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Design, synthesis, antibacterial activity and docking study of some new trimethoprim derivatives

Umer Rashid [a,b,*], Waqas Ahmad [a], Syed Fahad Hassan [c], Naveeda Akhtar Qureshi [d], Basit Niaz [a], Bakhtiar Muhammad [a], Sameera Imdad [e], Muhammad Sajid [e]

[a] Department of Chemistry, Hazara University, Mansehra 21120, Pakistan
[b] Department of Chemistry, COMSATS Institute of Information Technology, Abbottabad 22060, Pakistan
[c] Department of Pharmacy, The University of Lahore, Defense Road Campus, Lahore 53700, Pakistan
[d] Department of Animal Sciences, Quaid-i-Azam University, 45320 Islamabad, Pakistan
[e] Department of Biochemistry, Hazara University, Mansehra 21120, Pakistan

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ABSTRACT

In present study, nineteen novel trimethoprim (TMP) derivatives were designed, synthesized and evaluated for their antibacterial potential. Hydroxy trimethoprim **2** (HTMP) was synthesized by following the demethylation of 4-methoxy group at trimethoxy benzyl ring of TMP. Structure–activity relationship (SAR) studies were explored on HTMP by incorporating various substituents leading to the identification of some new compounds with improved antibacterial activities. The results revealed that the introduction of benzyloxy (**4a**–**e**) and phenyl ethanone (**5a**–**e**) group at 4-position of dimethoxy benzyl ring leads to overall increase in the antibacterial activity. The most potent antibacterial compound discovered is benzyloxy derivative **4b** with MIC value of 5.0 μM against *Staphylococcus aureus* and 4.0 μM against *Escherichia coli* strains higher than the standard TMP (22.7 μM against *S. aureus* and 55.1 μM against *E. coli*). Substitution at 4-NH_2 group was not tolerated and the resulting Schiff base derivatives **3a**–**h** demonstrated very little or no antibacterial activity in the tested concentration domain. We further performed exploratory docking studies on dihydrofolate reductase (DHFR) to rationalize the in vitro biological data and to demonstrate the mechanism of antibacterial activity. For the ability to cross lipophilic outer membrane, log*P* was computed. It was found that the compounds possessing high hydrophobicity have high activity against *E. coli*.

The infectious diseases are associated with high rates of deaths worldwide. The discovery of antibacterial chemotherapeutic agents has been considered as one of the most important achievements since the past fifty years. However, resistance to current clinical antimicrobial drugs has certainly increased the global health concern. Bacteria have the ability to render all the current anti-infective treatments useless. This stimulates drug discovery researchers to develop new antimicrobial agents. Since the last decade, a number of antibacterial drug discovery strategies have emerged.[1–3] Among these strategies, structural manipulation or chemical alteration and exploitation of the biochemical pathways inhibited by known and previously characterized clinical antimicrobial drugs are the important strategies in drug design.[4,5]

Trimethoprim (TMP) is a small drug molecule containing 2-aminopyrimidine scaffold (Fig. 1). TMP was initially considered as antimalarial agent, but now considered as highly effective against bacterial infections, especially in synergistic combination with sulfamethoxazole (Co-trimoxazole). TMP binds to dihydrofolate reductase (DHFR) and its affinity for bacterial DHFR is 3000 time greater than human DHFR.[6,7] Dihydrofolate reductase (DHFR, EC 1.5.1.3) is a critical enzyme that catalyses the reduction of dihydrofolate to tetrahydrofolate. DHFR has been the subject of much research as the target of antimicrobial agents. Methotrexate (MTX), trimethoprim (TMP) and pyrimethamine, having 2-aminopyrimidine moiety, are the selective inhibitors of DHFR. Increase resistance to TMP has limited its use. The two rings of TMP allow for the structural changes which may resulting in different lipophilicity and resistance profiles.[8,9]

The experience achieved by our research group in the field of computer aided drug design and synthesis of chemical entities as putative drugs for the treatment of various diseases prompted us

* Corresponding author. Tel.: +92 (992) 383591 6.
E-mail addresses: umerrashid@ciit.net.pk, umer_rashid39@hotmail.com (U. Rashid).

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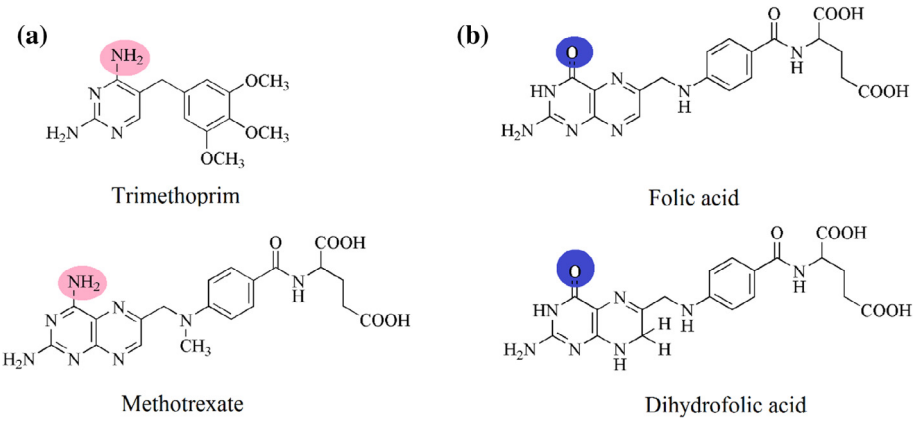


Figure 1. Comparison of the structures of the inhibitors and substrates of DHFR. Inhibitors differs from the folate substrates only in the replacement of 4-oxo group (blue circle) by 4-NH_2 group (pink circle). (a) Structures of two inhibitors of DHFR; trimethoprim and methotrexate; (b) structures of the two substrates of DHFR; Folic acid and dihydrofolic acid.

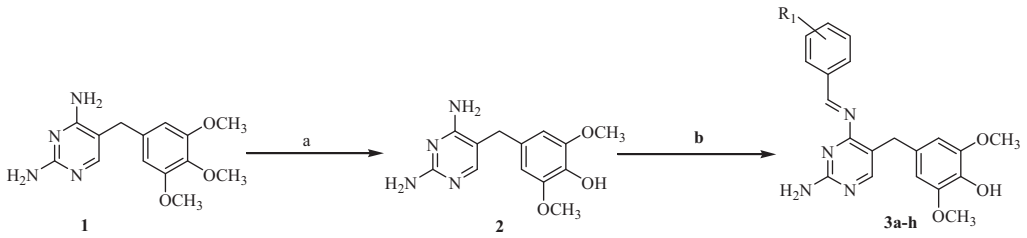
to further explore new compounds as antibacterial drugs.[10–13] Recently, we have reported medicinal chemistry approaches to design and synthesize antileishmanial and antibacterial agents.[10,11] Considering the pharmacological importance of TMP scaffold, it was planned to design and synthesize a variety of TMP-based potent antibacterial compounds to diversify the current antimicrobials.

Strong DHFR inhibitors like trimethoprim differs from the folate substrates only in the replacement of 4-oxo group by 4-NH_2 group (Fig. 1). Therefore, it is observed that the essential feature of the strong DHFR inhibitors is 2,4-diaminopyrimidine ring. 2,4-diaminopyrimidine moiety forms hydrogen bond interactions with Asp27, Leu5 and Phe92. While, the trimethoxy benzyl group is embedded in the hydrophobic pocket. It is evident that trimethoxy benzyl group was not tightly fitted into the active site as observed with para-aminobenzoate (pABA)-glutamate tail of methotrexate (MTX).[14] From these observations, we envisioned that the development of compounds that can bind tightly into the target may show the desirable effects on their antibacterial properties. With a view to synthesize potent antibacterial agents, we designed TMP derivatives by structural changes at both the TMP rings. Compound **2** (HTMP, Scheme 1) was designed by following the demethylation strategy of methoxy group at *para*-position trimethoxy benzyl ring. With an aim to design structural variants of HTMP (**2**) and to understand structural requirements for DHFR binding, compounds **3a–h** were designed by incorporating benzylidene group at 4-NH_2. Similarly, compounds **4a–e** and **5a–e** were designed to enhance the binding affinity through extra binding interactions with the DHFR.

In the first step, trimethoprim was reacted with 48% HBr to hydrolyse the ether into hydroxyl group.[15] This hydroxyl group could be further used in nucleophilic substitution reactions. In ^{1}H NMR spectrum of HTMP **2**, a broad singlet at 8.693 ppm of hydroxyl group indicates the hydrolysis (demethylation) of methoxy group at 4-position. Two broad singlet signals at 7.89 and 7.44 ppm designates the amino protons at 2- and 4-position respectively of 2,4-diaminopyrimidine ring. A singlet appears at 6.72 ppm shows the presence two aromatic protons at dimethoxy benzyl ring. Similarly, a proton singlet signal at 7.68 assignable for an aromatic proton at 2,4-diaminopyrimidine ring. A singlet with six proton integration, appears at 3.89 ppm, designates the methoxy protons. Another singlet at 3.49 ppm indicates the two protons of —CH_2 (Fig. S-1 in Supplementary data). In LC–MS spectrum, a peak present at *m*/*z* 277.12 confirms the protonated molecular ion $[M+H]^+$.

For the SAR studies, HTMP (**2**) was further used to design a set of novel antibacterials. First, we planned the synthesis of benzylideneamino derivatives to evaluate the effect of modification on 4-amino group. Eight Schiff base derivatives (**3a–h**) were synthesized by the reaction of different aryl aldehydes with HTMP (**2**) as shown in Scheme 1.[16] In ^{1}H NMR spectrum of **3a**, disappearance of peak of 4-NH_2 protons of HTMP (**2**) (at 7.44 ppm) and appearance of a downfield singlet signal at 8.30 ppm designates the imine proton (—N=CH) and confirms the formation of Schiff base derivative **3a**. A multiplet signal appears at 7.16 ppm shows the presence of five aromatic protons of the phenyl ring.

The synthesis of target molecules **4a–e** starts with the nucleophilic reaction of 4-OH group with substituted benzyl and acyl bromides in the presence of K_2CO_3 as base and acetonitrile as solvent (Scheme 2).[17] The synthesis of **5a–e** was carried out as depicted in Scheme 3. For the synthesis of **5a–e**, α-bromoketones (**3–7**) were synthesized using substituted acetophenones, *N*-bromosuccinimide (NBS) and *p*-toluenesulfonic acid (PTSA) under ultrasonic irradiations (Scheme 3).[18] Purified α-bromoketones (**3–7**) were used for the synthesis of target molecules **5a–e** (Scheme 3).[19] The reactions were monitored by using thin layer chromatography (TLC). The synthesized **4a–e** and **5a–e** were characterized by their melting points (mp) and spectroscopic analysis



Scheme 1. Synthesis of Schiff bases **3a–h**. Reagents and conditions: (a) 48% HBr. (b) Substituted aromatic aldehydes, ethanol, H_2SO_4, ultrasonic bath, 40 °C, 3–4 h.