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Synthesis of benzenepropanamine analogues as non-detergent spermicides with antitrichomonas and anticandida activities

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Abstract—Fifteen analogues of benzenepropanamine were synthesized and evaluated for their spermicidal as well as microbicidal activities against *Trichomonas vaginalis* and *Candida* spp. Several compounds showed appreciable dual activities. Compound **12** exhibited good spermicidal (MEC = 0.1%) along with substantial anticandidal (MIC = 0.05%) activities, while compounds **3** and **6** showed significant microbicidal activities with moderate spermicidal effect. The SAR of these structures is being discussed here in this communication. It is concluded that suitable structural modifications in this class of compounds at 3-amino position may lead to a potent spermicide with associated microbicidal activity.

1. Introduction

The current world population is expected to increase by more than 50% by the year 2050.[1] This is likely to be accompanied by an equally challenging rise in number of STD and HIV infections. Dually active, prophylactic vaginal contraceptives can effectively tackle both these problems simultaneously.[2] Nonoxynol-9 (N-9), the most widely used spermicide in contraceptive preparations displayed excellent microbicidal activity in vitro.[3–5] However, recent clinical trials have shown that N-9 does not offer any protection against STDs and HIV, but on the contrary it increases the risk of their transmission.[6–8] Since surfactant-type of action of N-9 has been held responsible for this anomaly,[9] efforts have been made to develop new dually active non-detergent agents that are devoid of the disadvantages of N-9.[10–18]

Acrylophenones, quinolines, and dithiocarbamates are the structural classes that have been reported[19] as potent, non-detergent spermicides. Moreover, (*E*)-4-hydroxy-2-nonenal, a lipid peroxide end-product, exhibits its spermicidal activity without disrupting the sperm membrane by reacting with free sulfhydryl groups of sulfur-bearing amino acids on sperm axonemal microtubules.[20] Similarly, specific sulfhydryl alkylating agents,[21] like *N*-alkylmaleimide derivatives, possess spermicidal activity.[22] Thus, sulfhydryl interactions play a vital role in spermicidal action. Second, paroxetine (**I**, Fig. 1), arylmethylaryl piperidine, and fluoxetine (**II**, Fig. 1), an aryloxyphenylpropylamine, selective serotonin reuptake inhibitor (SSRI) antidepressants, interact with neuronal 5-HT transporters via sulfhydryl binding[21] and have been recently reported by us as non-detergent spermicides.[23] Besides, several substituted aryloxyalkanols,[24] aminohydroxy alkyl derivatives,[25] and substituted arylethylamines[6] have also been shown to exhibit significant spermicidal activity.

These observations prompted us to synthesize some benzenepropanamine analogues (**III**, Fig. 1) for spermicidal activity. Since Trichomoniasis and Candidiasis are amongst the most common reproductive tract infections that cause morbidity,[26] it was thought worthwhile to evaluate these compounds for their anticandidal as well as antitrichomonas activity. Spermicidal action being the primary activity, the microbicidal effect even at sper-

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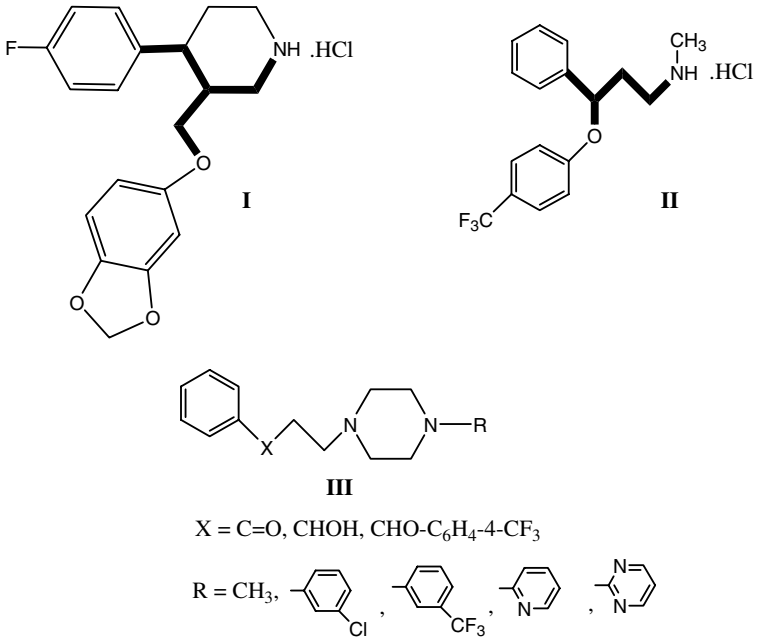


Figure 1.

micidal concentration would be an added advantage. Structural changes have been made at position-1 and at 3-amino function. Piperazine moiety has been incorporated at position-3 because some piperazine derivatives have shown spermicidal activity.[27,28]

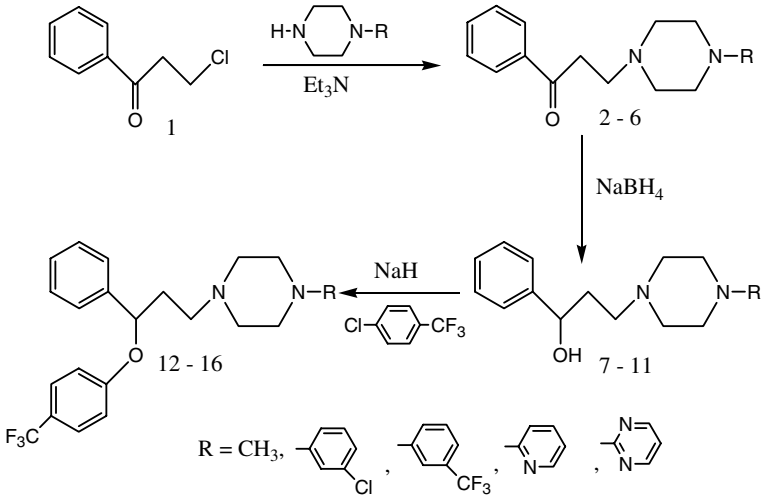
2. Chemistry

The compounds (**2**–**16**) were synthesized according to Scheme 1. 3-Chloropropiophenone (**1**) was reacted with appropriate 4-substituted piperazine in presence of triethyl amine in dry toluene to provide 3-(4-(substituted)-piperazin-1-yl)-1-phenylpropan-1-one hydrochloride salt (**2**–**6**). These propanones (**2**–**6**) were reduced to corresponding hydroxy compounds (**7**–**11**) with sodium borohydride in methanol which were condensed with 4-chlorobenzotrifluoride in dimethyl acetamide in presence of sodium hydride to give 1-(substituted)-4-[3-phenyl-3-(4-trifluoromethyl-phenoxy)-propyl]-piperazine hydrochloride salt (**12**–**16**). Physical data of these compounds (**2**–**16**) have been summarized in Table 1.

3. Results and discussion

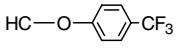
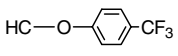
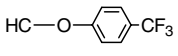
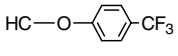
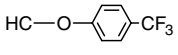
3.1. Spermicidal activity

Out of 15 compounds tested for their effect on sperm motility 10 compounds showed appreciable spermicidal activity at 1% concentration. Out of these 6 compounds (**7**–**9**, **12**–**14**) exhibited potent spermicidal activity with total immobilization of 100% spermatozoa within 60 s, while the other four compounds (**4**–**6** and **16**) showed substantial spermicidal activity by arresting the motility of ~99% sperm during this period. Compound **15** had



Scheme 1.

Table 1. Physical data of compounds **2**–**16**

Compound	X	R	Mol formula (mol wt)	Mp (°C)	Yield (%)	Analysis found/required		
						C	H	N
2	C=O	CH_3	$C_{14}H_{20}N_2O$·HCl 268.5	185–187	65	54.88/55.09	7.04/7.26	9.37/ 9.18
3	C=O		$C_{19}H_{21}ClN_2O$·HCl 365	180–182	73	62.29/62.47	6.19/6.07	7.93/ 7.67
4	C=O		$C_{20}H_{21}F_3N_2O$·HCl 398.5	195–197	68	60.09/60.23	5.37/5.56	7.23/ 7.02
5	C=O		$C_{18}H_{21}N_3O$·2HCl 368	230–235	85	58.56/58.70	6.07/6.29	11.69/11.41
6	C=O		$C_{17}H_{20}N_4O$·2HCl 369	187–189	89	55.11/55.29	5.87/6.00	15.35/15.17
7	HC–OH	CH_3	$C_{14}H_{22}N_2O$·HCl 270.5	260	58	54.67/54.73	7.59/7.87	9.36/ 9.12
8	HC–OH		$C_{19}H_{23}ClN_2O$·HCl 367	162–165	90	62.05/62.13	6.36/6.59	7.85/ 7.63
9	HC–OH		$C_{20}H_{21}F_3N_2O$·HCl 400.5	150–152	67	59.75/59.92	5.89/6.03	7.15/6.99
10	HC–OH		$C_{18}H_{23}N_3O$·2HCl 370	130–134	86	58.14/58.38	6.56/6.80	11.48/11.35
11	HC–OH		$C_{17}H_{20}N_4O$·2HCl 371	[a]	97	54.78/54.99	6.46/6.52	15.28/15.09
12		CH_3	$C_{21}H_{25}F_3N_2O$·2HCl 451	[a]	74	55.64/55.88	5.79/6.03	6.45 6.21
13			$C_{26}H_{26}ClF_3N_2O$·HCl 511	150–155	65	61.32/61.06	5.12/5.32	5.75/5.48
14			$C_{27}H_{26}F_6N_2O$·HCl 544.5	195–197	70	59.34/59.51	4.85/4.99	5.25/5.14
15			$C_{25}H_{26}F_3N_3O$·2HCl 574	218–220	78	58.25/58.37	5.25/5.49	8.32/8.17
16			$C_{24}H_{25}F_3N_4O$·2HCl 575	180–185	70	55.69/55.93	5.05/5.28	11.05/10.87

[a] Hygroscopic.

no effect on sperm motility at 1%. Compound **12** was most active in this series as the sperm completely lost motility even at 0.1% concentration. Sperm viability was almost in accordance to the sperm immobilization (Fig. 2) Table 2.

The results of effect on sperm motility of compounds (**2**–**16**) suggest that the substituents at position-1 and the group at 4-position of piperazine moiety play an important role in the sperm regulating motility. The 1-keto compounds (**2**–**6**) showed moderate spermicidal activity at 1% concentration that got further diluted at 0.1% concentration. Among 1-hydroxy compounds (**7**–**11**) there was a clear demarcation in the effect on the sperm motility according to substituents in piperazine moiety. Methyl (**7**), 3-chlorophenyl (**8**), and 3-(trifluoromethyl) phenyl (**9**) substitutions at position-4 of piperazine moiety completely immobilized the sperm at 1% concentration, while 2-pyridyl (**10**) and 2-pyrimidyl (**11**) substitutions had little effect on sperm motility. At 0.1% concentration, while compounds (**7**–**10**) had mild effect on sperm motility, compound (**11**) unexpectedly exhibited sperm hyperactivation. It has been suggested earlier that sperm membrane sulfhydryl groups can be used as tools for treatment of unexplained male infertility as well as targets for contraceptive research.[29] The 1-(4-trifluoromethyl-phenoxy) derivatives (**12**–**16**) showed similar demarcation in effect on sperm motility like the 2-hydroxy compounds. Methyl (**12**), 3-chlorophenyl (**13**), and 3-(trifluoromethyl) phenyl groups at position-4 of piperazine moiety completely inhibited the motility of human sperms at 1% concentration, whereas 2-pyridyl (**15**) and 2-pyrimidyl (**16**) groups in piperazine moiety had a lesser effect. The methyl

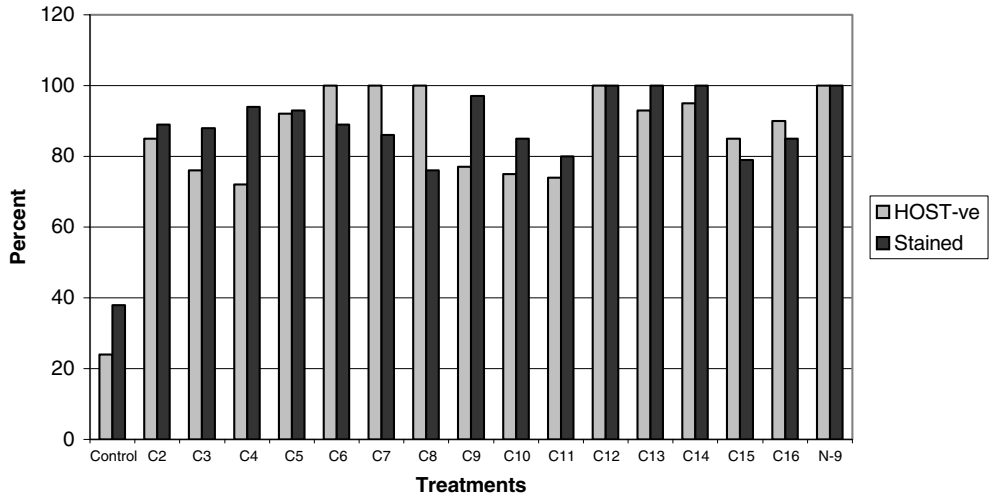


Figure 2. The effect of benzenepropanamine analogues (**2**–**16**) on sperm cell viability at 1.0% concentration.

Table 2. Spermicidal and antitrichomonas activity of the compounds (**2**–**16**)

Compound	Percent motility after 60 s (%)		Antitrichomonas activity MLC (μg/ml)
	1.0% concd	0.1% concd	
2	5	35%	50
3	16	45%	20
4	1	10%	100
5	~1	12%	100
6	<1	6%	25
7	NIL	2%	—
8	NIL	~1%	—
9	NIL	<1%	—
10	20	65%	—
11	50	80%	—
12	NIL	NIL	—
13	NIL	2%	—
14	NIL	25%	100
15	60	65%	—
16	<1	10%	100
N-9	NIL	NIL	20
Metronidazole	—	—	1.6
Vehicle (control)	70%	70%	NA

>200; NA, not applicable.

substitution (**12**) was most effective as complete immobilization of sperm was seen even at 10 times lower concentration of the compound, that is, 0.1%.

These results provide an interesting structure–activity relationship of the benzenepropanamine class of compounds for effect on sperm motility. A hydroxy or 4-trifluoromethyl phenoxy group at position-1 and a methyl, 3-chlorophenyl or 3-(trifluoromethyl) phenyl substituent at position-4 of 3-piperazino group impart sperm immobilizing activity, whereas if the group in piperazine moiety at position-4 is replaced by 2-pyridyl or 2-pyrimidyl groups, the sperm motility is increased. Notably, compound **12** showed potent spermicidal activity, whereas compound **11** exhibited mild sperm immobilization effect at 1.0% and motility-stimulating effect at 0.1%.

3.2. Anticandida activity

Compounds (**2**–**16**) were tested against nine strains of *Candida albicans* and activity was expressed in terms of MIC and IC_{50} values (Table 3). N-9 was taken as reference standard and Fluconazole as the standard antifungal drug.

The MIC of compounds (**2**–**16**) ranged from 12.5 to 50 μg/mL whereas that of N-9 was 50–50 μg/mL. Though these concentrations are higher than the standard antifungal drug fluconazole, still the MICs are lower than the spermicidal concentration. Moreover, in case of 1-keto compounds (**2**–**6**) the MIC was lower than that of N-9 indicating a desirable activity profile of these compounds as compared to N-9. The hydroxy compounds (**7**–**11**) and O-arylated analogues (**12**–**16**) were however less active with the MIC ranging from 50 to 50 μg/mL. The most active spermicidal compound (**12**) showed antifungal activity against five strains of *C. albicans* at a concentration of 50 μg/mL (0.05%), which is half its spermicidal concentration (0.1%).

3.3. Antitrichomonas activity

Compounds (**2**–**16**) were tested against *Trichomonas vaginalis* utilizing N-9 as reference standard and metronidazole as standard antitrichomonas drug. Out of which 7 compounds (**2**–**6**, **14**, **16**) showed antitrichomonas activity with MLC ranging from 20 to 100 μg/mL, whereas nonoxynol exhibited the activity at 20 μg/mL.

The results suggested that a keto group at position-1 was essential for antitrichomonas activity as compounds (**2**–**6**) showed good to moderate activity and two of them **3** and **6** had MLC comparable to nonoxynol-9. Whereas the reduction of the ketone to hydroxyl (**7**–**11**) resulted

Table 3. In vitro activity of compounds (**2**–**16**) against different species and strains of Candida

Compound	Anticandida activity (μg/ml)																	
	1		2		3		4		5		6		7		8		9	
	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
2	50	23.45	25	15.35	50	29.81	50	24.86	50	28.65	50	47.70	25	18.15	50	12.87	12.5	9.11
3	50	31.51	50	24.55	50	24.64	50	29.53	50	29.87	50	23.70	>50	>50	12.5	8.29	12.5	7.77
4	50	18.40	25	16.13	50	24.23	50	28.86	50	47.02	50	27.74	50	23.56	25	11.95	25	11.87
5	50	24.31	50	24.00	50	25.32	50	44.36	50	29.21	50	31.25	50	20.57	25	12.67	12.5	10.85
6	50	19.12	25	17.27	50	27.44	50	45.35	50	26.06	50	30.73	25	16.70	12.5	9.05	12.5	10.29
7	>50	>50	50	48.56	50	48.03	>50	>50	>50	>50	50	33.15	50	30.54	50	32.82	50	28.84
8	>50	28.74	50	27.64	50	26.15	>50	>50	50	27.83	>50	>50	>50	>50	50	24.14	50	17.54
9	50	13.86	50	40.97	>50	>50	>50	>50	>50	>50	50	25.50	50	17.18	50	28.78	50	37.84
10	>50	28.79	50	36.46	50	31.66	>50	>50	>50	>50	>50	49.49	50	30.04	50	34.11	50	49.21
11	>50	>50	50	48.24	50	47.76	>50	>50	>50	>50	>50	47.86	>50	48.11	50	37.81	>50	28.57
12	50	34.22	50	48.47	50	25.36	>50	>50	>50	>50	50	28.72	>50	>50	50	26.45	>50	>50
13	>50	>50	50	30.84	>50	>50	>50	>50	>50	>50	>50	49.14	>50	44.25	50	27.92	>50	>50
14	>50	>50	>50	>50	50	>50	>50	>50	>50	>50	>50	49.49	>50	>50	>50	29.94	>50	>50
15	>50	>50	>50	49.03	50	>50	>50	>50	>50	>50	>50	49.27	>50	>50	>50	36.08	>50	>50
16	>50	>50	>50	48.72	>50	>50	>50	>50	>50	>50	>50	49.24	>50	>50	>50	45.61	>50	>50
N-9	>50	46.48	50	47.84	>50	41.10	>50	>50	>50	>50	50	47.84	>50	>50	>50	>50	>50	>50
Std*	0.5	0.13	1.0	0.21	0.5	0.26	1.0	0.35	2.0	0.58	4.0	1.38	1.0	0.63	0.25	0.10	0.25	0.18

*Fluconazole; (1) *C. albicans*, (2) *C. parapsilosis* ATCC 22019, (3) *C. albicans* MTCC 183, (4) *C. albicans* ATCC 10231, (5) *C. albicans* MTCC 1346, (6) *C. krusei* ATCC 6258, (7) *C. albicans* ATCC 10453, (8) *C. albicans* ATCC 60193, and (9) *C. albicans* ATCC 66027.

in complete loss of activity. However, the arylation of the1-hydroxy group (**12**–**16**) also did not result in the desired activity.

It may be concluded from this study that modifications at position-1 and 3-amino function in benzenepropanamine class of compounds might lead to a potent spermicide with useful microbicidal activity.

4. Experimental

4.1. Chemistry

4.1.1. General. 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 FTIR 8201 PC spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker DRX-200 FT spectrometer in deuterated solvents with TMS as internal reference (chemical shifts in δ parts per million, J in hertz). Mass spectra were recorded on Jeol/SX-102/DA-6000 FAB-MS spectrometer. Elemental analyses were performed on Carlo Erba EA-1108 micro analyzer. All compounds were analyzed of C, H, N and the results obtained were within ±0.4% of calculated values. Thin layer chromatography was performed on precoated alumina plastic plates (Aldrich). Anhydrous sodium sulfate was used as drying agent.

4.2. Synthesis of 3-(4-(substituted)-piperazin-1-yl)-1-phenyl-propan-1-one hydrochloride salt (2–6)

4.2.1. General procedure. To a mixture of 3-chloro propiophenone (0.01 mol) and Triethylamine (0.012 mol) in dry toluene, substituted piperazine (0.01 mol) was added at room temperature and refluxed for 3 h. After the reaction was complete (as monitored by TLC), the separated triethylamine hydrochloride was filtered off and the residue was washed with distilled water (3× 5 ml) and the organic layer was dried over sodium sulfate. The sodium sulfate was filtered off and the solvent was evaporated on rotavapor. The formed oily residue was taken into methanol and cooled in icebath (0–5 °C) and concd HCl (2 equiv) was added dropwise and kept stirring in icebath for 3 h. Later the methanol was distilled off and the residue was taken into acetone. A white solid separated out which was filtered, washed with acetone, and dried.

4.2.2. 3-(4-Methyl-piperazin-1-yl)-1-phenyl-propan-1-one hydrochloride salt (2). Spectral data: IR (cm^{-1}): 3432, 2914, 1672, 1448, 1080, 966. Mass (*m/e*): 232, 212, 205, 193, 175, 145. ^1H NMR (δ ppm): 2.93 (s, 3H, N–CH_3), 3.58–3.65 (t, 2H, $COCH_2$), 3.73–3.81 (m, 10H, N–CH_2 piperazine), 7.46–7.63 (m, 3H, ArH of Ph), 7.97–8.01 (d, 2H, ArH ortho to CO).

4.2.3. 3-[4-(3-Chloro-phenyl)-piperazin-1-yl]-1-phenyl-propan-1-one hydrochloride salt (3). Spectral data: IR (cm^{-1}): 3084, 2914, 1676, 1250, 1092, 960. Mass (*m/e*): 328, 209, 188, 105, 77, 55. ^1H NMR (δ ppm): 3.07–3.24 (m, 2H, CH_2CO), 3.55–3.64 (m, 8H, N–CH_2 piperazine), 3.88 (t, 2H, N–CH_2), 6.76–6.80 (d, 1H, ArH para to Cl), 6.89–6.95 (m, 2H, ArH ortho, meta to Cl), 7.17–7.21 (m, 1H, ArH ortho to Cl), 7.46–7.53 (m, 2H, ArH meta to CO), 7.59–7.67 (dd, 1H, ArH para to CO), 8.00–8.04 (m, 2H, ArH ortho to CO).

4.2.4. 1-Phenyl-3-[4-(3-trifluoromethyl-phenyl)-piperazin-1-yl]-propan-1-one hydrochloride salt (4). Spectral data: IR (cm^{-1}): 3024, 2952, 1686, 1314, 1218, 1168, 1128, 946. Mass (*m/e*): 362, 230, 188, 145, 105, 91. ^1H NMR (δ ppm): 2.63–2.70 (m, 4H, N–CH_2 piperazine), 2.81–2.95 (t, 2H, CH_2CO), 3.17–3.27 (m, 6H, N–CH_2 pipera-

zine), 7.05–7.09 (d, 3H, ArH para, ortho, meta to CF_3), 7.27–7.41 (m, 1H, ArH ortho to CF_3), 7.44–7.58 (m, 3H, ArH meta, para to CO), 7.90–7.99 (m, 2H, ArH ortho to CO).

4.2.5. 1-Phenyl-3-(4-pyridin-2-yl-piperazin-1-yl)-propan-1-one hydrochloride salt (5). Spectral data: IR (cm^{-1}): 3076, 2962, 1684, 1602, 1280, 1184, 974. Mass (*m/e*): 296 (M^++1), 176, 121, 107. ^1H NMR (δ ppm): 3.75–3.82 (m, 8H, CH_2CO, N–CH_2 piperazine), 4.09–4.13 (m, 4H, N–CH_2 piperazine), 7.23–7.26 (t, 1H, ArH meta to N), 7.44–7.49 (d, 1H, ArH para to N), 7.62–7.70 (m, 2H, ArH ortho to CO), 7.77–7.81 (m, 1H, ArH meta to N), 8.09–8.13 (m, 3H, ArH meta, para to CO), 8.18–8.22 (m, 1H, ArH ortho to N).

4.2.6. 1-Phenyl-3-(4-pyrimidin-2-yl-piperazin-1-yl)-propan-1-one hydrochloride salt (6). Spectral data: IR (cm^{-1}): 3024, 2928, 1678, 1148, 980. Mass (*m/e*): 297, 279, 202, 189, 149, 122, 106, 91, 77. ^1H NMR (δ ppm): 2.55–2.60 (t, 4H, N–CH_2 piperazine), 2.84–2.92 (t, 2H, CH_2CO), 3.19–3.26 (t, 2H, N–CH_2, piperazine), 3.81–3.86 (t, 4H, N–CH_2 piperazine), 6.45–6.49 (t, 1H, ArH meta to N), 7.43–7.60 (m, 3H, ArH para, meta to CO), 7.95–7.99 (d, 2H, ArH ortho to CO), 8.28–8.31 (d, 2H, ArH ortho to N).

4.3. Synthesis of (±)-3-(4-(substituted)-piperazin-1-yl)-1-phenyl-propan-1-ol hydrochloride salt (7–11)

4.3.1. General procedure. The 3-(4-(substituted)-piperazin-1-yl)-1-phenyl-propan-1-one free base (0.01 mol) was taken into methanol and cooled in icebath (0–5 °C) and $NaBH_4$ (0.015 mol) was added in small portions with stirring. After completion (as monitored by TLC), the methanol was distilled off and the residue was taken into water and extracted with ethyl acetate. The combined organic layer was washed with water and dried over Na_2SO_4. The Na_2SO_4 was filtered off and washed with ethyl acetate and the solvent is evaporated on rotavapor. The formed oily residue was taken into methanol and cooled in icebath (0–5 °C) and concd HCl (2 equiv) was added dropwise and kept stirring in icebath for 3 h. Later the methanol was distilled off and the residue was taken into hexane. A white solid separated out which was filtered, washed with hexane, and dried.

4.3.2. (±)-3-(4-Methyl-piperazin-1-yl)-1-phenyl-propan-1-ol hydrochloride salt (7). Spectral data: IR (cm^{-1}): 3360, 3018, 2914, 2426, 1218, 1036, 758. Mass (*m/e*): 234,127, 113, 107, 83, 70. ^1H NMR (δ ppm): 1.74–1.89 (m, 2H, CH_2CHOH), 2.28 (s, 3H, N–CH_3), 2.51–2.76 (m, 10H, N–CH_2 piperazine), 4.90–4.95 (t, 1H, CHOH), 7.33–7.36 (m, 5H, ArH).

4.3.3. (±)-3-[4-(3-Chloro-phenyl)-piperazin-1-yl]-1-phenyl-propan-1-ol hydrochloride salt (8). Spectral data: IR (cm^{-1}): 3408, 3120, 2922, 2550, 1244, 1084, 898. Mass (*m/e*): 330, 209, 111, 83, 70. ^1H NMR (δ ppm): 2.25–2.31 (m, 2H, CH_2CHOH), 3.00 (m, 4H, N–CH_2 piperazine), 3.26–3.34 (t, 2H, N–CH_2), 3.64 (m, 4H, N–CH_2 piperazine), 4.79–4.89 (t, 1H, CHOH), 6.79–6.90 (m, 3H, ArH meta to Cl), 7.16–7.50 (m, 6H, ArH).

4.3.4. (±)-1-Phenyl-3-[4-(3-trifluoromethyl-phenyl)-piperazin-1-yl]-propan-1-ol hydrochloride salt (9). Spectral data: IR (cm^{-1}): 3318, 3024, 2929, 2557, 1610, 1351, 1251, 1166, 1125, 944. Mass (*m/e*): 365, 243, 188, 144, 107. ^1H NMR (δ ppm): 2.28–2.31 (m, 2H, CH_2CHOH), 3.17–3.32 (m, 4H, N–CH_2 piperazine), 3.48–3.96 (m, 6H, N–CH_2 piperazine), 7.14–7.24 (m, 3H, ArH para, ortho, meta to CF_3), 7.29–7.42 (m, 6H, ArH ortho to CF_3 Ph).

4.3.5. (±)-1-Phenyl-3-(4-pyridin-2-yl-piperazin-1-yl)-propan-1-ol hydrochloride salt (10). Spectral data: IR (cm^{-1}): 3575, 3024, 2938, 1596, 1126, 942. Mass (*m/e*): 297, 202, 189, 175, 121, 107, 92, 71. ^1H NMR (δ ppm): 1.88–1.96 (m, 2H, CH_2CHOH), 2.58–2.77 (m, 6H, N–CH_2 piperazine), 3.58–3.63 (t, 4H, N–CH_2 piperazine), 4.94–5.00 (t, 1H, CHOH), 6.61–6.67 (d, 2H, ArH meta to N), 7.28–7.35 (m, 1H, ArH para to N), 7.38–7.53 (m, 5H, ArH of Ph), 8.18–8.20 (d, 1H, ArH ortho to N).

4.3.6. (±)-1-Phenyl-3-(4-pyrimidin-2-yl-piperazin-1-yl)-propan-1-olhydrochloride salt (11). Spectral data: IR (cm^{-1}): 3214, 3024, 2895, 1582, 1130, 975. Mass (*m/e*): 299, 203, 177, 148, 122, 108. ^1H NMR (δ ppm): 1.87–1.96 (m, 2H, CH_2CHOH), 2.49–2.81 (m, 6H, N–CH_2 piperazine), 3.86–3.91 (t, 4H, N–CH_2 piperazine), 4.94–5.00 (t, 1H, CHOH), 6.47–6.52 (t, 1H, ArH meta to N), 7.35–7.41 (m, 5H, ArH of Ph), 8.30–8.32 (d, 2H, ArH ortho to N).

4.4. Synthesis of (±)-1-(substituted)-4-[3-phenyl-3-(4-trifluoromethyl-phenoxy)-propyl]-piperazine hydrochloride salt (12–16)

4.4.1. General procedure. To a precooled mixture of NaH (0.02 mol) and DMAC (5 ml), 3-(4-(substituted)-piperazin-1-yl)-1-phenyl-propan-1-ol (0.01 mol) in DMAC (5 ml) was added and the reaction mixture was brought to room temperature and then heated in oilbath to 90 °C for 2 h. The reaction mixture was cooled to room temperature and 4-chlorobenzotrifluoride (0.02 mol) was added dropwise and the reaction mixture was again heated to 110 °C until the reaction was complete (as monitored by TLC). The reaction mixture was then diluted with water and extracted with ethyl acetate. The organic layer was washed and dried over Na_2SO_4. The Na_2SO_4 was filtered off and washed with ethyl acetate and the solvent was evaporated on rotavapor. The formed oily residue was taken into methanol and cooled in icebath (0–5 °C) and concd HCl (2 equiv) was added dropwise and kept stirring in icebath for 3 h. Later the methanol was distilled off and the residue was taken into ethyl acetate/hexane (1:1) mixture. A white solid separated out which was filtered, washed with ethyl acetate/hexane (1:1), and dried.

4.4.2. (±)-1-Methyl-4-[3-phenyl-3-(4-trifluoromethyl-phenoxy)-propyl]-piperazine hydrochloride salt (12). Spectral data: IR (cm^{-1}): 3014, 2930, 1326, 1217, 1164, 1013, 764. Mass (*m/e*): 379, 251, 235, 127, 113, 105. ^1H NMR (δ ppm): 1.81–1.90 (m, 2H, CH_2CHOR), 2.28–2.30 (s, 3H, N–CH_3), 2.45–2.60 (m, 10H, N–CH_2 piper-

azine), 5.26–5.27 (t, 1H, CHOR), 6.88–6.92 (d, 2H, ArH meta to CF_3), 7.29–7.33 (m, 5H, ArH), 7.36–7.44 (d, 2H, ArH ortho to CF_3).

4.4.3. (±)-1-(3-Chloro-phenyl)-4-[3-phenyl-3-(4-trifluoromethyl-phenoxy)-propyl]-piperazine hydrochloride salt (13). Spectral data: IR (cm^{-1}): 3082, 2992, 1250, 1094, 956. Mass (*m/e*): 474, 354, 209, 196, 105, 70, 56. ^1H NMR (δ ppm): 2.58–2.67 (m, 2H, CH_2COR), 2.86–3.47 (m, 10H, N–CH_2 piperazine), 5.43–5.49 (t, 1H, CHOR), 6.75–6.79 (d, 1H, ArH para to Cl), 6.87–6.93 (m, 4H, ArH ortho, meta to Cl, meta to CF_3), 7.16–7.20 (m, 1H, ArH ortho to Cl), 7.31–7.84 (m, 7H, ArH ortho to CF_3, Ph).

4.4.4. (±)-1-[3-Phenyl-3-(4-trifluoromethyl-phenoxy)-propyl]-4-(3-trifluoromethyl-phenyl)-piperazine hydrochloride salt (14). Spectral data: IR (cm^{-1}): 3082, 2932, 1582, 1452, 1322, 1252, 1010, 952. Mass (*m/e*): 508, 364, 257, 243, 230, 200, 188, 145, 70, 56. ^1H NMR (δ ppm): 2.21–2.32 (m, 2H, CH_2COR), 2.52–2.71 (m, 6H, N–CH_2 piperazine), 3.22–3.32 (m, 4H, N–CH_2 piperazine), 5.28–5.34 (m, 1H, CHOR), 6.89–6.93 (d, 1H, ArH para to CF_3), 7.05–7.09 (m, 4H, ArH ortho, meta to CF_3, meta to CF_3), 7.31–7.59 (m, 8H, ArH ortho to CF_3, Ph).

4.4.5. (±)-1-[3-Phenyl-3-(4-trifluoromethyl-phenoxy)-propyl]-4-pyridin-2-yl-piperazine hydrochloride salt (15). Spectral data: IR (cm^{-1}): 3005, 2924, 1604, 1328, 1257, 1157, 1111, 970. Mass (*m/e*): 442, 322, 280, 176, 149, 121, 107. ^1H NMR (δ ppm): 2.59 (m, 2H, CH_2CHOR), 2.89 (m, 2H, N–CH_2), 3.17–4.28 (m, 8H, N–CH_2 piperazine), 5.43–5.49 (t, 1H, CHOR), 6.66–6.78 (m, 2H, ArH meta, para to N), 6.86–6.90 (d, 2H, ArH meta to CF_3), 7.30–7.34 (m, 5H, Ph), 7.42–7.46 (d, 2H, ArH ortho to CF_3), 7.51–7.55 (m, 1H, ArH meta to N), 8.18–8.21 (d, 1H, ArH ortho to N).

4.4.6. (±)-2-{4-[3-Phenyl-3-(4-trifluoromethyl-phenoxy)-propyl]-piperazin-1-yl}-pyrimidine hydrochloride salt (16). Spectral data: IR (cm^{-1}): 3024, 2914, 1617, 1327, 1239, 1157, 1114, 973. Mass (*m/e*): 443, 299, 281, 177, 148, 122, 108. ^1H NMR (δ ppm): 2.03–2.16 (m, 2H, CH_2CHOR), 2.54–2.77 (m, 6H, N–CH_2 piperazine), 3.85–3.88 (m, 4H, N–CH_2 piperazine), 5.28–5.34 (t, 1H, CHOR), 6.46–6.51 (t, 1H, ArH meta to N), 6.89–6.93 (d, 2H, ArH meta to CF_3), 7.29–7.59 (m, 7H, ArH ortho to CF_3, Ph), 8.29–8.39 (d, 2H, ArH ortho to N).

4.5. Biology

4.5.1. Spermicidal activity. Human semen samples were obtained from young, healthy, and fertile volunteers in a sterile vial by masturbation. The samples were allowed to liquefy at 37 °C for 30 min before use. Semen samples with >60% motility and normal sperm morphology were used in this study. The studies were carried out in a Computer-Assisted Semen Analyzer (CASA, HTM-IVOS, Hamilton Thorn Research, Beverly, USA) using a Makler Chamber and Phase Contrast Optics at acquisition rate of 30 Hz as previously reported.[15] One hundred microliters of semen was mixed with 500 μl of spermicide solution (normal saline in control) and vortexed for 20 s. A drop was immediately placed on the makler chamber and analyzed (0 s). The sperm suspensions were maintained at room temperature during the entire analysis (60 s). The observed results were also validated by visual scoring under a phase contrast microscope by two independent workers and the most appropriate data were selected.

4.5.2. Sperm viability assay. Supravital staining with fluorescent dye (propidium iodide) and the hypo-osmotic swelling test (HOST) were used to assess the effect of compounds on sperm cell viability.[30] A 0.2 ml aliquot of liquefied semen was treated with 1.0 ml of spermicide solution (1.0%) and incubated for 1 min at 37 °C. The spermatozoa were pelleted by centrifugation and 0.5 ml of 0.001% propidium iodide solution was added to the pellet and mixed gently. The mixture was incubated for 15 min at 37 °C. A wet mount for each compound was observed under the phase contrast microscope in normal light from a halogen lamp and the total number of sperm visible in the field was recorded. The same field was again visualized under blue light from a mercury lamp using a B2A (Nikon) filter and the number of fluorescent (red) sperm heads was recorded. The same was repeated for other fields of view. The HOST experiment was used to determine the effect on the physiological integrity of the sperm membrane. Human spermatozoa treated with spermicide solution (as in the supravital staining experiment) were pelleted, treated with hypo-osmotic solution (sodium citrate 25 mM/fructose 75 mM; 150 mosmol), and mixed gently. The suspension was incubated for 30 min at 37 °C. A wet mount was prepared for each compound solution and observed under a phase contrast microscope, and spermatozoa with and without tail curling were counted and recorded in different fields of view. The percentage of HOST-negative (HOST−ve) and PI-stained sperm (mean of three values) has been presented in Figure 2.

4.5.3. Anticandida activity. The MIC of each test compound was determined against 9 candida isolates by broth microdilution technique as per guidelines of NCCLS.[31,32] Minimum inhibitory concentrations (MIC) were measured in 96-well tissue culture plate (Cellstar Greiner Bio One, Germany) using RPMI 1640 media buffered with MOPS (3-[*N*-morpholino] propanesulfonic acid) (Sigma Chemical Co.). The inoculum of the test cultures was maintained at $1.0–5.0 \times 10^3$ cfu/ml. Microtiter plates were incubated at 35 °C in a moist, dark chamber, and MIC and IC_{50} values were recorded spectrophotometrically (Softmax pro® 4.3, Versamax microplate reader, Molecular Devices) after 48 h.

4.5.4. Antitrichomonas activity. *Trichomonas vaginalis* parasites were grown in TYI-S-33 medium.[33] Parasites to be used in drug susceptibility assays were grown for one day following regular subculturing and were in the log phase of growth. In vitro drug susceptibility assay was carried out using standard procedure.[34] The test agents were first dissolved in phosphate-buf-

fered saline (PBS) or ethanol and serially diluted with PBS to obtain the required concentrations. Nonoxynol-9 dissolved in PBS was used as reference standard. The compounds were tested in the concentration range of 0.001–0.01%. 5×10^3 Trophozoites/well/ml were taken for the assay. Parasites were cultured anaerobically at 37 °C in the presence or absence of the test agent. Parallel culture containing ethanol (final concentration: 0.01%)/PBS served as control. Trophozoite growth was monitored on a daily basis by comparing the cultures containing the test agent with the corresponding vehicle control cultures. Antitrichomonas activity was assessed by Trypan blue staining to determine viability of the cells and cell number score, and presented in terms of MLC.

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