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Dithiocarbamate–thiourea hybrids useful as vaginal microbicides also show reverse transcriptase inhibition: Design, synthesis, docking and pharmacokinetic studies ☆

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ABSTRACT

Prophylactic prevention is considered as the most promising strategy to tackle STI/HIV. Twenty-five dithiocarbamate–thiourea hybrids (**14**–**38**) were synthesized as woman controlled topical vaginal microbicides to counter *Trichomonas vaginalis* and sperm along with RT inhibition potential. The four promising compounds (**18**, **26**, **28** and **33**) were tested for safety through cytotoxic assay against human cervical cell line (*HeLa*) and compatibility with vaginal flora, *Lactobacillus*. Docking study of most promising vaginal microbicide (**33**) revealed that it docked in a position and orientation similar to known reverse transcriptase inhibitor Nevirapine. The preliminary in vivo pharmacokinetics of compound **33** was performed in *NZ*-rabbits to evaluate systemic toxicity in comparison to Nonoxynol-9.

The acquired immune deficiency syndrome (AIDS) epidemic continues to spread throughout the world and especially imposes a particular burden on women and girls. According to UNAIDS 2013 estimate nearly 52% of all individuals living with HIV are now women of reproductive age (15–44 years).[1] They acquire the virus largely by heterosexual contacts, documented as the dominant mode of this pandemic spread.[2] However, male condoms may reduce the risk of HIV infection by 80–90% but they are beyond the control of women.[3] Thus, women urgently need self controlled methods to protect themselves against HIV infection. In the absence of an effective prophylactic HIV vaccine, prevention of new infections has become a priority. It was thought worthwhile to integrate HIV prevention and reproductive health services including unintended pregnancy protection for women as both are related with unprotected sex. Topical vaginal microbicide is one of the most promising female-controlled approaches effective against STIs (Sexually transmitted infections) including HIV in conjunction with pregnancy protection via sperm immobilization.[4,5]

A safe, efficacious and women friendly vaginal microbicide has yet not been available because microbicides that are undergoing preclinical and human clinical trials possess detergent action. Nonoxynol-9 (N-9), the only recommended microbicide for protection against sexual transmission of HIV-1 resulted in compromised vagina owing to detergent action.[6,7] Moreover, studies found that with frequent use, N-9 causes mucosal erosion and local inflammation of the female reproductive tract, which increases susceptibility to HIV-1 and other viral infections. Thus, clinical safety becomes a serious liability for vaginal products.[8–10]

Anti-HIV-1 potential of reverse transcriptase (RT) inhibitors in genital tract secretions has already been established.[11,12] So, it was attempted to synthesize novel vaginal microbicides with RT inhibition as best suited prophylactic approach against vaginal

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HIV-1 transmission.[13,14] The success of thiourea derivatives as NNRTIs[15,16] and ongoing research interest in the development of dithiocarbamates (Fig. 1) as potent vaginal anti-trichomonal spermicides[17–22] led us to hybridize these scaffolds in one chemical entity (alkyl 4-(alkyl/arylcarbamothioyl)piperazine-1-carbodithioate, Fig. 1) to achieve dual action. Anti-HIV spermicidal potential of thiourea moiety has been recognized.[23–25] Considering the well-known 'butterfly' conformation of NNRTIs,[26] like piperazine derivative, Delavirdine (Fig. 1) these diverse scaffolds were synthesized and evaluated for their in vitro RT inhibition assay to ascertain their anti HIV-1 potential. Anti-*Trichomonas* and spermicidal activity of synthesized compounds was also evaluated against *Trichomonas vaginalis* and human sperm, respectively. The structure activity relationship (SAR) has also been discussed. The promising compounds were also assessed for their in vitro cytotoxicity profile on vaginal flora (*Lactobacillus*) and human cervical epithelium (*HeLa*) cells because they are intended to be used vaginally. Pharmacokinetics of most potent compound versus N-9 was evaluated in female Newzealand (*NZ*) rabbits to examine its absorption into systemic circulation and subsequent exposure in blood plasma through vaginal wall. Moreover, a docking study was also carried out to find a suitable correlation of most active compound with NNRTIs activity and inhibition of the prospective receptor.

The alkyl 4-(alkyl/arylcarbamothioyl)piperazine-1-carbodithioate (**14**–**38**) were synthesized according to the strategy depicted in Scheme 1. Sodium 4-(*tert*-butoxycarbonyl)piperazine-1-carbodithioate (**3**) was synthesized by reaction of *N*-Boc piperazine with carbon disulfide and sodium hydroxide. Reaction of **3** with different alkyl halide in the presence of triethylamine in methanol at rt yielded *tert*-butyl 4-(alkylthiocarbonothioyl)piperazine-1-carboxylate (**4**–**8**). Treatment with trifluoroacetic acid (TFA) in dichloromethane (DCM, 0–5 °C) and aqueous sodium bicarbonate ($NaHCO_3$) resulted in Boc deprotection and gave alkyl piperazine-1-carbodithioate (**9**–**13**). Compounds **9**–**13** afforded alkyl 4-(alkyl/arylcarbamothioyl)piperazine-1-carbodithioate (**14**–**38**) on treatment with isothiocyanates in ethanol at room temperature.

The structures of all newly synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, IR, mass spectrometry (ESMS and HRMS) and elemental analysis (see Supporting information).

The compounds presented in this study namely alkyl 4-(alkyl/aryl carbamothioyl) piperazine-1-carbodithioate (**14**–**38**) were evaluated for anti-*Trichomonas* and spermicidal potential along with RT inhibitory activity by using enzymatic RT assay (Table 1).

Trichomonas vaginalis causes punctuate hemorrhages giving HIV open access to its target T-lymphocytes. These hemorrhages leak the virus and cause them to concentrate in the infected area, which increase HIV transmission by 2 to 3 fold.[27] All the synthesized compounds (**14**–**38**) except **25** and **36** exhibited anti-*trichomonas* activity ranging from 7.78–500 μg/mL. Remarkable activity (better than N-9, MIC 20 μg/mL) was observed in compounds **28**, **33** and **14** which were active at 7.78 μg/mL, 15.56 μg/mL and 15.56 μg/mL, respectively.

A potent contraceptive (spermicidal) activity in microbicides would attract users, especially those at a high risk of acquiring HIV/STD, and may improve compliance in usage. Spermicides capable of killing 100% human sperm almost instantaneously at physiological concentrations in vitro are likely to provide adequate pregnancy protection in vivo. Among synthesized twenty five compounds, thirteen compounds (**14**, **16**, **18**, **23**, **26**–**31**, **33**, **36** and **38**) exhibited spermicidal activity (Table 1) at 0.025–2% (w/v) concentration and irreversibly immobilized 100% normal human spermatozoa. Out of these thirteen compounds, four compounds (**18**, **26**, **28** and **33**) were active at concentration 0.025–0.05% comparable or even more active than marketed spermicide N-9 (MEC, 0.05%). Two compounds (**18** and **33**) demonstrated extremely potent spermicidal activity at MEC 0.025%.

All the compounds inhibited the RT ranging 1.90–44.67% at 100 μg/mL concentration. The compounds that showed moderate inhibitory activity (>30%) were **16**, **18**, **20**, **29**, **31**, **33** whereas the control NNRTIs marketed drug Nevirapine (NVP) showed 99.6% inhibition. The enzyme assay results were visualized in combination with anti-*trichomonas* and spermicidal activity. The results are summarized in Table 1 along with standard drug NVP.

This study included 1,4-disubstituted piperazine compounds (**14**–**38**) having a substituted thiourea at the N^1-position while substituted dithiocarbamates at N^4-position. The thiourea substituents R^1 were benzyl, phenyl, 3-pyridyl, phenethyl and benzoyl while the dithiocarbamate substituents R^2 have been propyl, butyl, hexyl, octyl, and benzyl. The anti-*Trichomonas* activity

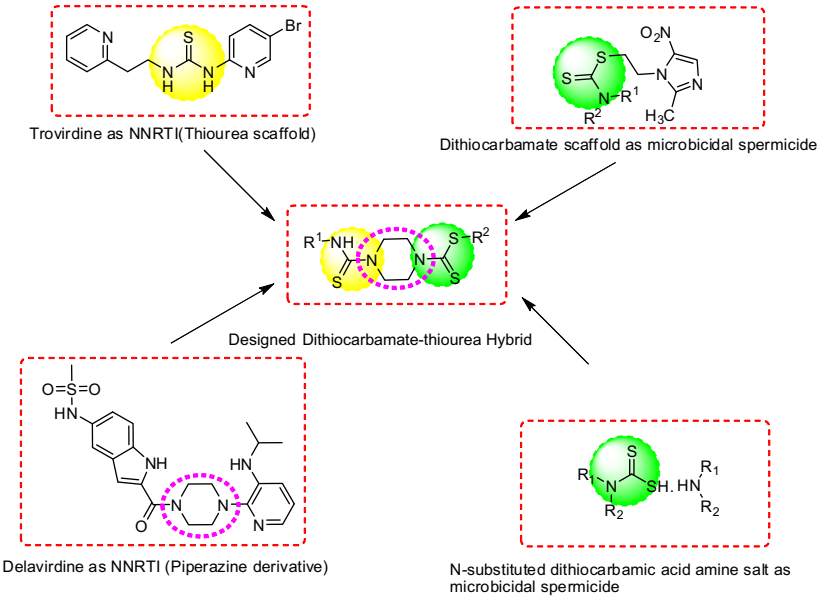

Figure 1. Designing of dithiocarbamate–thiourea hybrids.