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Synthesis of disulfide esters of dialkylaminocarbothioic acid as potent, non-detergent spermicidal agents☆

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Abstract—*S*,*S*′-[disulfanediylbis(dialkylaminopropane-2,1-diyl)]bis- (dialkylaminothiocarbamate) (**14**–**31**) were prepared and evaluated for the spermicidal activity and antifungal activity. Dialkyldithiocarbamates (**1**–**5**) were reacted with epichlorohydrin to give 1-dialkylaminocarbothioic acid *S*-[(2,3-epithio)propyl]ester (**7**–**11**), these on further reaction with a secondary amine gave *S*,*S*′-[disulfanediylbis(dialkylaminopropane-2,1-diyl)]bis- (dialkylaminothiocarbamate) (**14**–**31**). Some of these compounds (**16**, **19**–**21**, **23**, **30**, **31**) were found to be very potent spermicidal agents with marginal antifungal activity. Two compounds (**20**, **21**) were 25 times more active than nonoxynol-9 (N-9), the spermicide currently in the market.

1. Introduction

The global epidemics of unwanted pregnancies,[1] sexually transmitted diseases (STDs)[2,3] (including HIV[4]), and opportunistic *Candida* infection[5] have left women very limited options. While unwanted pregnancy has greater repercussions on female than male, the STD and HIV infections spread more rapidly from men to women than from women to men.[6] A condom, if used correctly and consistently, provides adequate dual protection against unwanted pregnancy as well as STD(s) and HIV. However, in many societies women do not have enough power to negotiate condom use with their partners, and are routinely subjected to nonconsensual coercive sex.[7] Hence, novel, women-controlled techniques of dual protection which are easy to use, safe, and affordable are required urgently. Microbicides can partly solve this problem by preventing or at least significantly reducing the transmission of STDs including HIV, but microbicides lacking contraceptive activity may not be used with the required compliance during most of the sexual contacts, especially during the ''most vulnerable'' sexual contacts (amongst promiscuous adults and adolescents) where the immediate worry of an unwanted pregnancy often overwhelms the bigger fret of STD and/or HIV acquisition.[8] The utility of microbicides in reproductive health of women has been reviewed recently.[9–11]

Vaginal contraceptive products have been available for many years and usually comprise of the membrane surfactant nonoxynol-9 (N-9) as one of the main ingredients.[12] However, the major drawback of using N-9 or other surfactants is their detergent-type cytotoxicity toward vaginal cells that has shown to increase the risk of HIV/STI transmission,[13–15] despite them possessing potent microbicidal activity in vitro.[16] Besides, N-9 is also known to inactivate Lactobacilli, which further enhances the chances of HIV/STI transmission.[17] Therefore, the development of spermicidal microbicides

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lacking detergent type membrane toxicity may offer a significant clinical advantage over the currently marketed products.[18]

The regulatory role of sulfhydryl (SH) groups in modulation of sperm membrane conformations is well established[19] and these free SH-groups are important for sperm motility and metabolism.[20] Evidences are also available for the involvement of these surface thiols in normal sperm functioning.[21] Thus compounds interacting with surface thiol groups may be detrimental for sperm as reported earlier for *N*-ethylmaleimide, a specific sulfhydryl alkylating agent.[22,23] Similarly SSRI antidepressants, which are known to bind with serotonin transporters by interaction with sulfhydryl groups,[22] exhibit spermicidal activity.[24] The inter-conversion of thiol groups to disulfide is an important biochemical event for the survival of sperm[25] under oxidative stress. Moreover, several antimicrobial peptides[26] with intramolecular disulfide linkages exhibit their activity against pathogens by channel formation in cell membrane and their reduced (thiol) form causes acute membrane permeabilization. Thus sulfhydryl-binding compounds with disulfide group may exhibit spermicidal as well as antimicrobial activity.

These observations prompted us to synthesize and evaluate the spermicidal and anti*candida* activities of various *S*,*S*′-[disulfandiyl bis(dialkylaminopropane-2,1-diyl) bis(dialkylaminothiocarbamate) (I, Fig. 1). The thiocarbamate group was also introduced into this structure as its microbicidal properties are documented[27,28] and bis(dimethylthiocarbamoyl)disulfide[29] has been used along with other compounds in microbicidal preparations. The details of our studies with respect to the synthesis, their spermicidal and antifungal activities, and the structure–activity relationship are presented here.

2. Results and discussion

2.1. Chemistry

Sodium dialkyl dithiocarbamates (**1**–**5**) were synthesized from the respective secondary amine as a common building block according to the literature.[30] These dithiocarbamates (**1**–**5**) on reaction with epichlorohydrin (**6**) yielded *S*-[(2,3-epithio)propyl]*N*,*N*-dialkylthiocarbamates (**7**–**11**) via an intramolecular rearrangement, the other probable compound, *S*-[2,3-epoxypropyl]*N*,*N*-dialkyldithiocarbamate (**12**) was not obtained.[31] The thiocarbamates (**7**–**11**) when reacted with a secondary amine in ethyl acetate gave free base *S*,*S*′-[disulfanediyl-bis(dialkylaminopropane-2,1-diyl)]bis-(dialkylaminothiocarbamate) without isolation of the thiol intermediate (**13**). These free bases were converted to tartrate salts (**14**–**31**, Scheme 1) by their treatment with tartaric acid in absolute alcohol.

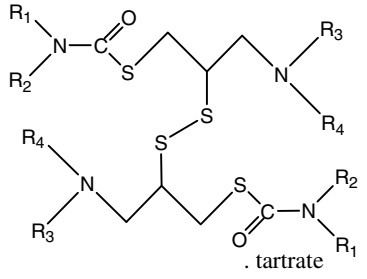


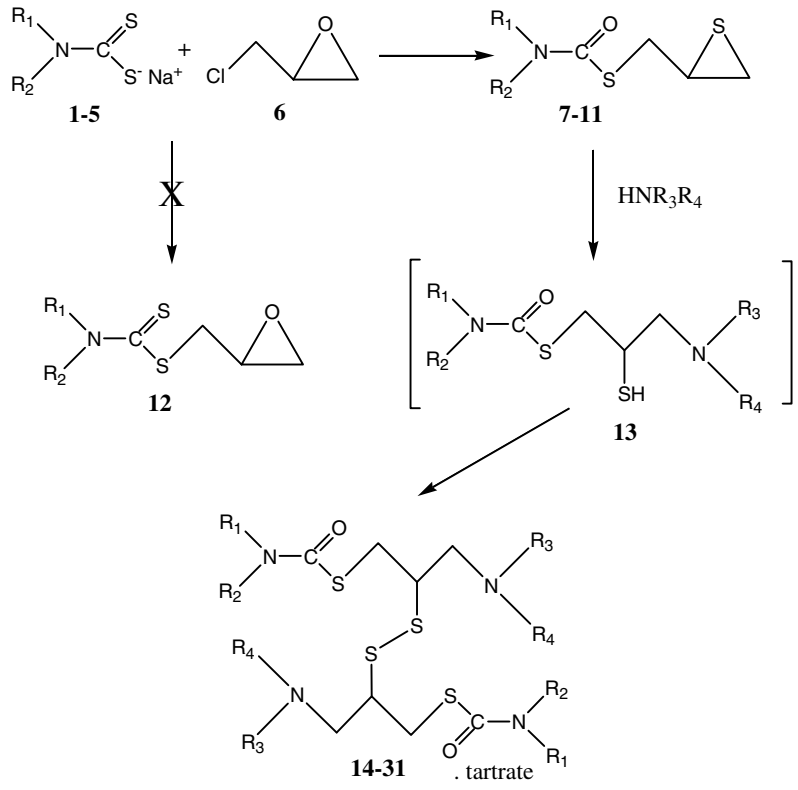
Figure 1.

2.2. Biology

Eighteen compounds (**14**–**31**) were tested in vitro for spermicidal activity against human spermatozoa (Table 1). Eleven compounds (**14**–**16**, **19**–**23**, **27**, **30**, **31**) showed 100% spermicidal activity at a concentration range 1.0%–0.002% w/w. Compounds **20** and **21** were found to be most active in the series with MEC ~0.002% w/w. Nonoxynol (N-9), the currently used vaginal microbicide, was taken as standard (MEC 0.05% w/w).

The structure–activity relationship indicated that the disulfide linkage is necessary for spermicidal activity. The cyclic amino group is essential at both the ends of the compounds for spermicidal activity because the replacement with of a dialkylamino group at any of the two positions resulted in loss of spermicidal activity (**24**–**26**, **28**, **29**). Compounds containing pyrrolidine or piperidine groups were more active than the corresponding morpholine containing compounds. Introduction of a morpholine moiety in the compounds (**14**–**18**) reduced the spermicidal activity. This confirms previous findings by Gupta et al.[32] The most plausible explanation for the spermicidal activity of several compounds in this series is the fact that the inter-conversion of intramolecular disulfide linkages to their reduced form (-SH) and vice versa may cause substantial membrane permeablization[26] resulting in immediate loss of membrane potential and a detergent-like, quick detrimental action on sperm. However, a preliminary study[8] conducted with compounds **20** and **21** has shown that this action does not involve gross structural damage to sperm membrane as seen in case of N-9 and other detergents, and consequently, these molecules are also much safer than detergents toward *Lactobacilli* and human cervical cells.

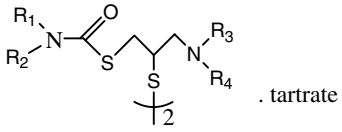
Some of the compounds (**16**, **19**–**21**, **23**, **30**, **31**) that were found highly active (MEC; $\leqslant$0.05%) in the spermicide assay were also checked for antifungal activity against different strains of *Candida albicans* (Table 2). The results showed that none of the compounds had an antifungal activity potent enough to be comparable to the standard antifungal drug, Fluconazole. Nevertheless, compounds (**16**, **19**–**21** and **30**) have shown better activity than N-9 against five or more strains of *Candida*. It is pertinent to note that the antifungal concentrations of compounds (**20** and **21**) are lower than their spermicidal MEC. Since these compounds are intended to be used at spermicidal concentrations in vaginal preparations, therefore what may appear as weak antifungal activity in comparison to fluconazole in vitro may actually provide useful prophylaxis against opportunistic *Candida* infections in vivo, as required for a dual action spermicide/microbicide agent.[33] Thus it may be concluded that



NR_1R_2= morpholino, pyrrolidino, piperidino, methyethylamino, diethylamino
NR_3R_4= pyrrolidino, piperidino, azepino, morpholino, 4-methyl piperazino

Scheme 1. Synthesis of *S,S′*-[disulfanediylbis(dialkylaminopropane-2,1-diyl)]bis(dialkylaminothiocarbamate)tartrates.

Table 1. In vitro spermicidal activity of the compounds (**14**–**31**) against human spermatozoa



Compound (lab code)	NR_1R_2	NR_3R_4	MEC[a], w/v (%)
14 (DSE-30)	Morpholino	Pyrrolidino	0.1
15 (DSE-31)	Morpholino	Piperidino	0.5
16 (DSE-32)	Morpholino	Azepino	0.05
17 (DSE-33)	Morpholino	Morpholino	Inactive at 1.0
18 (DSE-34)	Pyrrolidino	Morpholino	Inactive at 1.0
19 (DSE-35)	Pyrrolidino	Azepino	0.05
20 (DSE-36)	Pyrrolidino	Pyrrolidino	0.002
21 (DSE-37)	Piperidino	Pyrrolidino	0.002
22 (DSE-38)	Pyrrolidino	4-Methylpiperazino	1.0
23 (DSE-39)	Pyrrolidino	Piperidino	0.05
24 (DSE-40)	Methylethyl amino	Morpholino	Inactive at 1.0
25 (DSE-41)	Methylethyl amino	4-Methylpiperazino	Inactive at 1.0
26 (DSE-42)	Diethyl amino	Piperidino	Inactive at 1.0
27 (DSE-43)	Piperidino	Morpholino	0.1
28 (DSE-44)	Diethyl amino	Morpholino	Inactive at 1.0
29 (DSE-45)	Diethyl amino	Piperidino	Inactive at 1.0
30 (DSE-46)	Piperidino	4-Methylpiperazino	0.05
31 (DSE-47)	Piperidino	Piperidino	0.05
	Nonoxynol (N-9, standard)		0.05
	Placebo		Inactive at 1.0

[a]MEC denotes the minimum effective concentration that would kill 100% sperm in 'all' the semen samples and is not the mean value of the individual MECs for different samples.

Table 2. MIC (μg/ml) and IC_{50} (μg/ml) of compounds against different *Candida* strains

Strain	16		19		20		21		23		30		31		N-9		Fluconazole	
	MIC	$1C_{50}$	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
1	>50	>50	>50	50	>50	43	>50	47	>50	>50	50	47	>50	>50	>50	46	0.5	0.13
2	50	25	50	26	50	29	50	32	>50	42	50	40	>50	>50	>50	41	0.5	0.26
3	50	35	50	50	>50	>50	50	48	>50	>50	50	>50	>50	>50	>50	>50	2.0	0.58
4	>50	>50	>50	>50	>50	>50	50	48	>50	>50	50	>50	>50	>50	>50	>50	1.0	0.35
5	50	32	50	32	50	33	50	30	50	49	50	35	>50	>50	50	48	4.0	1.38
6	50	39	50	42	50	31	50	48	50	48	50	40	>50	>50	50	47	1.0	0.21
7	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	46	>50	>50	>50	>50	1.0	0.63
8	50	42	50	42	>50	>50	>50	41	>50	>50	>50	43	>50	>50	>50	>50	0.25	0.10
9	>50	>50	>50	>50	>50	42	>50	43	>50	>50	>50	40	>50	>50	>50	>50	0.25	0.18

MIC, minimum inhibitory concentration; IC_{50}, concentration at which 50% growth is inhibited; 1, *Candida albicans* (a clinical isolate maintained in our department); 2, *Candida albicans* MTCC 183; 3, *Candida albicans* MTCC 1346; 4, *Candida albicans* ATCC 10231; 5, *Candida krusei* ATCC 6258; 6, *Candida parapsilosis* ATCC 22019; 7, *Candida albicans* ATCC 10453; 8, *Candida albicans* ATCC 60193; 9, *Candida albicans* ATCC 66027.

terminal cycloalkyl amine at both the ends of the molecule plays an important role in spermicidal activity, resulting in compounds **20** and **21** (MEC: 0.002%, Table 1) displaying 25 times more efficacy than N-9 (0.05%). Their extreme safety toward human cervical cells in comparison to N-9 with exceedingly low toxicity to Lactobacilli[8] makes them ideal candidates for development as vaginal contraceptives. Further investigations are underway to check if these compounds induce a pro-inflammatory response in vaginal/cervical epithelial cells (as seen in case of N-9). Since target molecules/mechanisms on human sperm for these novel spermicides may be identical or similar to those in some common STD pathogens like *Trichomonas vaginalis*, *Chlamydia trichomatis*, HPV, HSV and HIV-1,[34] detailed evaluation of their microbicidal activity against these pathogens is warranted.

3. Experimental

3.1. Chemistry

The IR spectra were recorded in KBr or neat on Perkin-Elmer 157 and 133 spectrophotometers (υ_{max}, cm^{-1}). The PMR-spectra were recorded on a Bruker AVANCE DRX 300 MHz FT NMR spectrometer using TMS as internal reference (chemical shift in ppm). Mass spectra were obtained using a Jeol JMS D-300 instrument. Elemental analyses were estimated on Carlo Erba Model EA-1108 and Heraeus CHN rapid instruments. All compounds gave satisfactory IR, PMR, mass spectroscopic data and elemental analyses results. Melting points were taken on a precision melting point apparatus (IEC, Bombay, India). All standard chemicals and solvents were procured from Sigma–Aldrich Chemicals Pvt. Ltd, Bangalore, India, and Merck Ltd, Mumbai, India.

Sodium dialkyl dithiocarbamates (**1–5**)[30] and *S*-[(2,3-epithio)propyl]*N*,*N*-dialkylthiocarbamates (**7–11**)[31] were prepared by reported methods.

3.1.1. General method for the preparation of (±)-*S*,*S*′-[disulfanediylbis(dialkylaminopropane-2,1-diyl)]bis(dialkylaminothiocarbamate)tartrate (14–31; Scheme 1). The 1-dialkylaminocarbothioic acid *S*-(2,3-epithiopropyl) ester (**7–11**; 0.05 mol) and dialkylamine (0.05 mol) were dissolved in 75 ml ethyl acetate and stirred for 120 h at room temperature, solvent was distilled off and the resulting viscous mass was purified by column chromatography using basic alumina as stationary phase and ethyl acetate/hexane as eluents. The mass so obtained was identified as *S*,*S*′-[disulfanediylbis(dialkylaminopropane-2,1-diyl)]bis-(dialkylaminothiocarbamate) free base. The free base (0.05 mol) was dissolved in absolute alcohol (50 ml) and a solution of d-Tartaric acid (0.1 mol) in absolute alcohol (25 ml) was added with stirring at room temperature. The reaction mixture was further stirred for 2 h at room temperature. Adding dry diethyl ether precipitated the tartrate salts (**14–31**).

3.1.1.1. (±)-*S*,*S*′-[Disulfanediylbi(pyrrolidinopropane-2,1-diyl)]bis(morpholinothiocarbamate) (14). Yield: 84%; light yellow oil; eluents: 5% EtOAc/hexane; IR (neat) γ (cm^{-1}): 2966.9, 1651.1, 1405.0, 1214.2, 1115.7, 1019.3, 757.2; ^1H NMR ($CDCl_3$, δ ppm) δ 1.66–1.76 (m, 8, pyrrolidine CH_2 β to N), 2.40–2.71 (m, 8, pyrrolidine N–CH_2), 2.71–2.75 (m, 4, N–CH_2), 3.13–3.31 (m, 6, S–CH_2 and CH), 3.43–3.58 (m, 8, morpholine N–CH_2), 3.65–3.70 (m, 8, morpholine O–CH_2); MS (FAB): *m*/*z* 579 (M^++1), 580 (M^++2); Anal. Calcd for $C_{24}H_{42}N_4O_4S_4$: C, 49.80; H, 7.31; N, 9.68. Found: C, 49.49; H, 7.05; N, 10.01. Tartrate salt mp 100–102 °C.

3.1.1.2. (±)-*S*,*S*′-[Disulfanediylbis(piperidinopropane-2,1-diyl)]bis(morpholinothiocarbamate) (15). Yield: 77%; light yellow oil; eluents: 3% EtOAc/hexane; IR (neat) γ (cm^{-1}): 2931.4, 1652.5, 1402.9, 1211.7, 1115.4, 1018.9, 755.2; ^1H NMR ($CDCl_3$, δ ppm) δ 1.42 (m, 4, piperidine γ-CH_2), 1.56 (m, 8, piperidine CH_2 β to N), 2.40 (m, 8, piperidine N–CH_2), 2.53 (m, 4, N–CH_2), 3.16–3.32 (m, 6, S–CH_2 and CH), 3.44–3.56 (m, 8, morpholine N–CH_2), 3.68 (m, 8, morpholine O–CH_2); MS (FAB): *m*/*z* 607 (M^++1), 232; Anal. Calcd. for $C_{26}H_{46}N_4O_4S_4$: C, 51.45; H, 7.64; N, 9.23. Found: C, 51.78; H, 7.86; N, 9.02. Tartrate salt mp 88–90 °C.

3.1.1.3. (±)-*S*,*S*′-[Disulfanediylbis(azepinopropane-2,1-diyl)]bis(morpholinothiocarbamate) (16). Yield: 75%; light yellow oil; eluents: 4% EtOAc/hexane; IR (neat) γ (cm^{-1}): 2922.7, 1652.3, 1403.2, 1212.3, 1116.2, 1018.7, 755.8; ^1H NMR ($CDCl_3$, δ ppm) δ 1.58–1.69 (m, 16,

azepine CH_2 β and γ to N), 2.65–2.76 (m, 12, azepine N–CH_2), 3.05–3.12 (m, 2, S–CH_2), 3.24–3.47 (m, 4, S–CH_2 and CH), 3.56–3.58 (m, 8, morpholine N–CH_2), 3.65–3.70 (m, 8, morpholine O–CH_2); MS (FAB): *m/z* 635 (M^++1), 140; Anal. Calcd for $C_{28}H_{50}N_4O_4S_4$: C, 52.96; H, 7.94; N, 8.82. Found: C, 53.33; H, 8.35; N, 9.05. Tartrate salt mp 82–84 °C.

3.1.1.4. (±)-*S*,*S*′-[Disulfanediylbi(morpholinopropane-2,1-diyl)]bis(morpholinothiocarbamate) (17). Yield: 60%; light yellow oil; eluents: 2% EtOAc/hexane; IR (neat) γ (cm^{-1}): 2962.5, 1650.7, 1404.8, 1213.2, 1115.2, 1018.2, 754.7; ^{1}H NMR ($CDCl_3$, δ ppm) 2.49 (m, 8, morpholine N–CH_2), 2.58–2.61 (m, 4, N–CH_2), 3.15–3.32 (m, 6, S–CH_2 and CH), 3.40–3.57 (m, 8, morpholine N–CH_2), 3.61–3.70 (m, 16, morpholine O–CH_2); MS (FAB): *m/z* 612 (M^++2), 610 (M^+); Anal. Calcd for $C_{24}H_{42}N_4O_6S_4$: C, 47.19; H, 6.93; N, 9.17. Found: C, 47.33; H, 6.65; N, 8.95. Tartrate salt mp 93–95 °C.

3.1.1.5. (±)-*S*,*S*′-[Disulfanediylbis(morpholinopropane-2,1-diyl)]bis(pyrrolidinothiocarbamate) (18). Yield: 54%; light yellow oil; eluents: 5% EtOAc/hexane; IR (neat) γ (cm^{-1}): 2958.0, 1652.8, 1371.3, 1117.3, 751.7; ^{1}H NMR ($CDCl_3$, δ ppm) 1.88–1.95 (m, 8, pyrrolidine CH_2 β to N), 2.48–2.50 (m, 8, morpholine N–CH_2), 2.59–2.62 (m, 4, N–CH_2), 3.18–3.38 (m, 4, S–CH_2), 3.41–3.53 (m, 10, pyrrolidine N–CH_2 and CH), 3.68–3.72 (m, 8, morpholine O–CH_2); MS (FAB): *m/z* 578 (M^+); Anal. Calcd for $C_{24}H_{42}N_4O_4S_4$: C, 49.80; H, 7.31; N, 9.68. Found: C, 49.43; H, 7.65; N, 9.95. Tartrate salt mp 90–92 °C.

3.1.1.6. (±)-*S*,*S*′-[Disulfanediylbis(azepinopropane-2,1-diyl)]bis(pyrrolidinothiocarbamate) (19). Yield: 52%; light yellow oil; eluents: 6% EtOAc/hexane; IR (neat) γ (cm^{-1}): 2924.6, 1654.7, 1368.1, 1167.3, 1030.7, 770.9; ^{1}H NMR ($CDCl_3$, δ ppm) 1.58 (m, 16, azepine CH_2 β and γ to N), 1.82–1.90 (m, 8, pyrrolidine CH_2 β to N), 2.65–2.77 (m, 12, azepine N–CH_2), 3.06–3.20 (m, 6, S–CH_2 and CH), 3.24–3.52 (m, 8, pyrrolidine N–CH_2); MS (FAB): *m/z* 603 (M^++1); Anal. Calcd for $C_{28}H_{50}N_4O_2S_4$: C, 55.77; H, 8.36; N, 9.29. Found: C, 55.43; H, 8.65; N, 9.65. Tartrate salt mp 90–92 °C.

3.1.1.7. (±)-*S*,*S*′-[Disulfanediylbi(pyrrolidinopropane-2,1-diyl)]bis(pyrrolidinothiocarbamate) (20). Yield: 87%; light yellow oil; eluents: 4% EtOAc/hexane; IR (neat) γ (cm^{-1}): 2996.9, 1652.4, 1371.5, 1167.2, 754.1; ^{1}H NMR ($CDCl_3$, δ ppm) 1.75 (m, 8, pyrrolidine CH_2 β to N), 1.84–1.97 (m, 8, pyrrolidine CH_2 β to N), 2.45–2.65 (m, 8, pyrrolidine N–CH_2), 2.71–2.75 (m, 4, N–CH_2), 3.13–3.21 (m, 4, S–CH_2 and CH), 3.35–3.54 (m, 10, S–CH_2 and pyrrolidine N–CH_2); MS (FAB): *m/z* 547 (M^++1), 306; Anal. Calcd for $C_{24}H_{42}N_4O_2S_4$: C, 52.71; H, 7.74; N, 10.24. Found: C, 52.49; H, 8.05; N, 10.61. Tartrate salt mp 95–97 °C.

3.1.1.8. (±)-*S*,*S*′-[Disulfanediylbi(pyrrolidinopropane-2,1-diyl)]bis(piperidinothiocarbamate) (21). Yield: 71%; light yellow oil; eluents: 3% EtOAc/hexane; IR (neat) γ (cm^{-1}): 2937.4, 1649.7, 1409.6, 1247.0, 1124.0, 754.5; ^{1}H NMR ($CDCl_3$, δ ppm) 1.60 (m, 12, piperidine CH_2 β and γ to N), 1.76 (m, 8, pyrrolidine CH_2 β to N), 2.55 (m, 8, pyrrolidine N–CH_2), 2.67–2.74 (m, 4, N–CH_2), 3.06–3.30 (m, 6, S–CH_2 and CH), 3.41–3.50 (m, 8, piperidine N–CH_2); MS (FAB): *m/z* 575 (M^++1), 320, 287; Anal. Calcd for $C_{26}H_{46}N_4O_2S_4$: C, 54.32; H, 8.06; N, 9.74. Found: C, 54.05; H, 8.37; N, 9.58. Tartrate salt mp 83–85 °C.

3.1.1.9. (±)-*S*,*S*′-[Disulfanediylbis[(4-methyl)piperazino]propane-2,1-diyl]bis(pyrrolidinothiocarbamate) (22). Yield: 60%; light yellow oil; eluents: 2% EtOAc/hexane; IR (neat) γ (cm^{-1}): 3018.3, 1643.2, 1375.5, 1216.7, 1167.7, 759.5; ^{1}H NMR ($CDCl_3$, δ ppm) 1.91–1.98 (m, 8, pyrrolidine CH_2 β to N), 2.27 (s, 6, N–CH_3), 2.46–2.62 (m, 20, piperazine N–CH_2), 3.12–3.27 (m, 2, CH), 3.35–3.53 (m, 12, S–CH_2 and pyrrolidine N–CH_2); MS (FAB): *m/z* 605 (M^++1), 307; Anal. Calcd for $C_{26}H_{48}N_6O_2S_4$: C, 51.62; H, 8.00; N, 13.89. Found: C, 51.26; H, 8.29; N, 13.51. Tartrate salt mp 83–85 °C.

3.1.1.10. (±)-*S*,*S*′-[Disulfanediylbis(piperidinopropane-2,1-diyl)]bis(pyrrolidinothiocarbamate) (23). Yield: 84%; light yellow oil; eluents: 4% EtOAc/hexane; IR (neat) γ (cm^{-1}): 2933.1, 1653.7, 1370.9, 1114.3, 752.3; ^{1}H NMR ($CDCl_3$, δ ppm) 1.39–1.41 (m, 4, piperidine CH_2 γ to N), 1.55–1.64 (m, 8, piperidine CH_2 β to N), 1.90 (m, 8, pyrrolidine CH_2 β to N), 2.40 (m, 12, piperidine N–CH_2), 3.14–3.30 (m, 4, S–CH_2), 3.35–3.54 (m, 10, CH and pyrrolidine N–CH_2); MS (FAB): *m/z* 575 (M^++1), 307; Anal. Calcd for $C_{26}H_{46}N_4O_2S_4$: C, 54.32; H, 8.06; N, 9.74. Found: C, 53.98; H, 7.86; N, 10.02. Tartrate salt mp 68–70 °C.

3.1.1.11. (±)-*S*,*S*′-[Disulfanediylbis(morpholinopropane-2,1-diyl)]bis(methylethylaminothio-carbamate) (24). Yield: 66%; light yellow oil; eluents: 7% EtOAc/hexane; IR (neat) γ (cm^{-1}): 2930, 1384, 1118; ^{1}H NMR ($CDCl_3$, δ ppm) 1.15–1.48 (m, 6, C*H*$_3$–CH$_2$–N–C), 2.49–2.61 (m, 12H, 2× C*H*$_2$, N–C*H*$_2$), 3.07 (s, 6H, C*H*$_3$–N), 3.12–3.43 (m, 10H, morpholine N–CH_2, C–H, S–C*H*$_2$), 3.58–3.79 (t, 8H, morpholine O–CH_2); EIMS: 554 (M^+); Anal. Calcd for $C_{22}H_{42}N_4O_4S_4$: C, 47.65; H, 7.58; N, 10.11. Found: C, 47.78; H, 7.58; N, 10.47. Tartrate salt mp 50–52 °C.

3.1.1.12. (±)-*S*,*S*′-[Disulfanediylbis[(*N*-methyl)piperazino]propane-2,1-diyl]bis(methylethyl-aminothiocarbamate) (25). Yield: 30%; light yellow oil; eluents: 5% EtOAc/hexane; IR (neat) γ (cm^{-1}): 2932, 1384, 1116; ^{1}H NMR ($CDCl_3$, δ ppm) 1.15–1.30 (m, 6H, C*H*$_3$–CH$_2$–N–C), 2.27 (s, 6H, N–C*H*$_3$), 2.45–2.60 (m, 20H, C*H*$_2$–N), 2.97 (s, 6H, C*H*$_3$–N), 3.10–3.84 (m, 10H, C*H*$_2$–N, C*H*, S–C*H*$_2$); EIMS: 580 (M^+); Anal. Calcd for $C_{24}H_{48}N_6O_2S_4$: C, 49.66; H, 8.28; N, 14.48%. Found: C, 49.79; H, 8.64; N, 14.65. Tartrate salt mp138–142 °C.

3.1.1.13. (±)-*S*,*S*′-[Disulfanediylbis(piperidinopropane-2,1-diyl)]bis-(diethylaminothiocarbamate) (26). Yield: 44%; light yellow oil; eluents: 8% EtOAc/hexane; IR (neat) γ (cm^{-1}): 3020, 2936, 2810, 1640, 1236, 1216, 1120; ^{1}H NMR ($CDCl_3$, δ ppm) 1.16–1.25 (m, 12, C*H*$_3$–CH$_2$) 2.27 (m, 12, piperidine CH_2 β and γ to N) 2.45–2.63 (m, 20, C*H*$_2$–N) 3.11–3.16 (t, 4, S–C*H*$_2$) 3.26–3.67 (m, 10, C*H*$_2$–N, C*H*); EIMS: 578 (M^+); Anal.

Calcd for $C_{26}H_{50}N_4O_2S_4$: C, 53.98; H, 8.65; N, 9.69%. Found: C, 53.55; H, 9.00; N, 9.45. Tartrate salt mp 124–126 °C.

3.1.1.14. (±)-*S*,*S*′-[Disulfanediylbis(morpholinopropane-2,1-diyl)]bis(piperidinothiocarbamate) (27). Yield: 42%; light yellow oil; eluents: 4% EtOAc/hexane; IR (neat) γ (cm^{-1}): 2934, 1216, 1116; ^1H NMR ($CDCl_3$, δ ppm) 1.15–1.25 (m, 12, piperidine CH_2 β and γ to N), 2.41–2.54 (t, 12, 2× C*H*$_2$–N, N–C*H*$_2$), 3.13–3.19 (t, 4, S–C*H*$_2$), 3.24–3.90 (m, 10, C*H*$_2$–N, C*H*), 3.96–4.05 (t, 8H, morpholine O–C*H*$_2$); EIMS: 606 (M^+). Anal. Calcd for $C_{26}H_{46}N_4O_4S_4$: C, 51.49; H, 7.59; N, 9.24%. Found: C, 51.69; H, 7.97; N, 9.59. Tartrate salt mp 142–144 °C.

3.1.1.15. *S*,*S*′-[Disulfanediylbis(morpholinopropane-2,1-diyl)]bis(diethylaminothiocarbamate) (28). Light yellow oil; eluents: 6% EtOAc/hexane; IR (neat) γ (cm^{-1}) 2920, 1232, 1122; ^1H NMR ($CDCl_3$, δ ppm) 1.16–1.25 (m, 12, C*H*$_3$), 2.42–2.61 (m, 12, morpholine N–C*H*$_2$, N–C*H*$_2$), 3.12–3.18 (t, 4H, S–C*H*$_2$), 3.24–3.44 (m, 10H, C*H*$_2$–N, C*H*), 3.69–3.89 (t, 8H, morpholine O–C*H*$_2$); EIMS: 582 (M^+); Anal. Calcd for $C_{24}H_{46}N_4O_4S_4$: C, 49.48; H, 7.90; N, 9.62%. Found: C, 49.16; H, 7.61; N, 9.25. Tartrate salt mp 140–142 °C.

3.1.1.16. (±)-*S*,*S*′-[Disulfanediylbis(piperidinopropane-2,1-diyl)]bis(diethylaminothiocarbamate) (29). Light yellow oil; eluents: 7% EtOAc/hexane; IR (neat) γ (cm^{-1}) 2936, 1640, 1218, 1114; ^1H NMR ($CDCl_3$, δ ppm) 1.16–1.25 (m, 12, C*H*$_3$), 1.55–1.65 (m, 12, piperidine CH_2 β and γ to N), 2.38–2.40 (m, 8H, C*H*$_2$–N), 2.51–2.55 (m, 4, N–C*H*$_2$), 3.09–3.16 (t, 4, S–C*H*$_2$), 3.22–3.74 (m, 10H, C*H*$_2$–N, C*H*); EIMS: 578 (M^+); Anal. Calcd for $C_{26}H_{50}N_4O_2S_4$: C, 53.98; H, 8.65; N, 9.69. Found: C, 54.14; H, 9.01; N, 10.04. Tartrate salt mp 124–126 °C.

3.1.1.17. (±)-*S*,*S*′-[Disulfanediylbis[(*N*-methyl)piperazino]propane-2,1-diyl]bis(piperidinothiocarbamate) (30). Yield: 42%; viscous mass; eluents: 5% EtOAc/hexane; IR (neat) γ (cm^{-1}): 2936, 2854, 2804, 1248, 1126; ^1H NMR ($CDCl_3$, δ ppm) 1.60–1.64 (m, 12, piperidine CH_2 β and γ to N), 2.17 (s, 6, N–C*H*$_3$), 2.27–2.90 (m, 20, N–C*H*$_2$), 3.15–3.28 (m, 4, S–C*H*$_2$), 3.42–3.93 (m, 10, C*H*$_2$–N, C*H*); EIMS: 632 (M^+); Anal. Calcd for $C_{28}H_{52}N_6O_2S_4$: C, 53.16; H, 8.23; N, 13.29. Found: C, 53.44; H, 8.61; N, 13.04. Tartrate salt mp 110–112 °C (d).

3.1.1.18. (±)-*S*,*S*′-[Disulfanediylbis(piperidinopropane-2,1-diyl)]bis(piperidinothiocarbamate) (31). Yield: 38%; viscous mass; eluents: 3% EtOAc/hexane; IR (neat) γ (cm^{-1}): 2932, 2856, 2348, 1650, 1248, 1120; ^1H NMR ($CDCl_3$, δ ppm) 1.55–1.75 (m, 12, piperidine CH_2 β and γ to N), 2.40–2.57 (m, 12, N–*CH*$_2$), 3.20–3.30 (d, 4, S–*CH*$_2$), 3.40–3.50 (m, 10, *CH*$_2$–N, C*H*), EIMS: 602 (M^+); Anal. Calcd for $C_{28}H_{50}N_4O_2S_4$: C, 55.81; H, 8.31; N, 9.30. Found: C, 55.45; H, 8.51; N, 9.05. Tartrate salt mp 105–107 °C (d).

3.2. Biology

3.2.1. In vitro spermicidal activity.[35] Fresh human semen samples with normal sperm count, motility and morphology were obtained from healthy, young, fertile donors in a sterile vial by masturbation, liquefied for 45 min at 37 °C, and used for in vitro spermicidal activity. Samples having >60-million/ml sperm count with >65% motility were used in the study. The test-compounds or nonoxynol-9 (standard, a generous gift from Dr. N.M. Khanna, former Head of the Pharmaceutics Division, Central Drug Research India, Lucknow, India) were dissolved in a minimum volume of DMSO and diluted with physiological saline (0.9% NaCl) so as to make a 1.0% (10 mg/ml) solution. In control tubes, 10 mg glucose was dissolved in a minimum volume of DMSO and diluted with saline to make a 1.0% solution. The final concentration of DMSO was always $\leqslant$10%. These solutions were serially diluted with physiological saline. Spermicidal test was performed with each dilution starting from 1.0% till the minimum effective concentration (MEC) was arrived at, following the modified method of Sander and Cramer.[31] Briefly, 0.05 ml of human semen was added to 0.25 ml of spermicidal compound solution (saline in control) and vortexed for 10 s. A drop was immediately placed on a microscope slide, covered with a cover glass and examined under a phase contrast microscope. The results were scored positive if 100% spermatozoa became immotile in 20 s. However, if even one or two spermatozoa showed sluggish motility, the test concentration of the compound was scored negative. The MEC was determined in three individual semen samples from different donors.

3.2.2. In vitro antifungal activity.[36] *MIC determination:* The minimum inhibitory concentration (MIC) of compounds were determined by broth micro-dilution 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 and IC_{50} (at which 50% growth was inhibited) were recorded after 48 h of incubation.

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