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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

[e] Department of Biochemistry, Hazara University, Mansehra 21120, Pakistan

*Corresponding author. E-mail: umerrashid@ciit.net.pk; umer_rashid39@hotmail.com

Corresponding Author Tel. +92 (992) 383591-6

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 *S. aureus* and 4.0 µM against *E. 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*.

Key words: Trimethoprim, dihydrofolate reductase, antibacterial, hydrophobicity, docking

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 (**Figure 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 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 (**Figure 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 (**Figure 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 (m.p.) and spectroscopic analysis (^{1}H-NMR, ^{13}C-NMR and LC-MS). In ^{1}H NMR spectra, these compounds were confirmed by the disappearance of –OH peak of **HTMP 2** at 8.69 ppm. Singlet signals appeared at 4.7 ppm assignable to $–OCH_2$ protons for compounds **4a-e**.

In vitro antibacterial screening was carried out and TMP was used as a standard drug for comparison with the studied compounds. The compounds having antibacterial activity were then subjected to determine Minimum Inhibitory Concentration (MIC).[20] **Table 1** enlists the *in vitro* MIC values against gram positive and negative strains of bacteria. It is evident from **Table 1** that some analogues were found to have high potency than the standard drug trimethoprim (TMP). Demethylation of methoxy group at 4-position of TMP resulted in comparable activity to TMP. Except compound **3c**, all Schiff base derivatives (**3a-h**) showed very little or no antibacterial activity in the tested concentration domain and hence these are considered as poor antimicrobial compounds. 3-hydoxyphenyl containing compound (**3c**) has shown some little activity with MIC value of 336 µM (128 µg/ml) against *E. coli* strain. Optimization studies of the R^1-position revealed that the potency of the compounds with substituted benzyloxy derivatives (**4a-e**) was excellent. As can be seen, compound **4b** with $4\text{-}OCH_3$ group has shown remarkable potency with MIC value of 5 µM (2 µg/ml) for *S. aureus* and 4 µM (1.6 µg/ml) for *E. coli* strains. Replacement of methoxy with chlorine and methyl (**4c and 4d**) resulted in the slight decrease in activity against *S. aureus.* Derivatives with phenylethanone group (**5a-e**) have shown moderate-to-good activity with MIC value. Compounds with $4\text{-}OCH_3$ and methyl group (**5c** and **5d)** has shown excellent activity with MIC value of 18.7 µM (8 µg/ml) for both *S. aureus* and *E. coli* strains. Compound 2d with $4\text{-}CH_3$ group showed good activity against *E. coli* strains (MIC 15.5 µM).

We have analyzed the variations in the *in vitro* antibacterial results of both the strains. We were in opinion that these variations may be due to: 1) ability to enter the bacterial cytoplasm and even to cross nuclear envelope; 2) Interaction/fitting of the compounds into the active site. High value of log P has a direct effect with the potency of compounds against *S. aureus* and *E. coli.* Log P was predicted by ChemAxon software package (Marvin v15.4.6, 2015).[21] It is clear from the **Table 1** that the compounds with high log P value were found more active towards Gram negative strains than Gram positive strains. The reason is the presence of more lipophilic outer membrane in Gram negative bacteria (*E. coli*) which act as an efficient permeability barrier. This makes the results very promising against *E. coli*. The *in vitro* results in **Table 1** revealed that derivatives with high Log P values are found to be more potent against *E. coli* than standard drug and gram positive strain.

Interactions of the compounds with important residues were determined by docking studies. Computational docking studies were carried out using GOLD (Genetic Optimization for Ligand Docking) suit v5.4.1 to understand the types of interactions and binding orientations.[22-23] Based on the mechanism of action of TMP, DHFR was chosen as a bacterial target to deduce the interactions and binding mode of synthesized derivatives. X-ray structure of DHFR from *S. aureus* was retrieved from PDB (PDB ID 2W9S) with trimethoprim as co-crystallized ligand. The ability of the docking algorithm was validated to reproduce the co-crystallized pose of TMP in the 2W9S pocket. This yielded a good agreement between the docked and crystal structures as it further confirmed the interaction region of the ligand and a favorable interaction with DHFR. The binding mode of hydroxy trimethoprim (**2, HTMP**) resembles that of TMP. 2,4-diaminopyrimidine moiety forms hydrogen bond interactions with Asp27, Ile5 and Phe92. Ser49 forms hydrogen bond with hydroxyl group (**Figure 2a-b**). The GOLD fitness score of TMP (62.34) compared to HTMP (62.67) is in good agreement with bioactivity data.

We have found the different binding mode of these Schiff base derivatives (**3a-h**) due to their different orientation in the binding site. This orientation could explain the very little or no antibacterial activity of the Schiff base derivatives (**Table 1**). 2D binding orientations of 4-hydroxy **(3b)** and 3-hydroxy phenyl **(3c)**. These poses are generated by Discovery Studio Visualizer.[24] Compound **3c** with 4-hydroxy group oriented itself in such a way that it forms hydrogen bonds with Ala7 and Asn18. While, compound **3c** forms hydrogen bond interactions with Ala7, Asp27 and Ser49 **(Figure S-2 in Supplementary data)**.

These interactions along with high logP value may be reason of high activity of **3c** against *E. coli* strain. The good activity of **3c** compared to **3b** was further confirmed by Gold fitness score. The calculated GOLD fitness score for inhibitor **3b** is 41.05 and for **3c** is 45.42.

The designed compounds **4a-e** showed excellent activity and this was confirmed by the mode of orientation of these compounds and their Gold fitness score. The docking results showed that these compounds fit well in the binding cavity of DHFR. Compound **4b** is considered as the best compound into the binding site of 2W9S with the highest GOLD docking score of 79.89. It can be seen from the 2D diagram from Discovery Studio Visualizer that compound **4b** with exhibited four conventional hydrogen bonds with Ile5 (1.84 Å), Asp27 (1.49 Å), Phe92 (2.99 Å). Another hydrogen bond at a distance of 2.10 Å with Gln95 and 4-OCH_3 stabilizes the ligand-enzyme complex (**Figure 3a**). By replacing methoxy group (**4b**) with chlorine (**4c**), antibacterial activity decreased. Interaction pattern of compound **4c** is shown in **Figure 3b**. It can be seen that chlorophenyl moiety is located in the cavity space surrounded by Gly15, Gln19, Lys45, Gln95 and Thr121. The computed Gold fitness score for **4c** is 70.81.

Visual inspection of the docked poses of compounds **5a-e** showed that these compounds are also well stabilized in the cavity. Compound **5b** is properly fitted into the binding site of 2W9S with the GOLD docking score of 70.04. It can be seen from the 2D diagram from Discovery Studio Visualizer that compound **5b** exhibited six conventional hydrogen bonds with Asp27 (3.11 Å), Thr46 (3.78 Å), Phe92 (2.34 Å), Gln95 (2.25 Å) and with Lys45 (2.65 Å). On the other hand compounds with chloro and nitro groups showed less affinity and Gold fitness score. The methyl substituted analogue (**5d**) showed four conventional hydrogen bonds. 4-NH_2 group forms HB interactions with Ile5 and Phe92 at the distance of 3.72 Å and 2.95 Å respectively. Carbonyl oxygen forms HB interactions with Tyr16 and Gln19 at the distance of 1.90 Å and 3.21 Å respectively (**Figure S-3** in **Supplementary data**).

In conclusion, by using rational design approach, we have designed and synthesized some novel trimethoprim derivatives. Two strategies were adopted to understand structural requirements for DHFR binding. In the first strategy, we designed compounds by incorporating substituents at 4-NH_2 of 2,4-diaminopyrimidine ring. In the second strategy, trimethoxy benzyl ring was used for the SAR study and some benzyloxy and phenylethanone derivatives were synthesized. SAR studies have shown that

benzyloxy derivatives leads to excellent in vitro antibacterial against *S. aureus* and *E. coli* strains. Among the derivatives synthesized, **4a-d** exhibited the most potent activity. Based on the mechanism of action of TMP, a comparative docking study on DHFR as target proved useful to explain to rationalize the *in vitro* biological data and mechanism of antibacterial activity. Therefore, it is concluded that docking investigations and *in vitro* activity revealed a successful drug design.

Acknowledgments

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General procedure for synthesis of compound 2. 20.0 g of trimethoprim (68.88 mmol) was dissolved in 40 % HBr at room temperature. The mixture was then stirred at 100 ºC. After completion of reaction (TLC), the mixture was quenched addition of 50 % NaOH aq. (40-50 mL). After cooling to room temperature, it was placed at 4 ºC overnight to form crystals. The crystals were filtered and washed with ice-cold water (ca. 100 mL). After the crystals were dissolved in boiling water, the solution was neutralized to ca. pH 7 with 28% aqueous ammonia and placed at 4 ºC overnight for recrystallization. White crystals were filtered, washed with ice-cold water (ca. 100 mL), affording compound **2**.

White crystals. Yield 70%. Rf: 0.47 (n-hexane/ethyl acetate 1:1). 1H NMR (300 MHz, DMSO-d6): δ 8.69 (brs, 1H, OH), 7.89 (brs, 2H, NH2), 7.68 (s, 1H, Ar-H), 7.44 (brs, 2H, NH2), 6.73 (s, 2H, Ar-H), 3.89 (s, 6H, O-CH3), 3.49 (s, 2H, CH2). 13C-NMR (75 MHz, DMSO- d6): δ 162.0, 161.1, 160.1, 152.3, 130.9, 129.8, 106.8, 105.1, 56.2, 37.3. LC-MS for $C_{13}H_{16}N_4O_3$ (m/z): Found 277.12 [M+H].

16. **General procedure for synthesis of Schiff base derivatives of hydroxyl trimethoprim (HTMP, 3a-h).** In a pyrex tube containing 5 ml ethanol, add appropriate aromatic aldehydes (4 mmol) and compound 2 (4 mmol). Then one drop of sulphuric acid as catalyst was added. The reaction mixture was then irradiated under ultrasonic bath at 40 ^{o}C for 3-4 hr. The reaction was monitored by TLC. After the completion of reaction, cool the reaction mixture and evaporated the solvent and the resulting crude product was recrystallized from ethanol.

17. **General procedure for synthesis of benzyloxy derivatives of HTMP (4a-e):** To a solution of hydroxyl TMP (HTMP, 2) (4 mmol) in acetonitrile (25 mL) in a Pyrex tube was added potassium carbonate (8 mmol) and substituted benzyl halides (4 mmol). The mixture was stirred at 40-45 ºC for 30-45 minutes in ultrasonic bath. Reaction mixture was then filtered, diluted with ethyl

acetate. Organic layer was washed with 3 x 10 ml of NaHCO3 and then with brine (20 ml). The crude residue after drying over anhydrous $MgSO_4$, purified with silica gel column chromatography to afford the corresponding benzyloxy derivatives.

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General procedure of the synthesis of α–Bromination of acetophenones (3-7): To a solution of hydroxyl TMP (HTMP, 2) (4 mmol) in acetonitrile (25 mL) in a Pyrex tube was added potassium carbonate (8 mmol) and substituted benzyl halides (4 mmol). The mixture was stirred at 40-45 ºC for 30-45 minutes in ultrasonic bath. Reaction mixture was then filtered, diluted with ethyl acetate. Organic layer was washed with 3 x 10 ml of NaHCO3 and then with brine (20 ml). The crude residue after drying over anhydrous $MgSO_4$, purified with silica gel column chromatography to afford the corresponding benzyloxy derivatives.

19. **General procedure for synthesis of phenyl ethanone derivatives of HTMP (5a-e):** To a solution of hydroxyl TMP (**HTMP, 2**) (4 mmol) in acetonitrile (25 mL) in a Pyrex tube was added potassium carbonate (8 mmol) and α–bromoketones (**3-7**, 4 mmol). The mixture was stirred at 40-45 ºC for 30-45 minutes in ultrasonic bath. Reaction mixture was then filtered, diluted with ethyl acetate. Organic layer was washed with 3 x 10 ml of NaHCO3 and then with brine (20 ml). The crude residue after drying over anhydrous MgSO4, purified with silica gel column chromatography to afford the corresponding phenyl ethanone derivatives.

20. **Antibacterial Screening**: Trimethoprim derivatives were tested for their antibacterial activity. Antibacterial assay was used to find the effectiveness of newly synthesized compounds for inhibition of bacterial growth. The microorganisms Escherichia coli and Staphylococcus aureus were used for this study. This antibacterial activity was checked by agar well diffusion method. The compounds having antibacterial activity were then subjected to determine Minimum Inhibitory Concentration (MIC). MIC is the concentration of test compound at which the minimum or least inhibitory zone was detected. MIC was checked at lower concentrations of active test compounds (0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512 µg/mL). All the tests were performed in triplicate. MIC was expressed in µM against each concentration (**Table 1**, shown in

parenthesis in μg/mL). The reported MIC value are an average of at least three individual measurements.

21. www.chemaxon.com

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23. **Computational studies:** Log P was predicted by ChemAxon software package (Marvin v15.4.6, 2015). Docking experiment was carried using GOLD docking program 5.4.1. GOLD uses the Genetic algorithm (GA). This method allows a partial flexibility of protein and full flexibility of ligand. X-ray structure of DHFR from S. aureus was retrieved from PDB (PDB ID 2W9S) with TMP as co-crystallized ligand. As a first step, the ability of the docking algorithm was validated to reproduce the co-crystallized pose of TMP in the 2W9S pocket. This yielded a good agreement between the docked and crystal structures as it further confirmed the interaction region of the ligand. Gold Score was selected as the criteria for the selection of compounds because they serve as fitness function for the orientation, and estimates of binding affinity. Binding site was defined as the residues within 6 Å from the ligand. No water was present in any binding site. The default docking protocol was applied (1.09 auto settings, 10 GA) and the best pose saved. Each experiment was then repeated 10 times. The view of the docking results and analysis of their surface with graphical representations were done using Discovery Studio Visualizer.

24. Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 4.5, San Diego: Dassault Systèmes, 2015

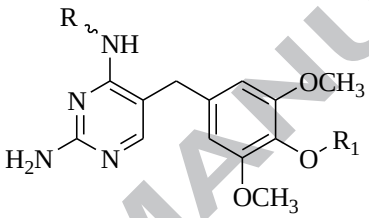
Figure Legends

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.

Figure 2: 2D interaction plot of: a) TMP (**1**) and HTMP (**2**) generated by Discovery Studio Visualizer showing interaction of with key amino acid residues. Conventional hydrogen bonding is shown by green color dotted lines.

Figure 3: 2D binding interaction plot of the most active compounds generated by Discovery Studio Visualizer s: a) Compound **4b;** b) Compound **4c**.

Table 1: MIC values (µM) of trimethoprim derivatives



Compounds	R	R[1]	MW	Log P	MIC (µM) (µg/ml)	
					S. aureus	*E. coli*
2	H	H	276.1	1.14	24.1 (6.6) [a]	48.2 (13.3)
3a	=CH-C_6H_5	H	364.1	3.84	NI	NI
3b	=CH-(4-OH-C_6H_4)	H	380.1	3.72	672 (256)	672 (256)
3c	=CH-(3-OH-C_6H_4)	H	380.1	3.72	672 (256)	336 (128)
3d	=CH-(3-NO_2-C_6H_4)	H	399.1	1.16	NI [b]	NI
3e	=CH-(4-CH_3-C_6H_4)	H	378.1	4.47	NI	NI
3f	=CH-(4-Cl-C_6H_4)	H	398.1	4.52	535.3 (213.3)	535.3 (213.3)
3g	=CH-(4-NMe_2-C_6H_4)	H	407.2	4.27	NI	NI
3h	=CH-(4-OCH_3-C_6H_4)	H	394.2	3.75	NI	NI
4a	H	-CH_2-C_6H_5	366.1	3.01	18.2 (6.6)	18.2 (6.6)
4b	H	-CH_2-(4-OCH_3-C_6H_4)	396.2	2.85	5 (2)	4.0 (1.6)
4c	H	-CH_2-(4-Cl-C_6H_4)	399.1	3.61	20 (8)	8.3 (3.3)
4d	H	-CH_2-(4-CH_3-C_6H_4)	380.2	3.52	10 (4)	5 (2)
4e	H	-CH_2-(4-NO_2-C_6H_4)	411.1	2.14	32.3 (13.3)	39 (16)
5a	H	-CH_2-CO-C_6H_5	394.1	2.52	81.2 (32)	81.2 (32)
5b	H	-CH_2-CO-(4-OCH_3-C_6H_4)	428.1	2.36	18.7 (8)	18.7 (8)
5c	H	-CH_2-CO-(4-Cl-C_6H_4)	408.2	3.12	78.4 (32)	78.4 (32)
5d	H	-CH_2-CO-(4-CH_3-C_6H_4)	424.2	3.03	37.7 (16)	15.5 (6.6)
5e	H	-CH_2-CO-(4-NO_2-C_6H_4)	439.1	3.04	72.9 (32)	72.9 (32)
Trimethoprim			**290.3**	**1.28**	**22.7 (6.6)**	**55.1 (16)**

[a] The reported MIC values in µg/ml (shown in parenthesis) are an average of at least three individual measurements.

[b] NI=No inhibition shown in the tested concentration domain

FIGURES

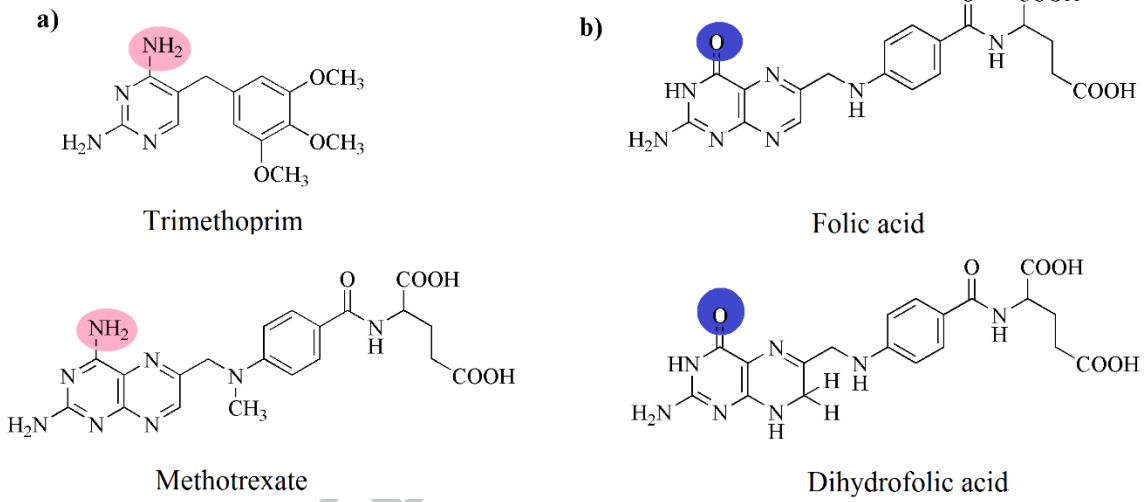


Figure 1: Comparison of the structures of the inhibitors and substrates of DHFR. Inhibitors differs from the folate substrates only in the replacement of 4-oxo group (blue circle) by 4-NH_2 group (pink circle). a) Structures of two inhibitors of DHFR; Trimethoprim and Methotrexate; b) Structures of the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

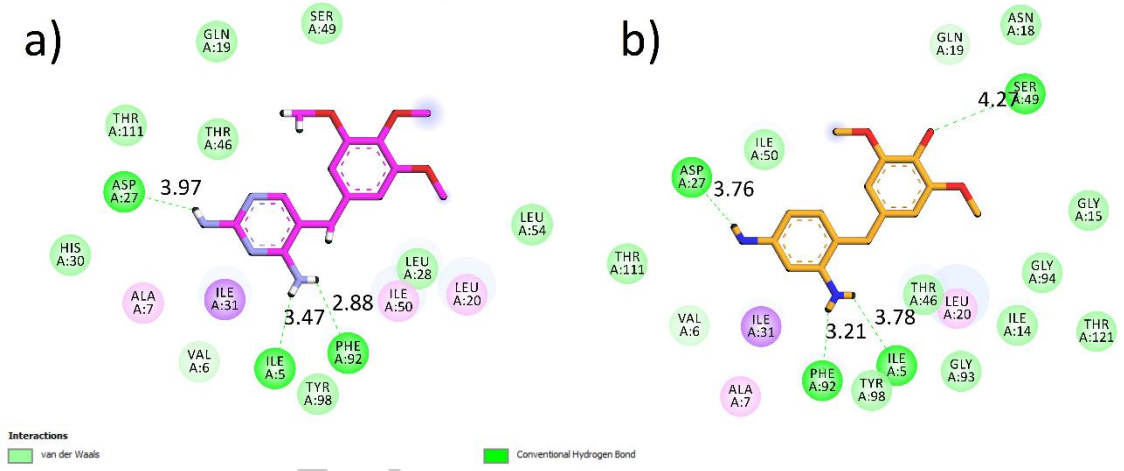


Figure 2: 2D interaction plot of: a) TMP (**1**) and HTMP (**2**) generated by Discovery Studio Visualizer showing interaction of with key amino acid residues. Conventional hydrogen bonding is shown by green color dotted lines.

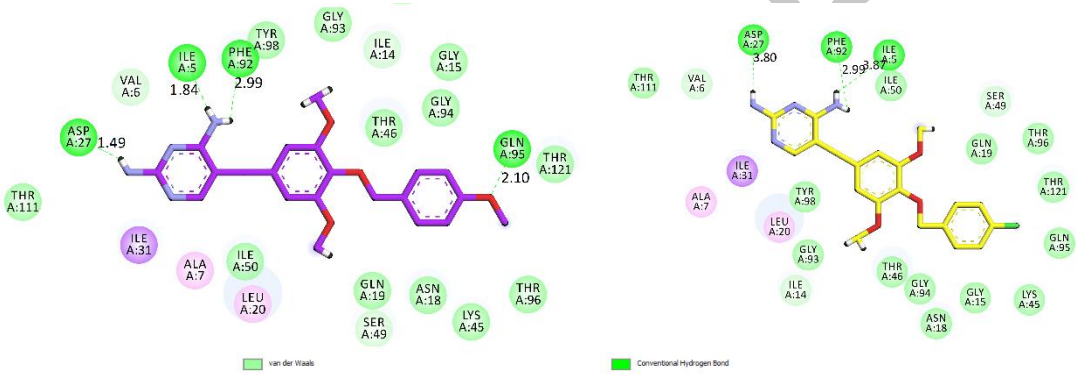
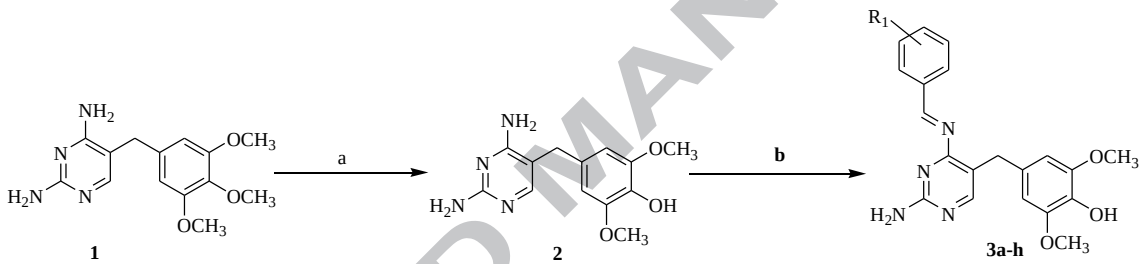
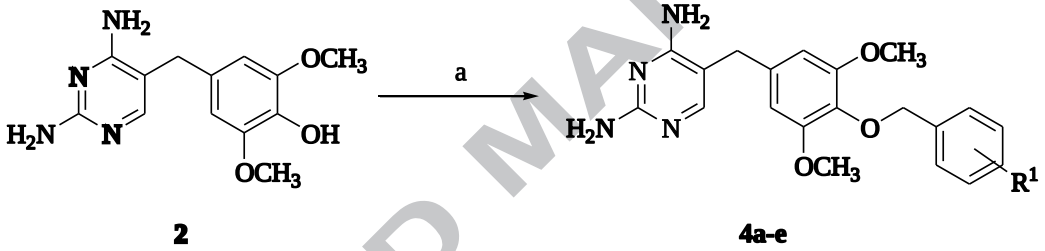


Figure 3: 2D binding interaction plot of the most active compounds generated by Discovery Studio Visualizer s: a) Compound **4b;** b) Compound **4c**.

Schemes

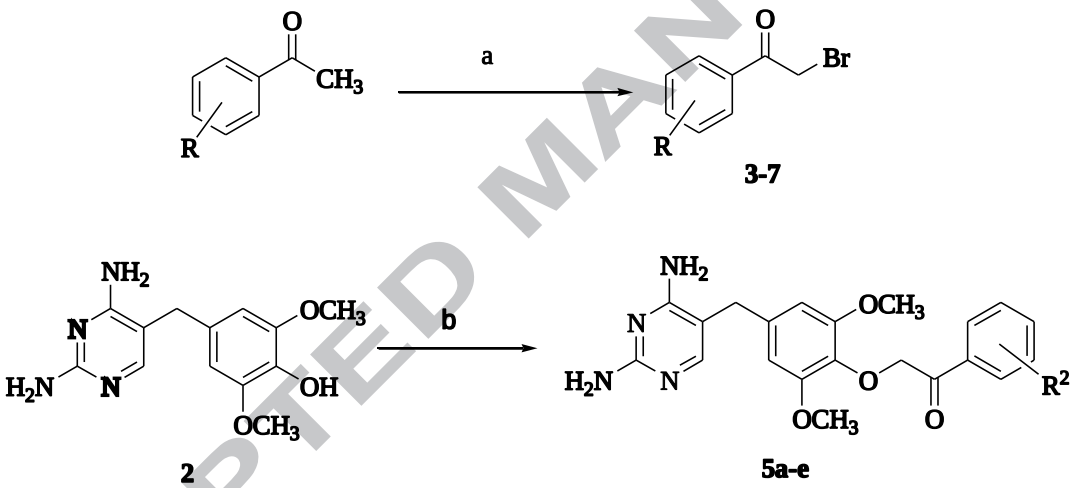


Scheme 1: Synthesis of Schiff bases **3a-h**. Reagents and conditions: a) 48% HBr b) Substituted aromatic aldehydes, ethanol, H_2SO_4, ultrasonic bath, 40 ^oC, 3-4 hr.



2 **4a-e**

Scheme 2: Synthesis of compounds **4a-e**. Reagents and conditions: a) Substituted benzyl bromides, K_2CO_3, ACN, ultrasonic bath, 40-45 ^{o}C, 30-45 min.



Scheme 3: Synthesis of compounds **5a-e**. Reagents and conditions: a) NBS, PTSA CH_2Cl_2, ultrasonic bath, 40-45 ^oC, 40 min; (b) α-bromoketones (**3-7**), K_2CO_3, ACN, ultrasonic bath, 40-45 ^oC, 30-45 min.

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

Umer Rashid*, Waqas Ahmad, Syed Fahad Hassan, Naveeda Akhtar Qureshi, Basit Niaz, Bakhtiar Muhammad, Sameera Imdad, Muhammad Sajid

*Corresponding author. E-mail: umerrashid@ciit.net.pk; umer_rashid39@hotmail.com

Corresponding Author Tel. +92 (992) 383591-6

Graphical Abstract