthesis of sodium 4-(benzylthiocarbonothioyl)piperazine-1-carbodithioate (15). Benzyl piperazine-1-carbodithioate (**9**, 3.61 g, 14.3 mmol) was dissolved in ethyl acetate (75 mL) and to this, aqueous sodium hydroxide (0.86 g, 21.4 mmol, 30%) was added whilst keeping the temperature below 5 °C. Carbon disulfide (1.2 mL, 28.6 mmol) dissolved in ethyl acetate (20 mL) was added dropwise with stirring below 5 °C. The reaction mixture was stirred at room temperature for a further 10 h to furnish a white solid. The solvent was distilled off and the crude was recrystallised in methanolic ether to obtain sodium 4-(benzylthiocarbonothioyl)piperazine-1-carbodithioate (83.7%) as a white powder; mp: >250 °C; IR (KBr) ν (cm^{-1}): 2918, 2850, 1643, 1509, 1468, 1417, 1221; ^1H NMR (300 MHz, DMSO-d_6): δ 7.13–7.04 (m, 5H), 4.29–4.28 (m, 2H), 4.15 (bs, 2H), 3.92–3.87 (m, 2H), 3.63–3.57 (m, 4H); ESI-MS: m/z 351 (MH^+); Anal. calcd for $C_{13}H_{15}N_2NaS_4$: C, 44.54; H, 4.31; N, 7.99; found, C, 44.36; H, 4.22; N, 7.87.

The following compounds (**19–20**) were prepared using a procedure similar to that described for compound (**15**).

Sodium 4-((2-(piperidin-1-yl)ethylthio)carbonothioyl)piperazine-1-carbodithioate (19). The title compound was synthesized from 2-(piperidin-1-yl)ethyl piperazine-1-carbodithioate (**10**), carbon disulfide and sodium hydroxide in 85.4% yield as white solid; mp: 205–206 °C; IR (KBr) ν (cm^{-1}): 2932, 2867, 1641, 1215; ^1H NMR (300 MHz, DMSO-d_6): δ 4.15 (bs, 4H), 3.92–3.67 (m, 4H), 3.14 (t, J = 7.2 Hz, 2H), 2.26 (bs, 3H), 2.14 (bs, 3H), 1.23–1.11 (m, 6H); ESI-MS: m/z 372 (MH^+); Anal. calcd for $C_{13}H_{22}N_3NaS_4$: C, 42.02; H, 5.97; N, 11.31; found, C, 42.14; H, 6.07; N, 11.45.

Sodium 4-(butylthiocarbonothioyl)piperazine-1-carbodithioate (20). The title compound was synthesized from butyl piperazine-1-carbodithioate (**13**), carbondisulfide and sodiumhydroxide in 89.2% yield as white solid; mp: >250 °C; IR (KBr) ν (cm^{-1}): 2962, 2931, 1638, 1216; ^1H NMR (300 MHz, DMSO-d_6): δ 4.39 (bs, 4H), 4.16–3.90 (m, 4H), 3.24 (t, J = 7.4 Hz, 2H), 1.63–1.56 (m, 2H), 1.41–1.33 (m, 2H), 0.88 (t, J = 7.3 Hz, 3H); ESI-MS: m/z 317 (MH^+); Anal. calcd for $C_{10}H_{17}N_2NaS_4$: C, 37.95; H, 5.41; N, 8.85; found, C, 38.12; H, 5.55; N, 8.97.

Synthesis of benzyl 4-allylpiperazine-1-carbodithioate (16). To the mixture of sodium 4-(benzylthiocarbonothioyl)-piperazine-1-carbodithioate (**15**, 0.58 g, 1.64 mmol), methanol (20.0 mL) and triethyl amine (0.34 mL, 2.46 mmol) was added 3-bromoprop-1-ene (0.14 g, 1.64 mmol) and stirred at room temperature for 3 h. Methanol from the reaction mixture obtained was evaporated under reduced pressure. EtOAc (15.0 mL) was added to the reaction mixture, and the solid salt was filtered off. The filtrate was washed with water (2 × 5 mL). The organic layer was collected, dried over sodium sulfate, concentrated under reduced pressure and purified by column chromatography using silica (60–120 mesh) to give the usual product 1-allyl 4-benzyl piperazine-1,4-bis(carbodithioate) (**22**, 0.32 g, 52%) white solid (mp: 67–68 °C) with title compound (**16**, 0.14 g, 23.8%) an unusual side product as brown semi solid; IR (KBr) ν (cm^{-1}): 2922, 2846, 1641, 1564, 1462, 1422, 1223; ^1H NMR (300 MHz, $CDCl_3$): δ 7.40–7.26 (m, 5H), 5.91–5.78 (m, 1H), 5.24–5.18 (m, 2H), 4.57 (s, 2H), 4.41–3.94 (m, 4H), 3.03 (d, J = 6.6 Hz, 2H), 2.54 (bs, 4H); ^{13}C (50 MHz, $CDCl_3$): δ 196.4 (C═S), 135.9, 134.0, 129.4, 128.6, 127.5, 118.9, 61.1, 52.3, 50.7, 42.2; HRMS m/z calcd for $C_{15}H_{20}N_2S_2$ (MH^+): 293.1146; found 293.1141; Anal. calcd for $C_{15}H_{20}N_2S_2$: C, 61.60; H, 6.89; N, 9.58; found, C, 61.48; H, 6.74; N, 9.46.

The unusual side products (**14**, **17** and **18**) were isolated using a procedure similar to that described for compound (**16**) with usual products (**21**, **23** and **24**). Compounds (**25–32**) were also synthesized by a similar method.

Benzyl 4-butylpiperazine-1-carbodithioate (17). The title compound was synthesized from sodium 4-(benzylthiocarbonothioyl)piperazine-1-carbodithioate (**15**) and 1-bromobutane in 25.7% yield as yellow oil; IR (neat) ν (cm^{-1}): 2953, 2814, 1632, 1553, 1465, 1425, 1220; ^1H NMR (300 MHz, $CDCl_3$): δ 7.39–7.25 (m, 5H), 4.56 (s, 2H), 4.38–3.92 (m, 4H), 2.50 (bs, 4H), 2.37–2.34 (m, 2H), 1.50–1.43 (m, 2H), 1.38–1.29 (m, 2H), 0.92 (t, J = 5.5 Hz, 2H); ^{13}C (50 MHz, $CDCl_3$): δ 196.3 (C═S), 136.0, 129.4, 128.6, 127.5, 57.9, 52.6, 42.1, 29.0, 20.6, 14.0; ESI-MS: m/z 309 (MH^+); Anal. calcd for $C_{16}H_{24}N_2S_2$: C, 62.29; H, 7.84; N, 9.08; found, C, 62.36; H, 7.92; N, 9.23.

Benzyl 4-(2-(piperidin-1-yl)ethyl) piperazine-1-carbodithioate (18). The title compound was synthesized from sodium 4-(benzylthiocarbonothioyl)piperazine-1-carbodithioate (**15**) and 2-chloroethylpiperidine in 28.5% yield as light yellow solid; mp: 78–79 °C; IR (KBr) ν (cm^{-1}): 2927, 2853, 1639, 1526, 1452, 1404, 1210; ^1H NMR (300 MHz, $CDCl_3$): δ 7.38–7.24 (m, 5H), 4.56 (s, 2H), 4.33–3.93 (m, 4H), 2.56–2.45 (m, 12H), 1.61–1.58 (m, 4H), 1.45–1.44 (m, 2H); ^{13}C (50 MHz, $CDCl_3$): δ 196.4 (C═S), 135.9, 129.4, 128.6, 127.6, 56.4, 55.2, 55.0, 53.0, 50.8, 50.0, 42.2, 25.6, 24.1; HRMS m/z calcd for $C_{19}H_{29}N_3S_2$ (MH^+): 364.1881; found 364.1875; Anal. calcd for $C_{19}H_{29}N_3S_2$: C, 62.77; H, 8.04; N, 11.56; found, C, 62.56; H, 7.91; N, 11.42.

Dibenzyl piperazine-1,4-bis(carbodithioate) (21). The title compound was synthesized from sodium 4-(benzylthiocarbonothioyl)piperazine-1-carbodithioate (**15**) and benzyl chloride in 51.2% yield as white solid; mp: 124–126 °C; IR (KBr) ν (cm^{-1}): 2923, 2851, 1640, 1562, 1455, 1401, 1215; ^1H NMR (300 MHz, $CDCl_3$): δ 7.39–7.24 (m, 10H), 4.57 (s, 4H), 4.32–4.20 (m, 8H); ^{13}C (75 MHz, $CDCl_3$): δ 197.5 (C═S), 135.6, 129.4, 128.7, 127.8, 48.7, 42.2; HRMS m/z calcd for $C_{20}H_{22}N_2S_4$ (MH^+): 419.0744; found 419.0754; Anal. calcd for $C_{20}H_{22}N_2S_4$: C, 57.38; H, 5.30; N, 6.69; found, C, 57.56; H, 5.45; N, 6.78.

1-Allyl 4-benzyl piperazine-1,4-bis(carbodithioate) (22). The title compound was synthesized from sodium 4-(benzylthiocarbonothioyl)piperazine-1-carbodithioate (**15**) and 3-bromoprop-1-ene in 52.0% yield as off-white solid; mp: 67–68 °C; IR (KBr)

ν (cm^{-1}): 2979, 2857, 1638, 1539, 1458, 1406, 1213; ^1H NMR (300 MHz, $CDCl_3$): δ 7.40–7.25 (m, 5H), 5.99–5.85 (m, 1H), 5.36–5.16 (m, 2H), 4.58 (s, 2H), 4.23–4.21 (m, 8H), 4.01 (d, J = 7.0 Hz, 2H); ^{13}C (50 MHz, $CDCl_3$): δ 197.4 (C═S), 197.2 (C═S), 135.6, 132.1, 129.4, 128.7, 127.7, 119.1, 48.6, 42.1, 40.3; ESI-MS: m/z 369 (MH^+); Anal. calcd for $C_{16}H_{20}N_2S_4$: C, 52.13; H, 5.47; N, 7.60; found, C, 52.26; H, 5.61; N, 7.72.

1-Benzyl 4-butyl piperazine-1,4-bis(carbodithioate) (23). The title compound was synthesized from sodium 4-(benzylthiocarbonothioyl)piperazine-1-carbodithioate (**15**) and 1-bromobutane in 56.3% yield as off-white solid; mp: 63–64 °C; IR (KBr) ν (cm^{-1}): 2965, 2812, 1639, 1543, 1456, 1401, 1213; ^1H NMR (300 MHz, $CDCl_3$): δ 7.39–7.25 (m, 5H), 4.58 (s, 2H), 4.25 (bs, 8H), 3.32 (t, J = 7.4 Hz, 2H), 1.74–1.64 (m, 2H), 1.51–1.39 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H); ^{13}C (50 MHz, $CDCl_3$): δ 198.3 (C═S), 197.3 (C═S), 135.6, 129.3, 128.6, 127.7, 48.5, 42.1, 37.0, 30.5, 22.1, 13.7; HRMS m/z calcd for $C_{17}H_{24}N_2S_4$ (MH^+): 385.0901; found 385.0895; Anal. calcd for $C_{17}H_{24}N_2S_4$: C, 53.08; H, 6.29; N, 7.28; found, C, 52.86; H, 6.11; N, 7.43.

1-Benzyl 4-(2-(piperidin-1-yl)ethyl)piperazine-1,4-bis(carbodithioate) (24). The title compound was synthesized from sodium 4-(benzylthiocarbonothioyl)piperazine-1-carbodithioate (**15**) and 2-chloroethylpiperidine in 49.7% yield as off-white solid; mp: 97–98 °C; IR (KBr) ν (cm^{-1}): 2933, 2851, 1640, 1552, 1464, 1425, 1226; ^1H NMR (300 MHz, $CDCl_3$): δ 7.40–7.26 (m, 5H), 4.58 (s, 2H), 4.39–4.27 (m, 8H), 3.51 (t, J = 7.3 Hz, 2H), 2.67 (t, J = 7.3 Hz, 2H), 2.50 (bs, 4H), 1.60–1.59 (m, 4H), 1.46–1.44 (m, 2H); ^{13}C (50 MHz, $CDCl_3$): δ 198.2 (C═S), 197.5 (C═S), 135.6, 129.4, 128.7, 127.8, 57.4, 54.4, 48.7, 42.2, 34.4, 25.9, 24.3; HRMS m/z calcd for $C_{20}H_{29}N_3S_4$ (MH^+): 440.1323; found 440.1315; Anal. calcd for $C_{20}H_{29}N_3S_4$: C, 54.63; H, 6.65; N, 9.56; found, C, 54.51; H, 6.42; N, 9.45.

Bis(2-(piperidin-1-yl)ethyl) piperazine-1,4-bis(carbodithioate) (25). The title compound was synthesized from sodium 4-((2-(piperidin-1-yl)ethylthio)carbonothioyl)piperazine-1-carbodithioate (**19**) and 2-chloroethylpiperidine in 79.1% yield as white solid; mp: 133–135 °C; IR (KBr) ν (cm^{-1}): 2926, 2857, 1658, 1249; ^1H NMR (300 MHz, $CDCl_3$): δ 4.28 (bs, 8H), 3.50 (t, J = 7.3 Hz, 4H), 2.65 (t, J = 7.3 Hz, 4H), 2.49–2.47 (m, 8H), 1.61–1.44 (m, 12H); ^{13}C (50 MHz, $CDCl_3$): δ 198.2 (C═S), 57.5, 54.4, 48.7, 34.5, 26.0, 24.3; HRMS m/z calcd for $C_{20}H_{36}N_4S_4$ (MH^+): 461.1901; found 461.1910; Anal. calcd for $C_{20}H_{36}N_4S_4$: C, 52.13; H, 7.87; N, 12.16; found, C, 52.36; H, 7.62; N, 12.05.

1-(2-(Piperidin-1-yl)ethyl)4-(2-(pyrrolidin-1-yl)ethyl)piperazine-1,4-bis(carbodithioate) (26). The title compound was synthesized from sodium 4-((2-(piperidin-1-yl)ethylthio)-carbonothioyl)piperazine-1-carbodithioate (**19**) and 2-chloroethylpyrrolidine in 82.7% yield as yellow solid; mp: 136–138 °C; IR (KBr) ν (cm^{-1}): 2927, 2801, 1640, 1227; ^1H NMR (300 MHz, $CDCl_3$): δ 4.28–4.18 (m, 8H), 3.54–3.47 (m, 4H), 2.80 (t, J = 7.0 Hz, 2H), 2.65 (t, J = 7.3 Hz, 2H), 2.59 (bs, 4H), 2.49–2.47 (m, 4H), 1.85–1.80 (m, 4H), 1.61–1.43 (m, 6H); ^{13}C (50 MHz, $CDCl_3$): δ 198.2 (C═S), 57.4, 54.7, 54.4, 54.1, 48.8, 36.3, 34.4, 25.9, 24.3, 23.5; ESI-MS: m/z 447 (MH^+); Anal. calcd for $C_{19}H_{34}N_4S_4$: C, 51.08; H, 7.67; N, 12.54; found, C, 51.26; H, 7.82; N, 12.65.

1-(2-Hydroxyethyl)4-(2-(piperidin-1-yl)ethyl)piperazine-1,4-bis(carbodithioate) (27). The title compound was synthesized from sodium 4-((2-(piperidin-1-yl)ethylthio)carbonothioyl)-piperazine-1-carbodithioate (**19**) and 2-bromoethanol in 76.3% yield as white solid; mp: 75–76 °C; IR (KBr) ν (cm^{-1}): 3436, 2930, 2851, 1640, 1217; ^1H NMR (300 MHz, $CDCl_3$): δ 4.51–4.20 (m, 8H), 3.90 (t, J = 5.9 Hz, 2H), 3.61 (t, J = 5.9 Hz, 2H), 3.52 (t, J = 7.4 Hz, 2H), 3.05 (bs, 1H), 2.69 (t, J = 7.4 Hz, 2H), 2.52 (bs, 4H), 1.62–1.60 (m, 4H), 1.47–1.41 (m, 2H); ^{13}C (50 MHz, $CDCl_3$): δ 198.0 (C═S), 197.9 (C═S), 61.2, 57.4, 54.3, 48.8, 39.5, 34.0, 29.7, 25.6, 24.2; HRMS m/z calcd for $C_{15}H_{27}N_3OS_4$ (MH^+): 394.1115; found 394.1120; Anal. calcd for $C_{15}H_{27}N_3OS_4$: C, 45.77; H, 6.91; N, 10.67; found, C, 45.62; H, 6.85; N, 10.79.

1-Allyl 4-(2-(piperidin-1-yl)ethyl)piperazine-1,4-bis(carbodithioate) (28). The title compound was synthesized from sodium 4-((2-(piperidin-1-yl)ethylthio)carbonothioyl)piperazine-1-carbodithioate (**19**) and 3-bromoprop-1-ene in 79.8% yield as off-white solid; mp: 88–89 °C; IR (KBr) ν (cm^{-1}): 2932, 2802, 1637, 1210; ^1H NMR (300 MHz, $CDCl_3$): δ 5.99–5.86 (m, 1H), 5.36–5.17 (m, 2H), 4.27 (bs, 8H), 4.02 (d, J = 6.9 Hz, 2H), 3.50 (t, J = 7.3 Hz, 2H)), 2.65 (t, J = 7.3 Hz, 2H), 2.47 (bs, 4H), 1.60–1.57 (m, 4H), 1.45–1.44 (m, 2H); ^{13}C (75 MHz, $CDCl_3$): δ 198.1 (C═S), 197.1 (C═S), 132.1, 119.0, 57.3, 54.3, 48.8, 40.2, 34.3, 25.8, 24.2; ESI-MS: m/z 390 (MH^+); Anal. calcd for $C_{16}H_{27}N_3S_4$: C, 49.32; H, 6.98; N, 10.78; found, C, 49.51; H, 7.12; N, 10.85.

1-Butyl 4-(2-(piperidin-1-yl)ethyl)piperazine-1,4-bis(carbodithioate) (29). The title compound was synthesized from sodium 4-((2-(piperidin-1-yl)ethylthio)carbonothioyl)piperazine-1-carbodithioate (**19**) and 1-bromobutane in 82.5% yield as light yellow solid; mp: 91–92 °C; IR (KBr) ν (cm^{-1}): 2935, 2865, 1638, 1218; ^1H NMR (300 MHz, $CDCl_3$): δ 4.40–4.14 (m, 8H), 3.53 (t, J = 7.3 Hz, 2H), 3.32 (t, J = 7.4 Hz, 2H), 2.69 (t, J = 7.3 Hz, 2H), 2.52 (bs, 4H), 1.75–1.60 (m, 6H), 1.52–1.42 (m, 4H), 0.95 (t, J = 7.3 Hz, 3H); ^{13}C (50 MHz, $CDCl_3$): δ 198.4 (C═S), 198.2 (C═S), 57.4, 54.4, 48.6, 37.1, 34.3, 30.6, 25.8, 24.3, 22.2, 13.8; HRMS m/z calcd for $C_{17}H_{31}N_3S_4$ (MH^+): 406.1479; found 406.1473; Anal. calcd for $C_{17}H_{31}N_3S_4$: C, 50.33; H, 7.70; N, 10.36; found, C, 50.10; H, 7.59; N, 10.21.

1-Butyl 4-(2-(pyrrolidin-1-yl)ethyl)piperazine-1,4-bis(carbodithioate) (30). The title compound was synthesized from sodium 4-(butylthiocarbonothioyl)piperazine-1-carbodithioate (**20**) and 2-chloroethylpyrrolidine in 85.2% yield as yellow solid; mp: 75–76 °C; IR (KBr) ν (cm^{-1}): 2922, 2865, 1642, 1211; ^1H NMR (300 MHz, $CDCl_3$): δ 4.26 (bs, 8H), 3.53 (t, J = 7.1 Hz, 2H), 3.32 (t, J = 7.4 Hz, 2H), 2.82 (t, J = 7.1 Hz, 2H), 2.61 (bs, 4H), 1.81 (4H, bs), 1.75–1.65 (m, 2H), 1.52–1.39 (m, 2H), 0.95 (t, J = 7.3 Hz, 3H); HRMS m/z calcd for $C_{16}H_{29}N_3S_4$ (MH^+): 392.1323; found 392.1315; Anal. calcd for $C_{16}H_{29}N_3S_4$: C, 49.06; H, 7.46; N, 10.73; found, C, 49.23; H, 7.62; N, 10.85.

1-Butyl 4-(2-hydroxyethyl)piperazine-1,4-bis(carbodithioate) (31). The title compound was synthesized from sodium 4-(butylthiocarbonothioyl)piperazine-1-carbodithioate (**20**) and 2-bromoethanol in 84.1% yield as white solid; mp: 78–79 °C; IR (KBr) ν (cm^{-1}): 3437, 2922, 2851, 1634, 1219; ^1H NMR (300 MHz, $CDCl_3$): δ 4.29 (bs, 8H), 3.90 (t, J = 5.9 Hz, 2H), 3.61

(t, J = 5.9 Hz, 2H), 3.33 (t, J = 7.4 Hz, 2H), 2.33 (bs, 1H), 1.75–1.65 (m, 2H), 1.52–1.39 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H); ^{13}C (50 MHz, $CDCl_3$): δ 198.4 (C═S), 197.7 (C═S), 61.3, 48.5, 39.3, 37.1, 30.5, 22.1, 13.7; ESI-MS: m/z 339 (MH^+); Anal. calcd for $C_{12}H_{22}N_2OS_4$: C, 42.57; H, 6.55; N, 8.27; found, C, 42.75; H, 6.68; N, 8.39.

1-Allyl 4-butyl piperazine-1,4-bis(carbodithioate) (32). The title compound was synthesized from sodium 4-(butylthiocarbonothioyl)piperazine-1-carbodithioate (**20**) and 3-chloroprop-1-ene in 78.1% yield as off-white solid; mp: 59–60 °C; IR (KBr) ν (cm^{-1}): 2963, 2870, 1639, 1211; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.10–5.86 (m, 1H), 5.36–5.17 (m, 2H), 4.27 (bs, 8H), 4.03 (d, J = 7.0 Hz, 2H), 3.33 (t, J = 7.4 Hz, 2H), 1.75–1.62 (m, 2H), 1.52–1.42 (m, 2H), 0.95 (t, J = 7.3 Hz, 3H); ^{13}C (75 MHz, $CDCl_3$): δ 198.5 (C═S), 197.3 (C═S), 132.2, 119.1, 48.7, 40.3, 37.1, 30.6, 22.2, 13.7 ; HRMS m/z calcd for $C_{13}H_{22}N_2S_4$ (MH^+): 335.0744; found 335.0741; Anal. calcd for $C_{13}H_{22}N_2S_4$: C, 46.67; H, 6.63; N, 8.37; found, C, 46.85; H, 6.77; N, 8.49.

Biology

Spermicidal activity[11]

The spermicidal assay was adapted from a standard procedure. 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% test solution. 0.05 mL of liquefied human semen was added to 0.25 mL of test solution and vortexed for 10 seconds 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 60 seconds and recorded (Table 1). The lowest concentration of compound which immobilized 100% human sperm irreversibly in all the three semen samples from different individuals is given in Table 1.

Antifungal activity[13]

The MICs of compounds were determined by broth microdilution technique as per the guidelines of the 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 × 10^3 CFU mL^{-1}. Micro titer plates were incubated at 35 °C. MICs were recorded after 48 h of incubation (Table 1).

Antitrichomonal activity[10]

Trichomonas vaginalis parasites to be used in drug susceptibility assays were grown in TYM medium supplemented with 10% FCS, vitamin mixture and 100 U mL^{-1} penicillin/streptomycin, at 37 °C in 15 mL tubes for one day, followed by regular subculturing, and were in the log phase of growth. The cultures routinely attained a concentration of 2 × 10^7 cells mL^{-1} in 48 h. Inoculums of 1 × 10^4 cells per tube were used for maintenance of the culture. *In vitro* drug susceptibility assays were carried out using the standard procedure. Stock solutions (100 μg mL^{-1}) 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^{-1} in 48-well plates. DMSO/TYM was used as vehicle in control wells. Parasites (5 × 10^4 trophozoites L^{-1}) 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 a microscope at 40× magnification. Viability of the cells was determined by trypan blue exclusion assay. Minimum conc. of the test agent at which all cells were found dead in 48 h was considered its MIC. The experiment was repeated three times to confirm the MIC (Table 1).

Cytotoxicity of compounds toward human cervical (HeLa) cells[10]

HeLa cells seeded at a density of 5 × 10^4 per well in 96-well plates were incubated with either the culture medium containing dilutions of test compounds, or vehicle (control), for 24 h. Thereafter 5 μL of MTT solution (5 mg mL^{-1} in buffer, pH 7.4) was added to each well. The formazan crystals formed inside the viable cells were solubilized in DMSO, and the optical density at 540 nm (OD_{540}) was recorded in a microplate reader (Microquant; BioTek, USA).

Compatibility of compounds with *Lactobacillus*[10]

Spores of *Lactobacillus jensenii* (ATCC 25258, strain 62G) were procured from ATCC and grown in 6% Rogosa SL broth medium (Hi Media, India) containing 0.132% acetic acid at 37 °C in microwell plates. Serial dilutions of test compounds were added to experimental wells, and vehicle was added to control wells in triplicate. Approximately 1000 CFU of *L. jensenii* were inoculated into each well. The plates were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 h. At the end of the experiment, the cultures were mixed thoroughly, 100 μL from each well was transferred to the corresponding well of a 96-well plate, and the numbers of lactobacilli were estimated by measuring the turbidity (OD_{610}) in a microplate reader.

Fluorescent labeling of sperm thiols

Free thiols on human sperm (after treatment with vehicle, and two most promising compounds **18** and **24**) were examined and imaged using a fluorescence microscope, after labeling with the thiols capturing dye mBBr. The semen sample (0.5 mL) was treated with 2.5 mL of compound **18** and **24** at MEC, as well as equal volume of saline (Control) in parallel and incubated for 15 min at room temperature. After incubation, sperm were pelleted at 700*g* for 10 min and washed 2–3 times with fresh PBS. To the pelleted sperm in 1 mL PBS, 0.5 mM (final concentration) mBBr was added and incubated for 15 min in the dark. After incubation the sperm was pelleted and washed with PBS, finally dissolved in 200 μL PBS. A drop of this sample was then put on a microscope slide, covered with a cover glass and imaged using the UV1A filter on a Nikon Eclipse 80i microscope equipped with epifluorescence

illumination. Exposure times were the same for all samples. The experimental results have been given in Fig. 2.

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