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# Identification and SAR of a new series of thieno[3,2-*d*]pyrimidines as Tpl2 kinase inhibitors

Yike Ni [a,*], Ariamala Gopalsamy [a], Derek Cole [b], Yonghan Hu [a], Rajiah Denny [a], Manus Ipek [a], Julie Liu [c], Julie Lee [c], J. Perry Hall [c], Michael Luong [c], Jean-Baptiste Telliez [c], Lih-Ling Lin [c]

[a] *Medicinal Chemistry, Pfizer, 200 Cambridge Park Drive, Cambridge, MA 02140, USA*
[b] *Medicinal Chemistry, Takeda San Diego, 10410 Science Center Drive, San Diego, CA 92121, USA*
[c] *Inflammation and Immunology, Pfizer, 200 Cambridge Park Drive, Cambridge, MA 02140, USA*

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ABSTRACT

We report here the synthesis and SAR of a new series of thieno[3,2-*d*]pyrimidines as potent Tpl2 kinase inhibitors. The proposed binding mode suggests the potential flipped binding mode depending on the substitution. Biacore studies show evidence of binding of these molecules to the protein kinase. The kinome inhibition profile of these molecules suggests good selectivity.



Tpl2 (Tumor Progression Locus-2) kinase is a serine/threonine kinase in the MAP3K family that activates the MEK-ERK pathway leading to the production of TNF-α and type 1 interferon.[1] Tpl2 was also shown to play a role in TNF-α signaling of TNF-α.[2] It has been well documented that TNF-α, a pro-inflammatory cytokine, is involved in the initiation and progression of inflammatory diseases such as rheumatoid arthritis (RA).[3] Tpl2 knock-out mice show reduced lipopolysaccharide (LPS)-induced TNF production.[4] Preliminary studies also demonstrated that Tpl2 knock-out mice show reduced disease severity in the murine collagen-induced arthritis (CIA) model.[5] Therefore, inhibition of Tpl2 kinase has the potential to be a novel and effective therapy for the treatment of RA and other human inflammatory diseases.

Considering that several mitogen-activated protein kinase (MAP kinase) inhibitors have advanced to clinical trials, the studies of Tpl2 inhibition are still their infancy giving the fact that to date only a handful of small molecule inhibitors of Tpl2 have been documented (Fig. 1). 1,7-Naphthyridine-3-carbonitriles **1** and quinoline-3-carbonitrile **2** are the earliest classes of compounds reported as reversible and ATP-competitive Tpl2 kinase inhibitors.[6] These Tpl2 inhibitors demonstrate a good correlation between cell-free Tpl2 inhibition and *p*-MEK and TNF inhibition in human monocytes, establishing a role of Tpl2 in TNF production in these cells. Thieno[2,3-*c*]pyridines **3** and **4** are the other two classes of Tpl2 inhibitors that were disclosed recently.[7]

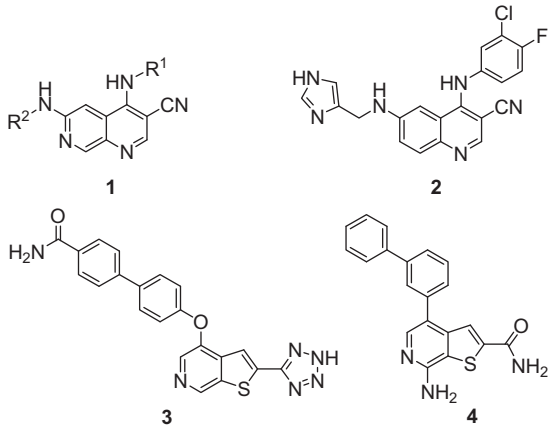


**Figure 1.** Literature reported Tpl2 inhibitors.

Tpl2 remains a challenging target for structure-based or in silico based drug design as no crystal structure of Tpl2 has been reported to date. It has been hypothesized that the difficulty of obtaining a crystal structure could be due to the instability of the active free form which is rapidly degraded in vitro.[7a] Albeit the lacking of crystal structure support, the low homology of Tpl2 to other kinases brings a unique opportunity to achieve selectivity across the kinome. We describe herein the identification and structure-activity relationship (SAR) of a new thieno[3,2-*d*]pyrimidine series

* Corresponding author. Tel.: +1 617 665 5673; fax: +1 617 665 5682.
*E-mail address:* yike.ni@pfizer.com (Y. Ni).

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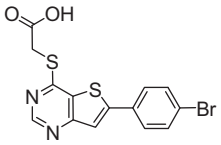


5

**Figure 2.** High throughput screening (HTS) hit.

**Table 1**
Profile of **5**.

| | |
|---|---|
| TPL2 $IC_{50}$ (μM) | 4.8 |
| MW | 381 |
| TPSA | 62 |
| *c* log *P* | 4.4 |
| Ligand efficiency | 0.35 |
| Aqueous solubility (μg/ml at pH 7.4) | >100 |
| Rat liver microsome stability (minute) | >30 |

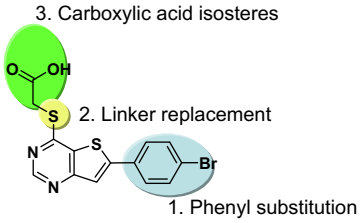
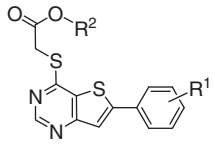



**Figure 3.** Medicinal chemistry strategy.

**Table 2**
SAR of phenyl substitution on inhibition of Tpl2 activity



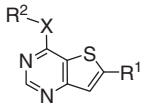
| Compound | $R^1$ | $R^2$ | Tpl2 $IC_{50}$ (μM) |
|---|---|---|---|
| 6 | 4-F | H | 8.1 |
| **7** | 4-Cl | H | 1.2 |
| **8** | 4-Me | H | 1.7 |
| **9** | 3-Me | H | 3.5 |
| **10** | 4-CN | H | 1.1 |
| **11** | 4-OMe | H | 1.7 |
| **12** | 4-SMe | H | 34 |
| **13** | 4-Ph | H | >100 |
| **14** | 4-$CONH_2$ | H | 1.5 |
| **15** | 3-$CONH_2$ | H | 7.1 |
| **16** | 4-CONHMe | H | >100 |
| **17** | 4-NHCOMe | H | 0.18 |
| **18** | 4-Cl | Et | >100 |

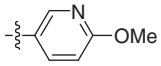
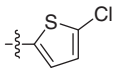
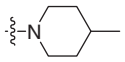
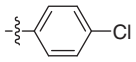
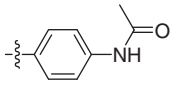
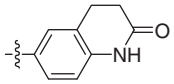
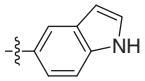
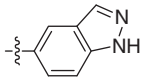
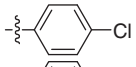
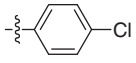
as Tpl2 inhibitors following traditional SAR approach and molecular modeling studies.

Thienopyrimidine **5** (Fig. 2, Tpl2 $IC_{50}$ = 4.8 μM) was identified as our lead compound following the high through-put screening of a compound library. Initial profile revealed that this compound has low micromolar Tpl2 potency, decent calculated properties and very good aqueous solubility (Table 1). Initial ADME profile of **5** also showed it is stable in rat liver microsomes.

As demonstrated in Figure 3, our medicinal chemistry strategy included (1) exploration of substitution on the phenyl ring, (2) replacement of the sulfur linker to eliminate potential side effect such as oxidation of the sulfur associated with the aryl thioether, and (3) identification of an optimal isostere of the carboxylic acid which has potential permeability issue.

**Table 3**
Substitutent pattern and inhibition of Tpl2 activity



| Compound | $R^1$ | X | $R^2$ | Tpl2 $IC_{50}$ (μM) |
|---|---|---|---|---|
| **19** | I | S | $CH_2CO_2Et$ | 5.1 |
| **20** | I | S | $CH_2CO_2H$ | 18 |
| **21** |  | S | $CH_2CO_2H$ | 22 |
| **22** |  | S | $CH_2CO_2H$ | >100 |
| **23** |  | S | $CH_2CO_2H$ | >100 |
| **24** |  | O | $CH_2CO_2H$ | 34 |
| **25** |  | O | $CH_2CO_2H$ | 10 |
| **26** |  | O | $CH_2CO_2H$ | 6.2 |
| **27** |  | O | $CH_2CO_2H$ | 2.3 |
| **28** |  | O | $CH_2CO_2H$ | 1.0 |
| **29** |  | NH | $CH_2CO_2H$ | >100 |
| **30** |  | NMe | $CH_2CO_2H$ | >100 |

Initial SAR of phenyl substitution indicated that relatively small groups on the phenyl ring can be tolerated (Table 2, compounds **6**–**11**). Large substitution in the $R^1$ position like –SMe, phenyl led to a dramatic loss of potency (compounds **12**, **13**). Interestingly, more polar hydrogen-bond donor groups, such as amide, directly attached to the phenyl ring also resulted in good potency (compound **14**). Our docking experiments suggest that these hydrogen-bond donor groups make a hydrogen-bond interaction with the Asp carboxylic acid residue in the DFG motif. However, hydrogen-bond acceptor or other polar groups may lead to steric or electrostatic clashes with the side chain (data not shown). Moving the substitution from 4- to 3-position resulted in a similar level or slight loss of potency (**9** vs **8**, **15** vs **14**). It is unclear at this stage why the methyl substituted amide resulted in compound **16** being inactive. The reversed amide **17** provided the highest potency in this series with a Tpl2 $IC_{50}$ of 0.18 μM. Replacement of the carboxylic acid with ester proved to be unfruitful as evidenced by the loss of activity of compound **18** when compared with compound **7**.

The two iodo intermediates **19** and **20** used for preparing the final targets were also tested in the enzymatic assay. It is interesting to see that the ester **19** is three fold more potent than the acid **20** (Table 3), unlike the activity relationship between **7** and **18**. Given the difficulty of obtaining a crystal structure of Tpl2, we used a homology model to gain some insight into the SAR and guide our

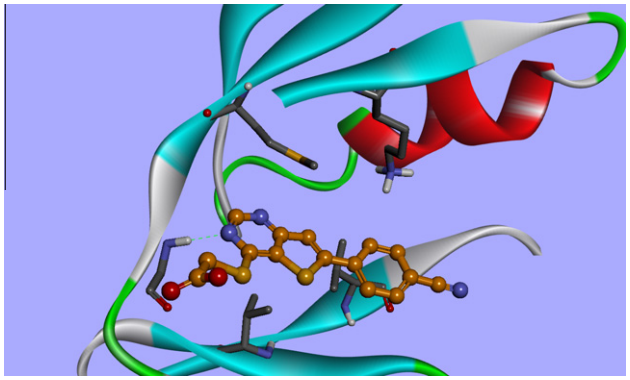
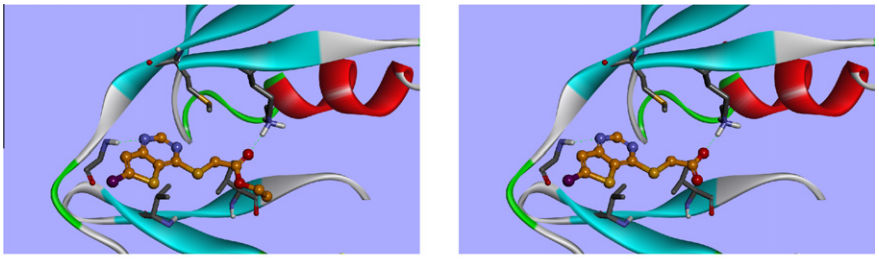


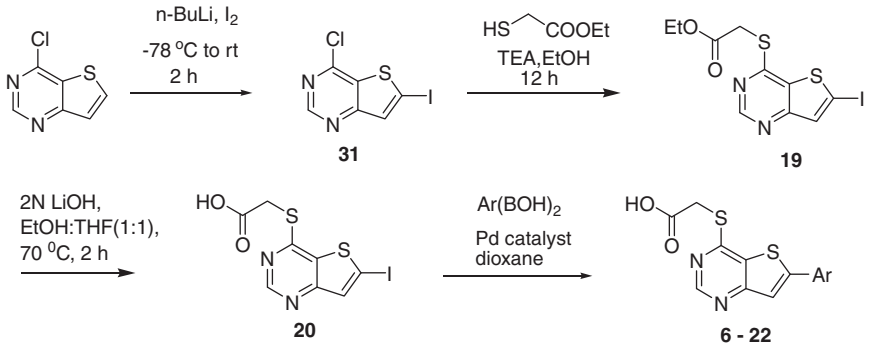
**Figure 4.** Model of **10** bound to homology model of Tpl2.

medicinal chemistry effort. Two binding modes were proposed in the literature for the thienopyridine series.[7] Based on the two proposed binding modes, it was hypothesized that the majority of targets **6**–**18** adopted the binding mode showed in Figure 4 in which the 3-N of the thieno[3,2-*d*]pyrimidine binds to the hinge region. The carboxylic acid chain extends to the solvent pocket and the substituted phenyl ring points to the Lys region. In contrast, when the phenyl ring was replaced with iodo, the binding mode could be flipped as indicated in Figure 5 where the 1-N of the thieno[3,2-*d*]pyrimidine interacts with the hinge NH (Ala), iodo on the thiophene ring points to the solvent pocket and the polar chain extends to the Lys region. In this case, the relative hydrophobic pocket near the Lys residue would favor the ester instead of the free carboxylic acid which is supported by the enzymatic potency of compounds **19** and **20**.

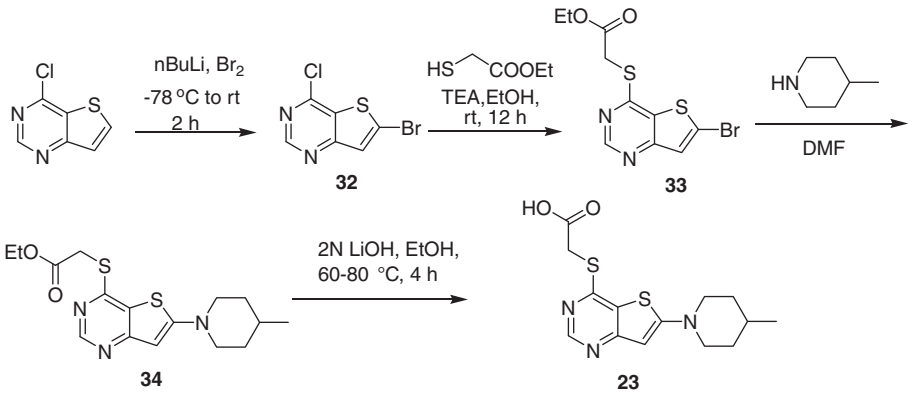


Compound **19**, Tpl2 $IC_{50}$ 5.1 μM Compound **20**, Tpl2 $IC_{50}$ 18 μM

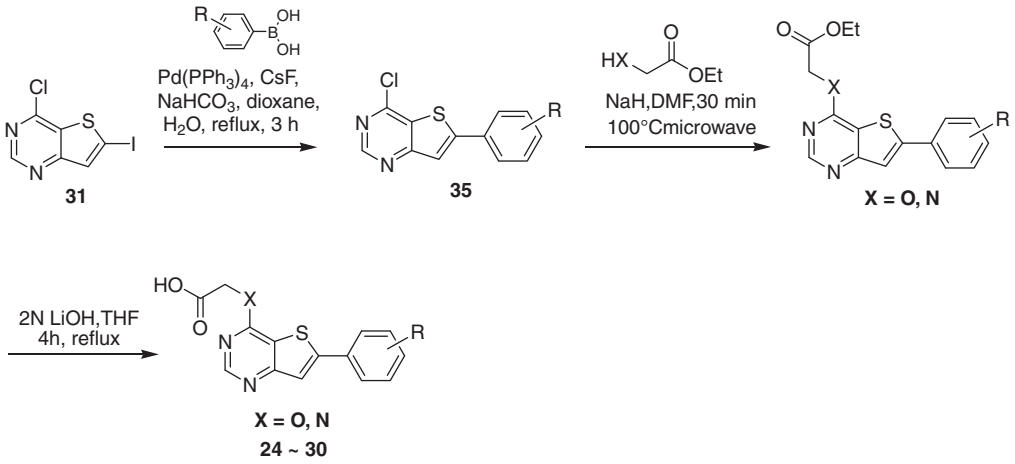
**Figure 5.** Flipped binding mode depending on the substitution.




**Scheme 1.** Synthesis aryl or heteroaryl substituted thienopyrimidines.




**Scheme 2.** Synthesis cyclic amino analog **23**.




**Scheme 3.** Synthesis of oxygen- and nitrogen-linker analogs.

It is of interest to investigate the SAR of different heterocycles in addition to the phenyl analogs. Unfortunately, as demonstrated in Table 3, replacement of the phenyl portion with various heterocycles resulted in significant loss of potency (compounds **21** and **22**).[8] Cyclic amines were also used as surrogates to the phenyl ring and all of these amino analogs led to a loss of activity (compound **23**). Given the potential side effect of the sulfur linker, we next turned out attention to replacing it with an oxygen linker. Although the *para*-chlorophenyl analogs with oxygen linker was much less potent than with sulfur linker (compound **24** vs **7**), the reversed amide analog **25** delivered improved potency with the new linker. The *N*-acetylaniline fragment in **25** would trigger the aniline structural alert,[9] which led us to modify this fragment. We were delighted to observe that modification to rigidify the *N*-acetylaniline forming a bicycle further improved the potency toward Tpl2 (compounds **26**–**28**). The indazole analog **28** proved to be the most potent compound bearing the oxygen linker with a Tpl2 $IC_{50}$ of 1.0 μM. Efforts trying to identify an optimal bioisosteres for the carboxylic acid were unfruitful. Analogs bearing ester, amide or heterocycles instead of the free carboxylic acid all turned out to be inactive (data not shown).[10] Replacement of the sulfur linker with nitrogen linker was also tried. All analogs bearing the nitrogen linkers proved to be inactive indicating electronic conflict in the linker region with more basic nitrogen (compounds **29**–**30**).

The general synthesis of the analogs **6**–**22** is highlighted in Scheme 1. Treatment of the commercially available 4-chlorothienopyrimidine with *n*-butyllithium followed by iodination provided 4-chloro-6-iodothieno[3,2-*d*]pyrimidine **31**, which reacted with ethyl 2-mercaptoacetate to give analog **19**. Hydrolysis of **19** afforded acid **20**, which coupled with various boronic acids or boronic esters provided the desired analogs.

A typical synthesis of cyclic amine substituted thienopyrimidines was demonstrated in Scheme 2. Failure of coupling between the iodo analog **19** and secondary amines led us to switch to the

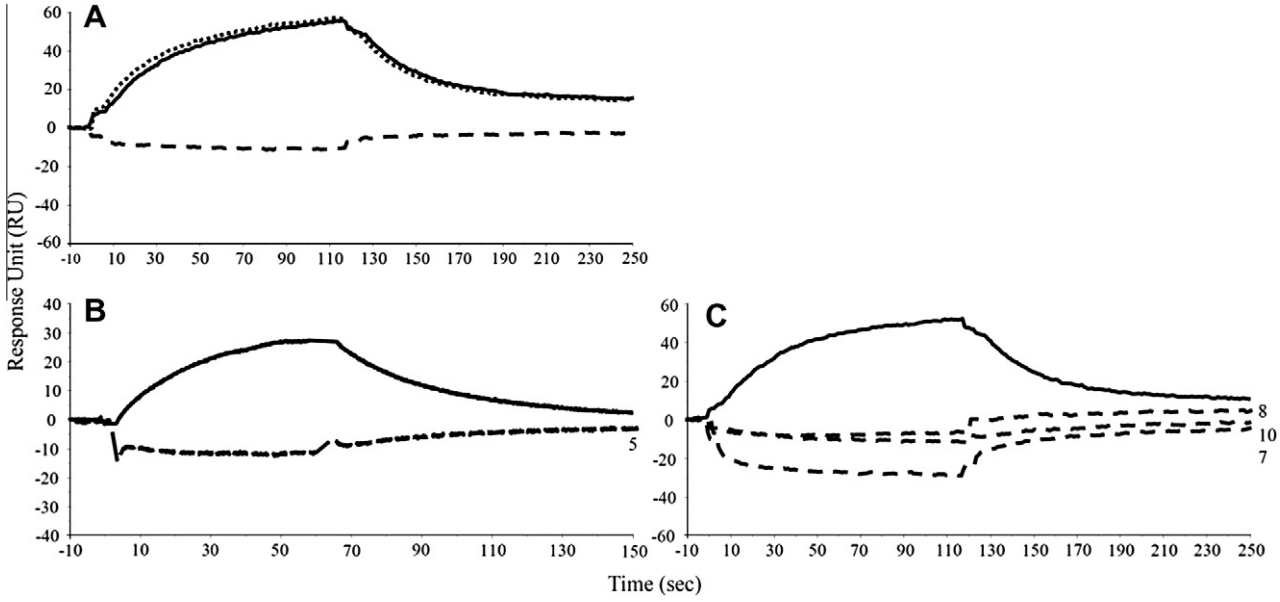



**Figure 6.** In solution competitive binding assay. Tpl2 (50 nM) was either injected alone (solid black lines) or in the presence of excess compound (50 μM, dashed lines). (A) Tpl2 competition in the presence of the reference compound (dashed line, positive control). Tpl2 binding in the presence of Staurosporine (dotted line, negative control). (B) Compound **5** and (C) compounds 7, 8 and 10 prevented Tpl2 binding to the reference compound surface. Data shown for each compound are representative of at least three independent surfaces where the same Tpl2 binding or competition was observed.

**Table 4**
Kinase selectivity profile (%inhibition at 1 μM)

| Kinase | CDK2 cyclinA | Aurora A | CHK1 | CHK2 | SRC | EGFR | FGFR1 | GSK3B | JAK3 | LCK |
|---|---|---|---|---|---|---|---|---|---|---|
| 17 | 46.8 | 50.5 | −0.8 | 10.8 | 17.0 | −3.8 | 11.0 | 50.5 | 43.3 | 13.8 |
| 28 | 27.3 | 88.5 | −7.8 | −2.3 | 29.0 | −1.3 | 3.3 | 56.5 | 29.5 | 6.0 |
| Kinase | MK2 | MARK1 | MET | MST2 | NEK2 | PAK4 | PKA | ROCK1 | CK1a1 | P38 |
| 17 | 3.3 | 23.0 | 9.8 | 27.3 | 3.3 | 11.0 | −3.3 | 17.0 | −1.3 | 5.3 |
| 28 | 25.3 | 1.3 | 21.8 | −3.3 | −3.5 | −9.0 | 6.8 | 26.0 | 4.5 | −0.8 |

use of bromo intermediate **33** which was synthesized from **32** following the same sequence as in Scheme 1. Coupling of **33** to 4-methylpiperidine led to the desired product **34** which was hydrolyzed to provide analog **23**.

To synthesize the oxygen- or nitrogen-linker analogs, intermediate **31** was coupled with corresponding boronic acids or boronic esters to give **35**, which reacted with 2-hydroxyl or 2-amino acetate, followed by hydrolysis to provide the desired products **24**–**30** (Scheme 3).

Several compounds (compounds **5**, **7**, **8**, **10**) were selected to investigate their binding to Tpl2. A Biacore-based competitive binding assay consisted of a reference compound immobilized on a sensor chip surface (250–470 RU) by amine coupling to measure Tpl2 binding in the presence or absence of compound. Tpl2 binds the reference compound as shown in Figure 6, solid lines by the increase in RU during the injection time of 2 min (Fig. 6A and C) or 1 min. (Fig. 6B). In the presence of excess soluble reference compound, no Tpl2 binding was observed (Fig. 6A, dashed line). As expected, Staurosporine (Fig. 6A, dotted line) did not compete for the binding of Tpl2. In the presence of compound **5** (Fig. 6B) or compounds **7**, **8** or **10** (Fig. 6C) no Tpl2 binding was observed. Thus, the compounds **5**, **7**, **8** and **10** bind Tpl2 and prevent Tpl2 binding to the reference compound surface. However, initial test of these compounds in cell assay (human monocyte) measuring the inhibition of phosphorylation of ERK did not show measurable inhibition, which might be due to the poor permeability associated with the carboxylic acids.

Compounds **17** and **28** were selected for kinase selectivity profiling with an invitrogen panel of kinases. As shown in Table 4, these compounds showed an overall good selectivity profile. Of the 39 kinases tested, only one kinase (Aurora A) showed >60% inhibition.

In summary, a new series of thieno[3,2-*d*]pyrimidines has been identified as potent and selective Tpl2 kinase inhibitors following traditional SAR approach and molecular modeling studies. The most potent compound was found to have a Tpl2 $IC_{50}$ of 0.18 μM. Proposed binding mode suggested that the binding mode could be flipped depending on the substitution. Primarily the carboxylic acid chain extends to the solvent pocket and the substituted phenyl ring points to the Lys region to engage interactions in the hydrophobic pocket. Biacore studies showed evidence of these molecules binding to the protein.

## Acknowledgment

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8. Several other heterocycles such as 3-pyridyl, 4-chloro-3-pyridyl, 5-methylthiophenyl, 3,5-dimethylisoxazol analogs were also prepared, but all resulted in the loss of activity (Tpl2 $IC_{50}$ >100 μM).
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