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Role of disulfide linkage in action of bis(dialkylaminethiocarbonyl)disulfides as potent double-Edged microbicidal spermicide: Design, synthesis and biology[#]

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Abstract:

Trichomoniasis and Candidiasis are amongst the most common morbidity-causing reproductive tract infections, generally treated by Metronidazole and Fluconazole respectively. Poor vaginal efficacy, drug-resistance and non-spermicidal nature limit their use as topical microbicidal contraceptives. Bis(dialkylaminethiocarbonyl)disulfides (**4–38**) were designed as dually active, non-surfactant molecules capable of eliminating *Trichomonas vaginalis* and *Candida* strains as well as irreversibly immobilizing 100% human sperm instantly, at doses non-cytotoxic to human cervical epithelial cells and vaginal microflora*invitro*. Compounds **12, 16, 17** were fifty times more active than nonoxynol-9, OTC vaginal spermicide, and compounds **12** and **17** have shown remarkable *in vivo* activity in rabbit model. Most promising compound **17** has shown promise for further development as a double-edged vaginal microbicide due to their improved activity and safety along with notable *in vivo* trichomonicidal activity. Role of disulfide group was established by loss of spermicidal activity on chemical modifications (**39–56**). These disulfides might be targeting thiol groups present over cell membrane of human sperm and *Trichomonas*as shown by fluorescence labeling of free thiols.

KEYWORDS: Dithiocarbamate, Disulfide, Disulfiram, Spermicide, Sulfhydryl, Contraceptive.

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1. Introduction:

Increasing sexually transmitted infections[1] (STIs) along with the population explosion is a global challenge[2-4] that cannot be overlooked. Growing drug-resistance of *Trichomonas* to Metronidazole, and fungal strains to Fluconazole, is a cause of serious concern.[5-6] Furthermore, nonoxynol-9 (N-9), the OTC vaginal spermicide does not protect against STIs and HIV in clinical situations but may in fact enhances their incidences due to its non-specific surfactant action.[7-9] Most heterosexual women would like to reduce the risk of acquiring STIs[10] and control their fertility. Trichomoniasis, the most prevalent, non-viral STI, affects 250–350 million people worldwide every year causing serious discomfort to women along with associated problems of adverse pregnancy outcomes, pre-term delivery, low-birth weight infants, infertility, and cervical cancer.[11] It is now well established that trichomoniasis[12] extensively raises the vulnerability to HIV[13,14] and therefore controlling trichomoniasis alone could significantly reduce the incidence of new HIV infections. Similarly, candidiasis caused by the fungus *Candida albicans*, is strongly associated with HIV-AIDS.[15]In spite of increased 'weapon store' for antifungal agents, currently available drugs do not suffice the growing demand of managing infections in complex patient populations.[16, 17] One of the major problems is increased drug resistance mainly due to chronic antimycotic therapy in HIV-infected and other immuno-compromised patients.[18] Usually, the female partner shoulders the primary responsibility of STI and pregnancy protection during most of the heterosexual contacts, including 'vulnerable' contacts amongst adolescents and promiscuous adults.[19] As prevention of pregnancy and infection is better than abortion and cure later, the best strategy would be to arrest the infection along with sperm in vagina during transmission, and therefore there is a need to develop a topically active medication against STIs (trichomoniasis and candidiasis) and sperm. Free thiol groups critically control the survival of predominantly anaerobic cells like *Trichomonas vaginalis*,[20] *Candida albicans*[21] and spermatozoa.[22] For example, the cleavage of disulfide bonds of sperm cell-specific hexokinase type 1 is associated with increased hexokinase activity and initiation of sperm motility.[24] The unique redox properties of protein thiols play an important role in enzyme catalysis, protein folding, and redox signaling, making it a key residue for chemical intervention[21,23] for manipulating cellular energy metabolism, motility and subsistence of *Trichomonas*, *Candida* and sperm cells. Consequently, sulfhydryl binding agents can impede sperm, *Trichomonas* and *Candida* cells to achieve prophylactic contraception as exemplified by *N*-ethyl maleimide[25], a

specific sulfhydryl alkylating agent, acrylophenone,[26] quinolines[27] and a variety of other thiol agents.[28]

Dithiocarbamate (DTC) is a desirable pharmacophore in various medicinally significant compounds and is widely exploited in microbicidal spermicides,[29-36] fungicides[37,38] and anti-HIV[39,40] agents. Thus, it was hypothesized that incorporating DTC and disulfide in a single chemical entity can make it to interact with multiple targets (*Trichomonas*, fungi and sperm) simultaneously. While investigating the DTC-disulfide hybrid framework, Disulfiram (DSF) molecule (Figure 1) was found to be the most appropriate structure. Disulfiram is a FDA approved deterrent to alcohol abuse,which is current in clinical use.[41] DSF was synthesized and as expected it exhibited sperm immobilizing activity, which was mild. Encouragedby this observation it was thought worthwhile to modify DSF framework and to synthesize bis(*N*-substituted piperazinethiocarbonyl) disulfides (Figure 1) as safe, multi-targeting microbicidal contraceptives.

(Figure 1.)

2. Results and Discussion:

2.1. Chemistry

The compounds **4**–**38** have been synthesized[42] according to Scheme 1 using different sodium salts of dithiocarbamic acid (**3**). Secondary amine was reacted with carbon disulfide under alkaline condition to furnish sodium dialkylcarbamodithioate (**3**) which was further treated with sodium nitrite and hydrochloric acid at 0-5 °C in water to provide corresponding bis(dialkylaminethiocarbonyl) disulfides (**4**–**38**, Table 1).

(Scheme 1)

2.2 Biological Evaluation

2.2.1.Spermicidal activity

The spermicidal activity of compounds (**4**–**38**, Table 1) was evaluated in comparison to N-9. Twenty-five compounds (**4**, **7**–**10**, **12**–**18**, **20**–**25**, **28**, **31** and **33**–**37**) irreversibly immobilized 100% human sperm at concentration ranging from 1–0.001% (MEC) within 30 seconds. Six

compounds (**4**, **12**, **16**–**18** and **35**) were found to be more potent than commercially available spermicide N-9.

The results of the effect on sperm motility of compounds (**4**–**38**, Table 1) propose that if -NR^1R^2 was dimethyl amine (**4**) the compound showed moderate spermicidal activity (MEC, 0.01%). If methyl group was replaced by cyclohexyl (**5**) or benzyl (**6**), the activity was completely lost, while introduction of cyclic amine (**7**–**11**) further decreases spermicidal activity. Among amines with single nitrogen like pyrrolidine (**7**), piperidine (**8**), 4-methylpiperidine (**10**) and azepane (**11**) activity remains unchanged in five (**7**) and six (**8**, **10**) membered ring while decreases when ring size increases to seven (**11**). Incorporation of one oxygen atom (**9**) into compound **8** increases activity by 10 folds. Whereas introducing additional nitrogen atom in amino residue remarkably increased spermicidal activity (**12**–**18**, MEC 1–0.001%). Among the alkyl substituted piperazines (**12**–**15**) chain length determine the spermicidal activity i.e., bulkier the alkyl group lesser the activity. While a substitution of the alkyl group with allyl (**16**), butyronitrile (**17**) and morpholino alkyl (**18**) retained high activity. Furthermore presence of aryl/heteroaryl group at NR^1R^2in this framework decreased the spermicidal action (**19**–**23**, MEC 0.1 and 1%) while a benzoyl group imparted mild activity (**24**, MEC 0.5%). A decrease or complete loss of spermicidal effect was observed when carboxylate (**25**–**27**), mesyl (**28**), tosyl (**29**), alkyl/benzyl carbodithioate (**30**–**34** and **38**) groups were introduced at NR^1R^2 (Table 1). Interestingly replacement of alkyl carbodithioate group with alkyl amino carbodithioate increased the spermicidal action (**35**–**37**, MEC, 0.5–0.002%).

(Table 1)

A close look at structure activity relationship (SAR) of bis(dialkylaminethiocarbonyl) disulfide (**4**–**38**) revealed that small alkyl group at N^4 position of piperazine (**12**, **16**, **17**, MEC 0.001%) is desirable for sperm immobilization activity.

2.2.2. Anti-Trichomonas activity

Seventeen compounds (**4**,**6**–**13**,**16**–**18**,**23**,**25**,**33**,**35**and**38**; Table 2) showed anti-trichomonal activity against Metronidazole (MTZ) susceptible strains with MIC ranging from 3.125–100 µg/mL (MTZ = 2.0 µg/mL),while fourteen compounds (**4**, **6**–**13**, **16**–**18**, **25** and **38**) among these exhibited trichomonicidal action against resistant strain at MIC 3.125–100 µg/mL (MTZ = 50.0

µg/mL). It is evident from the results (Table 2) that eleven compounds (**4**, **6**, **7**, **9**–**12**, **16**–**18** and **25**) illustrated better activity than MTZ against *Trichomonas* resistant strain. The comparison of anti-*Trichomonas* activity against susceptible and resistant strains revealed that MTZ lost its activity by 25 times against resistant strain while compounds (**4**, **7**, **9**, **10**, **17**and **25**) had better profile as there was no loss of activity.

The results of trichomonacidal activity against MTZ susceptible strain revealed that if -NR^1R^2 was alkyl substituted acyclic amines (**4**–**6**), dimethylamine (**4**) and benzylmethyl amine (**6**) were more preffered as their activity was comparable to standard drug MTZ. Whereas with cyclic amines (**7**–**11**) the activity was significant and pyrrolidine (**7**) and morpholine (**9**) seemed to be more desirable (MIC, 3.125 µg/mL) and an enhancement in ring size (**8**,**11**) resulted in decreased activity. An addition of a methyl group (**10**) in compound **8**at position 4 enhanced the activity by four fold. While a two nitrogen system i.e., alkyl substituted piperazine at -NR^1R^2 (**12**-**18**) smaller alkyl groups (**12** and **16**–**18**) were more desirable as the trichomonicidal activity was retained. When alkyl group was replaced by aryl/heteroaryl (**19**–**23**), benzoyl (**24**), carboxylate (**25**–**27**), mesyl/tosyl (**28**–**29**) and substituted carbodithioates (**30**–**38**) activity was decreased or completely lost. The results suggested that smaller alkyl group in cyclic/acyclic amine and piperazine framework was essential for anti-*Trichomonas* activity. The antitrichomonicidal activity against resistant strain of *Trichomonas* (Table 2) suggested that -NR^1R^2 substitution (**4**–**38**) had similar SAR as exhibited against susceptible strain. The small alkyl groups in one or two nitrogen scaffolds were more desirable in cyclic and acyclic amines as four compounds (**4**, **7**, **9** and **17**;3.125 µg/mL) were sixteen fold more active than MTZ while compound **25 (**6.25µg/mL), **6**, **10**–**12** (12.5 µg/mL) and **16**, **18 (**25.0 µg/mL) were eight, four and two times more active respectively. Compound **8** was equipotent and two compounds (**13** and **38**) were less active.

(Table 2)

The activity profile of compounds (**4**–**38**) against both the susceptible and resistant strains of *Trichomonas* suggested that compounds (**4**, **6**, **7**, **9** and**17**) exhibiting activity (3.125 µg/mL) comparable to MTZ (2 µg/mL) in susceptible strain were more effective against resistant strain. MTZ lost the activity by twenty five times while in compounds **4**, **7**, **9**and**17** activity was not decreased and in compound **6** the activity was lost by only four times. The result suggested that dimethylamino (**4**), pyrrolidino (**7**), morpholino (**9**) and 4-(3-cyanoproyl)piperazine (**17**) groups

in bis(dialkylaminethiocarbonyl) disulfides were more desirable to be effective against both susceptible and resistant strains of *Trichomonas*. Further a docking study was also carried out with less (**13**) and most active compounds (**4** and **17**) to ascertain their cysteine biosynthesis pathway.

Cysteine is indispensably vital for all living life forms as an amino acid for protein synthesis, as a precursor for glutathione and biomolecules such as coenzyme A, and as a source of sulphide for synthesis of iron-sulphur clusters. This series of compounds containing bis(*N*-substituted piperazinethiocarbonyl) disulfide moiety possibly exhibit anti-trichomonal activity by inhibiting the cysteine biosynthesis pathway. To better understand the mechanism of inhibition of these compoundsthe important component of cysteine biosynthesis pathway was explored.

The *Trichomonas vaginalis* cysteine synthase (TvCS) shows 44% of sequence identity with template. The super imposition of the modeled complex with template showed a root-mean-square deviation (RMSD) of 0.186Å.The validation of the resulting model was done with the Structural Analysis and Verification Server (SAVS)[43]. The model in which the majority of the residues (97%) occupy the most favorable region of Ramachandran plot and 2% and 1% residue lie in additionally allowed region and generously allowed region respectively was selected for further docking studies. Autodock4 tool was used to identify the possible binding site of the enzyme for inhibitory activity of our molecules. The structure preparation and minimization before studies were performed with compounds using SYBYL 7.1 program package[44] on silicon graphics fuel work station with IRIX 6.5 operating system. The structure of the compounds were prepared with the help of sketch module of Sybyl7.1 and geometry optimization was done using MMFF94 force field with Powell energy minimization algorithm, Gasteiger-Huckel charges, and 0.001 kcal/(mol.Å) energy gradient convergence criterion.

(Figure 2)

In a recent study[45] the analysis of the amino acid sequence of TvCS has been presented and it revealed that TvCS contains all the active site residues identified for CS of *S. Typhimurium* and *A. thaliana*. It was also suggested that the predicted active site lysine that covalently binds with PLP is Lys43. However, residues Asn73, Gln144, His154, Gly178, Thr179, Ser180, Thr182, and Ser259 are also connected to the co-factor with the help of hydrogen bonding. Therefore the docking studies were performed by considering this active site including the above mentioned

residues. The compounds docked well at the cavity that is suggested to involve in Pyridoxal-5'-phosphate (PLP) binding. Figure 2 shows the binding mode of docked complexes of the most active compounds with highest trichomonacidal activity. Most of the compounds in their docked conformation are interacting with Lys43 residue that is predicted to binds covalently with PLP. The compound **4** is also interacting with Gly178, Thr179, Ser180, Thr182 and Ser259. These interactions are reported to play important role in PLP binding to the CS, mentioned in the study done by Westrop *et al.*[45] The docked conformation of most and less potent molecules was analyzed to see how they differ from each other based on their binding affinity with pocket residues of PLP. Interestingly, in docking experiments, the less active compound **13** did not mimic the active analogue compound **17** and **4** (Figure 2). It is not involved in formation of hydrogen bond. Moreover, the thiocarbonyldisulfide moiety of compound **13** is protruding from the PLP binding region (Figure 2). These studies inferred that compound **17** and **4** are more potent compared to others due to its favorable H-bond interactions with Lys43 residue that may be responsible for binding with PLP.

2.2.3. Antifungal activity

The compounds (**4**–**38**) were screened against five fungal strains (Table 2) and thirty one of these (**4**–**26** and **28**–**35**) inhibited the growth of one or more fungal strains at MIC 0.78–50 µg/mL whereas eleven compounds (**4**, **7**–**13**, **15**, **25** and **35**) were active against all the strains. The subsequent SAR has been discussed on the basis of least MIC against any of the strain. The results (Table 2) suggested that a secondary amine with one nitrogen was more preferred scaffold as six compounds**4**, **6**–**8**, **10** and **11** of the eight (**4**–**11**) had remarkable activity (MIC, 0.78–3.125 µg/mL) whereas compound **5** and **9** were active at MIC 12.5 µg/mL. Among two nitrogen secondary amine derivatives i.e., piperazine scaffold (**12**–**38**) the activity decreased drastically as only three compounds (**14**, **20** and **25**) had activity at MIC 12.5 µg/mL and nine compounds (**12**, **16**, **21**, **22**, **28**, **30**, **31**, **33**, **34**) were marginally active (MIC 25 µg/mL) while rest of the compounds were either active at highest tested concentration (MIC 50 µg/mL) or inactive. The activity data of compounds **4**–**6** implied that if -NR^1R^2 was alkyl substituted acyclic amines the smaller alkyl groups were most suitable for antifungal activity as dimethylamino derivative (**4**, MIC, 0.78–12.5 µg/mL) exhibited significant activity. On the other hand, among cyclic amines (**7**–**11**), pyrrolidine (**7**) and piperidine (**8**) were most active and an addition of oxygen atom (**9**), methyl group (**10**) and enhancement of ring size (**11**) resulted in less active compounds.

2.2.4. Safety and compatibility of promising compounds (4, 12, 16, 17, 18 and 35)

The overall assessment of biological profile of all the compounds suggested that five compounds (**4**, **12**, **16**, **17**, **18** and **35**) of the series were most promising based on their spermicidal, anti-*Trichomonas* and antifungal activities as compared to vaginal microbicide available in the market N-9. To assess the potential of these compounds for the choice as a candidate[46] vaginal microbicide, the safety (Table 3) against vaginal epithelium (*HeLa* cells) and flora (*Lactobacillus*) was evaluated. The evaluated compounds (**4, 12, 16, 17, 18** and **35**; Table 3) displayed better safety towards *HeLa* cells (IC_{50} 156-1414 µg/mL) and much better compatibility with *Lactobacillus* (IC_{50} 361-3550 µg/mL) than N-9 (IC_{50} 32.5 and 33.2 µg/mL respectively). The selectivity index of compounds **4, 12, 16, 17, 18** and **35** was much higher (7–368) than that of N-9 (0.64), with compounds **4** and **17** exhibiting the highest selectivity index of 188 and 368, respectively.

(Table 3)

These compounds were three to forty three folds and eleven to ninety four folds safer against HeLa cells and *Lactobacillus* than N-9 and therefore appeared apparently much safer for vaginal use. Of these compounds **12**, **16** and **17** were most potent spermicides but the shelf-life of compound **16** was not good, therefore **12** and **17** were considered to be worthwhile in carrying out the *in vivo* spermicidal activity (Table 4) in rabbit model.

2.2.5. In vivo spermicidal activity[47] of compound 12 and 17

Apparently a dose dependent reduction in pregnancy rate was evident in rabbits receiving intravaginal instillation of compound **12** and **17**, however the effect was more prominent with compound **17**. With compound **12** about 50–60% animals did not conceive at vaginal dose of 15–20 mg and with compound **17** this efficacy was 86–100% at 10-50 mg doses, while this was only 20% in case of nonoxynol-9 (20 mg). The average litter size of animals receiving compound **12** before mating was reduced by 67%, 74% and 76% in 10, 15 and 20 mg groups, respectively. With compound **17** the average litter size was diminished by 90%, 100% and 100% respectively at doses 10, 25 and 50 mg groups. On the other hand, N-9 at 20 mg dose caused only 37% reduction in average litter size of rabbits (Table 4).

(Table 4)

2.2.6. In vivo Anti- Trichomonas efficacy of compounds 4 and 17 in the mouse subcutaneous abscess assay model[48]

The *in vivo* efficacy of compounds **4** and **17** was evaluated using the mouse abscess assay (Figure 3). Subcutaneous injection of live trichomonads resulted in a small pustule of ~70 mm^2 area on day-8 of injection (day-1 of treatment) in experimental and control animals that grew to ~109 mm^2 in area in controls but was reduced to ~15-25 mm^2 after 5-days of treatment with compounds **4**, **17** and Metronidazole. Thereafter the growth of abscess was exponential in controls and 7 days after treatment it was ~190 mm^2 in area while it was further subdued to ~5-10 mm^2 in treated animals. On the day of autopsy (i.e. the day following seven days of treatment), the abscess was ~190 mm^2 in area in untreated controls, 7.59 mm^2 in metronidazole, 4.45 mm^2 in compound **4** and 5.75 mm^2 in compound **17** treated groups. The spleen weight at autopsy was ~0.4 g in control, ~0.59 g in saline treated, ~0.50 g in metronidazole treated, ~0.30 g in compound **4** treated and ~0.30 g in compound **17** treated animals. From the results it was found that compound **4** and **17** were found more potent than MTZ in *In vivo*.

(Figure 3)

2.2.7.Mode of action of bis(dialkylaminethiocarbonyl) disulfides:

2.2.7.1. Role of disulfide group in sperm immobilization

The activity of compounds (**4**–**38**) may be due to the disulfide linkage of the molecule as it has been found that disulfide-sulfhydryl (S–S to SH) interconversion plays an important role for motility, membrane integrity and fluidity of sperm[49] and also very vital for the viability of *Candida*[32] and *Trichomonas*.[32] Therefore, to establish the role of disulfide group, modifications (Scheme 2) were carried out in bis(dialkylaminethiocarbonyl) disulfide scaffold. S-alkylated compounds **39**–**44** and **45**–**56** were synthesized and evaluated for spermicidal activity. Alkyl 4-alkylpiperazine-1-carbodithioates (**39**–**44**) were synthesized by reaction of sodium dialkylcarbamodithioate (**3**) with alkyl halides in methanol at room temperature. While alkylene bis(4-alkylpiperazine-1-carbodithioate) (**45**–**56**) were synthesized by reaction of **3** and dihaloalkanes in acetonitrile at room temperature. Alkyl 4-alkylpiperazine-1-carbodithioates (**39**–**44**, Table 5) were inactive at 1% concentration while compounds **45**–**56** (Table 6) immobilized the sperm at MEC 0.5–1%. These results suggested that any modification at

disulfide linkage resulted in 500 fold to 1000 fold decease or complete loss of activity. These findings confirmed the role of disulfide group in spermicidal activity of this active scaffold.

(Scheme 2)

(Table 5)

(Table 6)

2.2.7.2. Mode of action of compound 12: Fluoroscence labeling of sperm thiols

To study the mode of action of bis(dialkylaminethiocarbonyl) disulfides, free –SH groups were localized by fluorescence detection (after labeling with the thiols capturing dye mBBr) of human sperm that were either motile (control) or immobilized by compound **12** treatment, and digitally imaged for qualitative assessment. It became clearly evident by visual assessment of fluorescence intensities that control sperm (Figure 4) had remarkably higher number of free thiols as compared with sperm immobilized by compound **12** (Figure 4).

(Figure 4)

Even though the difference was marked throughout the structure of sperm, it was prominently noticeable in the tail region (principal piece). The diminished fluorescence with compound **12** suggested the interaction with free thiol might be the mechanism of spermicidal action.

2.2.7.3. Mode of action of compound 4 and 17: Fluorescence labeling of Trichomonas thiols

(Figure 5)

The compounds were designed to target free thiols over the *Trichomonas* and the inhibition was qualitatively assessed by fluorescence detection after labeling the free thiols with fluorimetric thiol detector which specifically binds free thiols (Figure 5). Motile *Trichomonasvaginalis* (control) and compounds **4** and **17**-treated *Trichomonas* were digitally imaged for qualitative assessment. It was clear from Figure 5 that there was reduction in free thiol fluorescence with compound **4** and **17** treated *Trichomonas*. Fluorescence intensities were higher in controls, due to the higher number of free thiols available than in compounds-treated samples. The decreased fluorescence with the compounds treatment suggested the interaction of compound **4** and **17**with free thiols present over *Trichomonas*, which could be the mechanism of trichomonicidal action.

3. Conclusion

Bis(dialkylaminethiocarbonyl) disulfide (**4**–**38**) designs were conceptualized, synthesized and evaluated for spermicidal, antifungal and anti-*Trichomonas* activities. 4-Substituted piperazine group with smaller alkyl chain at N^4 position (**12**, **16**, **17**, MEC 0.001%) was most desirable for spermicidal activity as these were fifty times more active than N-9. Whereas single nitrogen secondary amines (**4**–**11**) were more appropriate for antifungal and anti- *Trichomonas* action, though the activity was retained in two nitrogen secondary amines i.e., piperazine derivatives. Eleven compounds were 2-16 fold more active than MTZ against resistant *Trichomonas* strain. The findings suggested that bis(dialkylaminethiocarbonyl) disulfide is a very versatile scaffold. Most promising compounds (**4, 12, 16, 17, 18** and **35)** were found to be highly safe and compatible with cervico-vaginal epithelium and flora. Of these **12** and **17** exhibited remarkable *in vivo* contraceptive activity in rabbit model with percentage efficacies of 62 and 100%, respectively. Similarly the most active trichomonacidal compounds, **4** and **17** demonstrated better *in vivo*activities in mice model compared to the standard drug MTZ. Further a docking study was carried out with less (**13**) and most active trichomonicidal compounds (**4** and **17**) to determine their interaction with cysteine synthase. It was inferred that compound **4** and **17** were more potent compared to other compounds due to their favorable H-bond interactions with Lys43 residue, which may be responsible for binding with pyridoxal-5'-phosphate (PLP). The role of disulfide group was also established by the loss of spermicidal activity on carrying out chemical modification in this scaffold. These disulfide compounds might be imparting their activity by interacting with sulfhydryl groups[22] present over cell membrane of sperm and *Trichomonas* as shown by fluorescence labeling of thiols.[33,48] Thus bis(dialkylaminethiocarbonyl) disulfide frame work has evolved as a potent lead for development of double-edged vaginal microbicides.

4. Experimental

4.1 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. ^{1}H NMR &^{13}C NMR spectra were recorded on BrukerSupercon Magnet Avance DRX-300 spectrometers (operating at 300 and 400 MHz for ^{1}H; 50, 75, 100 and 125 MHz for ^{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 ± 0.3% of calculated values. The reaction progress was routinely monitored by thin layer chromatography (TLC) on pre-coated silica gel plates (Aldrich). Column chromatography was performed over Merck silica gel (100–200 Mesh). All compounds were characterized by TLC, ^{1}H and ^{13}C NMR, MS. Elemental analyses data meet the criteria of ≥95% purity. All chemicals and solvents were procured from Sigma-Aldrich / Merck India Ltd. Sodium dialkylcarbamodithioate (**3**) were prepared by known procedure.[30]

Bis(dimethylaminothiocarbonyl) disulfide (4). The mixture of sodium dimethylcarbamodithioate (0.38 g, 2.7 mmol), sodium nitrite (0.19 g, 2.7 mmol, dissolved in 1 mL methanol) and 5 mL distilled water was stirred at 0–5 °C for 5 minutes. Then 1 mL concentrated HCl was added drop wise and the reaction mixture was stirred at 0–5 °C for 20 minutes. White solid separated which was extracted with chloroform (10×2 mL). Combined organic layer was washed with distilled water (5×2 mL) and dried over sodium sulfate. Sodium sulfate was filtered off and filtrate was concentrated under reduced pressure. The crude product was purified after recrystallization with ethanol to afford compound as white crystals (0.18 g, 56%); mp: 116–118 °C; IR (KBr) ν (cm^{-1}): 2977, 2943, 1587, 1473, 1430; ^{1}H NMR (300 MHz, $CDCl_3$): δ3.63–3.61 (12H, m); ^{13}C NMR (50 MHz, $CDCl_3$): δ193.6 (C=S), 47.5, 42.1; ESI-MS: (*m/z*) 241 (MH^+); Anal. (%) calcd. for $C_6H_{12}N_2S_4$: C, 29.97; H, 5.03; N, 11.65; found, C, 29.89; H, 5.13; N, 11. 73.

The following compounds (**5**–**38**) have been synthesized according to general procedure using the corresponding sodium dialkylcarbamodithioate.

Bis(dicyclohexylaminothiocarbonyl) disulfide (5). The title compound was synthesized by oxidation of sodium dicyclohexylcarbamodithioate in 56% yield as semi-solid; IR (KBr) ν (cm^{-1}): 2946, 1645, 1434; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.35–4.90 (2H, m), 3.30–2.71 (2H, bs),

1.86–1.16 (40H, m); ESI-MS: (*m/z*) 513 (MH^+); Anal. (%) calcd. for $C_{26}H_{44}N_2S_4$: C, 60.88; H, 8.65; N, 5.46; found, C, 60.71; H, 8.82; N, 5.55.

Bis(benzylmethylaminothiocarbonyl) disulfide (6). The title compound was synthesized by oxidation of sodium benzyl(methyl)carbamodithioate in 67% yield as white solid; mp: 150–152 °C; IR (KBr) ν (cm^{-1}): 2978, 2927, 1596, 1487, 1444; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37 (10H, m), 5.40–3.30 (4H m) 3.54 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ195.5 (C=S), 134.8, 134.3, 128.9, 128.3, 128.0, 127.8, 127.5, 62.1, 58.5, 45.4, 39.3; ESI-MS: (*m/z*) 393 (MH^+); Anal. (%) calcd. for $C_{18}H_{20}N_2S_4$: C, 55.06; H, 5.13; N, 7.13; found, C, 55.14; H, 5.25; N, 7.03.

Bis(pyrrolidinylthiocarbonyl) disulfide (7). The title compound was synthesized by oxidation of sodium pyrrolidine-1-carbodithioate in 69% yield as white solid; mp:136–138 °C; IR (KBr) ν (cm^{-1}): 2948, 1657, 1434; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.11–3.96 (8H, m), 2.16–2.02 (8H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ188.2 (C=S), 56.0, 50.0, 25.6, 23.3; HRMS *m/z*calcd. for $C_{10}H_{16}N_2S_4$ (MH^+): 293.0275; found 293.0265; Anal. (%) calcd. for $C_{10}H_{16}N_2S_4$: C, 41.06; H, 5.51; N, 9.58; found, C, 41.15; H, 5.48; N, 9.51.

Bis(piperidinylthiocarbonyl) disulfide (8). The title compound was synthesized by oxidation of sodium piperidine-1-carbodithioate in 71% yield as white solid; mp: 124–126 °C; IR (KBr) ν (cm^{-1}): 2934, 1688, 1478, 1426; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.24 (8H, bs), 1.77 (12H, m); ^{13}C NMR (50 MHz, $CDCl_3$):δ 191.7 (C=S), 52.0, 25.0, 23.2; HRMS *m/z*calcd. for $C_{12}H_{20}N_2S_4$ (MH^+): 321.0588; found 321.0573; Anal. (%) calcd. for $C_{12}H_{20}N_2S_4$: C, 44.96; H, 6.29; N, 8.74; found, C, 44.86; H, 6.22; N, 8.79.

Bis(morpholinylthiocarbonyl) disulfide (9). The title compound was synthesized by oxidation of sodium morpholine-4-carbodithioate in 70% yield as white solid; mp: 118–120 °C; IR (KBr) ν (cm^{-1}): 2979, 2927, 1587, 1469, 1426; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.23 (8H, s), 3.78–3.76 (8H, m); ^{13}C NMR (50 MHz, $CDCl_3$): δ 193.8 (C=S), 66.4, 52.7; HRMS *m/z*calcd. for$C_{10}H_{16}N_2O_2S_4$ (MH^+): 325.0173; found 325.0160; Anal. (%) calcd. for $C_{10}H_{16}N_2O_2S_4$: C, 37.01; H, 4.97; N, 8.63; found, C, 37.16; H, 4.75; N, 8.56.

Bis(4-methylpiperidinylthiocarbonyl) disulfide (10). The title compound was synthesized by oxidation of sodium 4-methylpiperidine-1-carbodithioate in 68% yield as white solid; mp: 128–130 °C; IR ν(KBr) (cm^{-1}): 2929, 1587, 1480, 1437; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.39–4.88 (4H, m), 3.34–3.33 (4H, m) 1.83–1.80 (6H, m), 1.46–1.39 (4H, m), 1.01–1.00 (6H, m); ^{13}C NMR

(75 MHz, $CDCl_3$): δ192.6 (C=S), 54.9, 52.0, 51.8, 34.0, 33.4, 30.8, 21.2; ESI-MS: (*m/z*) 349 (MH^+); Anal. (%) calcd. for $C_{14}H_{24}N_2S_4$: C, 48.23; H, 6.94; N, 8.04; found, C, 48.15; H, 6.79; N, 8.12.

Bis(azepanylthiocarbonyl) disulfide (11). The title compound was synthesized by oxidation of sodium azepane-1-carbodithioate in 65% yield as white solid; mp: 112–114 °C; IR (KBr) ν (cm^{-1}): 2934, 1599, 1492, 1421; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.21–4.15 (8H, m), 2.04–2.01 (4H m), 1.94–1.86 (4H, m), 1.65–1.61 (8H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.2 (C=S), 58.1, 53.6, 28.0, 26.5, 26.3, 25.7; HRMS *m/z*calcd. for $C_{14}H_{24}N_2S_4$ (MH^+): 349.0876; found 349.0901; Anal. (%) calcd. for $C_{14}H_{24}N_2S_4$: C, 48.23; H, 6.94; N, 8.04; found, C, 48.31; H, 6.84; N, 8.16.

Bis(4-butyl-1-piperazinylthiocarbonyl) disulfide (12). The title compound was synthesized by oxidation of sodium 4-butylpiperazine-1-carbodithioate in 61% yield as yellow solid; mp: 105–107 °C; IR (KBr) ν (cm^{-1}): 2957, 1586, 1473, 1430; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.29 (8H, bs), 2.60 (8H, bs), 2.39 (4H, t, J = 7.2 Hz), 1.53–1.46 (4H, m), 1.43–1.33 (4H, m), 0.93 (6H, t, J = 7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.3 (C=S), 57.8, 52.7, 39.5, 28.9, 20.6, 14.0; HRMS *m/z*calcd. for $C_{18}H_{34}N_4S_4$ (MH^+): 435.1745; found 435.1739; Anal. (%) calcd. for $C_{18}H_{34}N_4S_4$: C, 49.73; H, 7.88; N, 12.89; found, C, 49.53; H, 7.98; N, 12.80.

Bis(4-octyl-1-piperazinylthiocarbonyl) disulfide (13). The title compound was synthesized by oxidation of sodium 4-octylpiperazine-1-carbodithioate in 55% yield as white solid; mp: 93–95 °C; IR (KBr) ν (cm^{-1}): 2930, 1585, 1471, 1430; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.29 (8H, bs), 2.60 (8H, bs), 2.38 (4H, t, J = 7.3 Hz), 1.49 (4H, bs), 1.28 (20H, bs), 0.88 (6H, t, J = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): 193.4 (C=S), 58.2, 52.8, 31.8, 29.5, 29.3, 27.5, 26.8, 22.7, 14.1; ESI-MS: (*m/z*) 547 (MH^+); Anal. (%) calcd. for $C_{26}H_{50}N_4S_4$: C, 57.09; H, 9.21; N, 10.24; found, C, 57.39; H, 9.18; N, 10.04.

Bis(4-hexadecyl-1-piperazinylthiocarbonyl) disulfide (14). The title compound was synthesized by oxidation of sodium 4-hexadecylpiperazine-1-carbodithioate in 56% yield as off white solid; mp: 95–97 °C; IR (KBr) ν (cm^{-1}): 2926, 1586, 1471, 1430; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.43–4.29 (8H, m), 2.60–2.49 (8H m), 2.40–2.35 (4H, m), 1.49 (4H, m), 1.25 (52H, m), 0.88 (6H, t, J = 7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.3 (C=S), 58.1, 52.7, 31.9, 29.6, 29.6, 29.5, 29.5, 29.3, 27.4, 26.8, 22.6, 14.1; ESI-MS: (*m/z*) 771 (MH^+); Anal. (%) calcd. for $C_{42}H_{82}N_4S_4$: C, 65.40; H, 10.71; N, 7.26; found, C, 65.55; H, 10.61; N, 7.19.

Bis(4-admentyl-1-piperazinylthiocarbonyl) disulfide (15). The title compound was synthesized by oxidation of sodium 4-adamentylpiperazine-1-carbodithioate in 59% yield as white solid; mp: 125–127 °C; IR (KBr) ν (cm^{-1}): 2927, 1590, 1433, 1374; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.27–3.58 (8H, m), 2.59–1.54 (24H, m), 1.25 (12H, bs), 1.07 (2H, bs); ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.5 (C=S), 58.2, 32.0, 29.8, 29.7, 29.7, 29.6, 29.4, 27.5, 26.9, 22.8, 14.2; ESI-MS: (*m/z*) 591 (MH^+); Anal. (%) calcd. for $C_{30}H_{46}N_4S_4$: C, 60.97; H, 7.85; N, 9.48; found, C, 61.15; H, 8.10; N, 9.25.

Bis(4-allyl-1-piperazinylthiocarbonyl) disulfide (16). The title compound was synthesized by oxidation of sodium 4-allylpiperazine-1-carbodithioate in 65 % yield as light yellow solid; mp: 105–107 °C; IR (KBr) ν (cm^{-1}): 2919, 1588, 1471, 1425; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.91–5.78 (2H, m), 5.25–5.18 (4H, m), 4.30 (8H, bs), 3.05 (4H, d, J = 6.5 Hz), 2.63–2.60 (8H, m); ^{13}C NMR (100 MHz, $CDCl_3$):δ 193.5 (C=S), 134.2, 118.8, 61.1, 54.0, 52.5, 51.3; HRMS *m/z*calcd. for $C_{16}H_{26}N_4S_4$ (MH^+): 403.1119; found 403.1115; Anal. (%) calcd. for $C_{16}H_{26}N_4S_4$: C, 47.72; H, 6.51; N, 13.91; found, C, 47.96; H, 6.31; N, 13.74.

Bis[4-(3-cyanoproyl)-1-piperazinylthiocarbonyl] disulfide (17). The title compound was synthesized by oxidation of sodium 4-(3-cyanopropyl)piperazine-1-carbodithioate in 62% yield as off white solid; mp: 108–110 °C; IR (KBr) ν (cm^{-1}): 2951, 1585, 1473, 1429; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.23 (8H, bs), 2.55 (8H, s), 2.47(4H, t, J = 6.6 Hz), 2.39 (4H, t, J = 7.0 Hz), 1.82–1.75 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.5 (C=S), 119.5, 55.6, 52.5, 51.2, 22.6, 14.9; HRMS *m/z*calcd. for $C_{18}H_{28}N_6S_4$ (MH^+): 457.1337; found 457.1337; Anal. (%) calcd. for $C_{18}H_{28}N_6S_4$: C, 47.34; H, 6.18; N, 18.40; found, C, 47.45; H, 6.10; N, 18.26.

Bis[4-(2-morpholinoethyl)-1-piperazinylthiocarbonyl] disulfide (18). The title compound was synthesized by oxidation of sodium 4-(2-morpholinoethyl)piperazine-1-carbodithioate in 60% yield as white solid; mp: 110–112 °C; IR ν (KBr) (cm^{-1}): 2955, 1589, 1471, 1430; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.28 (8H, bs), 3.72–3.69 (8H m), 2.67–2.47 (24H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.5 (C=S), 66.9, 56.4, 56.3, 55.4, 55.0, 54.1, 53.3, 53.0, 51.4; ESI-MS: (*m/z*) 549 (MH^+); Anal. (%) calcd. for $C_{22}H_{40}N_6O_2S_4$: C, 48.14; H, 7.35; N, 15.31; found, C, 48.32; H, 7.21; N, 15.45.

Bis[4-(4-fluorophenyl)-1-piperazinylthiocarbonyl] disulfide (19). The title compound was synthesized by oxidation of sodium 4-(4-fluorophenyl)piperazine-1-carbodithioate in 69% yield

as white solid; mp: 140–142 °C; IR (KBr) ν (cm^{-1}): 2924, 1586, 1474, 1429; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.04–6.99 (4H, m), 6.94–6.89 (4H, m), 4.46 (8H m), 3.32–3.29 (8H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.8 (C=S), 159.5, 147.0, 147.0, 118.7, 118.6, 116.1, 115.8, 50.3; ESI-MS: (*m/z*) 511 (MH^+); Anal. (%) calcd. for $C_{22}H_{24}F_2N_4S_4$: C, 51.74; H, 4.74; N, 10.97; found, C, 51.69; H, 4.81; N, 10.87.

Bis[4-(2-methoxyphenyl)-1-piperazinylthiocarbonyl] disulfide (20). The title compound was synthesized by oxidation of sodium 4-(2-methoxyphenyl)piperazine-1-carbodithioate in 60% yield as white solid; mp: 125–127 °C; IR (KBr) ν (cm^{-1}): 2998, 1592, 1474, 1429; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.08–6.88 (8H, m), 4.47 (8H m), 3.89 (6H, s), 3.24 (8H, bs); ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.6 (C=S), 152.2, 139.9, 123.8, 121.1, 118.6, 111.4, 55.5, 50.3; ESI-MS: (*m/z*) 535 (MH^+); Anal. (%) calcd. for $C_{24}H_{30}N_4O_2S_4$: C, 53.90; H, 5.65; N, 10.48; found, C, 53.98; H, 5.52; N, 10.39.

Bis[4-(4-nitro-2-(trifluoromethyl)phenyl)-1-piperazinylthiocarbonyl] disulfide (21). The title compound was synthesized from sodium 4 (4-nitro-2-(trifluoromethyl)phenyl)piperazine-1-carbodithioate in 56% yield as white solid; m.p: 146–148 °C; IR (KBr)ν (cm^{-1}): 2925, 2854, 1535, 1468, 1425,1327,1221; ^{1}H NMR (300 MHz, $CDCl_3$): δ8.14 (2H, d, *J* = 1.4 Hz), 7.75 (2H, dd, *J* = 8.64, 1.8 Hz), 7.26–7.20 (2H, m), 4.49 (8H, bs), 3.38 (8H, t, *J* = 4.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ198.6 (C=S), 156.8, 142.8, 128.0, 124.6, 124.5, 124.4, 124.4, 122.9, 52.6, 51.5, 50.5, 45.4; ESI-MS: *m/z* 701 (MH^+); Anal. (%) calcd. for $C_{24}H_{22}F_6N_6O_4S_4$: C, 41.14; H, 3.16; N, 11.99; found, C, 41.37; H, 3.19; N, 12.15.

Bis(4-pyridin-2-yl-1-piperazinylthiocarbonyl) disulfide (22). The title compound was synthesized by oxidation of sodium 4-(pyridin-2-yl)piperazine-1-carbodithioate in 61% yield as white-yellow solid; mp: 168–170 °C; IR (KBr) ν (cm^{-1}): 2944, 1657, 1561, 1474; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19–8.14 (2H, m); 7.57–7.47 (2H, m); 6.72–6.67 (4H, m); 4.41 (8H, bs), 3.78 (8H, bs); ESI-MS: (*m/z*) 477 (MH^+); Anal. (%) calcd. for $C_{20}H_{24}N_6S_4$: C, 50.39; H, 5.07; N, 17.63; found, C, 50.55; H, 5.32; N, 17.41.

Bis(4-pyrimidin-2-yl-1-piperazinylthiocarbonyl) disulfide (23). The title compound was synthesized by oxidation of sodium 4-(pyrimidin-2-yl)piperazine-1-carbodithioate in 62% yield as white solid; mp: 125–127 °C; IR (KBr) ν (cm^{-1}): 2991, 2861, 1586, 1552, 1481, 1420, 1352, 1221; ^{1}H NMR (300 MHz, $CDCl_3$): δ8.35 (4H, d, *J* = 4.7 Hz), 6.59–6.56 (2H, m), 4.38 (8H, bs),

4.06–4.03 (8H, m); ESI-MS: (*m/z*) 479 (MH^+); Anal. (%) calcd. for $C_{18}H_{22}N_8S_4$: C, 45.16; H, 4.63; N, 23.41; found, C, 45.29; H, 4.42; N, 23.24.

Bis(4-benzoyl-1-piperazinylthiocarbonyl) disulfide (24). The title compound was synthesized from sodium 4-benzoylpiperazine-1-carbodithioate in 52% yield as white solid; m.p: 150–152 °C; IR (KBr) ν (cm^{-1}): 2969, 1742, 1530, 1364, 1263, ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44–7.42 (10H, m), 4.32–3.70 (16H, m); ^{13}C NMR (75 MHz, $CDCl_3$): 197.5, 163.4, 135.6, 133.5, 132.1, 129.4, 129.1, 128.7, 127.9, 127.8, 50.1, 42.2; ESI-MS: *m/z* 531 (MH^+); Anal. (%) calcd. for $C_{24}H_{26}N_4O_2S_4$: C, 54.31; H, 4.94; N, 10.56; found, C, 54.12; H, 5.15; N, 10.69.

Bis(4-ethoxycarbonyl-1-piperazinylthiocarbonyl) disulfide (25). The title compound was synthesized by oxidation of sodium 4-(ethoxycarbonyl)piperazine-1-carbodithioate in 61% yield as white solid; mp: 120–122 °C; IR (KBr) ν (cm^{-1}): 2978, 2901, 1686, 1479, 1418, 1282, 1162, 1126; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.29 (8H, bs), 4.22–4.15 (4H, q, *J* = 7.1 Hz), 3.70–3.67 (8H, m), 1.29 (6H, t, *J* = 7.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ194.0 (C=S), 155.2 (C=O), 62.0, 52.0, 43.2, 14.6; ESI-MS: (*m/z*) 468 (MH^+); Anal. (%) calcd. for $C_{16}H_{26}N_4O_4S_4$: C, 41.18; H, 5.62; N, 12.01; found, C, 41.24; H, 5.41; N, 12.28.

Bis(4-isobutoxycarbonyl-1-piperazinylthiocarbonyl) disulfide (26). The title compound was synthesized from sodium 4-(isobutoxycarbonyl)piperazine-1-carbodithioate in 50% yield as white solid; m.p 130-132 °C; IR (KBr) ν (cm^{-1}): 2927, 2866, 1639, 1455, 1412; ^{1}H NMR (300 MHz, $CDCl_3$): δ4.30 (8H, bs), 3.92 (4H, d, *J* = 6.6 Hz), 3.69 (8H, bs), 2.02–1.89 (2H, m), 0.95 (12H, d, *J* = 6.7 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ194.0, 155.2, 62.0, 51.8, 49.2, 24.9, 14.7; ESI-MS: *m/z* 523(MH^+); Anal. (%) calcd. for $C_{20}H_{34}N_4O_4S_4$: C, 45.95; H, 6.56; N, 10.72; found, C, 46.12; H, 6.65; N, 10.85.

Bis(4-*tert*-butoxycarbonyl-1-piperazinylthiocarbonyl) disulfide (27).The title compound was synthesized by oxidation of sodium 4-(*tert*-butoxycarbonyl)piperazine-1-carbodithioate in 69% yield as white solid; mp: 163-165 °C; IR (KBr) ν (cm^{-1}): 2976, 2843, 1690, 1228; ^{1}H NMR (300 MHz, $CDCl_3$): δ4.27 (8H, bs), 3.64–3.61 (8H, m), 1.48 (18H, s); ^{13}C NMR (50 MHz, $CDCl_3$): δ193.9 (C=S), 154.4 (C=O), 80.7, 52.3, 43.1, 28.4;ESI-MS: (*m/z*) 545 (M + Na); Anal. (%) calcd. for $C_{20}H_{34}N_4O_4S_4$: C, 45.95; H, 6.56; N, 10.72; found, C, 46.14; H, 6.42; N, 10.61.

Bis(4-methylsulfonyl-1-piperazinylthiocarbonyl) disulfide (28). The title compound was synthesized from sodium 4-(methylsulfonyl)piperazine-1-carbodithioate in 46% yield as white

solid; m.p: 138–140 °C; IR (KBr) ν (cm^{-1}): 2969, 1742, 1530, 1364, 1263, ^{1}H NMR (300 MHz, CDCl3): δ4.41 (4H, t, *J* = 5.2 Hz), 3.96 (4H, t, *J* = 5.4 Hz), 3.50 (4H, t, *J* = 5.1 Hz), 3.24 (4H, t, *J* = 5.3 Hz), 2.84 (6H s); ^{13}C NMR (50 MHz, $CDCl_3$): δ194.0, 62.0, 51.8, 43.2; ESI-MS: *m/z* 479 (MH^+); Anal. (%) calcd. for $C_{12}H_{22}N_4O_4S_6$: C, 30.11; H, 4.63; N, 11.70; found, C, 30.40; H, 4.90; N, 11.52.

Bis(4-tosyl-1-piperazinylthiocarbonyl) disulfide (29). The title compound was synthesized from sodium 4-tosylpiperazine-1-carbodithioate in 48% yield as white solid; m.p: 140–142 °C; IR (KBr) ν (cm^{-1}): 2969, 1742, 1530, 1364, 1263, ^{1}H NMR (300 MHz, $CDCl_3$): δ7.64–7.61 (4H, m), 7.35–7.26 (4H, m), 4.38–4.32 (5H, m), 3.92–3.89 (2H, m), 3.27–2.97 (9H, m), 2.43 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ198.5, 146.7, 128.9, 128.8, 122.9, 48.9, 45.3, 22.7; ESI-MS: *m/z* 653 (M^+ + Na); Anal. (%) calcd. for $C_{24}H_{30}N_4O_4S_6$: C, 45.69; H, 4.79; N, 8.88; found, C, 45.58; H, 4.95; N, 8.70.

Bis(4-butylthiocarbonothioyl-1-piperazinylthiocarbonyl) disulfide (30). The title compound was synthesized by oxidation of sodium 4-(butylthiocarbonothioyl)piperazine-1-carbodithioate in 56% yield as light green solid; mp: 172–174 °C; IR (KBr) ν (cm^{-1}): 2927, 2866, 1639, 1216; ^{1}H NMR (300 MHz, $CDCl_3$): δ4.40–4.39 (16H, m), 3.33 (4H, t, *J* = 7.4 Hz), 1.75–1.66 (4H, m), 1.52–1.40 (4H, m), 0.95 (6H, t, *J* = 7.3 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ198.7 (C=S), 194.0 (C=S), 48.7, 37.2, 30.6, 22.2, 13.7;ESI-MS: (*m/z*) 587 (MH^+); Anal. (%) calcd. for $C_{20}H_{34}N_4S_8$: C, 40.92; H, 5.84; N, 9.54; found, C, 41.10; H, 5.97; N, 9.69.

Bis(4-hexylthiocarbonothioyl-1-piperazinylthiocarbonyl) disulfide (31). The title compound was synthesized by oxidation of sodium 4-(hexylthiocarbonothioyl)piperazine-1-carbodithioate in 69% yield as yellow solid; mp:158–160 °C; IR (KBr) ν (cm^{-1}): 2923, 2860, 1628, 1214; ^{1}H NMR (300 MHz, $CDCl_3$): δ4.40–4.39 (16H, m), 3.50 (4H, t, *J* = 7.4 Hz), 2.68–2.63 (4H, m), 2.47 (8H, bs), 1.59–1.57 (8H, m), 1.45–1.43 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ198.6 (C=S), 194.0 (C=S), 57.4, 54.4, 48.9, 34.6, 25.9, 24.4; ESI-MS: (*m/z*) 643 (MH^+); Anal. (%) calcd. for $C_{24}H_{42}N_4S_8$: C, 44.82; H, 6.58; N, 8.71; found, C, 44.65; H, 6.72; N, 8.86.

Bis(4-heptylthiocarbonothioyl-1-piperazinylthiocarbonyl) disulfide (32). The title compound was synthesized by oxidation of sodium 4-(heptylthiocarbonothioyl)piperazine-1-carbodithioate in 55% yield as white solid; mp: 169–171 °C; IR (KBr) ν (cm^{-1}): 2930, 2854, 1639, 1216; ^{1}H NMR (300 MHz, $CDCl_3$): δ4.41–4.39 (16H, m), 3.32 (4H, t, *J* = 7.4 Hz), 1.76–1.74 (4H, m),

1.42–1.25 (16H, m), 0.91–0.86 (6H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ198.7 (C=S), 193.9 (C=S), 48.8, 48.1, 37.5, 31.7, 29.0, 28.9, 28.5, 22.6, 14.1; ESI-MS: (*m/z*) 671 (MH^+); Anal. (%) calcd. for $C_{26}H_{46}N_4S_8$: C, 46.53; H, 6.91; N, 8.35; found, C, 46.74; H, 6.78; N, 8.46.

Bis(4-octylthiocarbonothioyl-1-piperazinylthiocarbonyl) disulfide (33). The title compound was synthesized by oxidation of sodium 4-(octylthiocarbonothioyl)piperazine-1-carbodithioate in 62% yield as pale yellow solid; mp: 147–149 °C; IR (KBr) ν (cm^{-1}): 2923, 2855, 1643, 1219; ^{1}H NMR (300 MHz, $CDCl_3$): δ4.66–4.24 (16H, m), 3.32 (4H, t, *J* = 7.4 Hz), 1.76–1.66 (4H, m), 1.44–1.28 (20H, m), 0.90–0.86 (6H, m); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6): δ197.6 (C=S), 193.2 (C=S), 47.2, 36.8, 36.5, 31.0, 28.4, 28.2, 27.8, 21.9, 13.4; ESI-MS: (*m/z*) 699 (MH^+); Anal. (%) calcd. for $C_{28}H_{50}N_4S_8$: C, 48.09; H, 7.21; N, 8.01; found, C, 47.93; H, 7.38; N, 8.16.

Bis(4-decylthiocarbonothioyl-1-piperazinylthiocarbonyl) disulfide (34). The title compound was synthesized by oxidation of sodium 4-(decylthiocarbonothioyl)piperazine-1-carbodithioate in 65% yield as light yellow solid; mp:138–140 °C; IR (KBr) ν (cm^{-1}): 2926, 2856, 1635, 1218; ^{1}H NMR (300 MHz, DMSO-d_6): δ4.49–4.46 (4H, m), 4.38–4.05 (8H, m), 3.80 (4H, t, *J* = 5.7 Hz), 3.25 (4H, t, *J* = 7.4 Hz), 1.68–1.58 (4H, m), 1.41–1.24 (28H, m), 0.87–0.83 (6H, m);ESI-MS: m/z 755 (MH^+); Anal. (%) calcd. for $C_{32}H_{58}N_4S_8$: C, 50.88; H, 7.74; N, 7.42; found, C, 51.03; H, 7.56; N, 7.28.

Bis[4-((2-(prrrolidin-1-yl)ethylthio)carbonothioyl)-1-piperazinylthiocarbonyl] disulfide (35). The title compound was synthesized by oxidation of sodium 4-((2-(pyrrolidin-1-yl)ethylthio)carbonothioyl)piperazine-1-carbodithioate in 63% yield as white solid; mp: 140–145 °d; IR (KBr) ν (cm^{-1}): 2921, 2863, 1642, 1219; ^{1}H NMR (300 MHz, $CDCl_3$): δ4.40–4.39 (16H, m), 3.53 (4H, t, *J* = 7.0 Hz), 2.82 (4H, t, *J* = 7.0 Hz), 2.60 (8H, bs), 1.80 (8H, bs); ^{13}C NMR (75 MHz, $CDCl_3$): δ198.5 (C=S), 194.0 (C=S), 54.7, 54.1, 50.8, 48.9, 36.4, 29.7, 23.6;ESI-MS: (*m/z*) 669 (MH^+); Anal. (%) calcd. for $C_{24}H_{40}N_6S_8$: C, 43.08; H, 6.03; N, 12.56; found, C, 42.93; H, 6.14; N, 12.33.

Bis[4-((2-(piperidin-1-yl)ethylthio)carbonothioyl)-1-piperazinylthiocarbonyl] disulfide (36). The title compound was synthesized by oxidation of sodium 4-((2-(pireidin-1-yl)ethylthio)carbonothioyl)piperazine-1-carbodithioate in 57% yield as white solid; mp:182–184 °C; IR (KBr) ν (cm^{-1}): 2926, 2854, 1638, 1217; ^{1}H NMR (300 MHz, $CDCl_3$): δ4.40–4.39 (16H, m), 3.50 (4H, t, *J* = 7.3 Hz), 2.66 (4H, t, *J* = 7.3 Hz), 2.47 (8H, bs), 1.59–1.57 (8H, m), 1.45–

1.43 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ198.6 (C=S), 194.0 (C=S), 57.4, 54.4, 48.9, 34.6, 25.9, 24.4; ESI-MS: (*m/z*) 697 (MH^+); Anal. (%) calcd. for $C_{26}H_{44}N_6S_8$: C, 44.79; H, 6.36; N, 12.05; found, C, 44.92; H, 6.14; N, 12.29.

Bis[4-((2-(morpholin-1-yl)ethylthio)carbonothioyl)-1-piperazinylthiocarbonyl] disulfide (37). The title compound was synthesized by oxidation of sodium 4-((2-(morpholin-1-yl)ethylthio)carbonothioyl)piperazine-1-carbodithioate in 54% yield as white solid; mp:170–172 °C; IR (KBr) ν (cm^{-1}): 2923, 2858, 1639, 1215; ^{1}H NMR (300 MHz, $CDCl_3$): δ4.41–4.39 (16H, m), 3.73–3.70 (8H, m), 3.51 (4H, t, *J* = 7.2 Hz), 2.70 (4H, t, *J* = 7.2 Hz), 2.55–2.52 (8H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ198.2 (C=S), 193.9 (C=S), 66.9, 57.0, 53.4, 49.4, 34.3;ESI-MS: (*m/z*) 701 (MH^+); Anal. (%) calcd. for $C_{24}H_{40}N_6O_2S_8$: C, 41.11; H, 5.75; N, 11.99; found, C, 41.27; H, 5.91; N, 12.13.

Bis(4-benzylthiocarbonothioyl-1-piperazinylthiocarbonyl) disulfide (38). The title compound was synthesized by oxidation of sodium 4-(benzylthiocarbonothioyl)piperazine-1-carbodithioate in 61% yield as white solid; mp:135–137 °C; IR (KBr) ν (cm^{-1}): 2918, 2850, 1639, 1534, 1494, 1452, 1219; ^{1}H NMR (300 MHz, $CDCl_3$): δ7.39–7.25 (10H, m), 4.58 (4H, s), 4.39–4.38 (16H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ197.5 (C=S), 135.6, 129.4, 128.7, 127.7, 48.6, 42.2; ESI-MS: (*m/z*) 655 (MH^+); Anal. (%) calcd. for $C_{26}H_{30}N_4S_8$: C, 47.67; H, 4.62; N, 8.55; found, C, 47.89; H, 4.75; N, 8.31.

Propyl 4-butylpiperazine-1-carbodithioate (39). To the mixture of sodium 4-butylpiperazine-1-carbodithioate (2.0 g, 8.33 mmol), triethyl amine (1.21 g, 12.49 mmol) and methanol (10 mL) was added propyl bromide (1.02 g, 8.33 mmol) drop wise through dropping funnel over 30 minutes at room temperature. The above reaction mixture was further stirred at room temperature for one hour. The reaction mixture was concentrated over rotavapor and 50 mL ethyl acetate was added to it. Ethyl acetate layer was washed with distilled water (5 x 3 mL) and dried over sodium sulfate. Sodium sulfate was filtered off and filtrate was concentrated under reduced pressure. The crude product was purified over column chromatography (100–200 mesh) using MeOH/$CHCl_3$ as eluent to afford compound as yellow liquid (0.70 g, 65%); IR (Neat) ν (cm^{-1}): 3019, 2400, 1632, 1523, 1215; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.34–3.96 (4H, m), 3.30–3.27 (2H, m), 2.51 (4H, t, *J* = 5.1 Hz), 2.38–2.34 (2H, m), 1.76–1.70 (2H, m), 1.51–1.44 (2H, m), 1.38–1.29 (2H, m), 1.02 (3H, t, *J* = 7.4 Hz), 0.92 (3H, t, *J* = 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 197.3,

58.0, 52.7, 49.9, 39.2, 29.0, 22.2, 20.7, 14.1, 13.6; ESI-MS *m/z*: 261 (MH^+); Anal. (%) calcd. for $C_{12}H_{24}N_2S_2$: C, 55.34; H, 9.29; N, 10.76; found C, 54.52; H, 9.46; N, 10.85.

The following compounds (**40**–**44**) were prepared using a procedure similar to that described for compound **39** from the corresponding sodium dialkylcarbamodithioate and haloalkane.

Hexyl 4-butylpiperazine-1-carbodithioate (40). The title compound was synthesized from sodium 4-butylpiperazine-1-carbodithioate and hexyl bromide in 62% yield as yellow liquid; IR (Neat) ν (cm^{-1}): 3020, 2932, 2401, 1634, 1466; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.34–3.74 (4H, m), 3.29 (2H, t, *J* = 7.5 Hz), 2.52–2.49 (4H, m), 2.38–2.34 (2H, m), 1.71–1.66 (2H, m), 1.49–1.28 (10H, m), 0.94–0.88 (6H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 197.3, 58.0, 52.7, 51.1, 37.3, 31.4, 29.0, 28.8, 28.6, 22.6, 20.7, 14.1, 14.1; ESI-MS *m/z*: 303 (MH^+); Anal. (%) calcd. for $C_{15}H_{30}N_2S_2$: C, 59.55; H, 9.99; N, 9.26; found C, 59.63; H, 10.14; N, 9.33.

Propyl 4-allylpiperazine-1-carbodithioate (41). The title compound was synthesized from sodium 4-allylpiperazine-1-carbodithioat and propyl bromide in 87% yield as yellow liquid;IR (Neat) ν (cm^{-1}): 3434, 2968, 2402, 1423, 1218; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.89–5.79 (1H, m), 5.23–5.16 (2H, m), 4.35–3.97 (4H, m), 3.30–3.26 (2H, m), 3.04–3.01 (2H, m), 2.52 (4H, t, *J* = 5.1 Hz), 1.77–1.70 (2H, m), 1.04–1.00 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 197.4, 134.3, 118.7, 61.2, 52.4, 49.8, 39.2, 22.1, 13.6; ESI-MS *m/z*: 245 (MH^+); Anal. (%) calcd. for $C_{11}H_{20}N_2S_2$: C, 54.05; H, 8.25; N, 11.46; found C, 54.03; H, 8.53; N, 11.41.

Hexyl 4-allylpiperazine-1-carbodithioate (42). The title compound was synthesized from sodium 4-allylpiperazine-1-carbodithioate and hexyl bromide in 80% yield as yellow liquid; IR (Neat) ν (cm^{-1}): 3079, 2859, 2402, 1641, 1463; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.88–5.84(1H, m), 5.23–5.17 (2H, m), 4.69–3.97 (4H, m), 3.29 (2H, t, *J* = 7.5 Hz), 3.04–3.01 (2H, m), 2.53 (4H, t, *J* = 5.1 Hz), 1.71–1.64 (2H, m), 1.42–1.28 (6H, m), 0.90–0.87 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 197.5, 134.3, 118.7, 61.2, 52.4, 51.0, 37.4, 31.4, 28.8, 28.6, 22.6, 14.1; ESI-MS *m/z*: 287 (MH^+); Anal. (%) calcd. for $C_{14}H_{26}N_2S_2$: C, 58.69; H, 9.15; N, 9.78; found C, 58.53; H, 9.25; N, 9.83.

Propyl 4-(3-cyanopropyl)piperazine-1-carbodithioate (43). The title compound was synthesized from sodium 4-(3-cyanopropyl)piperazine-1-carbodithioate and propyl bromide in 55% yield as yellow liquid; IR (Neat) ν (cm^{-1}): 3444, 3019, 2400, 1637, 1466, 1215; ^{1}H NMR (400 MHz, $CDCl_3$): δ4.35–3.94 (4H, m), 3.30–3.26 (2H, m), 2.53–2.49 (6H, m), 1.87–1.80 (2H, m), 1.79–1.70 (2H, m), 1.59 (2H, m), 1.02 (3H, t, *J* = 7.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ

197.6, 119.6, 55.8, 52.5, 50.8, 49.7, 39.2, 29.7, 22.7, 22.1, 14.9, 13.6; ESI-MS *m/z*: 272 (MH^+); Anal. (%) calcd. for $C_{12}H_{21}N_3S_2$: C, 53.10; H, 7.80; N, 15.48; found C, 53.34; H, 8.01; N, 15.27.

Hexyl 4-(3-cyanopropyl)piperazine-1-carbodithioate (44). The title compound was synthesized from sodium 4-(3-cyanopropyl)piperazine-1-carbodithioate and hexyl bromide in 58% yield as yellow liquid; IR (Neat) ν (cm^{-1}) 3020, 2931, 2401, 1637, 1424; ^{1}H NMR (400 MHz, $CDCl_3$) δ4.31–3.86 (4H, m), 3.31–3.27 (2H, m), 2.53–2.48 (4H, m), 1.86 (2H, t, J = 6.8 Hz), 1.82–1.80 (2H, m), 1.73–1.70 (4H, m), 1.69–1.65 (2H, m), 1.60–1.55 (4H, m), 0.90–0.86 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.7, 121.1, 57.7, 55.8, 52.5, 37.4, 31.4, 28.8, 28.6, 22.7, 22.6, 15.0, 14.1; ESI-MS *m/z*: 314 (MH^+); Anal. (%) calcd. for $C_{15}H_{27}N_3S_2$: C, 57.46; H, 8.68; N, 13.40; found C, 57.34; H, 8.51; N, 13.27.

Synthesis of methylene bis(4-methylpiperazine-1-carbodithioate) (45). The mixture of 4-methylpiperazine-1-carbodithioate (2.21 gm, 11.16 mmol) and diiodomethane (0.3 mL, 3.72 mmol) in CH_3CN (20 mL) was stirred at room temperature for overnight. The reaction mixture was concentrated under reduced pressure, crude product was treated with water (10 mL) and extracted withEtOAc (10 x 3 mL). EtOAc layer was washed with water (5 x 3 mL) and combined organic layers were dried on anhydrous sodium sulfate, filtered, and concentrated. The crude product was purified over column chromatography (100–200 mesh) using MeOH/$CHCl_3$ as eluent to afford compound as white solid (0.76 g, 56%); mp: 140–142 °C; IR (KBr) ν (cm^{-1}) 2929, 2799, 1658; ^{1}H NMR (300 MHz, $CDCl_3$): δ5.42 (2H, s), 4.32 (4H, bs), 3.90 (4H, bs), 2.48 (8H, bs), 2.31 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ195.7 (C=S), 54.3, 51.3, 49.9, 45.6; ESI-MS: (*m/z*)365 (MH^+); Anal. (%) calcd. for $C_{13}H_{24}N_4S_4$: C, 42.82; H, 6.63; N, 15.37; found C, 42.96; H, 6.75; N, 15.42.

The following compounds (**46**–**56**) were prepared using a procedure similar to that described for compound **45** from the corresponding sodium dialkylcarbamodithioate and dihaloalkane.

Ethane-1,2-diyl bis(4-methylpiperazine-1-carbodithioate) (46). The title compound was synthesized from sodium 4-methylpiperazine-1-carbodithioate and 1,2-dibromoethane in 53% yield as white solid; mp: 150–152 °C; IR (KBr) ν (cm^{-1}): 2931, 2854, 1630; ^{1}H NMR (300 MHz, $CDCl_3$): δ4.35 (4H, bs), 3.96 (4H, bs), 3.68 (4H, s), 2.51–2.48 (8H, m), 2.33 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ196.2 (C=S), 54.4, 51.2, 50.0, 45.7, 35.8; ESI-MS: (*m/z*)379 (MH^+); Anal. (%) calcd. for $C_{14}H_{26}N_4S_4$: C, 44.41; H, 6.92; N, 14.80; found C, 44.32; H, 7.16; N, 14.72.

Propane-1,3-diyl bis(4-methylpiperazine-1-carbodithioate) (47). The title compound was synthesized from sodium 4-methylpiperazine-1-carbodithioate and 1,2-dibromopropane in 53% yield as white solid; mp: 120–122 °C; IR (KBr) ν (cm^{-1}): 2927, 2853, 1654; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.32–3.97 (8H, m), 3.43 (4H, t, *J* = 7.2 Hz), 2.50–2.47 (8H, m), 2.32 (6H, s), 2.18–2.09 (2H, m); ESI-MS: (*m/z*) 393 (MH^+); Anal. (%) calcd. for $C_{15}H_{28}N_4S_4$: C, 45.88; H, 7.19; N, 14.27; found C, 45.67; H, 7.21; N, 14.47.

Methylene bis(4-allylpiperazine-1-carbodithioate) (48). The title compound was synthesized from sodium 4-allylpiperazine-1-carbodithioate and diiodomethane in 54% yield as white solid; mp: 125–127 °C; IR (KBr) ν (cm^{-1}): 2908, 2804, 1646; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.88–5.79 (2H, m), 5.42 (2H, s), 5.23–5.17 (4H, m), 4.34 (4H, bs), 3.90–3.89 (4H, m), 3.03 (4H, d, *J* = 6.5 Hz), 2.52 (8H, bs,); ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.8 (C=S), 134.2, 118.8, 61.1, 52.3, 51.6, 50.1, 45.7; ESI-MS: (*m/z*)417 (MH^+); Anal. (%) calcd. for $C_{17}H_{28}N_4S_4$: C, 49.00; H, 6.77; N, 13.45; found C, 49.25; H, 6.67; N, 13.59.

Ethane-1,2-diyl bis(4-allylpiperazine-1-carbodithioate) (49). The title compound was synthesized from sodium 4-allylpiperazine-1-carbodithioate and diiodoethane in 46% yield as white solid; mp: 140–142 °C; IR (KBr) ν (cm^{-1}): 2925, 2856, 1659; ^{1}H NMR (300 MHz, $CDCl_3$ + CCl_4): δ 5.87–5.78 (2H, m), 5.21–5.16 (4H, m), 4.36–3.89 (8H, m), 3.63 (4H, s), 3.02 (4H, d, *J* = 6.4 Hz), 2.52 (8H, bs); ^{13}C NMR (75 MHz, $CDCl_3$ + CCl_4): δ 196.2 (C=S), 134.6, 118.8, 61.4, 52.6, 51.0, 35.9; ESI-MS: (*m/z*)431 (MH^+); Anal. (%) calcd. for $C_{18}H_{30}N_4S_4$: C, 50.19; H, 7.02; N, 13.01; found C, 50.21; H, 7.32; N, 13.30.

Propane-1,3-diyl bis(4-allylpiperazine-1-carbodithioate) (50). The title compound was synthesized from sodium 4-allylpiperazine-1-carbodithioate and diiodopropane in 50% yield as white solid; mp: 108–110 °C; IR (KBr) ν (cm^{-1}): 2923, 2807, 1632; ^{1}H NMR (300 MHz, $CDCl_3$ + CCl_4): δ 5.89–5.77 (2H, m), 5.23–5.17 (4H, m), 4.33–3.97 (8H, m), 3.42 (4H, t, *J* = 7.2 Hz), 3.03 (4H, d, *J* = 6.5 Hz), 2.54–2.51 (8H, m), 2.18–2.08 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$ + CCl_4): δ 196.3 (C=S), 134.5, 118.6, 61.3, 52.5, 50.9, 50.0, 35.9, 28.2; ESI-MS: (*m/z*)445 (MH^+); Anal. (%) calcd. for $C_{19}H_{32}N_4S_4$: C, 51.31; H, 7.25; N, 12.60; found C, 51.38; H, 7.27; N, 12.63.

Methylene bis(4-butylpiperazine-1-carbodithioate) (51). The title compound was synthesized from sodium 4-butylpiperazine-1-carbodithioate and diiodomethane in 50% yield as white solid; mp: 116–118 °C; IR (KBr) ν (cm^{-1}): 2933, 2847, 1642; ^{1}H NMR (300 MHz, $CDCl_3$ + CCl_4): δ

5.38 (2H, s), 4.29–3.89 (8H, m), 2.50 (8H, bs), 2.37–2.32 (4H, m), 1.48–1.29 (8H, m), 0.92 (6H, t, J = 7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$ + CCl_4): δ 195.6 (C=S), 57.9, 52.7, 51.5, 50.2, 45.6, 29.1, 20.7, 14.2; ESI-MS: (*m/z*)449 (MH^+); Anal. (%) calcd. for $C_{19}H_{36}N_4S_4$: C, 50.85; H, 8.09; N, 12.48; found C, 50.76; H, 8.21; N, 12.29.

Ethane-1,2-diyl bis(4-butylpiperazine-1-carbodithioate) (52). The title compound was synthesized from sodium 4-butylpiperazine-1-carbodithioate and diiodoethane in 46% yield as white solid; mp: 120–122 °C; IR (KBr) ν (cm^{-1}): 2927, 2821, 1639; ^{1}H NMR (300 MHz, $CDCl_3$ + CCl_4): δ 4.28-3.96 (8H, m), 3.64 (4H, s), 2.52–2.49 (8H, m), 2.37–2.33 (4H, m), 1.49–1.44 (4H, m), 1.37–1.30 (4H, m), 0.95–0.90 (6H, m); ^{13}C NMR (75 MHz, $CDCl_3$ + CCl_4): δ 195.9 (C=S), 58.0, 52.8, 51.3, 50.3, 35.8, 29.1, 20.8, 14.2; ESI-MS: (*m/z*)463 (MH^+); Anal. (%) calcd. for $C_{20}H_{38}N_4S_4$: C, 51.90; H, 8.28; N, 12.11; found C, 52.11; H, 8.35; N, 12.20.

Propane-1,3-diyl bis(4-butylpiperazine-1-carbodithioate) (53). The title compound was synthesized from sodium 4-butylpiperazine-1-carbodithioate and diiodopropane in 40% yield as white solid; mp: 80–82 °C; IR (KBr) ν (cm^{-1}): 2926, 2816, 1643; ^{1}H NMR (300 MHz, $CDCl_3$ + CCl_4): δ 4.29–3.97 (8H, m), 3.40 (4H, t, J = 7.1 Hz), 2.52–2.49 (8H, m), 2.38–2.33 (4H, m), 2.17–2.07 (2H, m), 1.50–1.42 (4H, m), 1.38–1.30 (4H, m), 0.93 (6H, t, J = 7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$ + CCl_4): δ 196.4 (C=S), 58.0, 52.8, 50.6, 35.9, 29.1, 28.3, 20.8, 14.2; ESI-MS: (*m/z*) 477 (MH^+); Anal. (%) calcd. for $C_{21}H_{40}N_4S_4$: C, 52.90; H, 8.46; N, 11.75; found C, 52.88; H, 8.35; N, 11.55.

Methylene bis(4-(3-cyanopropyl)piperazine-1-carbodithioate) (54). The title compound was synthesized from sodium 4-(3-cyanopropyl)piperazine-1-carbodithioate and diiodomethane in 65% yield as white solid; mp: 130–132 °C; IR (KBr) ν (cm^{-1}): 2944, 2822, 2246, 1647; ^{1}H NMR (300 MHz, $CDCl_3$ + CCl_4): δ 5.36 (2H, s), 4.28–3.91 (8H, m), 2.53–2.41 (16H, m), 1.87–1.78 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$ + CCl_4): δ 195.7 (C=S), 119.1, 55.8, 52.6, 50.0, 45.7 , 22.8, 15.0; ESI-MS: (*m/z*)471 (MH^+); Anal. (%) calcd. for $C_{19}H_{30}N_6S_4$: C, 48.48; H, 6.42; N, 17.85; found C, 48.55; H, 6.32; N, 17.62.

Ethane-1,2-diyl bis(4-(3-cyanopropyl)piperazine-1-carbodithioate) (55). The title compound was synthesized from sodium 4-(3-cyanopropyl)piperazine-1-carbodithioate and diiodoethane in 51% yield as white solid; mp: 152–154 °C; IR (KBr) ν (cm^{-1}): 2929, 2814, 2248, 1632; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.33–3.96 (8H, m), 3.68 (4H, s), 2.55–2.43 (16H, m), 1.89–1.80 (4H, m);

^{13}C NMR (75 MHz, $CDCl_3$): δ196.3 (C=S), 119.6, 55.8, 52.5, 50.1, 35.8, 22.7, 15.0; ESI-MS: (*m/z*)485 (MH^+); Anal. (%) calcd. for $C_{20}H_{32}N_6S_4$: C, 49.55; H, 6.65; N, 17.34; found C, 49.75; H, 6.39; N, 17.19.

Propane-1,3-diyl bis(4-(3-cyanopropyl)piperazine-1-carbodithioate) (56). The title compound was synthesized from sodium 4-(3-cyanopropyl)piperazine-1-carbodithioate and diiodopropane in 56% yield as white solid; mp: 118–120 °C; IR (KBr) ν (cm^{-1}): 2942, 2823, 2248, 1646; ^{1}H NMR (300 MHz, $CDCl_3$ + CCl_4): δ4.34–4.05 (8H, m), 3.40 (4H, t, *J* = 7.1 Hz), 2.55–2.42 (16H, m), 2.16–2.07 (2H, m), 1.88–1.79 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$ + CCl_4): δ196.5 (C=S), 119.1, 55.9, 52.6, 50.7, 35.9, 28.2, 22.7, 15.0; ESI-MS: (*m/z*)499 (MH^+); Anal. (%) calcd. for $C_{21}H_{34}N_6S_4$: C, 50.57; H, 6.87; N, 16.85; found C, 50.68; H, 6.94; N, 16.75.

4.2. Biological materials and methods

4.2.1. Spermicidal activity[32]

Spermicidal assay was adapted from the 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 30 seconds and recorded (Table 1).

4.2.2. Anti-*Trichomonas* activity[32]

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 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 x 10^7 cells/mL in 48h. Inoculums of 1 x 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) of test compounds were prepared in DMSO. These stock solutions were serially diluted with TYM medium to obtain concentration up to 0.1 µg/mL in 48-well plates. DMSO/TYM was used as vehicle in control wells. Parasites (5 x 10^4trophozoites/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 40X 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 MIC. The experiment was repeated three times to confirm the MIC (Table 2)

4.2.3. Antifungal activity[30]

The MIC of compounds were determined by broth micro-dilution technique as per the guidelines 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 x 10^3 CFU/mL. Micro titer plates were incubated at 35 °C. MICs were recorded after 48h of incubation (Table 2).

4.2.4. Cytotoxicity towards human cervical (HeLa) cell line by lactate dehydrogenase – release assay[30]

A colorimetric assay for lactate dehydrogenase (LDH) release was used for the evaluation of the cytotoxicity of spermicidal compounds against the HeLa cell line. Exponentially growing HeLa cells were seeded into 96-well tissue culture plates at a density of 2 ×10^4 cells per well (in triplicate). After 24 h incubation in a CO_2 incubator at 37 °C in 5% CO_2 , 95% air atmosphere, the culture medium [Dulbecco's modified Eagle's medium (DMEM)] was replaced with 100.0 µL of fresh medium containing serially diluted spermicidal compounds. Control wells contained the medium only. Culture plates were incubated for another 5 h, and then 50.0 µL of the supernatant from each well of the assay plate was pipetted into the corresponding well of a flat-bottom 96-well plate. Colour reaction for LDH assay and IC_{50} measurement for cytotoxicity were performed using CytoTox-96 kit (Promega, Madison, WI, USA) by following the instructions of the manufacturer. Optical densities at 490 nm were measured in a micro-plate reader (µQuant, Bio-Tek, USA) (Table 3).

4.2.5. Effect on *Lactobacillus acidophillusin vitro*[30]

The effect of compounds exhibiting potent spermicidal activity on *Lactobacillus acidophillus* was determined by following the method published earlier. Briefly, Rogosa SL agar plates (7.5%; containing 0.132% acetic acid), prepared with (experimental) or without (control) the

addition of spermicidal compounds, were inoculated with *L. acidophillus* (~70 spores/10 cm^2) and incubated at 37 °C in 5% CO_2 and 95% air for 72 h. The number and size of colonies were recorded at the end of the experiment. The average colony size (% of control) was multiplied by the colony number and divided by 100 to arrive at the data presented. The aver-age colony size for the control was taken as 100% (Table 3).

4.2.6. Spermicidal activity (*in vivo*)[47]

All animal experiments were conducted in accordance and as per the approval of the Institutional Animal Ethics Committee of CSIR-Central Drug Research Institute (approval No. IAEC/2014/93). The rabbits were housed in stainless steel cages and kept in uniform husbandry conditions of temperature (25-26ºC), relative humidity (50-70%) and light/dark cycle (12/12 h). Young, adult female Belgian rabbits were given test compound through vaginal instillation. The test compounds were incorporated in K-Y-Jelly (Johnson & Johnson) through geometrical dilution. Pure K-Y-Jelly was used in control groups. Two mL of test/control jelly containing the test compounds at indicated doses (Table 4) was instilled 10–12 cm deep into the vagina of each rabbit with a catheter attached to the gavage needle of a syringe with the animal held in supine position. The animal was released after 5 min. Treated females were hand-mated once with a proven buck. Each buck was allowed one mating. Mating was re-confirmed by presence of sperm in vaginal smear. The mated rabbits were then kept in separate cages and allowed to complete gestation of 30-34 days. The pregnancies and litter size was recorded at completion of the gestation (Table 4).

4.2.7. Anti-Trichomonalactivity (*in vivo*)[48]

All animal experiments were conducted in accordance and as per the approval of the Institutional Animal Ethics Committee of CSIR-Central Drug Research Institute (approval No. IAEC/2014/94). The mice were housed in polypropylene cages and kept in uniform husbandry conditions of temperature (25-26ºC), relative humidity (50-70%) and light/dark cycle (12/12 h). The subcutaneous abscess assay of Krieger et al. (1983) was used. Briefly, the parasites (*T. vaginalis*) were cultured under partial anaerobic condition in TYM medium andon attaining concentration of approximately $2x10^6$ cells/ml (in ~48 hrs); trichomonads were harvested from the culture by centrifugation at 250xg for 10 min and then re-suspended in sterile saline. Six-week-old mice were inoculated subcutaneously with *T. vaginalis* (50 µl of 2×10^6 organisms per

ml) into the left hind flank. Control animals were injected with sterile saline only. Five groups were used for each experiment (*n*=3). The abscess / lesion formation was determined by palpation 7 days after injection, and measured daily thereafter.Fine needle biopsy specimens were taken from the lesion and examined microscopically to ensure infection**.**Infected animals were then treated with compounds (subcutaneously) with a dose of 5.0 µg in 50 µL of saline daily for 7 days and abscess size measured longitudinally and the area calculated as πr^2. Metronidazole was used as positive control. Control injections of sterile saline did not result in abscess formation.

4.2.8. Fluorescent labeling of sperm thiols[49]

Free thiols on human sperm (after treatment with vehicle, and two most promising compound **12** was 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 **12** 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 pelleted sperm in 1 ml PBS, 0.5mM (final concentration) mBBr was added and incubated for 15 min in the dark. After incubation sperm were pelleted and washed with PBS, finally dissolved in 200 µl PBS. A drop of this sample was then taken 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.

4.2.9. Qualitative estimation of inhibition of free sulfhydryl groups on *Trichomonasvaginalis*

The effect of test compound on Trichomonas free sulfhydryl groups was examined and imaged by a method published earlier with slight modification[33] using a fluorescence microscope, after labelling with the fluorometric thiol detector using a thiol –detection assay kit (Cayman). Trichomonas vaginalis was treated with the vehicle or the test compound at MIC and incubated for 24 h at 37°C. After incubation, trichomonads were pelleted at 700 xg for 10 min at 4°C and washed 2-3 times with PBS. Thereafter 50 µL fluorometric thiol detector (pre-diluted 100x with dilution buffer), was added and incubated for 5 min in the dark. A drop of this sample was taken

on a microscope slide, covered with a coverslip and imaged on a Nikon Eclipse 80i microscope equipped with epifluoresence illumination, using the UV-1A filter. Exposure times were the same for all samples.

4.2.10. Docking study

The sequence of *Trichomonas vaginalis* cysteine synthase (TvCS) was retrieved from Uniprot (A2GMG5). Since crystal structure of TvCS is not available, therefore a homology model was constructed usingcrystal structure of cysteine synthase from *Escherichia coli* (PDB ID-2BHS)[50] as template with the help of MODELLER package.[51] All docking studies were carried using AUTODOCK4.2 Package.[52]For molecular visualization and structure manipulation Chimera was used.[53]

ASSOCIATED CONTENT

Supporting Information:^{1}H NMR spectra of all the compounds, ^{13}C NMR spectra of forty eight compounds except (**5**, **22**, **23**, **34** and **37**) and HRMS data of compounds**7**, **8**, **9**, **11**, **12**, **16** and **19**. This material is available free of charge via internet at …….

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ABBREVIATIONS

STIs, sexually transmitted infections; N-9, nonoxynol-9; DTC, dithiocarbamate; DSF, disulfiram; SAR, structure activity relationship; MTZ, metronidazole; MOPS, [3-(*N*-morpholino)propanesulfonic acid]; LDH, lactate dehydrogenase; DMEM,dulbecco's modified eagle's medium; HCG, human chorionic gonadotrophin; TLC, thin layer chromatography.

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Table captions

Table 1: Spermicidal activity (MEC) of synthesized compounds (**4–38**)

Table 2: Antifungal and anti-*Trichomonas* activity of synthesized compounds (**4–38**)

Table 3: Toxicity and safety data of promising compounds (**4, 12, 16, 17, 18** and **35**)

Table 4:*In vivo* vaginal contraceptive efficacy of compound **12** and **17** in Belgian rabbits using K-Y Jelly (Johnson & Johnson) as vehicle

Table 5: Spermicidal activity of compounds **39–44**

Table 6: Spermicidal activity of compounds **45–56**

Figure captions

Figure 1: Structures of Disulfiram and synthesized compounds (**4–38**)

Figure 2: Docked conformations of compounds **17**(Grey) and **4** (Magenta). Hydrogen bonds are shown in black dashed lines and protein residues are shown in cyan colour; Docked conformations of less active compound **13** (Red), Protein residues are shown in cyan colour.

Figure 3: The subcutaneous abscess assay in mice using virulent, MTZ-susceptible *T.vaginalis*: [A] Abscess size in untreated mice (I), control untreated animal (II) and treated mice (III, IV, V) at days 1, 3, 7 of subcutaneous injections. [B] Statistical data of decrease in abscess size at days 1, 3, 5, 7 and 8 (autopsy day) [C] Variations in spleen size and weight per 100g body weight in mice of control, experimental-control and treated with MTZ or compounds **4, 17** [Mean ± SE of three independent experiment; significant difference from the control is indicated as *P<0.05, **P<0.01, ***P<0.001].

Figure 4: Fluorescence labeling of human sperm thiols; (A) Labelled with mBBr dye specifically binds with free thiols, (B) Phase contrast image, (C) Merged image.

Scheme captions

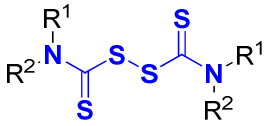
Scheme 1[a]. Synthesis of compounds(**4–38**)

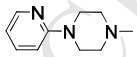
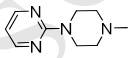
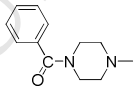
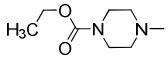
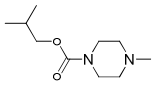
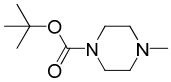
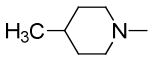
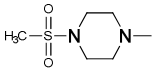
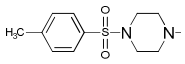
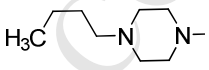
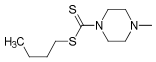
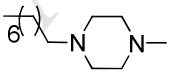
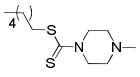
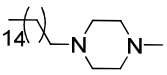
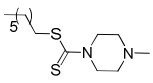
[a]Reagents and conditions: (a) NaOH, ethyl acetate, 0-5 °C; (b) $NaNO_2$, water, HCl, 20 min.

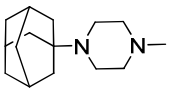
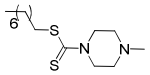
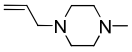
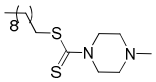
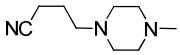
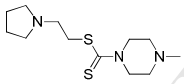
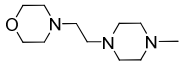
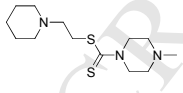
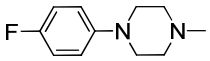
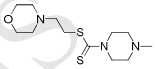
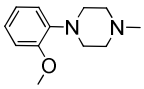
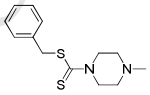
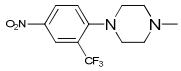
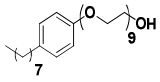
Scheme 2[a]. Synthesis of compounds(**45–56**)

[a]Reagents and conditions: (a) alkyl halide, methanol, room temperature, 3 h; (b) dihaloalkane, acetonitrile, room temperature, overnight.

1064 **Table 1.**



Comp.	NR^1R^2	Spermicidal Activity[a] MEC[b] (%)	Comp.	NR^1R^2	Spermicidal Activity[a] MEC[b] (%)
4		0.01%	**22**		1%
5		>1%	**23**		1%
6		>1%	**24**		0.5%
7		1%	**25**		1%
8		1%	**26**		>1%
9		0.1%	**27**		>1%
10		1%	**28**		1%
11		>1%	**29**		>1%
12		0.001%	**30**		>1%
13		0.1%	**31**		1%
14		1%	**32**		>1%

15		1%	**33**		1%
16		0.001%	**34**		1%
17		0.001%	**35**		0.002%
18		0.005%	**36**		0.5%
19		>1%	**37**		0.5%
20		1%	**38**		>1%
21		0.1%	**N-9[c]**		0.05%

[a] All the experiments were carried out in triplicate; [b]MEC, Minimum Effective Concentration; Vehicle (control) has >85%motility; [c] Nonoxynol-9 (positive control)

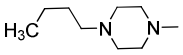
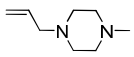
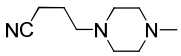
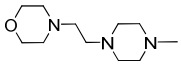
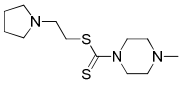
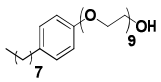
1068 **Table 2.**

Entry	Antifungal activity[a] MIC[b] μg/mL (μM)					Anti-*Trichomonas* Activity[a] MIC[b] μg/mL (μM)	
	c	***d***	***e***	***f***	***g***	**Clinical strain**	**Resistant strain[h]**
4	1.56 (6.5)	0.78 (3.2)	12.5 (51.8)	1.56 (6.5)	12.5 (51.8)	3.12 (12.9)	3.12 (12.9)
5	25 (48.7)	12.5 (24.3)	50 (97.4)	50 (97.4)	>50 (>97.4)	>100 (>194)	>100 (>194)
6	12.5 (31.8)	3.12 (7.9)	12.5 (31.8)	25 (63.6)	>50 (>127)	3.12 (7.9)	12.5 (31.8)
7	3.12 (10.6)	0.78 (2.6)	6.25 (21.3)	3.12 (10.6)	6.25 (21.3)	3.12 (10.6)	3.12 (10.6)
8	3.12 (9.7)	0.78 (2.4)	12.5 (38.9)	6.25 (19.4)	12.5 (38.9)	50 (155)	50 (155)
9	25 (76.9)	12.5 (38.4)	25 (76.9)	12.5 (38.4)	25 (76.9)	3.12 (9.6)	3.12 (9.6)
10	6.25 (17.9)	3.12 (8.9)	12.5 (35.8)	12.5 (35.8)	50 (143)	12.5 (35.8)	12.5 (35.8)
11	3.12 (8.9)	1.56 (4.4)	6.25 (17.9)	6.25 (17.9)	50 (143)	100 (286)	12.5 (35.8)
12	25 (57.4)	50 (114)	50 (114)	50 (114)	25 (57.4)	6.25 (14.3)	12.5 (28.7)
13	50 (91.4)	50 (91.4)	50 (91.4)	50 (91.4)	50 (91.4)	100 (182)	100 (182)
14	50 (64.8)	50 (64.8)	12.5 (16.2)	12.5 (16.2)	>50 (>64.8)	>100 (>129)	>100 (>129)
15	50 (84.6)	50 (84.6)	50 (84.6)	50 (84.6)	50 (84.6)	>100 (>169)	>100 (>169)
16	25 (62.0)	>50 (>124)	>50 (>124)	50 (124)	50 (124)	6.25 (15.5)	25 (62.0)
17	>50 (>109)	>50 (>109)	50 (109)	50 (109)	>50 (>109)	3.12 (6.8)	3.12 (6.8)
18	>50 (>91)	>50 (>91)	50 (91)	50 (91)	>50 (>91)	12.5 (22.7)	25 (45.5)
19	50 (97.8)	>50 (>97.8)	50 (97.8)	50 (97.8)	>50 (>97.8)	>100 (>195)	>100 (>195)
20	25 (46.7)	12.5 (23.3)	25 (46.7)	50 (93.4)	>50 (>93.4)	>100 (>186)	>100 (>186)
21	25 (35.6)	>50 (>71.3)	>50 (>71.3)	50 (71.3)	>50 (>71.3)	>100 (>142)	>100 (>142)
22	>50 (>104)	50 (104)	50 (104)	25 (52.4)	>50 (>104)	>100 (>209)	>100 (>209)
23	>50 (>104)	50 (104)	50 (104)	50 (104)	>50 (>104)	50 (104)	>100 (>208)
24	50 (94.1)	>50 (>94.1)	>50 (>94.1)	>50 (>94.1)	>50 (>94.1)	>100 (>188)	>100 (>188)

25	25 (53.4)	12.5 (26.7)	50 (106.8)	50 (106.8)	25 (53.4)	6.25 (13.3)	6.25 (13.3)
26	50 (95.6)	>50 (>95.6)	>50 (>95.6)	50 (95.6)	>50 (>95.6)	>100 (>191)	>100 (>191)
27	>50 (>91.7)	>50 (>91.7)	>50 (>91.7)	>50 (>91.7)	>50 (>91.7)	>100 (>183)	>100 (>183)
28	50 (104)	>50 (>104)	>50 (>104)	25 (52.1)	>50 (>104)	>100 (>208)	>100 (>208)
29	>50 (>76.5)	>50 (>76.5)	50 (76.5)	50 (76.5)	>50 (>76.5)	>100 (>153)	>100 (>153)
30	>50 (>85.1)	50 (85.1)	50 (85.1)	25 (42.5)	25 (42.5)	>100 (>170)	>100 (>170)
31	50 (77.7)	25 (38.8)	50 (77.7)	>50 (>77.7)	50 (77.7)	>100 (>155)	>100 (>155)
32	>50 (>74.5)	50 (74.5)	50 (74.5)	>50 (>74.5)	50 (74.5)	>100 (>149)	>100 (>149)
33	25 (35.7)	25 (35.7)	25 (35.7)	>50 (>71.5)	50 (>71.5)	62.5 (89.4)	>100 (>143)
34	50 (66.2)	25 (33.1)	50 (66.2)	>50 (>66.2)	50 (66.2)	>100 (>132)	>100 (>132)
35	50 (74.7)	50 (74.7)	50 (74.7)	50 (74.7)	50 (74.7)	31.25 (46.7)	>100 (>149)
36	>50 (>71.7)	>50 (>71.7)	>50 (>71.7)	>50 (>71.7)	>50 (>71.7)	>100 (>143)	>100 (>143)
37	>50 (>71.3)	>50 (>71.3)	>50 (>71.3)	>50 (>71.3)	>50 (>71.3)	>100 (>142)	>100 (>142)
38	>50 (>76.3)	>50 (>76.3)	>50 (>76.3)	>50 (>76.3)	>50 (>76.3)	62.5 (95.4)	62.5 (95.4)
FLU	0.12 (0.39)	0.06 (0.19)	0.12 (0.39)	0.12 (0.39)	0.12 (0.39)	-	-
MTZ	-	-	-	-	-	2.0 (11.6)	50 (292)
N-9	>50 (>81.1)	>50 (>81.1)	>50 (>81.1)	>50 (>81.1)	50 (81.1)	50.0 (81.1)	50.0 (81.1)

[a]All the experiments were carried out in triplicate,[b]MIC, minimum Inhibitory Concentration,[c]*Candidaalbicans*, [d]*Cryptococcusneoformans*, [e]*Sporothrixschenckii*, [f]*Trichophitonmentagrophytes*, [g]*Candidaparapsilosis (ATCC 22019)*,[h]ATCC 50143, Flu=Fluconazole, MTZ= Metronidazole, N-9= Nonoxynol-9.

Table 3.

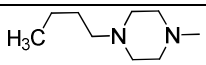
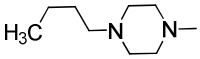
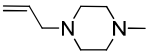
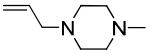
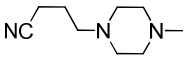
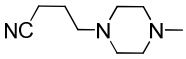
Comp.	NR^1R^2	*Lactobacillus jensenii* ATCC 25258 IC_{50} µM (µg/ml)	*HeLa* IC_{50} µM (µg/ml)	Anti-*Trichomonas* activity Selectivity index (IC_{50}/MIC µM)	
				Clinical strain	Resistant strain
4		8391.6 (2014)	2429.1 (583)	188	188
12		831.8 (361)	359.4 (156)	25	12
16		3614.4 (1453)	1338.3 (538)	86	21
17		6111.8 (2787)	2508.7 (1144)	368	368
18		6478.1 (3550)	168.6 (92.4)	7	3
35		6077.5 (3136)	2740.3 (1414)	58	>18
Nonoxynol-9		53.9 (33.2)	52.7 (32.5)	0.64	0.64

1077 **Table 4.**

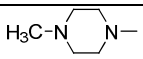
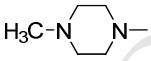
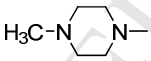
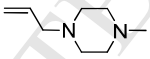
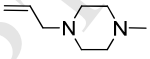
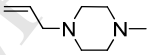
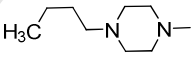
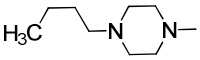
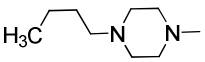
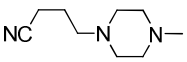
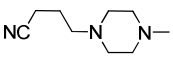
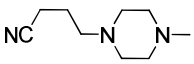
Treatment Group	Vaginal Dose (mg)	Number Pregnant/Mated	Percent Efficacy	Average Litter Size
Vehicle (K-Y Jelly)	0	9/9	0	7.5
12	10	5/8	37.5	2.5
12	15	3/8	62.5	2.0
12	20	3/6	50.0	1.83
17	10	1/7	85.7	0.71
17	25	0/7	100.0	0.0
17	50	0/6	100.0	0.0
Nonoxynol-9	20	4/5	20.0	4.8

1078

1079 **Table 5.**

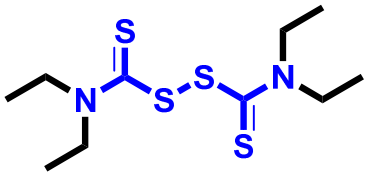
Compound	NR^1R^2	R^3	Spermicidal activity MEC (%)
39		propyl	>1%
40		hexyl	>1%
41		propyl	>1%
42		hexyl	>1%
43		propyl	>1%
44		hexyl	>1%

1080 **Table 6.**

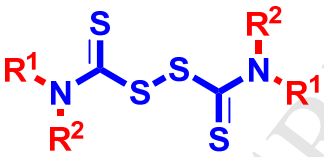
Compound	n	NR^1R^2	Spermicidal activity MEC (%)
45	1		0.5%
46	2		0.5%
47	3		0.5%
48	1		1.0%
49	2		0.5%
50	3		0.5%
51	1		0.5%
52	2		0.5%
53	3		0.5%
54	1		0.5%
55	2		0.5%
56	3		0.5%
Nonoxynol-9			0.05%

1081

Figure 1.



Disulfiram (DSF)



General structure of compounds synthesized

Figure 2.

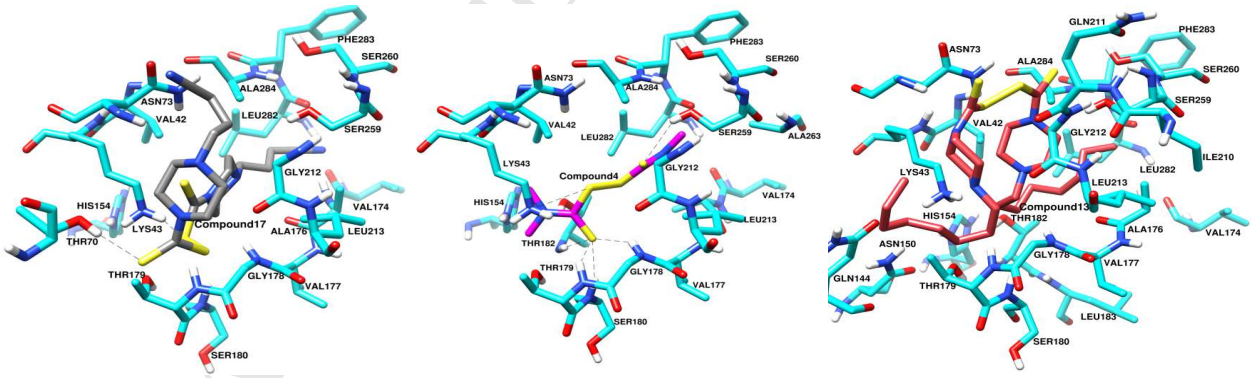


Figure 3.

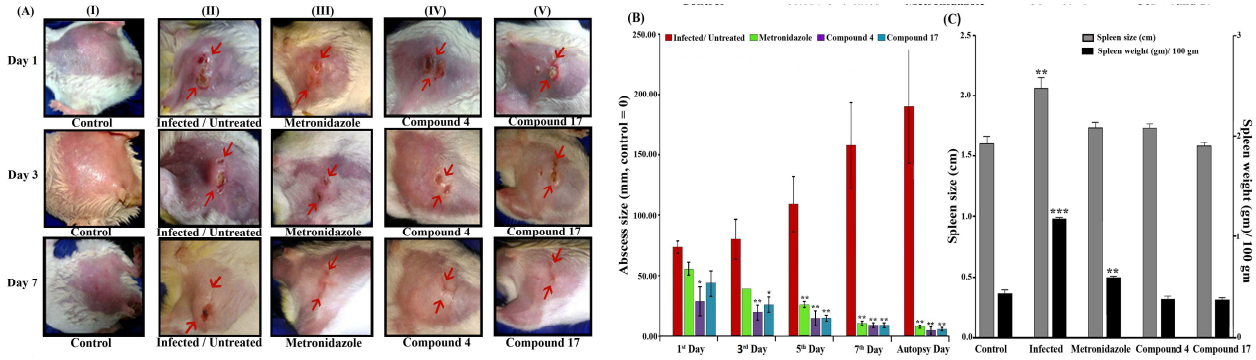
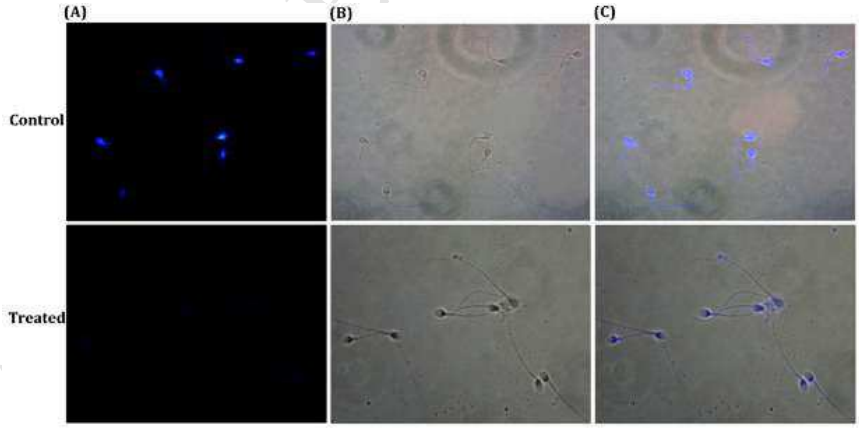


Figure 4.



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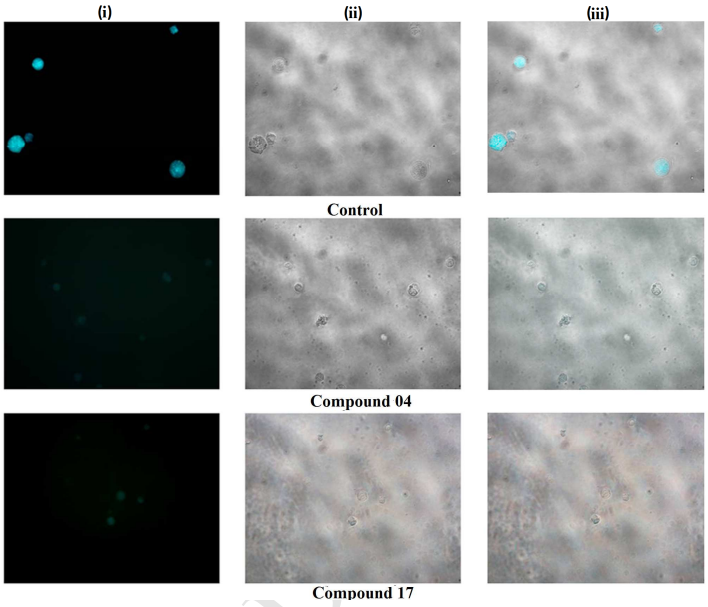
1111

1112 **Figure 5.**

1113



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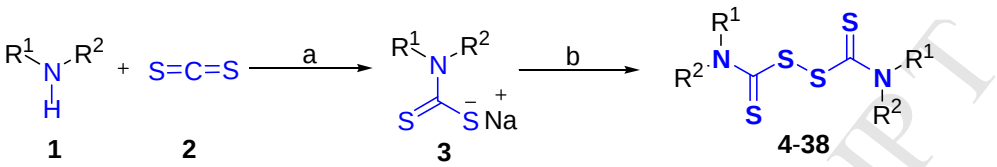
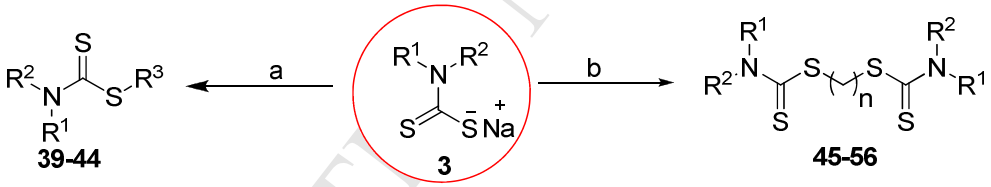
Scheme 1



1 **2** **3** **4-38**

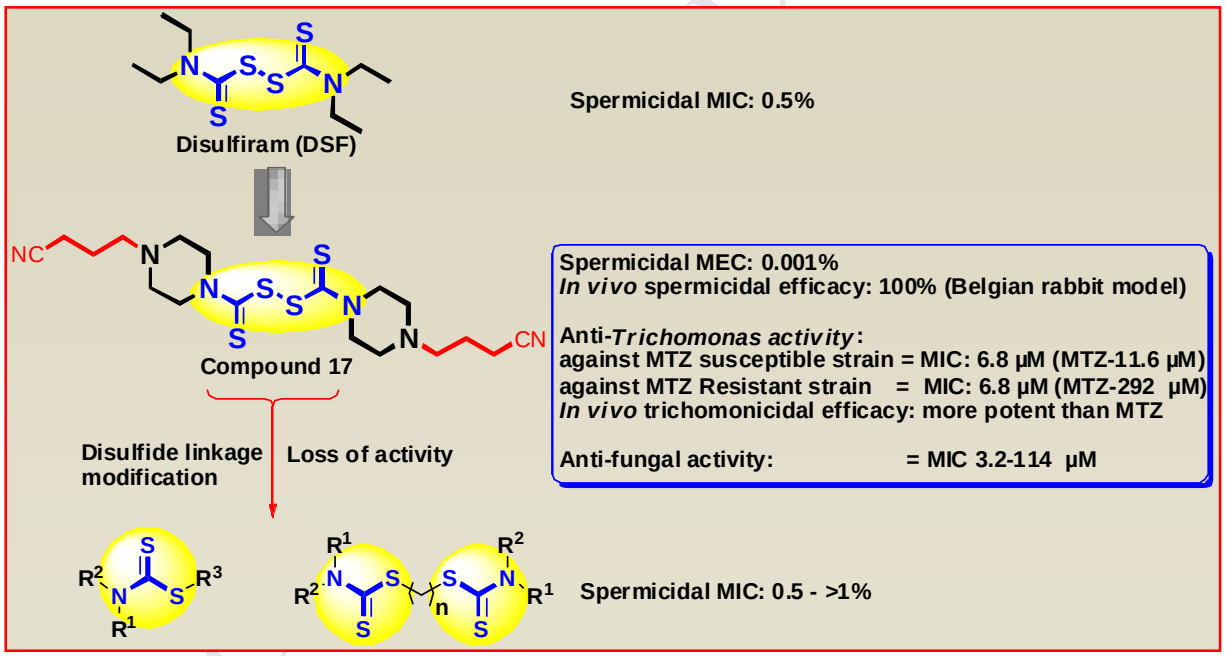
Scheme 2



39-44 **45-56**

Role of disulfide linkage in action of bis(dialkylaminethiocarbonyl)disulfides as potent double-Edged microbicidal spermicide: Design, synthesis and biology[#]

Nand Lal,[a] Santosh Jangir,[a] Veenu Bala,[a,e] Dhanaraju Mandalapu,[a] Amit Sarswat,[a] Lalit Kumar,[a] Ashish Jain,[b] Lokesh Kumar,[b] Bhavana Kushwaha,[b] Atindra K. Pandey,[c] Shagun Krishna,[d] Tara Rawat,[a] Praveen K. Shukla,[c] Jagdamba P. Maikhuri,[b] Mohammad I. Siddiqi,[d] Gopal Gupta,[b] and Vishnu L. Sharma*[,a,e]



Highlights:

- 34 compounds synthesized and evaluated for spermicidal and microbicidal activities.
- Spermicidal compounds were up to fifty fold more active than N-9 *in vitro*.
- Compounds **12** and **17** have exhibited remarkable *in vivo* efficacy.
- Role of S-S bond was established by the loss of activity on chemical modification.
- Sulfhydryl inhibition over sperm and *Trichomonas* shown by fluorescence labeling.