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4-Substituted Thieno[2,3-*d*]pyrimidines as Potent Antibacterial Agents: Rational Design, Microwave-Assisted Synthesis, Biological Evaluation and Molecular Docking Studies

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ABSTRACT

In an attempt to discover a new class of antibacterial agents with improved efficacy and to overcome the drug resistant problems, some novel 4-substituted thieno[2,3-*d*]pyrimidines have been synthesized *via* microwave assisted methodology and evaluated for their *in vitro* antibacterial activity against various pathogenic bacterial strains. Compounds **12*b*** and **13*c*** showed the promising inhibitory potencies against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli* with MICs ranging from 2 to 10 µg/ml. Compound **13*c*** was also found to be highly potent against methicillin resistant *S. aureus* (MRSA) with MIC value of 4 µg/ml. Docking simulation studies have been performed to unravel the mode of action and association study indicate the binding of

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potent compounds with DHPS enzyme. I*n silico* ADME studies suggest the drug like characteristics of the potent compounds.

KEYWORDS: Thieno[2,3-*d*]pyrimidines, Antibacterial activity, Molecular Docking studies, ADME.

A large number of pyrimidine derived drugs such as trimethoprim, piromidic acid, tetroxoprim and metioprim (1) are clinically used for antibacterial therapy. The fused pyrimidines such as thieno[2,3-*d*]pyrimidines have been attracted considerable attention due to their structural resemblance with biogenic pyrimidines and possess vast therapeutic properties such as anti-inflammatory (2), antiviral (3), analgesic (4), herbicidal (5), anti-proliferative (6), and antimicrobial (3, 7-8) activities.

Sulfonamides, which are widely prescribed antimicrobial agents, inhibits bacterial growth by dihydropteroate synthase (DHPS) inhibition (9), therefore, DHPS has been considered as a selective and promising target for developing antimicrobial agents. The widespread microbial resistance and severe side effects of sulphonamides, limit their use (9-10) clinically, which triggers an urgent need to develop new approaches that can overcome the problems of microbial resistance. In this context, Yun *et al.* reported that sulfathiazole-6-hydroxymethyl-7,8-dihydropterin-pyrophosphate (STZ-DHPP) adduct, which accommodates both *p*-aminobenzoic acid (pABA) and pterin binding pockets of DHPS (Figure 1), bound to Bacillus anthracis dihydropteroate synthase (BaDHPS) (11). Thus, bacterial resistance of sulphonamides may be prevented by synthesizing such analogues that will target both sites of DHPS enzyme. Inspired by this development, Nasr et al. reported some thiophene, pyrazole and pyridine comprised sulfisoxazole moiety as DHPS inhibitors by targeting both pABA and pterin binding pockets of the DHPS (12). Further, they also reported some 2,3-dihydrothiazoles and 4-thiazolidinones containing sulfisoxazoles for the same purpose (Figure 1) (13).

Inspired from the aforementioned facts to target DHPS enzyme by binding both pABA and pterin binding pockets, and taking cognizance of antimicrobial potential of pyrimidine analogs; it was decided to resynthesize 4-substituted thieno[2,3-*d*]pyrimidine analogues (**9-13**) (14) along with some novel compounds (**13*e*,*f***) and evaluated for antibacterial activity (Figure 1). Further, molecular docking study has been performed to explore their binding mode of action. Recently, we have reported the synthesis and biological activity of the similar compounds for antiproliferative activity (14).

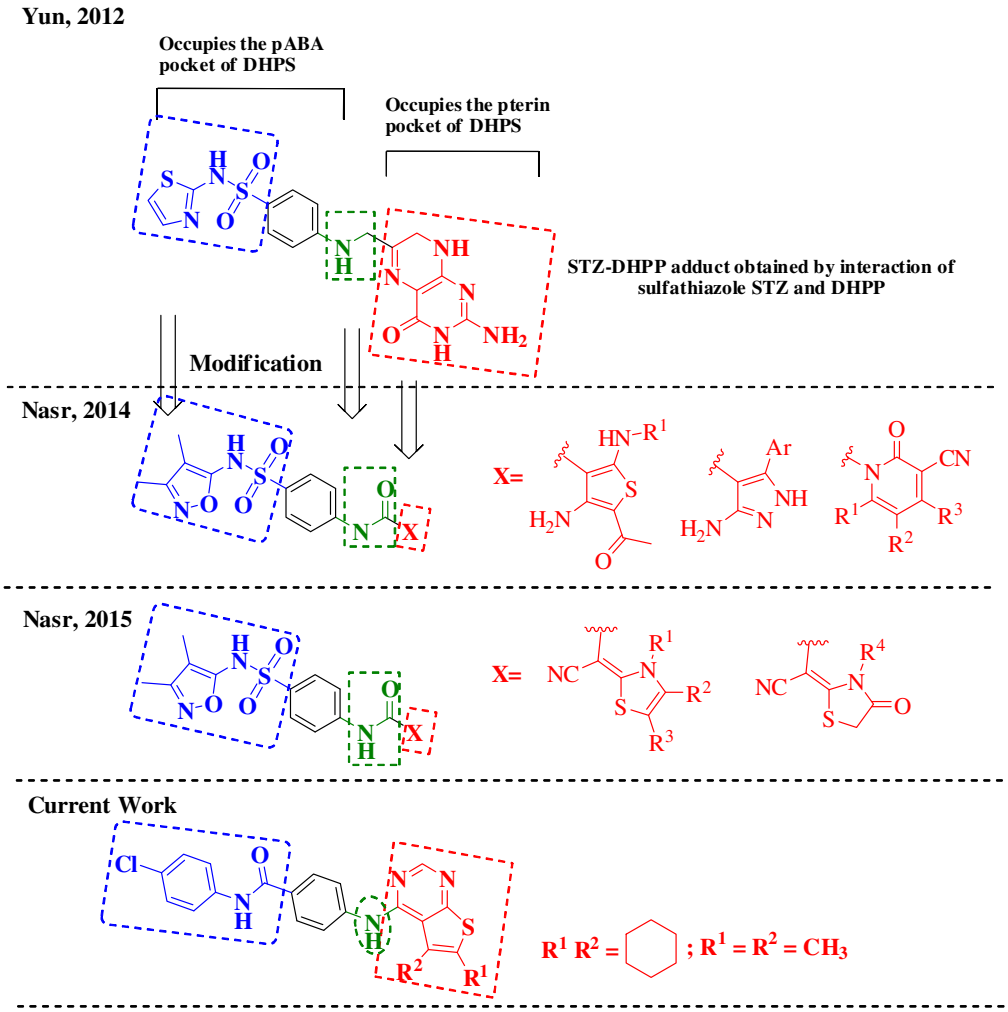


Figure 1. Design of antibacterial DHPS inhibitors.

Experimental

Chemistry

General Procedure for N-(4-substituted phenyl)-4-(thieno[2,3-d]pyrimidin-4-ylamino)benzamide (**13*a-d***)

To a microwave reaction vessel equipped with magnetic stirrer, a solution of appropriate 4-(thieno[2,3-*d*]pyrimidin-4-*yl*-amino)benzoic acid (**12*a*,*b***) (14) and DCC (1.2 equiv) in DMF was added and stirred vigorously for 15 min at 0 ^{o}C. Then substituted aniline (1.2 equiv, **5*a*,*e***) was added in microwave vessel and irradiated in microwave reactor at a ceiling temperature of 110 ^{o}C and maximum power of 80 W for 15-18 min. After completion of reaction, the reaction mixture was poured on ice water and extracted with ethyl acetate. The organic layer separated was concentrated under reduced pressure to obtain the desired product (**13*a*,*b***).

N-(4-chlorophenyl)-4-(5,6,7,8-tetrahydrobenzothieno[2,3-d]pyrimidin-4-ylamino)benzamide (**13*a***)

Yield 86%; mp 158-162 ^{o}C; IR KBr, cm-1: 3460 (γCONH), 3314 (γNH), 2928 (γCH), 1647 (γCO); ^{1}H NMR (400 MHz, DMSO-d_6): 10.32 (s, 1H, CONH), 8.48 (s, 1H, NH), 8.45 (s, 1H, C2-H), 8.05 (d, 2H, *J* = 7.8 Hz, Ar-Hs), 7.98 (d, 2H, *J* = 7.8 Hz, Ar-Hs), 7.84 (d, 2H, *J* = 8.6 Hz, 2H, Ar-Hs), 7.74 (d, 2H, *J* = 8.6 Hz, Ar-Hs), 3.16 (dist. s, 2H, C5-H), 2.85 (dist. s, 2H, C6 & C7-Hs), 1.88-1.84 (m, 4H, C8-Hs); ^{13}C NMR (125 MHz, DMSO-d_6): 165.89, 154.54, 151.85, 148.66, 145.57, 141.21, 135.67, 130.09, 127.90 (X2), 126.52 (X2), 124.56, 123.29 (X2), 120.25 (X2), 117.01, 116.40, 25.57, 24.90, 22.52, 22.36. HRMS (microTOF-QII, MS, ESI): *m/z* $[M+Na]^+$ Calculated for $C_{23}H_{19}ClN_4OS$: 457.9412, Obsd 457.9415.

The compounds (**9-13**) were synthesized following the general procedure as described above and the characterization data of other compounds has been provided in the SI.

Biological Evaluation

All the synthesized compounds were screened for their antibacterial activity against various bacterial strains, namely *S. aureus* (MTCC-740), *B. subtilis* (MTCC-441), *P. aeruginosa* (MTCC- 741), *E. coli* (MTCC-119) and MRSA (MTCC-1430). The MICs of synthesized compounds were determined by broth microdilution method [15, SI] .

Molecular Docking Study

Docking studies have been performed with the various module of Schrodinger Suite 2013.

Fluorescence Spectroscopy

Steady-state fluorescence was performed using a PerkinElmer Luminescence spectrometer LS-55 using pyrene as an external fluorescent probe (2×10^{-6} M) at an excitation wavelength of 300 nm at 298.15 K. The temperature was controlled using built-in temperature controller within ±0.1 K by using circulating water bath. The spectra were recorded between 311 and 320 nm using an excitation and emission slit width of 2.5 nm, each.

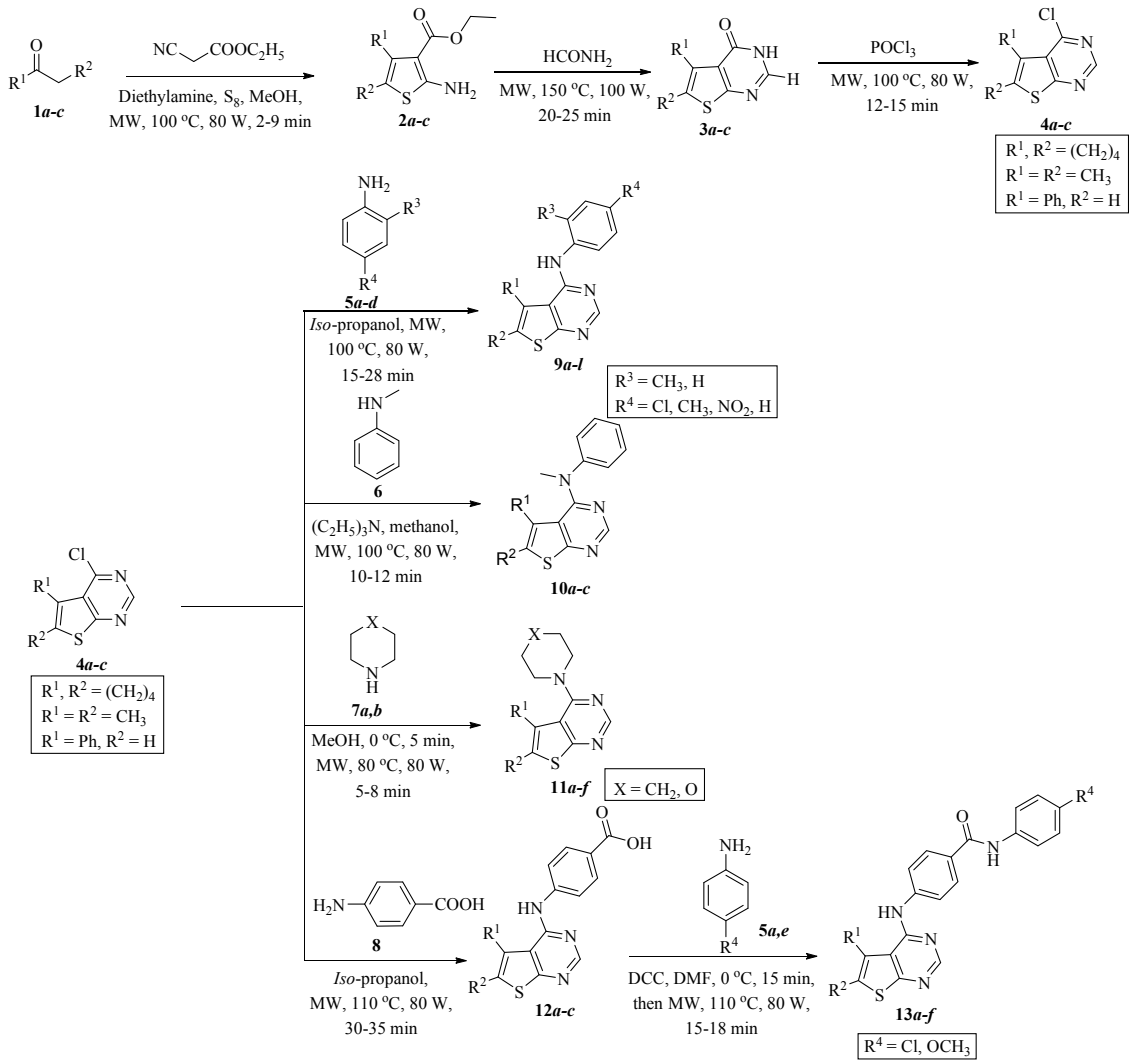
Results and Discussion

Chemistry

The synthetic strategy adopted for the synthesis of substituted thieno[2,3-*d*]pyrimidines is outlined in scheme 1. The substituted 4-anilino thieno[2,3-*d*]pyrimidines (**9*a-l***, **12*a-c***) were synthesized by employing our previously reported methods *via* microwave irradiation (Scheme1) (14). Initially, 2-amino-3-carbethoxy thiophenes **2*a-c*** have been prepared using Gewald reaction followed by cyclization with formamide and chlorination of the resultant intermediate **3*a-c*** with phosphorus oxychloride yielded **4*a-c***. Then, the nucleophilic displacement of chloride atom at 4th position of **4*a-c*** with appropriate amines (**5-8**) afforded

the desired 4-anilino thieno[2,3-*d*]pyrimidines **9*a-l***, **10*a-c***, **11*a-f*** and **12*a-c***, respectively, in excellent yields (Table 1).

Finally, the synthesis of substituted amide analogues **13*a-f*** was achieved in excellent yield by reacting substituted benzoate **12*a-c*** with 4-chloroaniline **5*a*,*e*** in presence of *N*,*N*-dicyclohexylcarbodiimide (DCC) under microwave-irradiation conditions.



Scheme 1. Synthesis of 4-substituted thieno[2,3-*d*]pyrimidines (**9-13**).

Antibacterial Activity

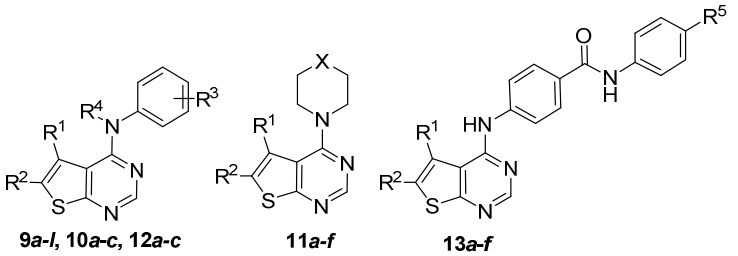
All the synthesized compounds were evaluated for antibacterial activity against Gram +ve, *S. aureus* (MTCC-740) and *B. subtilis* (MTCC-441); and Gram -ve, *P. aeruginosa* (MTCC-741) and *E. coli* (MTCC-119) strains using ciprofloxacin and sulfathiazole as standards (15). From the results of antibacterial study, it was depicted that the most sensitive strain was *S.*

aureus followed by *B. subtilis*, while, both *P. aeruginosa* and *E. coli* were least sensitive towards the evaluated molecules (Table 1).

On observing the antibacterial activity of 4-substituted anilino thieno[2,3-*d*]pyrimidines **9*a-l***, it was found that compounds **9*e***, **9*f***, **9*g***, **9*h*** and **9*l*** showed moderate inhibitory potential against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli*. Among the various substituents present at C-4 position of aniline group in 4-anilino-thieno[2,3-*d*]pyrimidines, methyl group derivative **9*f*** possesses significant activity, specifically against *S. aureus* and *B. subtilis*, while, on shifting methyl substitution from 4^{th} to 2^{nd} position as in **9*h***, further improvement of the activity was observed. On the other hand, replacement of the anilino scaffold with azacyclic ring (morpholine or piperidine) in **11*a-f***, significantly improved the antibacterial activity against all pathogenic strains, with the only exception of compound **11*a***. Further, it was apparent that 4-carboxyanilino substituted derivatives **12*a-c*** displayed pronounced effect against *S. aureus* and *B. subtilis*, while, compound **12*b*** and **12*c*** also showed significant activity against *P. aeruginosa* and *E. coli*. The increased activity of **12*b*** compared to **12*c*** and **12*a*** suggests the importance of 5,6-dimethyl substitution on thieno[2,3-*d*]pyrimidine than cyclopentane fused tricyclic derivative. Benzamide derivatives **13*a-f*** exhibited significant antibacterial activity. Particularly, the *N*-4-chlorophenyl benzamide derivatives **13*a***, **13*c*** and **13*e*** exhibited better activity than *N*-4-methoxyphenyl benzamide derivatives **13*b***, **13*d*** and **13*f***. The *N*-4-chlorophenyl benzamide derivatives **13*a***, **13*c*** and **13*e*** showed remarkable inhibitory potential against *S. aureus* (MIC 12, 2 and 8 µg/ml, respectively), *B. subtilis* (MIC 12, 6 and 10 µg/ml, respectively), *P. aeruginosa* (MIC 6, 6 and 10 µg/ml) and *E. coli* (MIC 16, 10 and 12 µg/ml, respectively), which is also superior to the standard sulfathiazole and comparable to the ciprofloxacin against *S. aureus*.

These results clearly indicated that compounds bearing benzamide **13*a-f*** or 4-carboxyanilino **12*a-c*** functionality displayed excellent inhibitory potential and the 5,6-dimethyl substituted thieno[2,3-*d*]pyrimidines **9*e-h***, **12*b***, **13*c*** and **13*d*** possess higher antibacterial activity followed by 5-phenyl substituted derivatives **9*i-l***, **12*c***, **13*e*** and **13*f*** and then the cyclopentane fused tricyclic derivatives **9*a-d***, **12*a***, **13*a*** and **13*b***.

Table 1. Yield %, reaction time, *in vitro* antibacterial activities and G-score of the synthesized compounds (**9-13**).



Code	R^1	R^2	R^3	X	R^4	R^5	Yield (%)	Time (min)	MIC (µg/ml)[a]				*In-silico Analysis*
									S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*	G-Score[b]
9*a*	$(CH_2)_4$		4-Cl	-	H	-	85	15	76	80	68	66	-4.25
9*b*	$(CH_2)_4$		4-CH_3	-	H	-	92	15	70	78	70	76	-3.84
9*c*	$(CH_2)_4$		4-NO_2	-	H	-	80	15	>100	>100	>100	**8**	-3.20
9*d*	$(CH_2)_4$		2-CH_3	-	H	-	62	20	78	86	80	92	-3.89
9*e*	CH_3	CH_3	4-Cl	-	H	-	85	24	**16**	**20**	30	68	-3.64
9*f*	CH_3	CH_3	4-CH_3	-	H	-	87	24	**14**	**18**	>100	>100	-3.99
9*g*	CH_3	CH_3	4-NO_2	-	H	-	79	24	**20**	**18**	**12**	70	-3.03
9*h*	CH_3	CH_3	2-CH_3	**-**	H	-	57	28	**4**	**12**	>100	>100	-6.02
9*i*	Ph	H	4-Cl	-	H	-	73	15	26	28	54	68	-4.14
9*j*	Ph	H	4-CH_3	-	H	-	75	18	24	20	>100	>100	-3.70
9*k*	Ph	H	4-NO_2	-	H	-	66	18	28	34	>100	74	-3.13
9*l*	Ph	H	2-CH_3	**-**	H	-	55	20	**18**	**16**	80	>100	-4.05
10*a*	$(CH_2)_4$		H	**-**	CH_3	-	68	10	88	>100	74	>100	-4.34
10*b*	CH_3	CH_3	H	-	CH_3	-	62	12	84	>100	78	>100	-3.77
10*c*	Ph	H	H	-	CH_3	-	65	10	76	88	>100	>100	-3.81
11*a*	$(CH_2)_4$		-	CH_2	-	-	64	07	72	76	>100	>100	-4.65
11*b*	$(CH_2)_4$		-	O	-	-	61	5	22	**20**	>100	64	-6.06
11*c*	CH_3	CH_3	**-**	CH_2	**-**	-	56	08	**6**	**14**	40	**8**	-5.80
11*d*	CH_3	CH_3	-	O	-	-	53	07	**8**	**14**	88	40	-4.93
11*e*	Ph	H	**-**	CH_2	**-**	-	65	07	**12**	**18**	36	**16**	-5.74
11*f*	Ph	H	-	O	-	-	66	05	**10**	**12**	84	48	-5.41
12*a*	$(CH_2)_4$		4-COOH	-	H	-	60	30	**6**	**12**	>100	>100	-4.86
12*b*	CH_3	CH_3	4-COOH	-	H	-	58	35	**4**	**8**	**10**	**6**	-4.7
12*c*	Ph	H	4-COOH	-	H	-	54	32	**6**	**10**	**18**	**18**	-4.74
13*a*	$(CH_2)_4$		-	-	-	Cl	86	15	**12**	**12**	**6**	16	-4.47
13*b*	$(CH_2)_4$		-	-	-	OCH_3	92	15	**16**	**14**	36	28	-4.26
13*c*	CH_3	CH_3	**-**	**-**	**-**	Cl	84	18	**2**	**6**	**6**	**10**	**-6.11**
13*d*	CH_3	CH_3	**-**	**-**	**-**	OCH_3	86	18	**8**	**14**	24	32	-4.32
13*e*	Ph	H	**-**	**-**	**-**	Cl	75	15	**8**	**10**	**10**	**12**	-4.64
13*f*	Ph	H	**-**	**-**	**-**	OCH_3	78	15	**16**	**20**	28	34	-4.20
Ciprofloxacin									2	1.6	0.6	1.2	-4.61
Sulfathiazole									64	32	32	16	-3.77

[a] MIC values determination was performed in triplicate.
[b] calculated theoretically using Glide 5.9.

The active compounds were further evaluated against methicillin resistant strain of *S. aureus* (MRSA) to determine whether they were also susceptible to resistant strain. It was noteworthy that all the tested compounds were found to show significant effect against MRSA (Table 2).

Table 2. MIC (µg/ml) value of compounds against methicillin resistant *S. aureus* (MRSA).

Compd.	**11*c***	**11*d***	**12*a***	**12*b***	**12*c***	**13*a***	**13*c***	**13*d***	**13*e***	**13*f***	**Ciprofloxacin (17)**
MRSA	10	12	10	6	14	10	4	8	12	20	4

Molecular Docking Studies

Considering the potent activity of synthesized compounds against various bacterial strains, molecular docking studies were performed to investigate whether the synthesized compounds fit well in the active site of *Bacillus anthracis* dihydropteroate synthase (BaDHPS, PDB ID: 3TYE, Figure 2) (11). The reliability of proposed docking algorithm was validated by redocking of the crystallographic ligand, STZ-DHPP adduct, in the BaDHPS crystal structure and the top ranked pose showed heavy-atom root-mean-square deviation (RMSD) value of 0.84 Å (Figure S1).

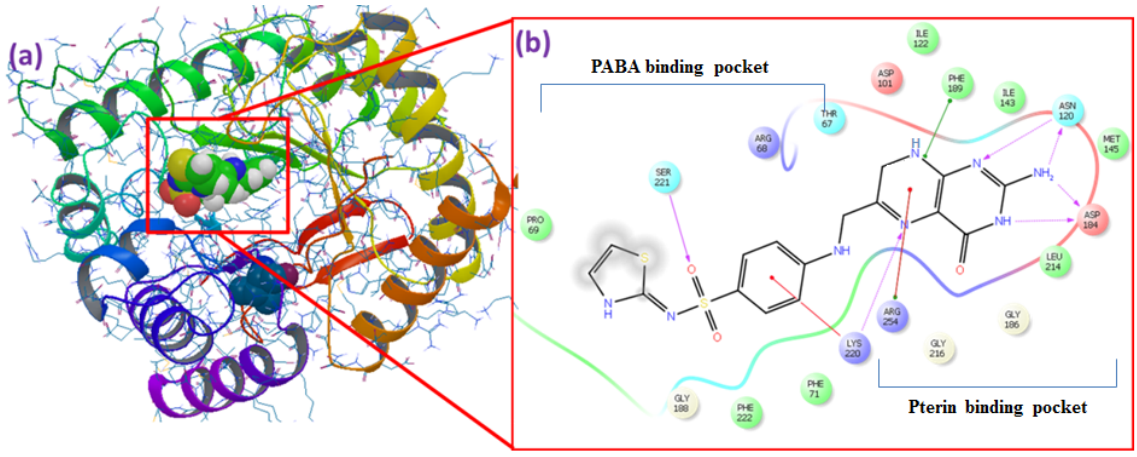


Figure 2. (a) The crystal structure *of Bacillus anthracis* dihydropteroate synthase with its grid by receptor grid generation. **(b)** The hypothetical binding pose of STZ-DHPP adduct (STZ: sulfathiazole, DHPP: 6-hydroxymethyl-7,8-dihydropterin-pyrophosphate) showing the active amino acids around it after extraction.

The affinities of all the synthesized compounds were evaluated in terms of G-score (Glide score), which is in the range from -6.11 to -3.03 (Table 1). The docking of BaDHPS with compound **13*c*** revealed that its dock score (-6.11) was quite comparable to ciprofloxacin (-4.61) and sulfathiazole (-3.77). The hydrophilic and hydrophobic maps in Figure 3a represent the important interactions of compound **13*c*** and it was observed that it targets both

pterin and pABA binding pockets of BaDHPS. Moreover, it was disclosed that compound **13*c*** occupies the same position in pABA binding pocket as observed in STZ-DHPP-BaDHPS complex i.e. carbonyl and NH of amide group forms hydrogen bond with Ser221 (3.39Å) and Gly188 (2.22Å), respectively (Figure S2). Additionally, thienopyrimidine ring occupies the pterin binding pocket by forming hydrogen bond between terminal nitrogen and Lys220 with distance of 3.50Å and thiophene forms co-ordination bond to positively charged nitrogen of histidine with distance of 3.21Å. 2D-picture clearly depicts the surrounding amino acids within the cavity of the enzyme (Figure 3b). Therefore, docking results suggest that the 4-substituted thieno[2,3-*d*]pyrimidines, particularly compound **13*c*** may act as inhibitors of bacterial dihydropteroate synthase.

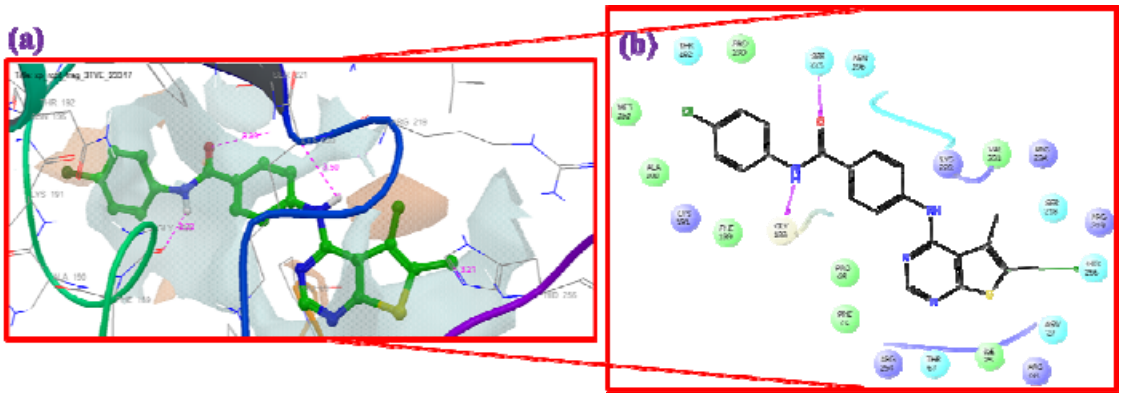


Figure 3. (a) The postulated binding mode of the most potent compound **13*c*** within the cavity showing the hydrophilic and hydrophobic maps of the crystal structure of the receptor. **(b)** The binding interactions of the same compound with surrounding amino acids within the cavity of the gorge.

Association constant using fluorescence spectroscopy

To further investigate the binding of potent compounds **12*b*** and **13*c*** with DHPS enzyme, association constant or binding constant (Ka) of potent compounds **12*b*** and **13*c*** with DHPS enzyme was determined using sensitive fluorescence spectroscopic technique (Figure S3-S5) (18-22). The results of investigations revealed that both compounds **12*b*** and **13*c*** showed appreciable K_a = 13.08 and 19.71 x 10^3 M^{-1} (Table S1), respectively, which, indicates that **13*c***-[DHPS] binding is stronger than **12*b***-[DHPS].

Physicochemical and ADME properties

It was evident that most of the potential drug candidates usually fail to reach the clinic due to their unfavourable ADME (Adsorption, Distribution, Metabolism, Excretion and Toxicity) profile; thus, initial evaluation of ADME parameters is a best way to avoid this problem. For this purpose, computational study of the synthesized compounds was performed by using Qikprop v3.6 tool of Schrodinger software (Table S2 and 3) (23). Absorption (% ABS) was

calculated by: % ABS = 109- (0.345 X TPSA) (24). It was observed that the potent compounds showed good % absorption in a range of 79.34–84.31% (Table 3). Further, Lipinski's rule of 5 states that for a molecule to develop as active drug candidate, it should possess no more than one violation of the criteria's given in Table S1 and 3 (25). The result of ADME study revealed that none of the compounds violated the Lipinski's rule of five, which indicates that 4-substituted thieno[2,3-*d*]pyrimidines may be utilized to develop drug-like antibacterial candidates.

Table 3. In silico predicted physicochemical pharmacokinetic properties.

Entry	% ABS	n-ON acceptors	n-OHNH donors	ROF	TPSA ($Å^2$)	QP logP	QP logS	QP logK	QP logBB	MW
Rule	-	<10	<5	≤1	≤140	≤5	≤5	≤5	≤5	<500
12*b*	79.45	4	2	0	85.65	2.70	-3.91	-0.09	-0.98	299.34
13*c*	84.31	5	2	0	71.55	4.72	-6.71	0.70	-0.49	408.90

% ABS: percentage absorption, n-ON acceptors: number of H-bond acceptors; n-OHNH donors: number of H-bond donors; ROF: Rule of Five: number of violations; TPSA: topological polar surface area; QP logP: predicted octanol/water partition coefficient; QP logS: predicted aqueous solubility; QP logK: prediction of binding to human serum albumin; QP logBB: prediction of Blood/Brain partition coefficient; MW: molecular weight.

Conclusion

Variedly substituted 4-substituted thieno[2,3-*d*]pyrimidines were synthesized *via* microwave assisted methodology and evaluated for *in vitro* antibacterial activity. Compounds **13*c*** and **12*b*** were found to exhibit the excellent inhibitory potential against various susceptible and resistant strains; this might be attributed to the presence of *N*-4-chlorophenyl benzamide and benzoate moiety, respectively, at 4^{th} position of anilino thienopyrimidine. Molecular docking studies showed that thienopyrimidines accommodate both pABA and pterin binding pocket of DHPS, in the same manner as reported for STZ-DHPP adduct, suggesting that they may act as DHPS inhibitors. Further, binding study using fluorescence spectroscopy revealed that both the potent compounds **13*c*** and **12*b*** showed appreciable association with DHPS enzyme. *In silico* ADME analysis of synthesized compounds indicated that the thienopyrimidine derivatives have prospective to develop as drug candidate. These studies suggests that the potent compounds such as **13*c*** and **12*b*** may serve as potent "lead" for the drug design and drug development as dihydropteroate synthase inhibitor.

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Conflict of interest

The authors declare they have no conflict of interests.

Figure Legends

Figure 1. Design of antibacterial DHPS inhibitors.

Figure 2. (a) The crystal structure *of Bacillus anthracis* dihydropteroate synthase with its grid by receptor grid generation. **(b)** The hypothetical binding pose of STZ-DHPP adduct (STZ: sulfathiazole, DHPP: 6-hydroxymethyl-7,8-dihydropterin-pyrophosphate) showing the active amino acids around it after extraction.

Figure 3. (a) The postulated binding mode of the most potent compound **13*c*** within the cavity showing the hydrophilic and hydrophobic maps of the crystal structure of the receptor. **(b)** The binding interactions of the same compound with surrounding amino acids within the cavity of the gorge.

Scheme 1. Synthesis of 4-substituted thieno[2,3-*d*]pyrimidines (**9-13**).

References

1. Selvam T.P., James C.R., Dniandev P.V., Valzita S.K. (2012) A mini review of pyrimidine and fused pyrimidine marketed drugs. Res Pharm; 2(4):01-09.
2. Modica M., Santagati M., Santagati A., Cutuli V., Mangano N., Caruso A. (2000) Synthesis of new [1, 3, 4] thiadiazolo [3, 2-*a*] thieno [2, 3-*d*] pyrimidinone derivatives with antiinflammatory activity. Die Pharmazie; 55(7):500-502.
3. Hafez H.N., Hussein H.A., El-Gazzar A.R. (2010) Synthesis of substituted thieno [2, 3-*d*] pyrimidine-2, 4-dithiones and their *S*-glycoside analogues as potential antiviral and antibacterial agents. Eur J Med Chem; 45(9):4026-4034.
4. Santagati N., Caruso A., Cutuli V., Caccamo F. (1995) Synthesis and pharmacological evaluation of thieno [2, 3-*d*] pyrimidin-2, 4-dione and 5H-pyrimido [5, 4-*b*] indol-2, 4-dione derivatives. Farmaco (Societa chimica italiana: 1989); 50(10):689-695.
5. Kawaguchi S., Kumata S., Ota C. (2003) Novel herbicides, usage thereof, novel thienopyrimidine derivatives, intermediates of the same, and process for production thereof. Google Patents: EP1544202A1.
6. Meyer M.D., Altenbach R.J., Basha F.Z., Carroll W.A., Condon S., Elmore S.W., Kerwin J.F., Sippy K.B., Tietje K., Wendt M.D. (2000) Structure-activity studies for a novel series of tricyclic substituted hexahydrobenz[*e*]isoindole α1A adrenoceptor

antagonists as potential agents for the symptomatic treatment of benign prostatic hyperplasia (BPH). J Med Chem; 43(8):1586-1603.

7. Aly H.M., Saleh N.M., Elhady H.A. (2011) Design and synthesis of some new thiophene, thienopyrimidine and thienothiadiazine derivatives of antipyrine as potential antimicrobial agents. Eur J Med Chem; 46(9):4566-4572.
8. Kanawade S.B., Toche R.B., Rajani D.P. (2013) Synthetic tactics of new class of 4-aminothieno [2, 3-*d*] pyrimidine-6-carbonitrile derivatives acting as antimicrobial agents. Eur J Med Chem; 64:314-320.
9. Argyropoulou I., Geronikaki A., Vicini P., Zanib F. (2009) Synthesis and biological evaluation of sulfonamide thiazole and benzothiazole derivatives as antimicrobial agents. Arkivoc; 6:89-112.
10. Anand N. (1996) In Burger's Medicinal Chemistry and Drug Discovery, Vol. 2, Therapeutic Agents, Wolff, M. E. Ed. (Wiley-Interscience, New York, ed. 5), p. 527.
11. Yun M., Wu Y., Li Z., Zhao Y., Waddell M.B., Ferreira A.M., Lee R.E., Bashford D., White S.W. (2012) Catalysis and Sulfa Drug Resistance in Dihydropteroate Synthase. Science; 335:1110-1114.
12. Nasr T., Bondock S., Eid S. (2014) Design, synthesis, antimicrobial evaluation and molecular docking studies of some new thiophene, pyrazole and pyridone derivatives bearing sulfisoxazole moiety. Eur J Med Chem; 84:491-504.
13. Nasr T., Bondock S., Eid S. (2015) Design, synthesis, antimicrobial evaluation and molecular docking studies of some new 2,3-dihydrothiazoles and 4-thiazolidinones containing sulfisoxazole. J Enzyme Inhib Med Chem; DOI: 10.3109/14756366.2015.1016514.
14. Gill R.K., Kumar V., Bishnoi M., Yadav K., Kondepudi K.K., Bariwal J. (2016) Design and Green Synthesis of Thieno [2,3-*d*] pyrimidine Analogues as Potential Antiproliferative Agents. Anti-Cancer Agents Med Chem; 16 (DOI: 10.2174/1871520616666160817142647)
15. Fromtling R., Galgiani J., Pfaller M., Espinel-Ingroff A., Bartizal K., Bartlett M., Body B., Frey C., Hall G., Roberts G. (1993) Multicenter evaluation of a broth macrodilution antifungal susceptibility test for yeasts. Antimicrob Agents Chemother; 37(1):39-45.
16. LigPrep version 2.6, Schrödinger, LLC, New York, **2013**.
17. Shalit T., Berger S.A., Gorea A., Frimerman H. (1989) Widespread Quinolone Resistance among Methicillin-Resistant *Staphylococcus aureus* Isolates in a General Hospital. Antimicrob Agents Chemother; 33:593-594.
18. Zhao Y., Hammoudeh D., Lin W., Das S., Yun M-K., Li Z., Griffith E., Chen T., White S. W., Lee R.E. (2011) Development of a Pterin-Based Fluorescent Probe for Screening Dihydropteroate Synthase. Bioconjugate Chem; 22: 2110-2117.
19. Levy C., Minnis D., Derrick J.P. (2008) Dihydropteroate synthase from *Streptococcus pneumoniae*: structure, ligand recognition and mechanism of sulfonamide resistance. Biochem J; 412:379-388.
20. Liu S.Q., Xu J.J., Chen H.Y. (2004) A reversible adsorption–desorption interface of DNA based on nano-sized zirconia and its application. Colloids Surf B; 36:155–159.

21. Kaur G., Raj T., Kaur N., Singh N. (2015) Pyrimidine-based functional fluorescent organic nanoparticle probe for detection of Pseudomonas aeruginosa. Org Biomol Chem; 13:4673-4679.
22. Vashishat R., Chabba S., Mahajan R.K. (2017) Surface active ionic liquid induced conformational transition in aqueous medium of hemoglobin. RSC Adv; 7:13041-13052.
23. QikProp, version 3.6, (2013), Schrödinger, LLC, New York.
24. Zhao Y., Abraham M.H., Lee J., Hersey A., Luscombe N.C., Beck G., Sherborne B., Cooper I. (2002) Rate-Limited Steps of Human Oral Absorption and QSAR Studies. Pharm Res; 19:1446.
25. Lipinski C.A., Lombardo L., Dominy B.W., Feeney P.J. (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev; 64:4-17.