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Discovery of thieno[2,3-*c*]pyridines as potent COT inhibitors

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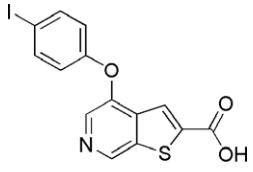
ABSTRACT

Evaluation of hit chemotypes from high throughput screening identified a novel series of 2,4-disubstituted thieno[2,3-*c*]pyridines as COT kinase inhibitors. Structural modifications exploring SAR at the 2- and 4-positions resulting in inhibitors with improved enzyme potency and cellular activity are disclosed.

COT/Tpl2/MAP3K8 is a mitogen-activated serine/threonine kinase kinase kinase (MAP3K) that is essential for lipopolysaccharide (LPS)-induced activation of the ERK MAPK cascade in macrophages.[1] COT kinase activation is believed to play an integral role in the production of pro-inflammatory cytokines such as TNF and IL-1β.[2] Elevated levels of these cytokines have been clinically implicated as mediators of a number of autoimmune diseases,[3] in particular, the pain and joint destruction characteristic of rheumatoid arthritis. By inference, pharmaceutical agents that inhibit COT kinase have the potential to be a novel and effective therapy for the treatment of a range of immunological diseases, such as rheumatoid arthritis. To date, only 1,7-naphthyridine-3-carbonitriles[4] and quinoline-3-carbonitriles[5] have been reported as COT kinase inhibitors. Herein, we report a new class of potent COT inhibitors based on a thieno[2,3-*c*]pyridine core.



1

Our initial hit came from a high throughput screen of the Abbott compound collection using a ^{33}P gel-permeation assay in the presence of 30 μM ATP. The resulting thieno[2,3-*c*]pyridine, **1,** was selected based upon its moderate potency for the COT HTRF kinase assay (IC_{50} = 1 μM),[6] good selectivity across a panel of 15 kinases (representing all branches of the human kinome), and its concurrence with internal hit criteria. Interestingly, **1** had been identified internally as a member of a series that inhibited TNF stimulated ICAM-1 and E-selectin expression; however, the specific molecular target was unknown.[7]

While COT has a low homology to other kinases, providing a unique opportunity to achieve selectivity across the kinome, it also presented a challenge in terms of applying structure-based drug design techniques. To date, no crystal structure of this protein is known. The optimization of **1** focused primarily upon SAR exploration of the 2- and 4-substituents of the thieno[2,3-*c*]pyridine core based on modeling hypotheses. In this letter, we describe the optimization of these thienopyridines that resulted in the identification of cell-active inhibitors of COT.

Preparation of the versatile intermediates **3–6** was carried out from commercially available 3,5-dibromo-4-pyridine carboxaldehyde (Scheme 1). Cyclization of aldehyde **2** with methyl thioglycolate or 2-mercaptoacetamide provided either the ester **3**[7] or primary amide **4**, respectively. The tetrazole moiety **6** was introduced by dehydration of the amide to the nitrile **5** and by subsequent reaction with sodium azide.

Functionalization of the thienopyridines at the 4-position provided access to a wide variety of substituted aryl ether analogs. This transformation was accomplished via an Ulmann

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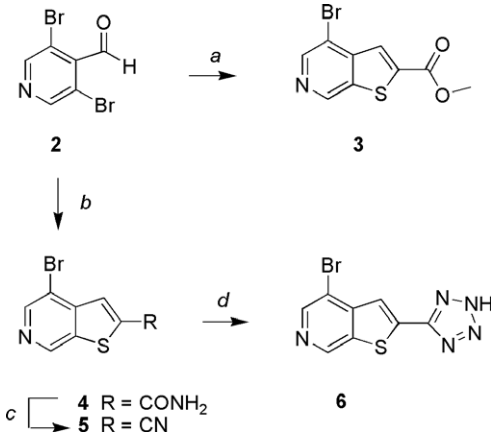


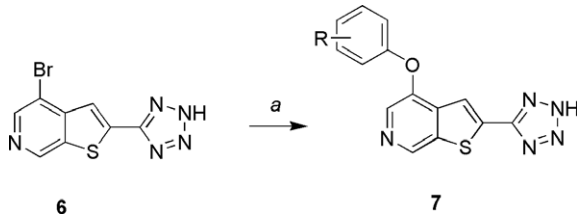
Scheme 1. Reagents and conditions: (a) $HSCH_2CO_2CH_3$, Cs_2CO_3, THF, 60 °C, 2 h; (b) $HSCH_2CONH_2$, Cs_2CO_3, THF, 60 °C, 2 h; (c) TFAA, pyridine, rt, 6 h; (d) 1.3 equiv NaN_3, 1.3 equiv NH_4Cl, DMF, 80 °C, 4–18 h.

coupling[8] of bromide **6** with substituted phenols, as shown in Scheme 2.

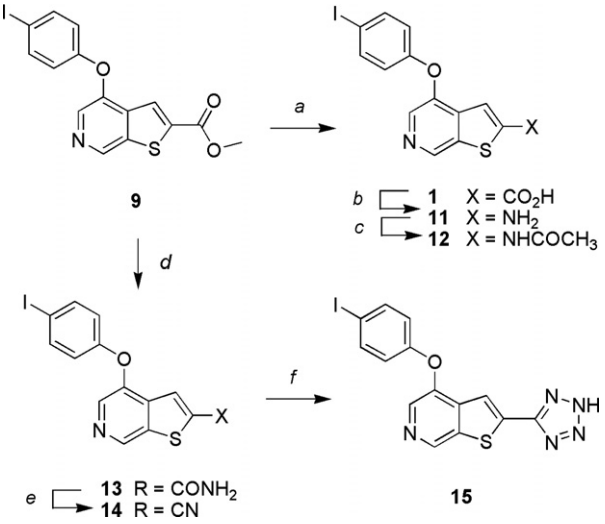
An alternate route was utilized to access halogen-substituted aryl ether moieties off C4 of the thienopyridine core. Treatment of 3,5-dichloro-4-pyridine carboxaldehyde with a substituted phenol and subsequent reaction with methyl thioglycolate provided direct access to phenoxy compound **9** (Scheme 3). Suzuki coupling of the aryl halide with boronic acids generated biphenyl-substituted analogs **10**.

Final product elaboration to the acid **1** was accomplished by ester hydrolysis. Curtius rearrangement of the acid provided the C2 amine **11** which could be further elaborated by acylation as depicted in Scheme 4. The corresponding nitrile **14** was prepared by treatment of ester **9** with ammonia to generate amide **13** followed by dehydration. Reaction with sodium azide generated tetrazole **15**.

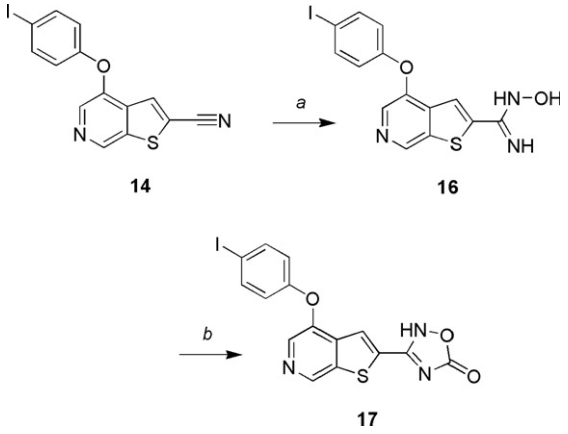
Alternatively, oxazolidinone **17** was synthesized via reaction of hydroxylamine hydrochloride with nitrile **14** followed by cyclization in the presence of CDI (Scheme 5). Reaction of acid chloride



Scheme 2. Reagents and conditions: (a) RPhOH, Cu(I)Cl, Cs_2CO_3, 2,2,6,6-tetramethyl-3,5-heptanedione, NMP, 220 °C, microwave, 10 min.



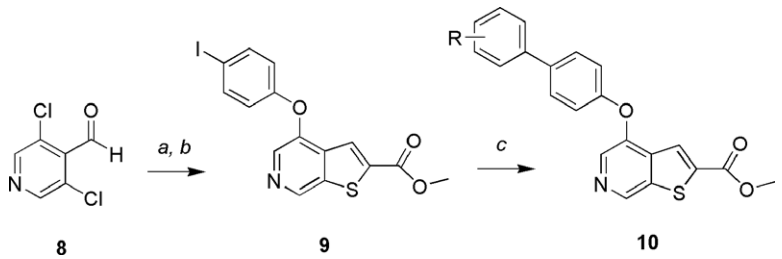
Scheme 4. Reagents and conditions: (a) NaOH, water, dioxane, 100 °C, 1 h; (b) $(PhO)_2OPN_3$, Et_3N, ^tBuOH, reflux, 16 h; (c) Ac_2O, $EtPr_2N$, THF, rt, 16 h; (d) NH_3, MeOH, sealed tube, 100 °C, 1 h; (e) TFAA, pyridine, rt, 6 h; (f) 1.3 equiv NaN_3, 1.3 equiv NH_4Cl, DMF, 80 °C, 4–18 h.



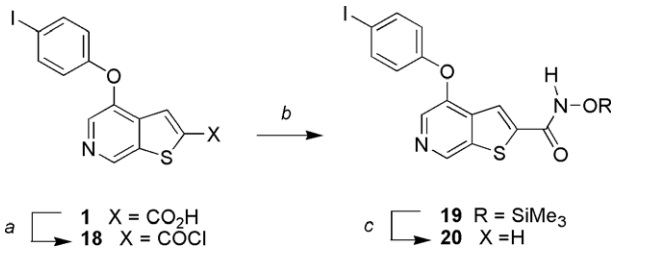
Scheme 5. Reagents and conditions: (a) NH_2OH-HCl, *i*-Pr_2EtN, DMSO, 70 °C, 1.5 h; (b) CDI, DMF, 100 °C, 1.5 h.

18 with trimethylsilyl hydroxylamine and subsequent liberation of the silyl group during reverse-phase (RP) HPLC purification generated the hydroxamic acid **20** (Scheme 6).

Our initial binding mode hypothesis for **1** (Fig. 1) had the 6-N of the thieno[2,3-*c*]pyridine core bound to the hinge of COT kinase (Gly 210), thus presenting the carboxylic acid toward the water-accessible ribose pocket and the 4-substituent in a hydrophobic domain abutting the gatekeeper residue (Met 207). In order to test



Scheme 3. Reagents and conditions: (a) 4-I-PhOH, KO^tBu, THF, rt, 16 h; (b) $HSCH_2CO_2CH_3$, Cs_2CO_3, THF, 60 °C, 2 h; (c) $RPhB(OH)_2$, $Pd(PPh_3)_4$, Cs_2CO_3, DME/EtOH/H_2O, 135 °C, microwave, 6 min.



Scheme 6. Reagents and conditions: (a) $(COCl)_2$, CH_2Cl_2, cat DMF, rt, 1 h; (b) Me_3SiONH_2, CH_2Cl_2, 60 h; (c) RP-HPLC.

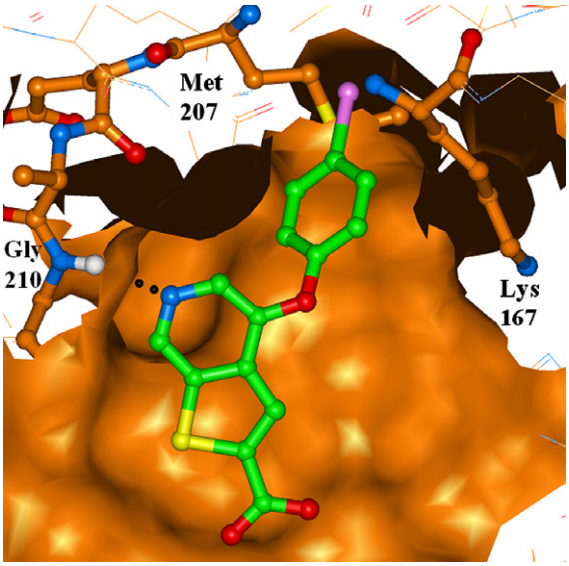
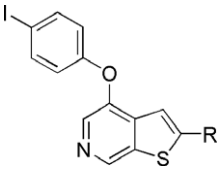


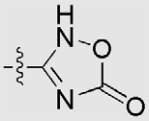
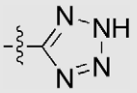
Figure 1. Model of **1** bound to homology model of COT/Tpl-2 kinase. A hydrogen bond between the ligand and the backbone NH of Gly 210 (3.1 Å) is shown as a black dotted line.

this proposal, we initially evaluated the properties of the presumably solvent exposed 2-substituent and its impact on enzyme potency (Table 1). Compounds were evaluated for inhibition of COT kinase activity using an HTRF-based assay.[6] The neutral 2-amino analogs **11** and **12** were inactive against COT; however, replacement of the acid group with small acidic heterocycles[9] was tolerated. For example, the oxazolidinone (estimated pK_a ~6.1) **17** and tetrazole (estimated pK_a ~5.3) **15** maintained potency similar to that of the carboxylic acid **1.** The hydroxamic acid moiety in **20** (estimated pK_a ~6.9) also served as an equitable replacement of the carboxylic acid.

Optimization of the 4-position of the thieno[2,3-*c*]pyridine focused on a range of hydrophobic residues. To the best of our knowledge, no kinase inhibitors have successfully circumvented a bulky methionine gatekeeper to access the rear 'hydrophobic' selectivity pocket, hence we focused primarily on optimizing the van der Waal interactions between the 4-substituent and the Gly-rich loop. For comparative purposes, the tetrazole moiety was held constant at the 2-position, and initially a range of substituted phenoxy analogs were prepared (Table 2). The unsubstituted phenoxy derivative **21** was less potent than the lead compound **1**. Preparation of the 2-, 3-, and 4-phenyl-substituted analogs (**22–24**) established the preference for substitution at the 4-position. Small lipophilic acyclic substituents were also examined in this region. Compared to the 4-Ph derivative **24**, the 4-Me and 4-Br analogs **25** and **26** had significantly reduced potency. Interestingly, both

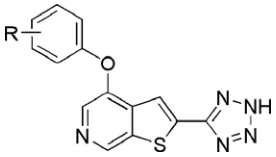
Table 1
Inhibition of COT kinase by representative C-2 analogs



Compound	R	COT HTRF IC_{50}, μM[a]
1	$-CO_2H$	0.94
11	$-NH_2$	>50
12	$-NHCOCH_3$	>50
17		1.12
15		0.41
20	–CONHOH	0.54

[a] See Ref. 6 for assay conditions.

Table 2
Inhibition of COT kinase by selected C-4 analogs



Compound	R	COT HTRF IC_{50}, μM[a]
21	H	2.2
22	2-Ph	15
23	3-Ph	1.7
24	4-Ph	0.15
25	4-Me	7.5
26	4-Br	1.6
27	4-Cl	0.71
28	4-^{t}Bu	0.61

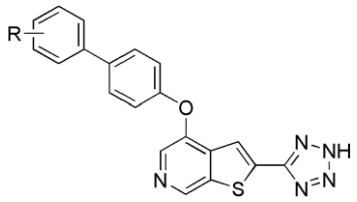
[a] See Ref. 6 for assay conditions.

the 4-Cl and 4-^{t}Bu analogs (**27**, **28**) were moderately active but still less potent than **24**.

Since the effort to replace the distal phenyl group failed to maintain or improve potency, the biphenyl moiety was retained within the core pharmacophore and further optimization was focused on the substitution pattern of the distal ring (Table 3). In general, substitution at the 4-position (**31**, **34–41**) afforded analogs with a trend toward greater potency compared to those substituted at either the 2- or 3-positions (comparative groupings are **29–31** as well as **32–34**). Both electron-donating and electron-withdrawing groups were tolerated. Of the substituents evaluated at the 4-position of the distal phenyl ring, the analogs that provided a marked improvement in potency were those capable of forming H-bonds with the protein, such as **39–41**. Since the primary amide showed improved potency over the *N,N*-dimethyl derivative, we speculate that both an H-bond donor and an H-bond acceptor are required for optimal potency.

A proposed model for an alternative binding mode of compound **1** bound to COT kinase is shown in Figure 2. The inhibitor was docked manually to optimize hydrogen bonds between the ligand and both the hinge and salt-bridge Lys residue within the ATP-

Table 3
Inhibition of COT kinase by selected C-4 analogs



Compound	R	COT IC_{50}, μM[a]	MEK IC_{50}, μM[b]	ERK IC_{50}, μM[b]	COT cell IC_{50}, μM[b]
24	H	0.15	9.7	12	4.3
29	2-OMe	0.32	21.8		
30	3-OMe	0.24	23		
31	4-OMe	0.13	49		
32	2-Cl	0.15	1.5	>50	
33	3-Cl	0.12	1.9	>50	
34	4-Cl	0.09	2.4	>50	2.1
35	4-CN	0.31	21.2	47	
36	4-F	0.17	7.6	11.3	6.5
37	4-NH_2	0.23	1.5	1.1	
38	4-CF_3	0.28	1.8	1.7	0.56
39	4-$CONMe_2$	0.05	48	>50	
40	4-CH_2OH	0.04	14		
41	4-$CONH_2$	0.01	18		0.13

[a] See Ref. 6 for assay conditions.
[b] See Ref. 10 for assay conditions.

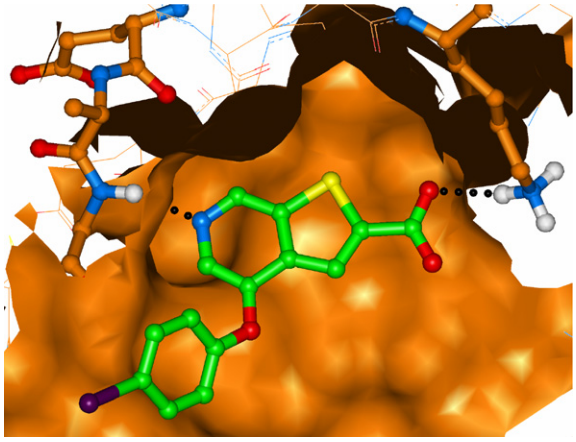


Figure 2. Model of **1** bound to homology model of COT/Tpl-2 kinase.

binding site. The aryl-oxy unit at the 4-position of the thienopyridyl core ring projects along the protein surface and has some exposure to solvent, in accord with the SAR in which a wide variety of substituents gave rise to active analogs. We speculate that the high activity of compounds **39**–**41** was due to polar interactions in this extended hinge region, but the exact binding mode and interactions will need confirmation by crystallographic analysis of an inhibitor bound to the enzyme.

Several compounds were evaluated for selectivity over MEK and ERK, two kinases downstream of COT in its pathway.[10] In general, the thienopyridines showed good selectivity over these kinases, suggesting that cell potency is due to the inhibition of COT (Table 3).

Selected compounds were profiled in a cellular activity assay measuring the inhibition of pERK production in murine peritoneal exudates cells (PECs) stimulated with lipopolysaccharide (LPS), Table 3.[10] Parent compound **24** demonstrated low micromolar potency in agreement with enzyme data. This was also the case for the 4-Cl and 4-F congeners **34** and **36**. However, the 4-CF_3-substituted analog **38** showed approximately a 10-fold increase in cellular potency, possibly indicating better cell penetration or reduced plasma protein binding. Further improvement with the 4-carboxamide (**41**) was observed, again in line with enzyme data.

In summary, we have described a novel series of substituted thieno[2,3-*c*]pyridines as potent COT enzyme inhibitors. Further profiling and optimization of this chemotype will be the discussion of a subsequent publication.

Acknowledgment

The authors thank Kent Stewart for helpful discussions about the COT model.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.08.037.

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10. Details of the MEK1 cascade HTRF assay, the ERK HTRF assays, and the COT cellular assays are provided in the supporting information.