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Quinoline and thiazolopyridine allosteric inhibitors of MALT1

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ABSTRACT

Quinolines and thiazolopyridines were developed as allosteric inhibitors of MALT1, with good cellular potency and exquisite selectivity. Mouse pharmacokinetic (PK) profiling showed these to have low *in vivo* clearance, and moderate oral exposure. The thiazolopyridines were less lipophilic than the quinolines, and one thiazolopyridine example was active in our hIL10 mouse pharmacodynamic (PD) model upon oral dosing.

The paracaspase MALT1 is involved in the activation of the NF-κB signaling pathway through both its protease and scaffolding functions. MALT1 is considered to be a therapeutically relevant target for several B cell lymphomas, including ABC-DLBCL, MALT lymphoma and mantle cell lymphoma, and its role in autoimmune and inflammatory diseases is also being explored. The biological functions of MALT1 and the potential therapeutic value of MALT1 inhibitors have been reviewed recently.[1,2]

We previously described a series of covalent substrate-based inhibitors of MALT1, derived from a literature tool compound Z-VRPR-fmk.[3,4] Our substrate-based inhibitors contained a fluoromethyl ketone warhead, which formed a covalent bond to the active site cysteine residue. Compounds were quite selective across a panel of proteases, and examples had good anti-proliferative activity in a subset of ABC-DLBCL cell lines. The PK profile supported their use *in vivo* upon IV or IP dosing, and one representative example showed moderate efficacy in xenograft studies. This result, combined with the relatively rapid turnover of MALT1, suggested that optimal *in vivo* efficacy may require 24 h coverage of the target. We therefore chose to develop small molecule MALT1 inhibitors with increased potential for good oral PK.

Phenothiazines were reported as allosteric MALT1 inhibitors,[5] and a crystal structure of ligand-free dimeric MALT1 in complex with thioridazine was obtained.[6] The inhibitor was found to bind far from the active site in a hydrophobic pocket at the interface between the caspase domain and the Ig3 domain, and this prevented the conformational changes required to transform MALT1 into its active form. Other series of non-substrate-based MALT1 inhibitors have been reported in both the primary[7] and patent[8,9] literature, and given their structures, these are also likely to be allosteric inhibitors. We followed up on these reports to develop bis-aryl urea MALT1 inhibitors, with the objective of excellent protease selectivity, and the potential for sustained oral exposure. During the course of our work, a patent application containing thiazolopyridine MALT1 inhibitors similar to those described here was published.[10] Very recently, examples from the pyrazolopyrimidine urea series were confirmed to bind in the same allosteric pocket as the phenothiazines.[11]

We prepared a set of urea derivatives from commercially available mono- and bicyclic aryl acids and 1,2,3-triazolo-3-aminopyridine **3** (Scheme 1). The carboxylic acids were converted to isocyanates prior to coupling with aminopyridine **3**. We also prepared dimethylpyridine urea **6** from 3-amino-4,6-dimethylpyridine and **3**, activated as the phenyl carbamate. We retained nitrogen in the mono- and bicyclic rings at the *meta* position to the urea, with the assumption that it was critical for binding.

As reported previously, we used a GloSensor luciferase reporter assay, which measures cellular MALT1 protease activity as our primary screening assay.[4] Compounds were also tested in an OCI-Ly3 (a MALT1-dependent DLBCL cell line) proliferation assay. Cell data for the initial set of ureas is shown in Table 1. To benchmark the assays, we used compound **9** (example 10 from reference 8) as a positive control. Of the

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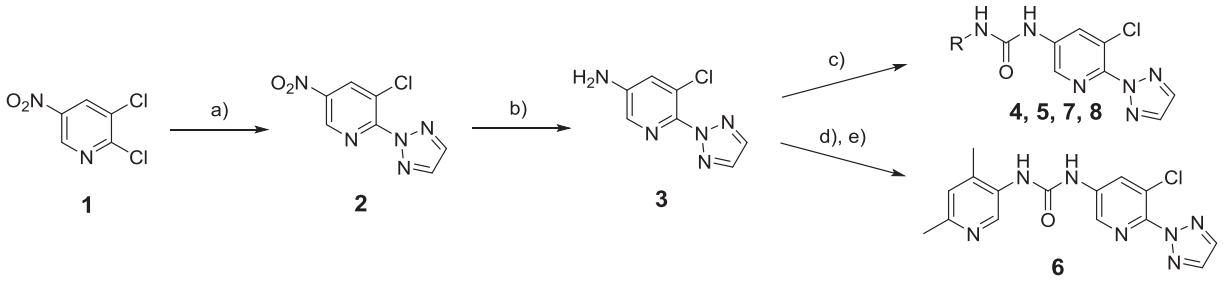
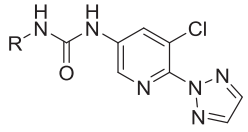


Scheme 1. Preparation of compounds **4–8**. Reagents and conditions: (a) 1,2,3-triazole, K_2CO_3, THF, 44%; (b) $SnCl_2$, c.HCl, EtOH, 97%; (c) RCO_2H, DPPA, Et_3N, dioxane, 80 °C, then amine 3, 100 °C, 9–46%; (d) phenyl chloroformate, pyridine, 0 °C – rt; (e) 3-amino-4,6-dimethylpyridine, Et_3N, DMF, 50 °C, 44% over two steps.

Table 1
MALT1 cell data for compounds **4–9**.



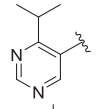
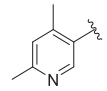
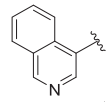
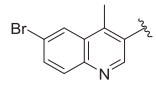
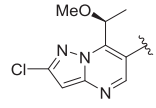
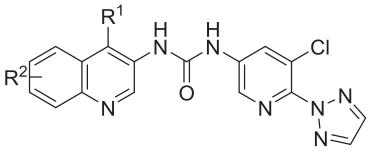
Example	R	GloSensor (μM)	OCI-Ly3 (μM)
4		> 20	> 20
5		1.98	> 20
6		19.3	> 20
7		6.02	> 20
8		0.16	12.9
9		0.006	0.12

Table 2
MALT1 cell data for quinolines **10–27**.

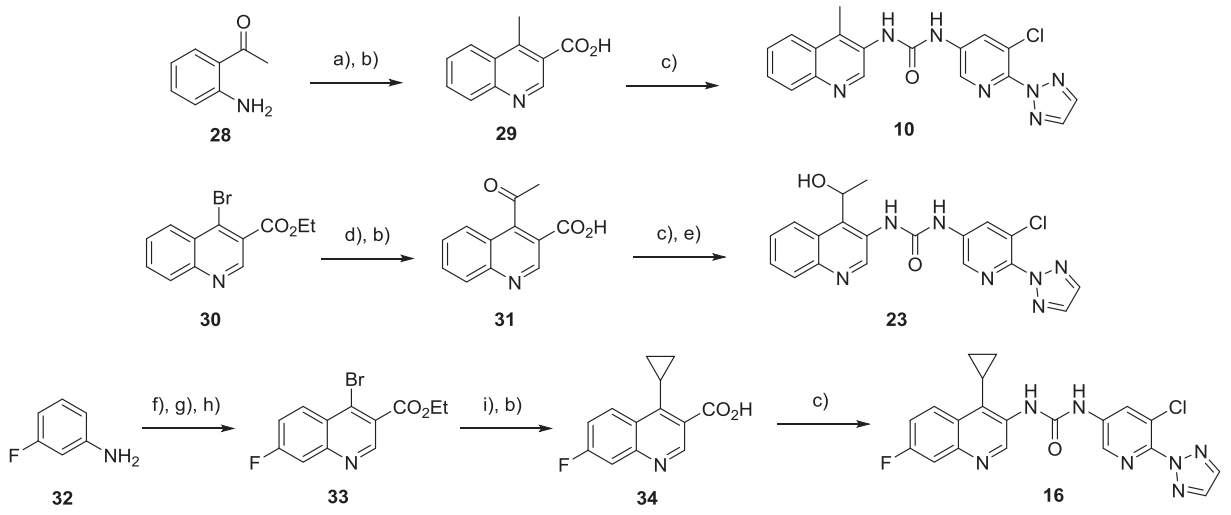


Example	R^1	R^2	GloSensor (μM)	OCI-Ly3 (μM)
10	Methyl	–	0.237	7.17
11	Isopropyl	–	0.078	2.42
12	Cyclopentyl	–	0.451	7.02
13	Cyclopropyl	–	0.007	1.58
14	Cyclopropyl	7-Cl	0.432	> 20
15	Cyclopropyl	6-Cl	0.002	0.99
16	Cyclopropyl	7-F	0.092	0.90
17	Cyclopropyl	6-F	0.006	0.72
18	Cyclopropyl	7-MeO	> 20	> 20
19	Cyclopropyl	6-MeO	0.013	2.39
20	1-Methoxyethyl	–	0.033	0.62
21	1-Ethoxyethyl	–	0.055	1.53
22	1-Methoxyisopropyl	–	0.165	4.43
23	1-Hydroxyethyl	–	0.028	0.73
24	1-Methoxyethyl	6-F	0.018	0.27
25	1-Ethoxyethyl	6-F	0.027	0.59
26	1-Methoxyisopropyl	6-F	0.035	0.41
27	1-Hydroxyethyl	6-F	0.007	0.56

monocyclic derivatives, only the isopropyl pyrimidine **5** was moderately active (IC_{50} 1.98 μM) in our GloSensor assay, however this did not translate into anti-proliferative effects. Quinoline **8** was more promising, with an IC_{50} of 0.16 μM in the GloSensor assay and some modest activity (EC_{50} 12.9 μM) in the OCI-Ly3 proliferation assay, and we focused our efforts on exploring the SAR in this series.

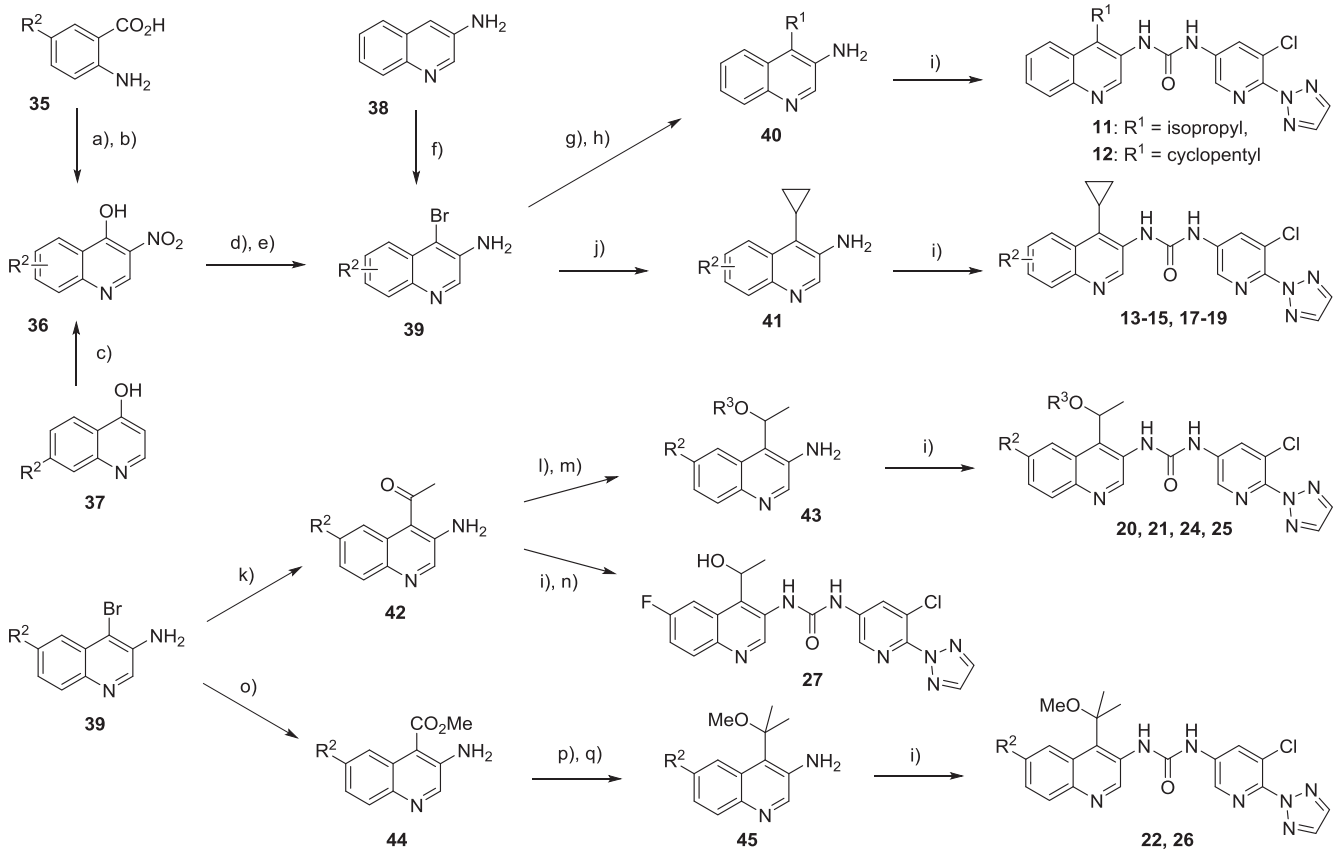
We first explored substituents at the quinoline 4-position. The 4-cyclopropyl compound **13** showed good potency in both cell assays, with an IC_{50} of 0.007 μM in the GloSensor assay, and an EC_{50} of 1.58 μM in the OCI-Ly3 proliferation assay. We retained the 4-cyclopropyl group to explore substituents at the 6- and 7-positions of the quinoline, while continuing to develop the SAR at the 4-position. The 6-substituted quinolines were potent in the GloSensor assay, with 6-F giving slightly better activity in the OCI-Ly3 assay (**17**, 0.72 μM) compared to 6-Cl (**15**, 1.0 μM) and 6-MeO (**19**, 2.39 μM). Only fluorine was well-tolerated at the 7-position. The 7-MeO compound **18** was inactive in both cell assays, implying limited space at this position of the molecule. At the quinoline 4-position, methoxyethyl (**20**) and hydroxyethyl (**23**) groups gave compounds with enhanced potency compared with the cyclopropyl quinoline **13** in the OCI-Ly3 assay. Compounds which combined the preferred 4-substituents with the 6-F quinoline core gave a further improvement in potency, with methyl ether **24** the most potent of these in the OCI-Ly3 assay (0.27 μM). Cell data for quinolines **10–27** is shown in Table 2.

Quinolines **10–27** were prepared from the corresponding quinoline acids (Scheme 2) or 3-aminoquinolines (Scheme 3). 4-Methyl quinoline acid **29** was prepared by treatment of 2-aminoacetophenone with methylpropiolate and copper(II) triflate to generate the quinoline ester, which was then hydrolyzed to the acid. The acid was converted to the isocyanate with DPPA and Et_3N, followed by addition of aminopyridine **3** to generate urea **10** (Scheme 2). Ureas **11–13** and **20–22** were prepared from the corresponding aminoquinolines **40**, **41**, **43** and **45** (Scheme 3). Bromination of 3-aminoquinoline with NBS gave **39**. Suzuki coupling of **39** with the isopropenyl and cyclopentenyl boronates followed by hydrogenation gave the 4-isopropyl and 4-cyclopentyl quinoline amines **40**, while reaction with cyclopropyl boronic acid gave **41**. Amines **40** and **41** were treated with the pyridine isocyanate **48** generated from the 1,2,3-triazolopyridine acid (Scheme 4) to give ureas **11–13**. Stille coupling of the bromoquinoline intermediate **39** with tributyl(1-ethoxyvinyl)tin followed by acidic work-up gave the ketone **42**. This was reduced to the alcohol with sodium borohydride, followed by alkylation with methyl or ethyl iodide to the ethers **43**, and converted to ureas **20** and **21** as before. Palladium-catalyzed carbonylation of **39** in methanol gave aminoester **44**, which was converted to the

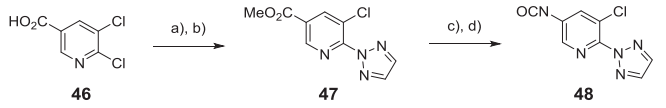


Scheme 2. Preparation of ureas via quinoline acids. Reagents and conditions: (a) methyl propiolate, $Cu(OTf)_2$, toluene, reflux, 9%; (b) LiOH, H_2O, THF, 67–73%; (c) DPPA, Et_3N, dioxane, rt then 80 °C, then amine **3**, 100 °C, 7–10%; (d) tributyl(1-ethoxyvinyl) stannane, $Pd(PPh_3)_2Cl_2$, DMF, then 2 N HCl, dioxane, 87%; (e) $NaBH_4$, MeOH, 42%; (f) diethyl ethoxymethylenemalonate, ethanol, reflux; (g) PPA, $POCl_3$, 75 °C, 18% over two steps; (h) $POBr_3$, $^iPr_2EtN$, CH_3CN, reflux, 73%; (i) cyclopropylboronic acid, Cs_2CO_3, $PdCl_2(dppf)$, dioxane:water 5:1, 80 °C, 54%.

tertiary alcohol with MeMgBr. Methylation and reaction with the isocyanate gave urea **22**. Preparation of alcohol **23** through coupling of the corresponding aminoquinoline alcohol and isocyanate **48** was complicated by an intramolecular reaction during the urea-formation step. Instead, **23** was prepared via conversion of the ketone acid **31** to the isocyanate for reaction with aminopyridine **3**, and a final reduction



Scheme 3. Preparation of ureas via 3-aminoquinolines. Reagents and conditions: (a) 2-nitroacetaldehyde oxime, c. HCl, H_2O, 78–99%; (b) K_2CO_3, Ac_2O, 90 °C, 39–60%; (c) c. HNO_3, propionic acid, 90 °C, 61–74%; (d) $POBr_3$, $^iPr_2EtN$, CH_3CN, reflux, 38–83%; (e) Fe, AcOH, 60 °C, 41–83%; (f) NBS, DMF, 72%; (g) 2-isopropenyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane or 2-cyclopentenyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, Cs_2CO_3, $Pd(dppf)Cl_2$, dioxane: water 4:1, 80 °C; (h) 10% Pd/C, EtOH, H_2, 37–63% over two steps; (i) isocyanate **48**, toluene or dioxane, 100 °C, 6–48%; (j) cyclopropylboronic acid, Cs_2CO_3, $Pd(dppf)Cl_2$, dioxane:water 5:1, 80 °C, 49–74%; (k) tributyl(1-ethoxyvinyl)stannane, $Pd(PPh_3)_4$, dioxane, 90 °C, then 2 N HCl, dioxane, 91–98%; (l) $NaBH_4$, MeOH or EtOH, 0 °C to rt, 58–77%; (m) NaH, MeI or EtI, THF, 77–90%; (n) $NaBH_4$, MeOH, 48%; (o) CO, $Pd(dppf)Cl_2$, Et_3N, MeOH:THF (1:1), 100 °C, 77–93%; (p) MeMgBr (3 M in THF), THF, −78 °C to rt; (q) NaH, MeI, THF, 0 °C to rt, 25–30% over two steps.



46 47 48

Scheme 4. Preparation of triazolopyridine isocyanate **48**. Reagents and conditions: (a) $SOCl_2$, MeOH, 0 °C then reflux, 35%; (b) 1,2,3-triazole, CsF, DMSO, 100 °C, 25%; (c) LiOH, H_2O, MeOH; (d) DPPA, Et_3N, toluene, rt then 80 °C.

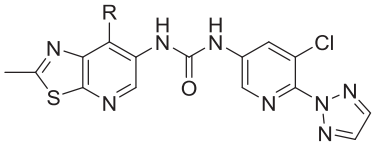
step (Scheme 2). The ketone acid was obtained by a Stille coupling of the bromoester **30** with tributyl(1-ethoxyvinyl)tin and subsequent hydrolysis.

4-Cyclopropyl quinoline ureas substituted at the 6 and 7-positions (**14–19**) were prepared using similar procedures from the corresponding aminoquinolines (Scheme 3), with the exception of **16**, prepared from the bromoester **33** (Scheme 2). The 4-hydroxy-3-nitroquinoline intermediates **36** were prepared by one of two routes. Nitration of the 4-hydroxyquinolines **37** generated the 7-chloro and 7-methoxy derivatives. The 6-substituted 4-hydroxy-3-nitroquinolines were prepared by treatment of the corresponding anthranilic acids **35** with 2-nitroacetaldehyde oxime, followed by cyclization with acetic anhydride. The 4-hydroxy-3-nitroquinolines were converted to the 3-amino-4-bromoquinolines **39** with phosphoryl bromide followed by reduction with iron in acetic acid. Compounds combining the preferred 4-substituents and the 6-F group (**24–27**) were also prepared using the chemistry described in Scheme 3. Experimental procedures and characterization data for the quinolines are described in Ref. 12.

We explored additional 6,5 and 6,6 bicyclic scaffolds with a view to reducing the lipophilicity of the compounds relative to the quinoline series, initially retaining the 4-cyclopropyl group to compare with **13**. Thiazolopyridine **49** showed similar activity to quinoline **13** in the cell assays, and this series was pursued further. Ethers **50** and **51** were more potent than the cyclopropyl analog, while alcohol **52** was similar. The synthesis of compounds **49–52** is shown in Scheme 5, using similar chemistry to that described for the preparation of the quinolines, and their cell data is shown in Table 3.

In addition to the OCI-Ly3 proliferation assay, we ran a counter-screen in an OCI-Ly1 assay to check for cellular effects driven by off-target activity. None of the quinolines and thiazolopyridines showed significant activity up to 20 μM in this assay. Compounds **26** and **51** were profiled in a panel of 28 proteases, and showed minimal effects on

Table 3
MALT1 cell data for thiazolopyridines **49–52**.



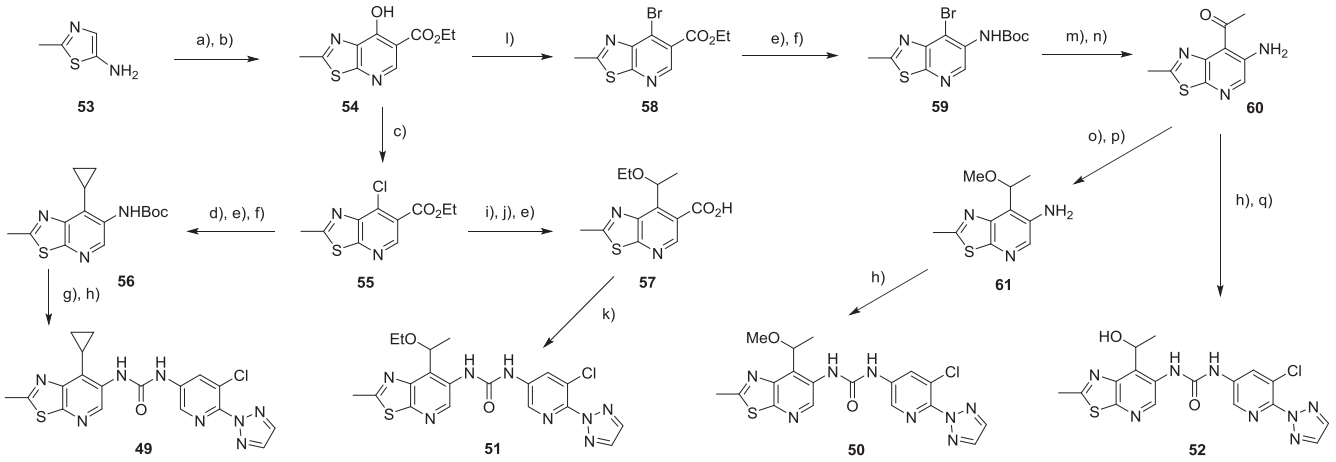
Example	R	GloSensor (μM)	OCI-Ly3 (μM)
49	Cyclopropyl	0.046	1.35
50	1-Methoxyethyl	0.030	0.73
51	1-Ethoxyethyl	0.036	0.36
52	1-Hydroxyethyl	0.069	1.46

all the targets at a concentration of 10 μM,[13] as anticipated for an inhibitor binding to an allosteric pocket specific to MALT1. Compound **26** was also profiled in a panel of 468 kinases,[14] as the quinoline urea series bears a structural resemblance to some Type II kinase inhibitors.[15] However, at a concentration of 10 μM, it showed no significant activity (> 20% inhibition) against any kinase targets.

Microsome stability of the series was generally very good, (Table 4), but the compounds were poorly soluble and highly protein bound. Higher unbound levels were observed for the thiazolopyridine **51**. Quinolines **24** and **26** and thiazolopyridine **51** were profiled in mouse *in vivo* PK studies and found to have very low *in vivo* Cl, and moderate oral exposure.

Methoxyethyl thiazolopyridine **50** was profiled in our PD model,[4] which measured serum levels of human IL-10 from mice xenografted with OCI-Ly3 cells. Secretion of IL-10 in ABC-DLBCL has been reported to be dependent upon MALT1 protease activity.[16,17] Oral dosing of **50** at 30 mpk resulted in a 50% reduction in hIL-10 levels after 12 h, while at a dose of 100 mpk, near-complete suppression of hIL-10 was observed.

In conclusion, we have developed two series of allosteric MALT1 inhibitors with good cell potency and excellent target class selectivity. While the physical properties of the series are non-optimal, the overall PK profile is promising, and we were able to demonstrate significant effects on a PD marker of MALT1 inhibition upon oral dosing in mice.



Scheme 5. Preparation of thiazolopyridine ureas. Reagents and conditions: (a) diethyl ethoxymethylenemalonate, EtOH, 90 °C, 32%; (b) Ph_2O, 250 °C, 98%; (c) $SOCl_2$, cat. DMF, DCM, reflux, 64%; (d) tributyl(cyclopropyl)stannane, $Pd(PPh_3)Cl_2$, dioxane, 100 °C, 39%; (e) LiOH, THF, MeOH, 60 °C, 59–75%; (f) DPPA, Et_3N, ^tBuOH, 100 °C, 28–96%; (g) TFA, DCM; (h) isocyanate **48**, toluene or dioxane, 100 °C, 17–52% (i) tributyl(1-ethoxyvinyl)stannane, $Pd(PPh_3)Cl_2$, dioxane, 100 °C, 62%; (j) H_2, 10% Pd/C, EtOH, 99%; (k) DPPA, Et_3N, dioxane, rt, then amine **3**, 100 °C, 30%; (l) $POBr_3$, DMF, 80 °C, 51%; (m) tributyl(1-ethoxyvinyl)stannane, $Pd(PPh_3)_4$, dioxane, 110 °C, 62%; (n) 3 M HCl, dioxane, 60 °C, 46%; (o) $NaBH_4$, MeOH; (p) NaH, MeI, THF, 67% over two steps; (q) NaH, MeI, THF, 13%.

Table 4
Microsome stability (mouse, human), aqueous solubility, PPB and *in vivo* mouse PK data (1 mpk IV, 5 mpk PO).

Compound	Microsome stability $T_{1/2}$ (min)	Sol pH 7 (μM)	PPB (% free)	IV Cl (ml/min/kg)	T1/2 (h)	F %	Auc inf (ng*h/mL)
24	> 180 (m), > 180 (h)	–	0.6 (m), 0.2 (h)	1.4	12.2	7	3371
26	> 180 (m), 118 (h)	< 4	1.2 (m)	4	2.7	30	8199
51	127 (m), 43 (h)	< 4	2.2 (m), 2.5 (h)	1.6	3.2	19	10,130

Acknowledgments

Synthetic chemistry was partially conducted by Peptech Corporation (Shanghai). Microsome stability, aqueous solubility and PPB measurements were conducted by Janssen Pharmaceuticals. Mouse PK studies were conducted at the Drug Metabolism and Pharmacokinetics laboratory at The Scripps Research Institute, under the supervision of Dr Mike Cameron. Protease profiling was conducted at Genscript USA. This work was supported by funding from Janssen Pharmaceuticals and the NIH (R01 CA182736). We thank Milka Kostic for assistance with the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.05.040.

References

1. Jaworski M, Thome M. *Cell Mol Life Sci.* 2016;73:459.
2. Hachmann J, Salvesen GS. *Biochimie.* 2016;122:324.
3. Fontan L, Qiao Q, Hatcher JM, et al. *J Clin Invest.* 2018;128:4397.
4. Hatcher JM, Du G, Fontan L, et al. *Bioorg Med Chem Lett.* 2019;29:1336.
5. Nagel D, Spranger S, Vincendeau M, et al. *Cancer Cell.* 2012;22:825.
6. Schlauderer F, Lammens K, Nagel D, et al. *Angew Chem Int Ed Engl.* 2013;52:10384.
7. Schlapbach A, Revesz L, Pissot Soldermann C, et al. *Bioorg Med Chem Lett.* 2018;28:2153.
8. Pissot Soldermann C, Quancard J, Schlapbach A, Simic O, Tintelnot-Blomley M, Zoller T. PCT Int. Appl. WO2015181747.
9. Lu T, Allison BD, Barbay JK, et al. PCT Int. Appl. WO2018119036.
10. Kukreja G, Irlapati NR, Jagdale AR, et al. PCT Int. Appl. WO2018020474.
11. Quancard J, Klein T, Fung SY, et al. *Nat Chem Biol.* 2019;15:304.
12. Gray NS, Scott DA, et al. PCT Int Appl WO 2018165385.
13. www.genscript.com. See for data.
14. DiscoverX, KinomeScan. www.discoverx.com.
15. Zhao Z, Wu H, Wang L, et al. *ACS Chem Biol.* 2014;9:1230.
16. Hailfinger S, Lenz G, Ngo V, et al. *Proc Natl Acad Sci USA.* 2009;106:19946.
17. Ferch U, Kloo B, Gewies A, et al. *J Exp Med.* 2009;206:2313.