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Design and synthesis of 3-(azol-1-yl)phenylpropanes as microbicidal spermicides for prophylactic contraception ☆

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ABSTRACT

We designed a series of 25 3-(azol-1-yl)phenylpropanes which yielded 10 compounds (**3**, **4**, **7**, **8**, **13**, **14**, **19**, **21**, **23**, **26**) that irreversibly immobilized 100% human sperm at 1% (w/v) concentration in 60 s; 12 compounds (**8**, **9**, **15**, **16**, **19–21**, **23–25**, **27**, **28**) that showed potent microbicidal activity at 12.5–50 μg/mL against *Trichomonas vaginalis*; and 17 compounds (**3–11**, **13**, **15**, **19**, **21**, **23**, **26**, **28**, **30**) that exhibited potent anticandida activity with minimum inhibitory concentration (MIC) of 12.5–50 μg/mL. Almost all the compounds exhibited high level of safety towards normal vaginal flora (*Lactobacillus*) and human cervical (HeLa) cells in comparison to the marketed spermicide nonoxynol-9 (N-9). All the biological activities were evaluated in vitro. Two compounds (**4, 8**) with good safety profile exhibited multiple (spermicidal, antitrichomonas and anticandida) activities, warranting further lead optimization for furnishing a prophylactic vaginal contraceptive.

Good reproductive health is the basis for intimate relationship, happy family and healthy children, which ensures that every child is wanted, every birth is safe, and every person is free of sexually transmitted infection (STI) and human immunodeficiency virus (HIV).[1] On the other hand, some 340 million new cases of curable STIs occur every year [excluding HIV and other viral sexually transmitted diseases (STDs)], amongst which trichomoniasis has the highest incidence. The presence of one or more STI increases the risk of becoming infected with HIV by 2–9 times. Furthermore, women's health can also be affected adversely by reproductive tract infections like candidiasis that are not sexually transmitted. Therefore, developing user-friendly, self-administrable, anti-STI vaginal spermicides has become an urgent global priority.[2]

The inter-conversion of sulfhydryl groups to disulfide plays an important role in sperm maturation, its motility and viability.[3–5] This free SH to S–S interchange protects sperm from oxidative damages.[6] Thus a sulfhydryl binding agent should possess spermicidal activity as exemplified by *N*-ethylmaleimide,[7] acrylonitriles,[8] acrylophenones,[9] quinolines,[10] 5,5′-dithiobis(2-nitrobenzoic acid),[11] dithiocarbamates[12] and disulfide esters of carbothioic acid.[13–16]

In our efforts to design dually active spermicides, benzenepropanamines[17] and selective serotonin reuptake inhibitor (SSRI) antidepressants fluoxetine and paroxetine[18] exhibited potent spermicidal activity, possibly by interacting with sulfhydryl groups present over sperm cell membrane.[19,20] Metronidazole[21–23] is the only FDA approved drug for trichomonasiasis. On the other hand various azoles being used clinically as microbicidal agents.[24–28]

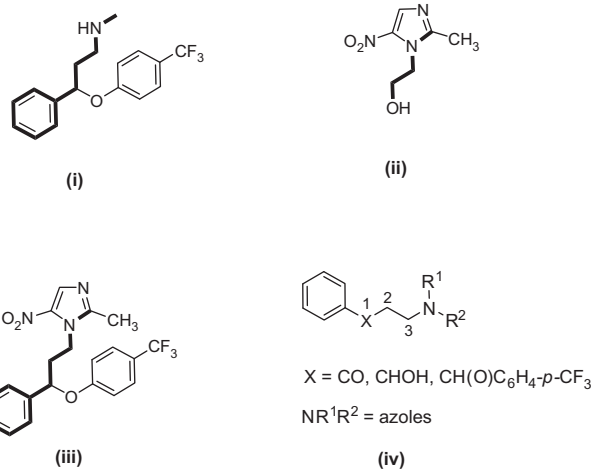

Figure 1. Chemical structures of phenylpropanamine (i), metronidazole (ii), hybrid compound (iii) and general structures of synthesized compounds (iv).

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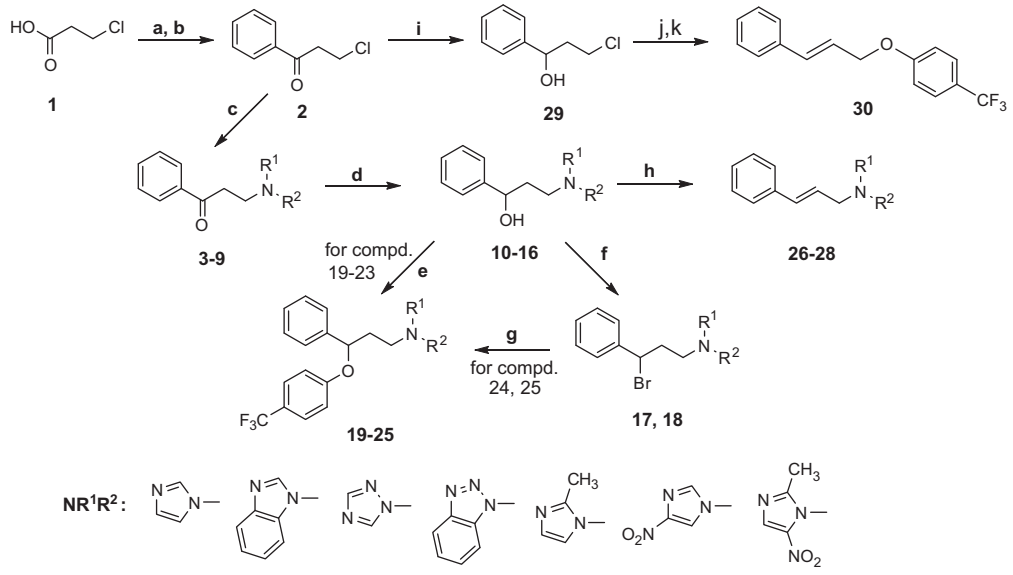
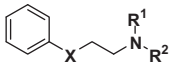
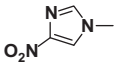
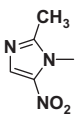


Scheme 1. Synthetic route of the compounds. Reagents and conditions: (a) $SOCl_2$, C_6H_6, reflux, 4 h; (b) C_6H_6, $AlCl_3$, 0–5 °C; (c) HNR^1R^2, toluene, Et_3N, reflux, 5 h; (d) $NaBH_4$, MeOH, 0–5 °C, 2 h; (e) NaH, *p*-chlorobenzotrifluoride, DMAc/DMF, 110–115 °C, 7–10 h; (f) PBr_3, diethylether, rt, 3 h; (g) K_2CO_3, dry acetone, 4-(trifluoromethyl)phenol, rt, 16 h; (h) PBr_3, toluene, reflux, 1 h; (i) $NaBH_4$, MeOH, 0–5 °C, 1 h; (j) PBr_3, diethylether, rt, 3 h; (k) 4-(trifluoromethyl)phenol, NaH, DMF, 110–115 °C, 8 h.

Table 1
Spermicidal and antitrichomonas activity of the compounds (**3**–**16**) against human spermatozoa and *Trichomonas vagainalis*

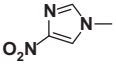
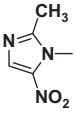


3-16

Compound	X	NR^1R^2	Immobilization of human spermatozoa[a,b] (%)	Antitrichomonas activity (MIC in μg/mL) at 48 h
3			100	>200
4			100	200
5			60	>200
6			80	200
7			100	>200
8			100	25
9			90	50
10			90	>200
11			90	>200
12			70	>200
13			100	>200

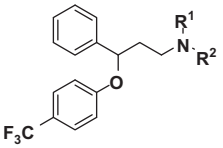
(*continued on next page*)

Table 1 (*continued*)

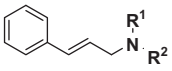
Compound	X	NR^1R^2	Immobilization of human spermatozoa[a,b] (%)	Antitrichomonas activity (MIC in μg/mL) at 48 h
14			100	>200
15			95	25
16			70	50
Metronidazole	—	—	—	2.0
Nonoxynol-9	—	—	100	37.4

[a] Vehicle (control) has 100% motility at the time of testing.
[b] At 1% concentration of test compound.

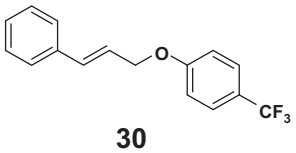
Table 2
Spermicidal and antitrichomonas activity of the compounds (**20**–**29**, **31**) against human spermatozoa and *Trichomonas vagainalis*



19-25



26-28



30

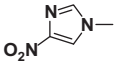
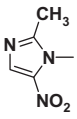
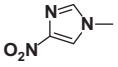
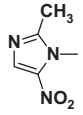
Compound	NR^1R^2	Immobilization of human spermatozoa[a,b] (%)	Antitrichomonas activity (MIC in μg/mL) at 48 h
19		100	12.5
20		50	25
21		100	50
22		70	>200
23		100	25
24		40	12.5
25		70	25
26		100	>200
27		80	25

Table 2 (*continued*)

Compound	NR^1R^2	Immobilization of human spermatozoa[a,b] (%)	Antitrichomonas activity (MIC in μg/mL) at 48 h
28		90	25
30	—	90	>200
Metronidazole	—	—	2.0
Nonoxynol-9	—	100	37.4

[a] Vehicle (control) has 100% motility at the time of testing.
[b] At 1% concentration of test compound.

We hypothesized that hybrid molecules (Fig. 1, iii) incorporating both phenylpropanamine (Fig. 1, i) and metronidazole (Fig. 1, ii) structures may yield dually active spermicidal microbicides. Thus the compounds synthesized (Fig. 1, iv) were evaluated in vitro for their spermicidal, antitrichomonas and anticandida activities as well as their safety against vaginal micro flora and cervical cells. The structure activity relationship (SAR) is being discussed in this communication.

The compounds (**3**–**16**, **19**–**28**, **30**) were synthesized according to Scheme 1. Compounds **19**–**25** were obtained by using the published reaction procedure,[17] however, nitro group containing compounds **24** and **25** could not be obtained by this method. Furthermore, unusual reactions were observed while preparing compounds **24** and **25**, resulting in formation of compounds **26**–**28** and **30** due to dehydrobromination.

The study included the incorporation of four phenylpropane moieties viz., 1-oxo (**3**–**9**), 1-hydroxyl (**10**–**16**), 1-[4-(trifluoromethyl)]phenoxy (**19**–**25**) and 1,2-dehydro (**26**–**28**, **30**) with five diazoles and two triazoles. All the 25 compounds exhibited spermicidal activity (Tables 1 and 2) as these compounds immobilized 40–100% normal human spermatozoa. Ten compounds (**3**, **4**, **7**, **8**, **13**, **14**, **19**, **21**, **23**, **26**) caused 100% immobilization while six compounds (**9**–**11**, **15**, **28**, **30**) caused 90–95% immobilization of human sperm at 1% concentration (w/v). The spermicidal activity of **3**, **10**, **19** and **26** among the imidazolo derivatives ranged between 90% and 100%, which suggested that imidazole group imparts the activity. Whereas in benzimidazolo derivatives (**4**, **11**, **20**), the sperm immobilizing potential reduced progressively from oxo (100%) and hydroxy (90%) to 1-[4-(trifluoromethyl)]phenoxy (50%). Conversely, in triazolo derivatives (**5**, **12**, **21**) the spermicidal activity increased from 60% (oxo) to 70% (hydroxyl) and 100% 1-[4-(trifluoromethyl)]phenoxy. Among benzotriazolo compounds (**6**, **13**, **22**), the hydroxyl derivative was 100% active while the other two showed 70–80% activity. The three 2-methylimidazolyl compounds (**7**, **14**, **23**) caused 100% immobilization of human sperms. Among 4-nitroimidazolyl compounds, the oxo (**8**) and hydroxyl (**15**) derivatives immobilized 95–100%, whereas 1-[4-(trifluoromethyl)]phenoxy (**24**) and 1,2-dehydro (**27**) exhibited reduced spermicidal activity of 40% and 80%, respectively. The compounds with 2-methyl-5-nitroimidazolyl group exhibited 90% (**9**, **28**) and 70% (**16**, **25**) spermicidal activity. On the other hand, 1,2-dehydro compound (**30**) in which the 3-azol-1-yl group was replaced by 1-[4-(trifluoromethyl)]phenoxy moiety; the spermicidal activity was 90%. Thus it could be inferred that in 3-azol-1-ylphenylpropanes an imidazole or 2-methylimidazole group at position-3 with an oxo group at position-1 was more desirable for spermicidal activity.

All the 25 compounds were evaluated for antitrichomonas activity, out of which 14 compounds (**4**, **6**, **8**, **9**, **15**, **16**, **19**–**21**, **23**–**25**, **27**, **28**) were found to be active against *Trichomonas vaginalis* with MIC ranging from 12.5 to 200 μg/mL (Tables 1 and 2). The standard drugs metronidazole and nonoxynol-9 inhibited the growth of *T. vaginalis* at 2.0 and 37.5 μg/mL, respectively. Among 1-oxo (**3**–**9**) and 1-hydroxyl (**10**–**16**) derivatives of phenylpropanes, significant antitrichomonas activity was exhibited by compounds substituted with 4-nitroimidazolyl (**8**, **15**) and 2-methyl-5-nitroimidazolyl (**9**, **16**) groups at position-3 (MIC 25–50 μg/mL). Whereas amongst 1-[4-(trifluoromethyl)]phenoxy (**19**–**25**) derivatives, six compounds exhibited significant activity (MIC 12.5–50 μg/mL) while compound **22** was inactive (MIC >200 μg/mL). The compounds with 4-nitroimidazolyl (**27**) and 2-methyl-5-nitroimidazolyl groups (**28**) at position-3 were more active among 1,2-dehydrophenylpropanes (**26**–**28**). The significant activity in compounds containing nitroimidazole group confirmed the role of nitro group in antitrichomonas activity of metronidazole.[29]

All the compounds were tested against five strains of *candida*. Seventeen compounds (**3**–**11**, **13**, **15**, **19**, **21**, **23**, **26**, **28**, **30**) exhibited anticandida activity (Table 3) with MIC of 25–50 μg/mL against one or more strains. Out of these, two compounds (**19**,

Table 3
Anticandida activity of compounds (**3**–**16**, **19**–**28**, **30**) against five different strains of *candida*

Compound	Anticandida activity (MIC in μg/mL)				
	1	**2**	**3**	**4**	**5**
3	>50	>50	>50	50	>50
4	25	50	>50	25	50
5	>50	>50	>50	50	>50
6	>50	50	>50	>50	50
7	>50	>50	>50	50	>50
8	50	>50	>50	50	>50
9	>50	>50	>50	50	>50
10	>50	>50	>50	50	>50
11	>50	>50	>50	50	>50
12	>50	>50	>50	>50	>50
13	>50	>50	>50	50	>50
14	>50	>50	>50	>50	>50
15	>50	>50	>50	50	>50
16	>50	>50	>50	>50	>50
19	12.5	50	50	50	50
20	>50	>50	>50	>50	>50
21	50	>50	50	>50	>50
22	>50	>50	>50	>50	>50
23	25	50	25	50	50
24	>50	>50	>50	>50	>50
25	>50	>50	>50	>50	>50
26	>50	50	>50	25	>50
27	>50	>50	>50	>50	>50
28	50	>50	>50	>50	>50
30	>50	>50	>50	50	>50
Fluconazole	0.5	0.5	0.25	0.25	0.25
Nonoxynol-9	>50	50	>50	>50	>50

1, *Candida albicans*; **2**, *Candida parapsilosis*; **3**, *Candida albicans MTCC-183*; **4**, *Patient isolates CDRI*; **5**, *NCIM-3557*; MIC, minimum inhibitory concentration.

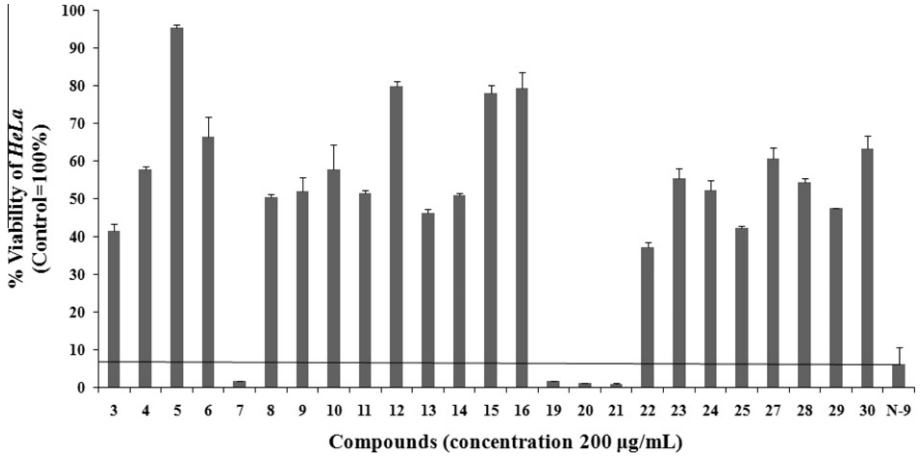


Figure 2. Cytotoxicity of compounds (**3**–**16**, **19**–**28**, **30**) towards human cervical (HeLa) cells.

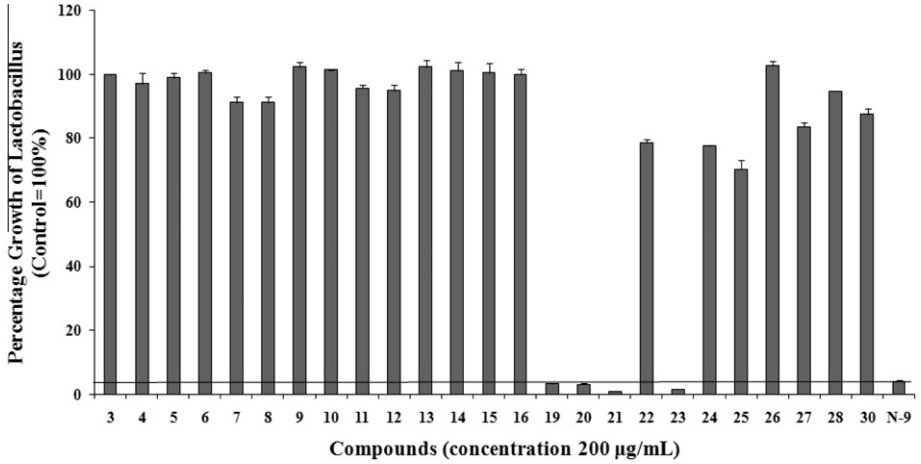


Figure 3. Compatibility of compounds (**3**–**16**, **19**–**28**, **30**) with normal vaginal flora *Lactobacillus*.

23) inhibited the growth of all the five *candida* strains tested. The activity was found to be much better than nonoxynol-9 (MIC >50 µg/mL). This suggests that imidazole and 2-methylimidazole was important for anticandida activity.

All the compounds were evaluated for their safety towards human cervical cell line (HeLa, Fig. 2) and vaginal flora (*Lactobacillus*, Fig. 3) at 200 µg/mL concentration by cell-viability assay. Most of the compounds were highly safe as compared to nonoxynol-9. However, the toxicity of four compounds (**19**–**21**, **23**) towards HeLa cells and *Lactobacillus* indicates that incorporation of 1-[4-(trifluoromethyl)]phenoxy group at position-1 may not be desirable.

Of the 10 compounds (**3**, **4**, **7**, **8**, **13**, **14**, **19**, **21**, **23**, **26**) that immobilized 100% human sperms, nine of these have been found to be dually active (except compound **14**). Compounds **3**, **13** and **26** exhibited anticandida activity as well and were safe towards both HeLa cell and *Lactobacillus* as compared to nonoxynol-9. However, compound **7** showed safety only towards *Lactobacillus*. Five compounds (**4**, **8**, **19**, **21**, **23**) exhibited spermicidal, anticandida and antitrichomonas activities. However, amongst these compounds **4** and **8** had high safety profile against HeLa cells and *Lactobacillus*, compound **26** was safe towards HeLa cells only while the remaining two (**19**, **21**) exhibited toxicity towards both the HeLa cells and *Lactobacillus*. The activities and safety profile of compounds **4** and **8** suggested that a keto group at position-1 with an imidazole or a nitroimidazole at position-3 in phenylpropane framework were most suited for dual activity and safety. The study has resulted into the discovery a structural class that may lead to a potent dually active microbicidal spermicide.

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Supplementary data

Supplementary data (the general procedures for the synthesis and biological evaluation of compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.042.

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