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C–N bond formation under Cu-catalysis: Synthesis and in vitro evaluation of *N*-aryl substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones against chorismate mutase

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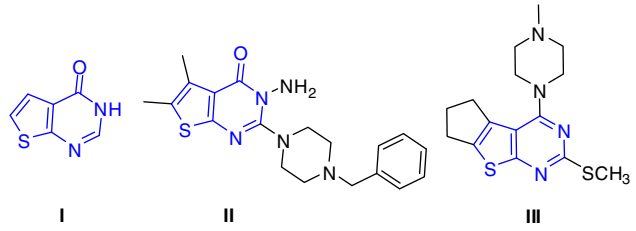
ABSTRACT

A series of novel *N*-aryl substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones were designed and synthesized as potential inhibitors of chorismate mutase. Synthesis of this class of compounds was carried out by using Cu-mediated C–N bond forming reaction between thieno[2,3-*d*]pyrimidin-4(3*H*)-ones and aryl boronic acids. The reaction can be performed in an open flask as the conversion was found to be not sensitive to the presence of air or atmospheric moisture. A range of compounds were prepared by using this method and single crystal X-ray diffraction study was performed using a representative compound. In vitro pharmacological data of some of the compounds synthesized along with dose response studies using active molecules are presented. In silico interactions of these molecules with chorismate mutase are also presented.

1. Introduction

Pyrimidine nucleus fused with another heterocycle has been found to be an integral part of many natural products, agro chemicals and veterinary products.[1,2] This class of compounds has also found wide applications in the design and discovery of novel bioactive molecules and drugs.[3] Recently, thienopyrimidine **I** (Fig. 1) that belongs to this class has attracted considerable interest because of their various pharmacological properties such as antimicrobial, [4] antiviral, [5] anti inflammatory, [6] antidiabetic[7] and anticancer activities. [8] Among thieno[2,3-*d*]pyrimidine derivatives, 3-amino-5,6-dimethyl-2-[4-(1-phenyl-methyl)-1-piperazinyl]thieno[2,3-*d*]pyrimidin-4(3*H*)-one[9] **II** and 4-(4-methyl-1-piperazinyl)-2-methyl thio-6,7-dihydro-5*H*-cyclopenta [4,5]thieno[2,3-*d*]pyrimidine[10] **III** (Fig. 1) exhibited remarkable affinity and selectivity for the 5-HT3 receptor. Due to our continuing interest in the identification of novel anti-tubercular agents, [11] we became interested in exploring the *N*-aryl substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one **C** framework for the design of small molecules[12] as potential inhibitors of *Mycobacterium tuberculosis* H37Rv chorismate mutase (CM).[13] Indeed, the framework **C** was derived from the general structure of inhibitors represented by **A** (reported by us earlier[11d]) via **B** (Fig. 2). In view of the fact that tuberculosis (TB) kills more than two million people a year worldwide and chorismate mutase being considered as a promising target for the development of anti tubercular agents the generation of a library of small molecules based on the framework **C** was undertaken.

Preparation of *N*-aryl substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives usually involves (i) the construction of the six membered pyrimidone ring by using 2-amino-*N*-aryl substituted thiophene-3-carboxamide via a multi-step process[14a] or (ii) condensation reaction of aryl amine with substituted thieno[2,3-*d*][1,3]oxazin-4-one.[14b,c] However, all these methods require tedious steps along with harsh reaction conditions and afforded the desired product in low yield. [14] Moreover, we required a more flexible, robust and high yielding method for the preparation of our target molecules based on **C**. Copper promoted C–N bond



I II III

Figure 1. Thieno[2,3-*d*]pyrimidine (**I**) and its biologically active derivatives (**II** and **III**).

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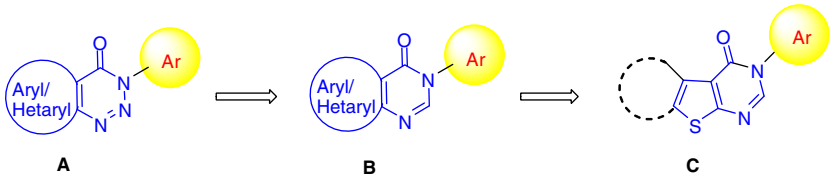
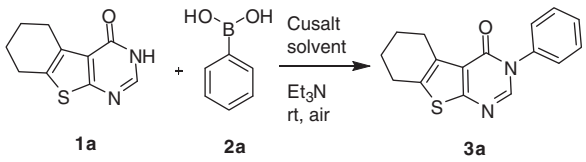


Figure 2. Design of novel and potential inhibitors (**C**) of chorismate mutase from **A**.

formation between the –NH– moiety of a heteroaryl derivative and organoboronic acids via cross coupling reactions have emerged as a powerful synthetic tool for the preparation of *N*-(hetero)aryl substituted derivatives.[15] Recently we have reported[11d] efficient *N*-arylation of 1,2,3-triazin-4-ones successfully under mild reaction conditions in the presence of a copper catalyst and a base at ambient temperature. Additionally, the reactions were carried out without using any inert atmosphere as the methodology was found to be not sensitive to air oxygen. We adopted a similar strategy to generate the compound library based on thieno[2,3-*d*]pyrimidin-4(3*H*)-one framework **C** where Cu-mediated N-arylation was used as a key synthetic step. Thus, aryl boronic acids (**2**) were coupled with various substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones (**1**) in the presence of a Cu-salt to give the corresponding N-arylated products (**3**) (Scheme 1). Herein we report the preliminary results of this study and in vitro pharmacological evaluation of a series of *N*-aryl substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**3**) as potential inhibitors of CM. Notably, only few heteroaryl based inhibitors of CM has been reported so far[11] and to the best of our knowledge synthesis and CM inhibiting properties of this class of compounds has not been reported earlier.

Table 1
Effect of reaction conditions on Cu-mediated coupling of **1a** with **2a**[a]



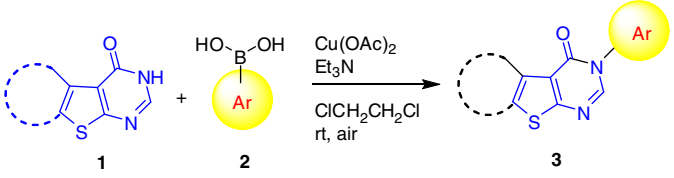
Entry	Cu salts	Solvent	Time (h)	Yield[b] (%)
1	$Cu(OAc)_2$	DCM	1.0	82
2	$Cu(OAc)_2$	DCE	1.0	90
3	$Cu(OAc)_2$	DMF	3.0	65
4	$Cu(OAc)_2$	THF	3.0	55
5	$Cu(OAc)_2.H_2O$	DCE	5.0	50
6	CuI	DCE	6.0	35
7	CuBr	DCE	6.0	30
8	$Cu(OTf)_2$	DCE	3.0	75

[a] All the reactions were carried out using **1a** (0.485 mmol), **2a** (0.728 mmol), a Cu-salt (0.485 mmol) and Et_3N (0.970 mmol) in a solvent (5 mL) at room temperature without using inert or anhydrous atmosphere (DCM = dichloromethane; DCE = 1,2-dichloroethane).
[b] Isolated yield.

2. Results and discussion

2.1. Chemistry

Initially, the coupling of 4,5,6,7-tetrahydrobenzo[*b*]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**1a**) with phenyl boronic acid (**2a**, 1.5 equiv) was chosen as a model reaction to establish the optimized reaction conditions. Thus the reaction was performed in the presence of various copper catalysts and solvents using Et_3N as a base at room temperature under anhydrous conditions (Table 1). All the reactions were performed in the presence of air. The desired product **3a** was isolated in 82% yield when the reaction was carried out using anhydrous $Cu(OAc)_2$ in dichloromethane (DCM) (Table 1, entry 1). The reaction was completed within 1 h. A further improvement of yield was observed when 1,2-dichloroethane (DCE) was used in place of DCM (Table 1, entry 2). The use of DMF and THF not only decreased the product yield but increased the reaction time from 1 to 3 h (Table 1, entry 3 and 4). The use of other catalysts e.g. $Cu(OAc)_2{\cdot}H_2O$, CuI, CuBr and $Cu(OTf)_2$ was also examined (entries 5–8, Table 1) but found to be inferior in terms of product yield. It is worthy to mention that while significant decrease in product yield was observed when hydrated $Cu(OAc)_2$ was used in place of anhydrous one the reaction

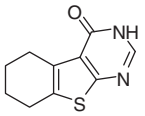
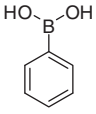
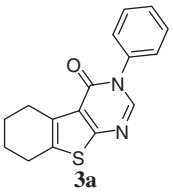
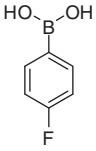
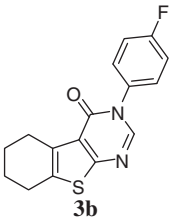
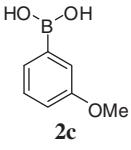
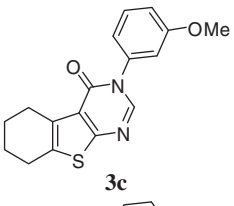
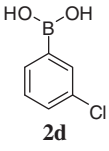
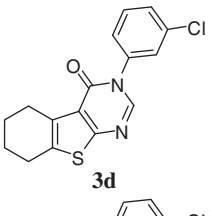
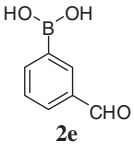
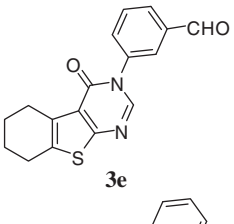
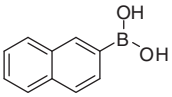
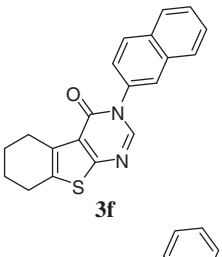
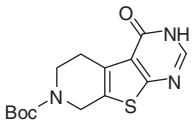
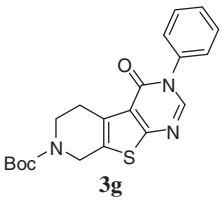
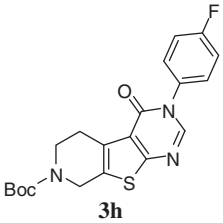


Scheme 1. Cu-mediated coupling of substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**1**) with aryl boronic acids (**2**).

however was found to be not sensitive to the atmospheric moisture. The methodology therefore does not require the use of an inert or anhydrous atmosphere. Overall, the combination of anhydrous $Cu(OAc)_2$ and Et_3N in DCE was found to be optimum for the N-arylation of compound **1a**.

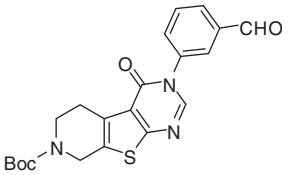
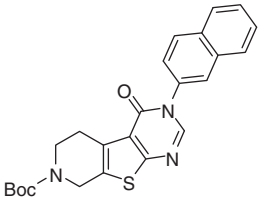
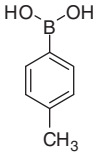
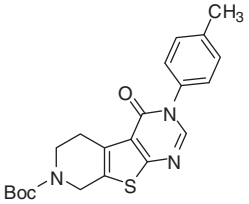
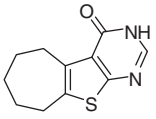
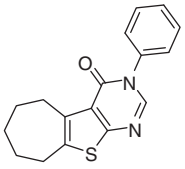
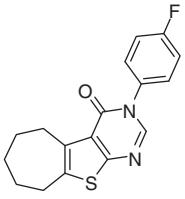
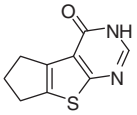
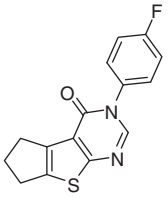
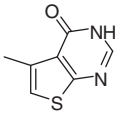
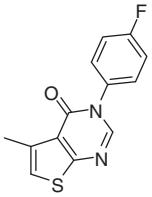
We then used the optimized conditions for the coupling of a range of thieno[2,3-*d*]pyrimidin-4(3*H*)-ones (**1**) with a variety of aryl boronic acids (**2**) and the results are summarized in Table 2. It is evident from Table 2 that the reaction proceeded well irrespective of the nature of thieno[2,3-*d*]pyrimidin-4(3*H*)-ones (**1a–d**) employed. Thus 4,5,6,7-tetrahydrobenzothieno[2,3-*d*] pyrimidin-4(3*H*)-one[16a] (**1a**), tert-butyl-4-oxo-3,4,5,6,7,8-hexahydropyrido[4′,3′:4,5]thieno-[2,3-*d*]pyrimidine-7-carboxylate[16b] (**1b**), 6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one[16c] (**1c**) and 5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one[16a] (**1d**) were employed successfully to afford the desired products **3a-o** in good yields (Table 2). The substituent like F, Cl, OCH_3, CH_3 and CHO group present in aryl boronic acids were well tolerated and the use of naphthalen-2-yl boronic acid (Table 2, entries 6 and 10) was found to be effective. It is worthy to mention that no desired product was isolated when pyridin-2-yl boronic acid was reacted with **1a** under the optimized reaction conditions. Additionally, alkyl boronic acid such as cyclopropyl boronic acid was also found to be ineffective in the present C–N bond forming reaction. All the compounds synthesized were well characterized by spectral (NMR, MS and IR) data. The molecular structure of a representative compound **3m** was established unambiguously by single crystal X-ray diffraction study (Fig. 3).[17] The molecule **3m** crystallized in the monoclinic space group $P2_1/c$ with one molecule in the asymmetric unit ($Z = 4$) (Fig. 3). The molecule in the asymmetric unit does not possess any conventional functional groups to form the strong hydrogen bonding. Interestingly, the molecule in the asymmetric unit form tetramer via C–H···O (C16–H16···O1: 2.58 Å, 134°:

Table 2
Synthesis of *N*-aryl substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives (**3**) via $Cu(OAc)_2$ mediated C–N bond forming reaction between **1** and **2** (Scheme 1)[a]

Entry	Thieno[2,3-*d*]pyrimidin-4(3H)-one (**1**)	$ArB(OH)_2$ (**2**)	Products (**3**)	Time (h)	Yield[b] (%)
1	 **1a**	 **2a**	 **3a**	1.0	90
2	**1a**	 **2b**	 **3b**	1.0	88
3	**1a**	 **2c**	 **3c**	1.2	88
4	**1a**	 **2d**	 **3d**	1.0	85
5	**1a**	 **2e**	 **3e**	1.5	80
6	**1a**	 **2f**	 **3f**	1.2	82
7	 **1b**	**2a**	 **3g**	1.0	90
8	**1b**	**2b**	 **3h**	1.0	88

(*continued on next page*)

Table 2 (*continued*)

Entry	Thieno[2,3-*d*]pyrimidin-4(3H)-one (**1**)	$ArB(OH)_2$ (**2**)	Products (**3**)	Time (h)	Yield[b] (%)
9	**1b**	**2e**	 **3i**	1.5	80
10	**1b**	**2f**	 **3j**	1.2	82
11	**1b**	 **2g**	 **3k**	1.5	85
12	 **1c**	**2a**	 **3l**	1.0	88
13	**1c**	**2b**	 **3m**	1.0	85
14	 **1d**	**2b**	 **3n**	1.0	86
15	 **1e**	**2b**	 **3o**	1.0	82

[a] All the reactions were carried out using **1** (0.485 mmol), **2** (0.728 mmol), $Cu(OAc)_2$ (0.485 mmol) and Et_3N (0.970 mmol) in DCE (5 mL) at room temperature without using inert or anhydrous atmosphere.

[b] Isolated yield.

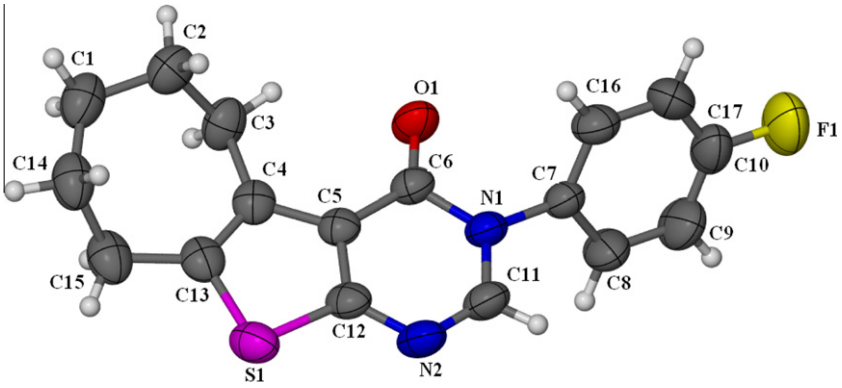


Figure 3. ORTEP representation of the compound **3m** (Thermal ellipsoids are drawn at 50% probability level).

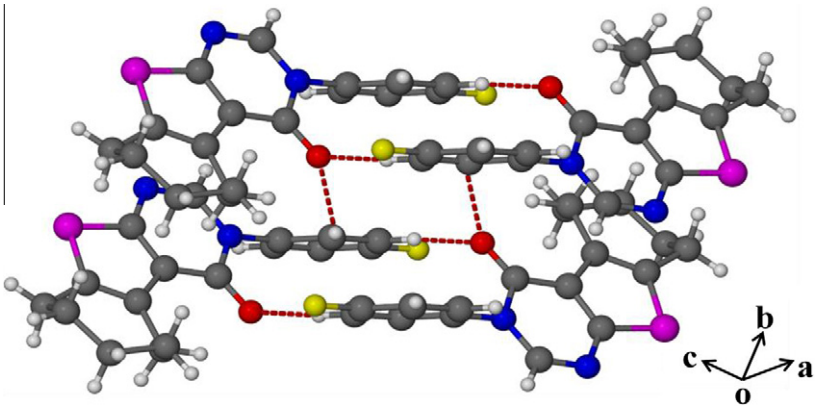


Figure 4. Showing the tetramer formation via C–H. . .O interactions (compound **3m**).

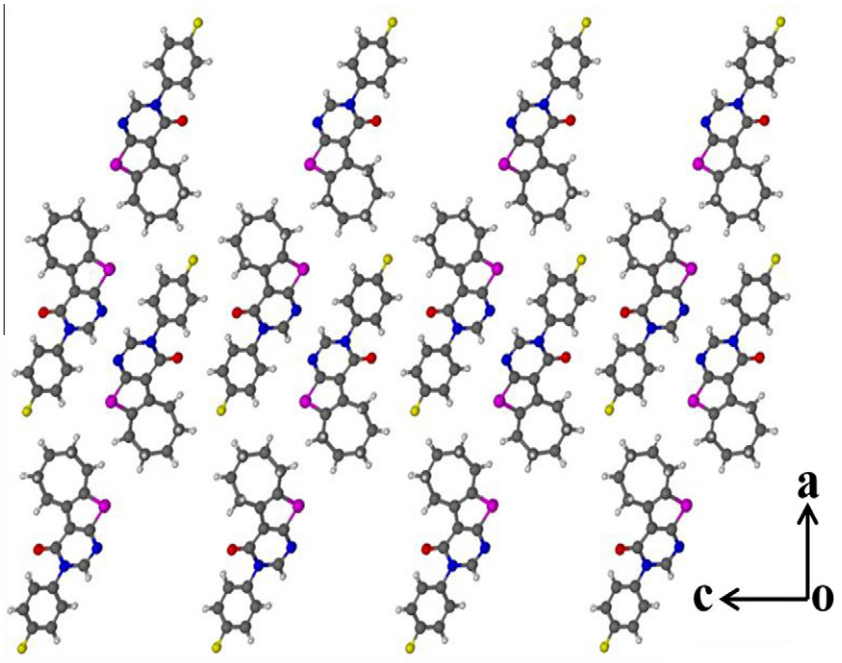
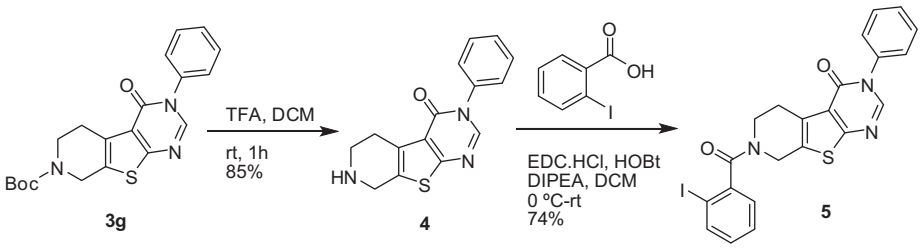


Figure 5. Showing the packing arrangement along the ac axis (compound **3m**).



Scheme 2. Structural elaboration of compound **3g**.

C17–H17...O1: 2.42 Å, 156°: C3–H3B...O1: 2.38 Å, 132°) synthon as shown in the Figure 4 which is although a very weak interaction. These interactions propagated in a 2D zipper fashion and made the packing as much as strong in both the directions on the ac plane as shown in Figure 5.

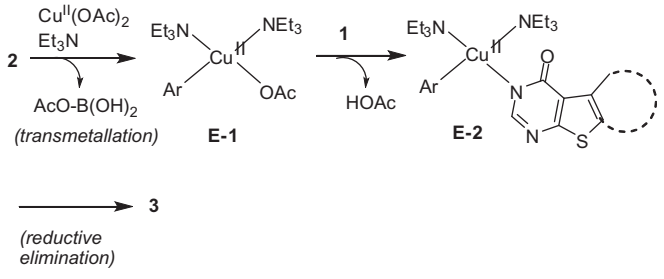
In order to demonstrate the further scope of the present methodology, further structure elaboration of compound **3g** was carried out using a two-step method (Scheme 2). Thus, the Boc group of compound **3g** was removed in the presence of trifluoroacetic acid to give the secondary amine **4** in good yield (Scheme 2). The amine obtained was then reacted with 2-iodobenzoic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) hydrochloride (EDC·HCl), *N*-hydroxybenzotriazole (HOBt) and *N,N*-diisopropylethylamine (DIPA) in dichloromethane to give the corresponding amide **5**. The iodo group of amide **5** is amenable for further functionalization via transition metal catalyzed C–C or C–N bond forming reactions. [18]

A plausible mechanism can be proposed for the present Cu-mediated coupling reaction between thieno[2,3-*d*]pyrimidin-4(3*H*)-ones (**1**) and aryl boronic acids (**2**) (Scheme 3).[19] Thus transmetallation of aryl boronic acid (**2**) with $Cu(OAc)_2$ provides the Cu(II) complex **E-1**. Subsequent displacement of OAc moiety of **E-1** by the reactant **1** through its amide NH provides the Cu(II) complex **E-2** which undergoes reductive elimination to give the product **3**. In the presence of air oxygen, as reported earlier, [19] it is possible that the Cu(II) species **E-2** might undergo change of its oxidation state (prior to reductive elimination) leading to the corresponding Cu(III) species which then afford the product **3** via reductive elimination.

2.2. Pharmacology

Having prepared a range of *N*-aryl substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones (**3**) via $Cu(OAc)_2$ mediated C–N bond forming reaction between **1** and **2** some of the compounds synthesized were tested for CM inhibiting properties in vitro. The CM (EC5.4.99.5) an enzyme in the shikimate pathway is responsible for the production of tyrosine and phenylalanine via the Claisen rearrangement of chorismate to prephenate. Due to the absence of this pathway in animals but not in bacteria CM is considered as an attractive target for the identification of novel antibacterial agents. Furthermore, the low sequence homology amongst known CM provides a new opportunity for developing unique inhibitors targeted to specific microorganisms. The pharmacological assay[20] involved determination of activity of enzyme CM which catalyzes the conversion of chorismate to prephenate. Thus determination of activity of CM is based on the direct observation of conversion of chorismic acid to prephenate spectrophotometrically at OD_{274}. This reaction was performed in the presence of test compounds to determine their CM inhibiting properties. A known inhibitor of CM that is 4-(3,5-dimethoxyphenethylamino)-3-nitro-5 sulfamoyl benzoic acid[12a] was prepared and used as a reference compound, the IC_{50} value of which was found to be less than 10 μM. The compounds were tested at 50 μM and the results are summarized in Table 3. It is evident from Table 3 that a number of compounds showed significant inhibition (>30%) of CM. In general, a *p*-substituent on the *N*-aryl ring of **3** was unfavorable in terms of activity (**3a** vs **3b**, Table 3) whereas a *m*-substituent enhanced the activity (**3a** vs **3c–e**, Table 3). A *N*-naphthyl group though appeared as better than *p*-substituent (**3f** vs **3b**, Table 3) was found to be inferior to a *m*-substituent. A trend similar to that of **3a** versus **3e** was observed in case of compound **3g** or **4** versus **3i**. Notably, a six membered saturated carbocyclic/N-heterocyclic ring was fused with the thiophene moiety in all these cases for example **3a–g** and **3i** and **4**. Replacing the 6-membered ring by a 7-membered one (**3l–m**) or its removal (e.g. **3o**) was found to be detrimental. Nevertheless, based on the preliminary data generated compounds **3c** and **3i** were chosen for further evaluation in vitro. In a dose–response study, compounds **3c** and **3i** showed inhibition of CM in a dose dependent manner with an IC_{50} value of 19.80 ± 1.02 and 23.88 ± 0.90 μM respectively (Fig. 6). Thus, *N*-aryl thieno[2,3-*d*]pyrimidin-4(3*H*)-one has been identified as a new scaffold for the development of novel inhibitors of chorismate mutase.

To understand the nature of interactions of these molecules with CM protein docking studies were carried out using the compounds **3c** and **3i**. The results showed (i) an H-bonding interaction



Scheme 3. Proposed mechanism for the Cu-mediated coupling of **1** with **2.**

Table 3
Inhibition of chorismate mutase by *N*-aryl pyrimidones (**3**) in vitro

N-Aryl thieno[2,3-*d*]pyrimidin-4(3*H*)-ones (**3**)	% Inhibition[a] @ 50 μM
3a	35.6
3b	12.8
3c	47.0
3d	42.5
3e	45.3
3f	33.0
3g	37.2
3i	49.9
3l	13.0
3m	14.6
3o	17.7
4	36.2
5	12.6

[a] Data presented are average of three experiments.

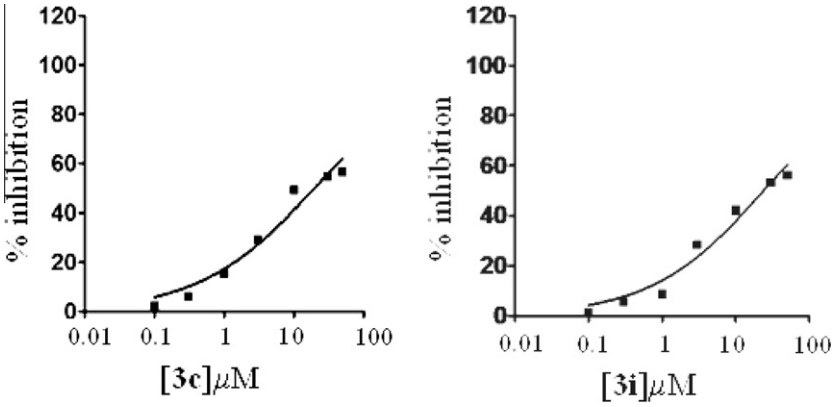


Figure 6. Dose dependent inhibition of CM by compounds **3c** and **3i**.

between the pyrimidine nitrogen of **3c** and arginine 49 residue of CM and (ii) a π-cation interaction between the benzene ring of **3c** and arginine 134 residue (Fig. 7). In the case of compound **3i** an H-bonding interaction was observed between the carbonyl oxygen of the ester moiety and the lysine 60 residue of CM (Fig. 8).

3. Conclusions

In conclusion, synthesis of novel *N*-aryl substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones was carried out for the first time by using Cu-mediated C–N bond forming reaction between thieno[2,3-*d*]pyrimidin-4(3*H*)-ones and aryl boronic acids. The methodology does not require the use of inert or anhydrous atmosphere and can be performed in an open flask. A range of compounds were prepared in good to excellent yields by using this direct N-arylation process and the molecular structure of a representative compound was established unambiguously by single crystal X-ray diffraction. Among all the compounds synthesized two showed promising inhibitory properties when tested against chorismate mutase in vitro. In silico interactions of these molecules with CM are presented. Overall, Cu-mediated direct access to *N*-aryl substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones has resulted in identification of novel small molecules as inhibitors of chorismate mutase for the potential treatment of tuberculosis.

4. Experimental section

4.1. Chemistry

4.1.1. General methods

Unless stated otherwise, reactions were performed under nitrogen atmosphere using oven dried glassware. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230–400 mesh) using distilled hexane, ethyl acetate, dichloromethane. ^{1}H NMR and ^{13}C NMR spectra were determined in $CDCl_3$ or DMSO-d_6 solution by using 400 or 100 MHz spectrometers, respectively. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, $\delta = 0.00$) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants (J) are given in hertz. Infrared spectra were recorded on a FT-IR spectrometer. Melting points were determined using melting point apparatus and are uncorrected. MS spectra were obtained on a mass spectrometer. Chromatographic purity by HPLC (Agilent 1200 series Chem Station software) was determined by using area normalization method and the condition specified in each case: column, mobile phase (range used), flow rate, detection wavelength, and retention times.

4.1.2. Typical procedure for the synthesis of *N*-aryl substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives (3a–o)

A mixture of the substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one **1** (0.485 mmol), aryl boronic acid **2** (0.728 mmol), anhydrous $Cu(OAc)_2$ (0.485 mmol) and Et_3N (0.970 mmol) in 1,2-dichloroethane (5 mL) was stirred at ambient temperature for 1.0–2.0 h. Progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was filtered through celite. The filtrate was concentrated under vacuum, and the residue was purified by column chromatography on silica gel using hexane/ethyl acetate to afford the desired product.

4.1.3. 3-Phenyl-5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*] pyrimidin-4(3*H*)-one (3a)

Yield: 90% (0.12 g); Light brown solid; mp: 187–189 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.54–7.45 (m, 3H), 7.38 (d, $J = 7.2$ Hz, 2H), 3.01 (t, $J = 5.8$ Hz, 2H), 2.80 (t, $J = 5.8$ Hz, 2H), 1.90–1.82 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.7, 157.4, 145.1, 137.1, 134.5, 132.0, 129.4, 129.0 (2C), 127.1 (2C), 122.8, 25.5, 25.2, 22.8, 22.2; HPLC: 97.8%, column: Zorax XDB C-18 150 × 4.6 mm 5μ, mobile phase A: 0.1% Formic Acid in water, mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 9/98, 14/98, 16/50, 18/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 7.47 min; IR (KBr) υ_{max} 2924, 2866, 1682, 1562 cm^{-1}; m/z (CI) 282.9 (M+1).

4.1.4. 3-(4-Fluorophenyl)-5,6,7,8-tetrahydrobenzo[*b*]thieno [2,3-*d*]pyrimidin-4(3*H*)-one (3b)

Yield: 88% (0.13 g); White solid; mp: 171–173 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.14–7.12 (m, 2H), 7.00–6.96 (m, 2H), 2.78 (br s, 2H), 2.58 (br s, 2H), 1.65–1.62 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6, 162.1 (C-F $J = 248.1$ Hz), 157.4, 144.8, 134.7, 133.0 (C-F $J = 3.0$ Hz), 130.8, 128.9 (C-F $J = 8.3$ Hz, 2C), 122.7, 116.4 (C-F $J = 22.9$ Hz, 2C), 25.5, 25.2, 22.8, 22.1; HPLC: 99.4%, column: Zorax XDB C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.1% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 9/98, 14/98, 16/50, 18/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 7.56 min; IR (KBr) υ_{max} 2948, 2889, 1692, 1560, 1508 cm^{-1}; m/z (CI) 300.9 (M+1).

4.1.5. 3-(3-Methoxyphenyl)-5,6,7,8-tetrahydrobenzo[*b*]thieno [2,3-*d*]pyrimidin-4(3*H*)-one (3c)

Yield: 88% (0.13 g); White solid; mp: 169–171 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.71–7.69 (m, 2H), 7.54–7.51 (m, 2H), 3.83 (s, 3H), 3.03 (t, $J = 5.6$ Hz, 2H), 2.80 (t, $J = 5.4$ Hz, 2H),

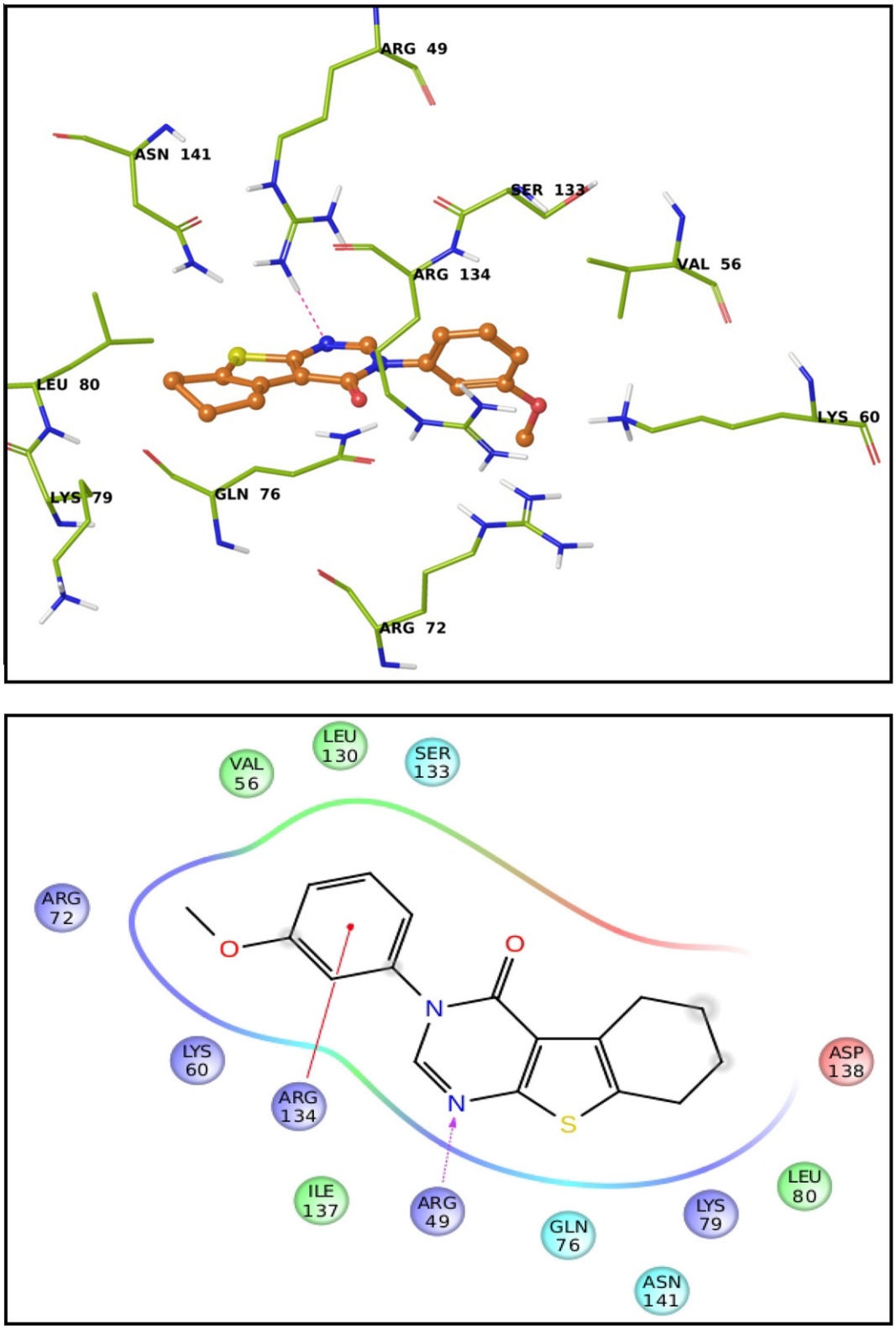


Figure 7. Docking of compound **3c** at the active site of CM.

1.90–1.82 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.7, 160.3, 157.4, 145.0, 138.1, 134.5, 132.3, 130.8, 128.7, 122.8, 115.0, 112.9, 55.4, 25.5, 25.2, 22.8, 22.2; HPLC: 98.1%, column: Zorax XDB C-18 150 × 4.6 mm 5μ, mobile phase A: 0.1% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 9/98, 14/98, 16/50, 18/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 7.75 min; IR (KBr) υ_{max} 2936, 2862, 1679, 1600, 1560 cm^{-1}; m/z (CI) 312.9 (M+1).

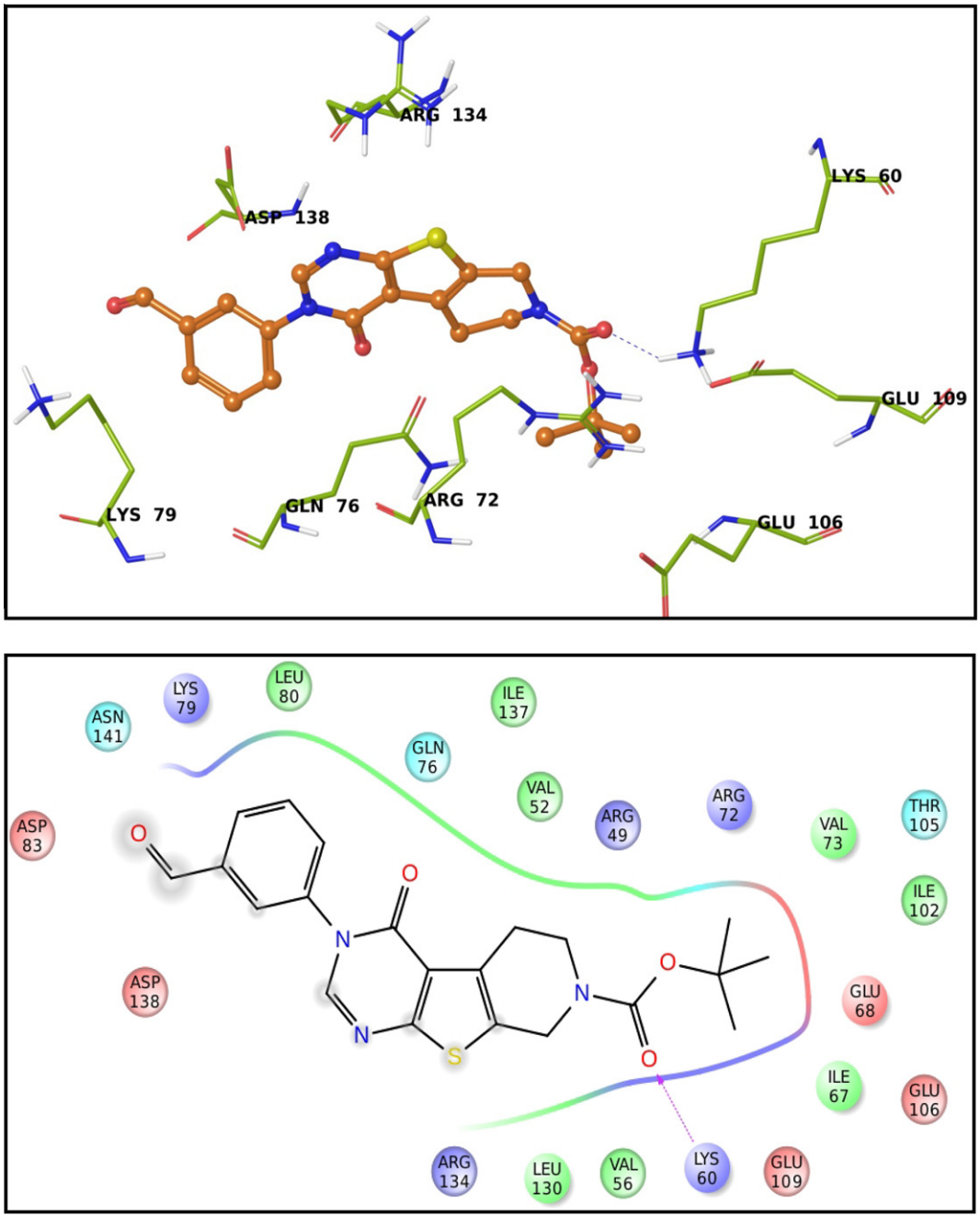


Figure 8. Docking of compound **3i** at the active site of CM.

4.1.6. 3-(3-Chlorophenyl)-5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (3d)

Yield: 85% (0.13 g); White solid; mp: 207–209 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.47–7.46 (m, 2H), 7.43 (s, 1H), 7.30–7.27 (m, 1H), 3.01 (t, J = 5.8 Hz, 2H), 2.81 (t, J = 5.6 Hz, 2H), 1.91–1.82 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.7, 157.1, 144.5, 138.1, 134.9, 132.0, 130.4, 129.3, 128.7, 127.6, 125.4, 122.8, 25.5, 25.2, 22.8, 22.1; HPLC: 93.9%, column: Zorax XDB C-18 150 × 4.6 mm 5µ, mobile phase A: 0.1% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 9/98, 14/98, 16/50, 18/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 8.84 min; IR (KBr) υ_{max} 2936, 2868, 1678, 1558 cm^{-1}; m/z (CI) 316.8 (M+1).

4.1.7. 3-(4-Oxo-5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)benzaldehyde (3e)

Yield: 80% (0.12 g); White solid; mp: 204–206 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.01 (s, 1H), 8.01–7.98 (m, 2H), 7.92 (s, 1H), 7.74–7.67 (m, 2H), 3.01 (t, J = 6.0 Hz, 2H), 2.81 (t, J = 6.0 Hz, 2H), 1.93–1.81 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.6, 161.7, 157.2, 144.4, 138.1, 137.6, 135.2, 133.0 (2C), 132.1, 130.3, 130.2, 128.0, 25.6, 25.3, 22.8, 22.2; HPLC: 99.3%, column: X Bridge C-18 150 × 4.6 mm 5µ, mobile phase A: 0.1% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 9/98, 14/98, 16/50, 18/50; flow rate: 1.0 mL/min; UV 245 nm, retention time 5.84 min; IR (KBr) υ_{max} 3049, 2932, 2845, 1705, 1669, 1556 cm^{-1}; m/z (CI) 310.8 (M+1).

4.1.8. 3-(Naphthalen-2-yl)-5,6,7,8-tetrahydrobenzo[*b*]thieno [2,3-*d*]pyrimidin-4(3*H*)-one (3f)

Yield: 82% (0.13 g); White solid; mp: 204–206 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.97 (d, J = 8.4 Hz, 1H), 7.92–7.87 (m, 2H), 7.83 (d, J = 1.6 Hz, 1H), 7.58–7.56 (m, 2H), 7.52–7.50 (m, 1H), 3.03 (t, J = 5.4 Hz, 2H), 2.82 (t, J = 5.6 Hz, 2H), 1.91–1.83 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.8, 157.7, 145.2, 134.8, 134.6, 133.2, 133.0, 132.0, 129.3, 128.1, 127.8, 127.2, 126.9, 125.8, 124.8, 122.9, 25.6, 25.2, 22.8, 22.2; HPLC: 97.0%, column: Zorax XDB C-18 150 × 4.6 mm 5μ, mobile phase A: 0.1% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 9/98, 14/98, 16/50, 18/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 9.49 min; IR (KBr) υ_{max} 3053, 2927, 2864, 1677, 1560 cm^{-1}; m/z (CI) 333.0 (M+1).

4.1.9. *tert*-Butyl-3-phenyl-4-Oxo-3,4,5,6,7,8-hexahydropyrido [4',3':4,5]thieno-[2,3-*d*]pyrimidine-7-carboxylate (3g)

Yield: 90% (0.17 g); White solid; mp: 98–100 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.56–7.48 (m, 3H), 7.38 (d, J = 6.8 Hz, 2H), 4.66 (s, 2H), 3.71 (t, J = 5.4 Hz, 2H), 3.11 (br s, 2H), 1.49 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.6, 157.4, 145.8, 136.8 (2C), 129.6 (3C), 129.3 (2C), 127.1 (2C), 122.5, 80.4, 42.9, 42.3, 28.4 (3C), 25.8; HPLC: 95.6%, column: X Bridge C-18 150 × 4.6 mm 5μ, mobile phase A: 5 mM NH_4OAc in water mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 9/95, 13/95, 15/50, 18/50; flow rate: 1.0 mL/min; UV 218 nm, retention time 6.78 min; IR (KBr) υ_{max} 2974, 2928, 1689, 1558 cm^{-1}; m/z (CI) 384.2 (M+1).

4.1.10. *tert*-Butyl-3-(4-fluorophenyl)-4-oxo-3,4,5,6,7,8-hexahy dropyrido[4',3':4,5]thieno-[2,3-*d*]pyrimidine-7-carboxylate (3h)

Yield: 88% (0.17 g); White solid; mp: 129–131 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.38–7.35 (m, 2H), 7.26–7.20 (m, 2H), 4.66 (s, 2H), 3.71 (t, J = 4.2 Hz, 2H), 3.10 (br s, 2H), 1.49 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.9 (C-F J = 248.6 Hz), 162.6, 157.3, 154.3, 145.3, 136.2, 132.8 (C–F J = 3.1 Hz, 2C), 130.8, 129.1 (C–F J = 8.8 Hz, 2C), 116.7 (C–F J = 23.0 Hz, 2C), 80.4, 42.9, 41.4, 28.4 (3C), 25.8; HPLC: 96.7%, column: X Bridge C-18 150 × 4.6 mm 5μ, mobile phase A: 5 mM NH_4OAc in water mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 10/95, 15/95, 18/50, 20/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 7.06 min; IR (KBr) υ_{max} 2974, 2931, 1690, 1566 cm^{-1}; m/z (CI) 402.2 (M+1).

4.1.11. *tert*-Butyl-3-(3-formylphenyl)-4-oxo-3,4,5,6,7,8-hexahy dropyrido[4′,3′:4,5]thieno-[2,3-*d*]pyrimidine-7-carboxylate (3i)

Yield: 80% (0.16 g); White solid; mp: 94–96 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.08 (s, 1H), 8.03–8.01 (m, 2H), 7.92 (s, 1H), 7.75–7.67 (m, 2H), 4.67 (s, 2H), 3.72 (t, J = 4.2 Hz, 2H), 3.11 (br s, 2H), 1.49 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.6, 162.5, 157.1, 154.6, 145.0, 137.8, 137.6 (2C), 132.9 (2C), 130.4 (2C), 130.3, 127.8, 80.4, 43.1, 42.9, 28.4 (3C), 25.8; HPLC: 95.0%, column: X Bridge C-18 150 × 4.6 mm 5 μ, mobile phase A: 5 mM NH_4OAc in water mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 10/95, 15/95, 18/50, 20/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 5.87 min; IR (KBr) υ_{max} 2974, 2929, 1690, 1599, 1556 cm^{-1}; m/z (CI) 412.2 (M+1).

4.1.12. *tert*-Butyl-3-(naphthalen-2-yl)-4-oxo-3,4,5,6,7,8-hexahy dropyrido[4′,3′:4,5]thieno-[2,3-*d*]pyrimidine-7-carboxylate (3j)

Yield: 82% (0.17 g); White solid; mp: 174–176 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.15 (s, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.93–7.88 (m, 2H), 7.13 (d, J = 1.2 Hz, 1H), 7.60–7.55 (m, 2H), 7.49 (dd, J = 8.2, 1.8 Hz, 1H), 4.68 (s, 2H), 3.72 (t, J = 7.4 Hz, 2H), 3.13 (br s, 2H), 1.49 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 162.6, 157.5, 154.6, 145.7, 134.5, 133.3, 133.1, 129.5 (2C), 128.1 (2C), 127.8 (2C), 127.3, 127.1, 125.8, 124.6, 80.4, 43.0, 42.9, 28.4 (3C), 25.8; HPLC: 97.3%, column: X Bridge C-18 150 × 4.6 mm 5μ, mobile phase A: 5 mM NH_4OAc in water mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 10/95, 15/95, 18/50, 20/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 8.89 min; IR (KBr) υ_{max} 2981, 2895, 1680, 1563 cm^{-1}; m/z (CI) 434.2 (M+1).

4.1.13. *tert*-Butyl-3-*p*-tolyl-4-oxo-3,4,5,6,7,8-hexahydropyrido [4′,3′:4,5]thieno-[2,3-*d*]pyrimidine-7-carboxylate (3k)

Yield: 85% (0.16 g); White solid; mp: 87–89 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.93 (s, 1H), 7.25 (d, J = 8.0 Hz, 2H), 7.17 (d, J = 8.0 Hz, 2H), 4.58 (s, 2H), 3.63 (t, J = 5.2 Hz, 2H), 3.03 (br s, 2H), 2.35 (s, 3H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.5, 157.4, 154.6, 145.7, 139.4 (2C), 134.3, 130.1 (2C), 129.8, 126.8 (2C), 115.1, 80.3, 43.0, 42.9, 28.4 (3C), 25.8, 21.2; HPLC: 94.8%, column: X Bridge C-18 150 × 4.6 mm 5 μ, mobile phase A: 5 mM NH_4OAc in water mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 10/95, 15/95, 18/50, 20/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 8.06 min; IR (KBr) υ_{max} 2973, 2927, 1689, 1560 cm^{-1}; m/z (CI) 397.8 (M+1).

4.1.14. 3-Phenyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno [2,3-*d*]pyrimidin-4(3*H*)-one (3l)

Yield: 88% (0.13 g); White solid; mp: 91–93 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.55–7.49 (m, 3H), 7.39–7.37 (m, 2H), 3.36–3.34 (m, 2H), 2.89–2.87 (m, 2H), 1.93–1.88 (m, 2H), 1.76–1.64 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.1, 157.9, 144.8, 138.9, 137.7, 137.3, 129.5 (2C), 129.1, 127.3 (3C), 32.6, 30.0, 27.9, 27.7, 27.2; HPLC: 95.5%, column: X Bridge C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.1% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 9/98, 14/98, 16/50, 18/50; flow rate: 1.0 mL/min; UV 215 nm, retention time 7.93 min; IR (KBr) υ_{max} 3057, 2920, 2846, 1678, 1563 cm^{-1}; m/z (CI) 296.9 (M+1).

4.1.15. 3-(4-Fluorophenyl)-6,7,8,9-tetrahydro-5*H*-cyclohepta [4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (3m)

Yield: 85% (0.13 g); White solid; mp: 144–146 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.38–7.34 (m, 2H), 7.21 (t, J = 8.4 Hz, 2H), 3.35–3.32 (m, 2H), 2.89–2.87 (m, 2H), 1.93–1.88 (m, 2H), 1.75–1.64 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.9 (C-F J = 248.2 Hz), 160.0, 157.9, 144.6, 144.5, 139.2, 137.7, 133.2 (C-F J = 3.3 Hz), 129.2 (C-F J = 8.8 Hz, 2C), 116.6 (C-F J = 22.9 Hz, 2C), 32.5, 29.9, 27.9, 27.7, 27.1; HPLC: 99.2%, column: X Bridge C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.1% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 9/98, 14/98, 16/50, 18/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 7.97 min;IR (KBr) υ_{max} 3072, 2924, 2852, 1686, 1566 cm^{-1}; m/z (CI) 314.8 (M+1).

4.1.16. 3-(4-Fluorophenyl)-6,7-dihydro-5*H*-cyclopenta[4,5] thieno[2,3-*d*]pyrimidin-4(3*H*)-one (3n)

Yield: 86% (0.12 g); White solid; mp: 179–181 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.40–7.36 (m, 2H), 7.21 (t, J = 8.4 Hz, 2H), 3.06 (t, J = 7.2 Hz, 2H), 2.98 (t, J = 7.2 Hz, 2H), 2.51–2.44 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.7, 162.5 (C-F J = 248.0 Hz), 157.3, 144.6, 140.7, 133.0 (C-F J = 3.3 Hz) 132.4, 128.9 (C-F J = 8.8 Hz, 2C), 120.4, 116.4 (C-F J = 22.9 Hz, 2C), 30.3, 28.9, 23.7; HPLC: 99.7%, column: Zorax XDB C-18 150 × 4.6 mm 5μ, mobile phase A: 0.1% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 9/98, 14/98, 16/50, 18/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 6.23 min; IR (KBr) υ_{max} 2909, 2853, 1698, 1563, 1510 cm^{-1}; m/z (CI) 286.8 (M+1).

4.1.17. 3-(4-Fluorophenyl)-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (3o)

Yield: 82% (0.10 g); White solid; mp: 181–183 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.40–7.36 (m, 2H), 7.22 (t, J = 8.2 Hz, 2H), 6.89 (s, 1H), 2.58 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 162.5 (C–F J = 248.4 Hz), 158.0, 145.7, 135.3, 132.8 (C–F J = 3.1 Hz), 129.0 (C-F J = 8.8 Hz, 2C), 122.8, 119.0, 116.5 (C-F J = 23.0 Hz, 2C), 16.5; HPLC: 99.5%, column: Zorbax XDB C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.1% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 9/95, 14/95, 16/50, 18/50; flow rate: 0.8 mL/min; UV 220 nm, retention time 5.93 min; IR (KBr) υ_{max} 2923, 2857, 1692, 1581, 1513 cm^{-1}; m/z (CI) 260.9 (M+1).

4.1.18. 3-Phenyl-3,4,5,6,7,8-hexahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (4)

To a mixture of compound **3g** (200 mg, 0.52 mmol) in dichloromethane (5 mL) at 0 °C was added trifluoroacetic acid (TFA) (1 mL) and then warmed to room temperature. The reaction mixture was stirred for 2 h, then removed the solvent under vacuum, diluted the reaction mixture with ethyl acetate (20 mL) and washed with saturated $NaHCO_3$ solution (2 × 10 mL) followed by brine solution (10 mL). The organic layer dried over anhydrous Na_2SO_4 and concentrated under low vacuum to provide the desired compound **4** (125 mg, 85%) as a brown solid; mp: 144–146 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.56–7.47 (m, 3H), 7.38 (d, J = 6.8 Hz, 2H), 4.19 (s, 2H), 3.40 (br s, 1H), 3.28 (t, J = 5.8 Hz, 2H), 3.18–3.16 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.4, 157.4, 145.7, 136.9, 131.8, 130.3, 129.6 (2C), 129.3, 127.1 (2C), 122.6, 44.1, 42.4, 25.9; HPLC: 92.1%, column: X Bridge C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.1% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/10, 2/10, 9/95, 14/95, 16/10, 18/10; flow rate: 1.0 mL/min; UV 220 nm, retention time 6.06 min; IR (KBr) υ_{max} 3304, 3060, 2944, 1677, 1555 cm^{-1}; m/z (CI) 283.8 (M+1).

4.1.19. 7-(2-Iodobenzoyl)-3-phenyl-3,4,5,6,7,8-hexahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (5)

A mixture of compound **4** (100 mg, 0.35 mmol), 2-iodo benzoic acid (104 mg, 0.42 mmol), EDC.HCl (80 mg, 0.42 mmol), HOBT (56 mg, 0.42 mmol) and DIPEA (0.12 ml, 0.70 mmol) in dry CH_2Cl_2 (5 ml) was stirred at 0 °C under a nitrogen atmosphere. Then, the reaction mixture was allowed to stir at room temperature for 14 h. After completion of the reaction, the mixture was concentrated under reduced pressure, diluted with ethyl acetate (25 mL) and washed with water (2 × 20 mL) followed by brine (20 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated under low vacuum. The residue was purified by column chromatography to give the desired product **5** (132 mg, 74%) as a white solid; mp: 143–145 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.88–7.84 (m, 1H), 7.56–7.49 (m, 3H), 7.44–7.37 (m, 3H), 7.23–7.21 (m, 1H), 7.14–7.10 (m, 1H), 5.22 (d, J = 17.2 Hz, 1H), 4.84 (d, J = 17.2 Hz, 1H), 3.54 (d, J = 6.0 Hz, 2H), 3.31–3.23 (m, 2H) (extra signals due to rotamers); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 162.9, 157.4, 145.9, 142.0, 139.3, 136.8, 131.3, 130.5, 130.1, 129.9, 129.6 (2C), 129.4, 128.5, 127.1 (2C), 122.3, 92.4, 44.0, 41.3, 26.3 (Extra signals due to rotamers); HPLC: 95.4%, column: X Bridge C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.1% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 9/98, 14/98, 16/50, 18/50; flow rate: 1.0 mL/min; UV 310 nm, retention time 5.43 min; IR (KBr) υ_{max} 3059, 2930, 2851, 1682, 1642, 1555 cm^{-1}; m/z (CI) 513.7 (M+1).

4.2. Single crystal X-ray data for compound 3m

Single crystals suitable for X-ray diffraction of **3m** were grown from dimethyl formamide. The crystals were carefully chosen using a stereo zoom microscope supported by a rotatable polarizing stage. The data was collected at room temperature on Bruker's KAPPA APEX II CCD Duo with graphite monochromated Mo-Kα radiation (0.71073 Å). The crystals were glued to a thin glass fibre using FOMBLIN immersion oil and mounted on the diffractometer. The intensity data were processed using Broker's suite of data processing programs (SAINT), and absorption corrections were applied using SADABS.[22a] The crystal structure was solved by direct methods using SHELXS-97 and the data was refined by full matrix least-squares refinement on F^2 with anisotropic displacement parameters for non-H atoms, using SHELXL-97.[22b] Crystal data of (**3m**): Molecular formula = $C_{17}H_{15}FN_2OS$, Formula weight = 314.38, Crystal system = Monoclinic, space group = $P2_1/c$, a = 13.849 (6) Å, b = 4.433 (2) Å, c = 24.677 (10) Å, V = 1514.5 (11) Å^3, T = 296(2) K, Z = 4, D_c = 1.379 Mg m^{-3}, μ(Mo-Kα) = 0.23 mm^{-1}, 12508 reflections measured, 3626 independent reflections, 2057 observed reflections [$I > 2.0\sigma(I)$], R_1_obs = 0.037, Goodness of fit = 1.01.

4.3. Pharmacology[12a,21]

4.3.1. Chorismate mutase activity assay

Mycobacterium tuberculosis Chorismate Mutase (MtCM) gene was PCR amplified and cloned into expression vector pET22b. MtCM was purified from over expressed culture of BL21 (DE3) harboring pET22b/MtCM by Ni-NTA affinity chromatography.

Activity of chorismate mutase enzyme is based on the direct observation of conversion of chorismate to prephenate spectrophotometrically at OD_{274}. The reaction volume of 100 μl contained 50 mM Tris–HCl (pH 7.5), 0.5 mM EDTA, 0.1 mg/mL bovine serum albumin, and 10 mM β-Mercaptoethanol, and chorismic acid 4 mM. The reaction was started by adding 180 pmol of purified protein to the pre-warmed chorismic acid solution. Inhibitory screening of the test compounds against Chorismate Mutase activity was measured at 50 μM concentration of the effectors. The reaction was allowed to proceed at 37 °C and was terminated after 5 min with 100 μl of 1 N HCl. A blank with no enzyme for every reaction was kept as a control to account for the non enzymatic conversion of chorismate to prephenate.

The percentage of enzyme inhibition caused by the test compound is calculated by the following formula

$$\%\text{inhibition} = 100 - \text{residual activity of CM}$$

$$\left[\text{Residual activity of CM} = \frac{A_{274}(S + E' + C) - (A_{274}S + C)}{A_{274}(S + E) - A_{274}(S)} \times 100\right]$$

S = Absorbance of the Substrate (Chorismic Acid) at 274 nm; E′ = Absorbance of the Enzyme (Chorismate Mutase) at 274 nm with Compound; E = Absorbance of the Enzyme (Chorismate Mutase) at 274 nm without Compound; C = Test Compound; A274 = Absorbance at 274

Dose–response study of the compound **3c** and **3i** against chorismate mutase activity was carried out using the concentration from 1 to 100 μM.

4.4. Docking studies

The docking studies were carried out by using schrodinger 2012 software. The PDB ID 2F6L was used for this study. The protein was prepared by giving preliminary treatment like adding hydrogen, adding missing residues, refining the loop with prime and finally minimized by using OPLS 2005 force field. The search grid was generated by picking the active site residues manually and extended up to 20 Å. The hydroxyl groups of search area were allowed to move. The molecules were minimized by using macromodule

application. We used 1000 iteration for minimization using OPLS 2005 force field and charges were also added from force field only. The PRCG (Polak-Ribier conjugate gradient) method was used for minimization. All the molecules were docked by using glide XP (extra precision) dock application. We performed flexible docking by allowing sample ring conformations and sample nitrogens to move to possible extent in docking.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.07.011.

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