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Synthesis and antibacterial activity evaluation of metronidazole–triazole conjugates

Beena [a], Nitin Kumar [a], Rajesh K. Rohilla [b], N. Roy [b], Diwan S. Rawat [a,*]

[a] Department of Chemistry, University of Delhi, Delhi 110007, India
[b] Department of Biotechnology, National Institute of Pharmaceutical Education and Research, SAS Nagar, Punjab 160062, India

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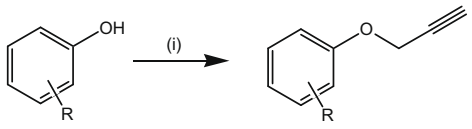
ABSTRACT

Synthesis and antibacterial activity of metronidazole–triazole conjugates are reported. Total 21 hybrid compounds have been synthesized with different substitution pattern on the triazole ring in order to study their influence on the antibacterial activity. These compounds demonstrated potent to weak antibacterial activity against Gram-positive, and Gram-negative bacteria. Six compounds have shown equal or better antibacterial activity against Gram-negative strains than the reference compound.

Metronidazole, (MTZ, 1-[2-hydroxyethyl]-2-methyl-5-nitroimidazole) has been a drug of choice for the treatment of anti-infectious diseases against protozoa such as *Trichomonas vaginalis*, *Entamoeba histolytica*, *Giardia intestinalis*, and infections caused by Gram-negative anaerobes such as bacteroides and Gram-positive anaerobes such as *clostridia*.[1–5] MZT has extensively been used for the treatment of anaerobic infections after bowel surgery, and infections caused by *Clostridium difficile*.[6,7] MZT in combination of other drugs has been used for the treatment of infections caused by *Helicobacter pylori*[8] and has also been used for the radiosensitization of hypoxic tumors and Crohn's disease.[9–11] Since its discovery in 1959, metronidazole has been among the top 100 most prescribed drugs in the US and one of the 10 most used drugs during pregnancy.[12,13] Even today, it remains one of the best drug for the treatment of *Trichomonas vaginalis* infections, a common disease of the pregnant women with a cure rate of approximately 95%.[14,15] However, resistance to these compounds have been demonstrated in *trichomonads* and Bacteroides *fragilis*, in both natural and in vitro under drug pressure-induced populations.[16,17] We anticipated that hybrid molecule that contains metronidazole and triazoles, one of the most active and widely studied pharmacophore,[18–20] will lead to a molecule of biomedical importance. For this we decided to functionalize hydroxyl functionality of MTZ in such a way that MTZ can be covalently linked to triazoles. As part of our ongoing efforts towards the synthesis of biologically active compounds,[21–23] we report herein synthesis and antibacterial activity of metronidazole–triazole conjugates. Synthesis of metronidazole–triazole conjugates started from the preparation of substituted alkynes by the literature method (scheme 1).[24–26] 1-(2-Azido-ethyl)-2-methyl-5-nitro-*1H*-imidazole (**5**)[27] was prepared by literature method (scheme 2). Subsequent reaction of compound **5** with substituted terminal alkynes (**2a**–**2k**) in presence of sodium ascorbate, and $CuSO_4{\cdot}5H_2O$ in *t*-BuOH/H_2O (1:1), lead to the formation of desired hybrid molecules in good yield (**6a**–**6o**, and **7a**–**7f**). All of the reported compounds were purified over silica gel column and characterized spectroscopically.[28]

In vitro antibacterial activity. Antimicrobial susceptibility testing was carried out using National Committee for Clinical Laboratory Standards (NCCLS) microdilution assay. Briefly, the bacterial strains were grown in standard media until exponential growth was achieved. Tests were performed in a 96-well microtiter plate in a final volume of 100 μl. Test compounds were dissolved in 5% DMSO at an initial concentration of 500 μg and serially diluted in



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2a, R = H; **2b,** R = *o*-methyl;
2c, R = *p*-methyl; **2d,** R = *m*-methyl;
2e, R = *o*-NO_2; **2f**, R = *p*-NO_2;
2g, R = *o*-CHO; **2h**, R = *p*-CHO;
2i, R = *p*-$COCH_3$; **2j**, R = *o*-Cl;
2k, R = *p*-Cl

Scheme 1. Reagents and condition: (i) Propargyl bromide, K_2CO_3, DMF, rt.

* Corresponding author. Tel.: +91 11 27667465; fax: +91 11 27667501.
E-mail address: dsrawat@chemistry.du.ac.in (D.S. Rawat).

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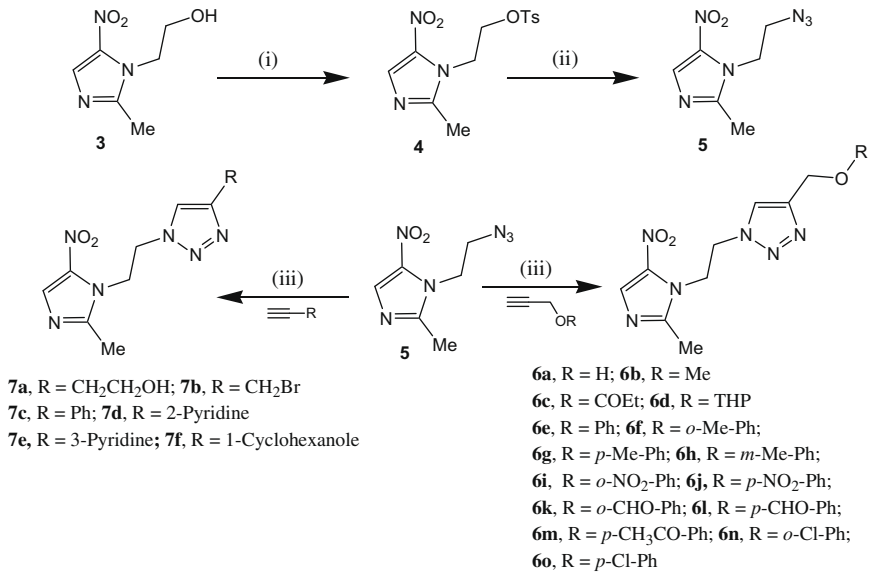

Scheme 2. Reagents and conditions: (i) Pyridine, *p*-TsCl, rt, 12 h; (ii) NaN_3, DMF, 0 °C, and 70–80 °C; 1 h; (iii) *t*-BuOH:H_2O (1:1), Sodium ascorbate, $CuSO_4{\cdot}5H_2O$.

plate. Each well was then inoculated with $\sim 2–5 \times 10^5$ bacterial cells and incubated at 37 °C for 24 h with shaking at 200 rpm. One well containing cells and 5% DMSO without any test compound (growth control), and one well containing only growth medium (sterility control) were used as controls. Growth of bacteria was determined using Power wave200 microplate scanning spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA). Percent survival was calculated using growth without any compound as 100% survival. IC_{50} values are calculated using Grafit 4.0 software (Erithacus Software Ltd., Horley, Surrey, UK).

The compounds were evaluated against Gram-positive and Gram-negative bacterial strains. IC_{50} values of each compounds are shown in Table 1.

Compound **6a** has shown potent antibacterial activity against Gram-positive as well as Gram-negative bacteria with IC_{50} value of 0.091–0.35 μg/mL. Introduction of one more CH_2 group between OH and CH_2 of compound **6a**, results in total loss of antibacterial activity of the compound (entry **7a**). Similarly substitution of OH by OCOEt, OMe, tetrahydropyran or Br functional group in compound **6a** leads devoid of antibacterial activity of the compounds (entry **6b**–**6d**, and **7b**). Compound **6l** having CHO group at the *para* position of the phenyl ring exhibit potent antibacterial activity against *E. Coli, P. aerogenosa* while it has been inactive against Gram-positive bacteria. Substitution at *para* position of the phenyl ring by methyl, CHO or chloro group have shown very good antibacterial activity of these compounds (**6g, 6l,** and **6o**). Presence of NO_2 group at any position of the phenyl ring results in the devoid of antibacterial activity of the compounds (entry **6i**, and **6j**). From structural subset of 21 compounds, it is clear from Table 1 that substitution at *ortho* position of the phenyl ring has negative effect on the antibacterial activity (entry **6f, 6n**) and unsubstituted phenyl ring containing compound also shows good antibacterial activity (entry **6e**) Similarly, compounds having phenyl or pyridyl groups directly attached with the triazole ring exhibit potent antibacterial activity (entry **7c**–**7e**). Six compounds (**6a, 6f**–**6h, 6l, 6o, 7c**–**7f**) have shown better or equal antibacterial activity against Gram-negative strains than tetracycline, which has been used as a standard drug in this study. Further structural modification of the most active compounds (**6a, 6f**–**6h, 6l, 6o, 7c**–**7f**) is under progress and results will be published in due course of time. All of these compounds have also been tested against *G. candidum, C. galbrata* and *C. albicans* and found to be inactive.

Table 1
Antibacterial activity of metronidazole–triazole conjugates

Compound	IC_{50} (μg/mL)			
	E. Coli	*P. aerogenosa*	*S. aeureus*	*E. epidermidis*
6a	0.091	0.180	0.350	0.350
6b	*	*	*	*
6c	*	*	*	*
6d	*	*	*	*
6e	0.670	0.130	*	*
6f	0.028	*	*	*
6g	0.060	0.030	0.130	*
6h	0.067	0.068	0.260	*
6i	*	*	*	*
6j	*	*	*	*
6k	*	*	*	*
6l	0.070	0.070	*	*
6m	*	*	*	*
6n	*	*	*	*
6o	0.003	0.020	0.130	*
7a	*	*	*	*
7b	*	*	*	*
7c	0.130	0.130	*	*
7d	0.120	0.130	0.130	*
7e	*	*	0.310	*
7f	*	0.180	*	*
Tet	0.090	0.060	0.120	0.080

[Tet]Tetracycline was used as a reference compound.
* IC_{50} > 500 μg/ml.

In conclusion, we have reported the synthesis of 21 new metronidazole–triazole conjugates and evaluated their antibacterial activity. Some of the compounds exhibit weak to potent anti-bacterial activity against Gram-positive bacteria, but have shown potent activity against Gram-negative bacteria. In particular six of the reported compounds shows better antibacterial activity against Gram-negative strains than the tetracycline which has been used as a reference compound.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.01.037.

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28. Synthetic methods and analytical data of all of the compounds reported in this paper can be found at supporting information section.