Bioorganic & Medicinal Chemistry Letters 20 (2010) 3863–3867

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Activity of substituted thiophene sulfonamides against malarial and mammalian cyclin dependent protein kinases

Diana Caridha, April K. Kathcart, Dayadevi Jirage, Norman C. Waters *

Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Silver Spring, MD 20910, USA

ARTICLE INFO

Article history:
Received 5 April 2010
Revised 10 May 2010
Accepted 12 May 2010
Available online 21 May 2010

Keywords:
Malaria
Plasmodium
Pfmrk
CDK inhibitor
CDK7
Kinase
Cancer
Cell cycle

ABSTRACT

Cyclin dependent protein kinases (CDKs) are pursued as drug targets for several eukaryotic pathogens. In this study, we identified thiophene and benzene sulfonamides as potent inhibitors of Pfmrk, a *Plasmodium falciparum* CDK with sequence homology to human CDK7. Several of the compounds demonstrated inhibitor selectivity for CDK7 over CDK1, CDK2, and CDK6. The compounds are moderate antimalarial agents against drug resistant parasites and possess encouraging in vitro therapeutic indices as determined against human cell lines. One particular sub-class of compounds, bromohydrosulfonylacetamides, was specific for Pfmrk with IC_{50} values in the sub-micromolar range. These compounds represent the most potent Pfmrk inhibitors reported and provide support for further characterization and derivation as potential antimalarial agents.

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Cyclin dependent protein kinases (CDKs) are highly conserved regulators of cell cycle control among most eukaryotic organisms.[1] Multiple mechanisms regulate the activity CDKs to ensure the precise and ordered progression of the mitotic cell cycle. When these mechanisms fail, uncontrolled growth can result in neoplasticity. Interestingly, a few mammalian cells and eukaryotic single cell organisms grow and differentiate via an alternate mitotic cell cycle known as endoreduplication, in which multiple rounds of DNA synthesis occurs in the absence of mitosis or cytokinesis.[2] The primary difference between a mitotic cell cycle and an endocycle depends on the timing and alternation between CDK activation and inactivation.[3] Some cancer cells can exit the normal mitotic cell cycle and undergo an endocycle process in an effort to escape drug pressure and/or develop resistance.[4] This implication of a direct role in cell cycle progression, differentiation and development of drug resistance make CDKs attractive drug targets. In fact, there are currently 11 compounds in various stages of clinical develop for the treatment of cancer.[5] CDKs are also pursued as drug targets for the development of anti-infective agents against fungal, viral, and protozoan infections. *Plasmodium falciparum* is a protozoan intracellular parasite that is responsible for approximately two million malaria-related deaths annually.[6] In the absence of an effective vaccine, antimalarial drugs must be prescribed for treatment and prophylaxis. *P. falciparum* has developed resistance to most of the antimalarial drugs currently in use today at an alarming rate and therefore new drugs are urgently needed. It is expected that target-based approaches may introduce novel chemotypes into the antimalarial drug development pipeline.

Several plasmodial CDKs have been identified and among these, PfPK5 and Pfmrk have been pursued as antimalarial drug targets.[7,8] PfPK5 shares significant sequence homology with CDK1 and is believed to play a role in DNA synthesis.[9,10] Pfmrk shares similarities with human CDK7 by virtue of its sequence similarity, substrate specificity and effector molecule binding (cyclin H and MAT1).[11–13] Pfmrk is localized to the nucleus where it is believed to play a role in either DNA replication or transcriptional control.[14] Direct comparison, however, of the cell cycle-dependent functions of plasmodial and human CDKs is difficult since the malaria cell cycle operates under the confines of an endomitotic cycle (schizogony) however it is likely that CDKs play a key regulatory role in the malaria cell cycle.[15,16] The identification of inhibitors for Pfmrk and PfPK5 has been aided by crystal structures, QSAR pharmacophores and molecular docking approaches.[17–20]

In the search for inhibitors against malarial CDKs, selectivity must be addressed to avoid toxicity associated with cross-reactivity with human CDKs. Reports have demonstrated that inhibitors can be developed that are selective for malarial CDKs. Differences in the architecture of the active sites provide opportunities to design selective malarial CDK inhibitors. Several unique amino

* Corresponding author. Tel.: +1 61 7 3332 4817; fax: +1 61 7 3332 4800.
E-mail address: norman.waters@us.army.mil (N.C. Waters).

0960-894X/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2010.05.039

acids occupy the active site of Pfmrk that is believed to dictate a specific inhibitor profile. Many of the broad-spectrum CDK inhibitors that are commercially available fail to inhibit Pfmrk.[18,21] Using a QSAR pharmacophore specific for Pfmrk,[19] several chemotypes, (oxindoles, chalcones, tryptanthrins, and phenyl-quinolinones), have been identified as inhibitors.[22–24] Compounds in these chemical classes demonstrate selectivity for Pfmrk over human and additional plasmodial CDKs.

In this study, we explore the Pfmrk inhibiting properties of several sulfonamides. Previous reports demonstrated that isoquinoline sulfonamides are weak Pfmrk inhibitors and that variation within the quinoline moiety influences Pfmrk inhibition.[21] In this study, we investigate additional sulfonamides, which contain substituted moieties of thiophene (Fig. 1) and benzene (Fig. 2). These compounds were tested for inhibition of Pfmrk using an in vitro kinase assay as previously reported.[23] As shown in Table 1, many of these compounds inhibit Pfmrk at low to sub-micromolar concentrations. This is the first reported class of compounds with this level of potency against Pfmrk. Although we grouped these compounds into separate chemical classes, three compounds have the sulfonamide linker replaced with a carbamoylformamide linker (**1**), formylacetohydrazide linker (**2**), or an aminoacetonitrile linker

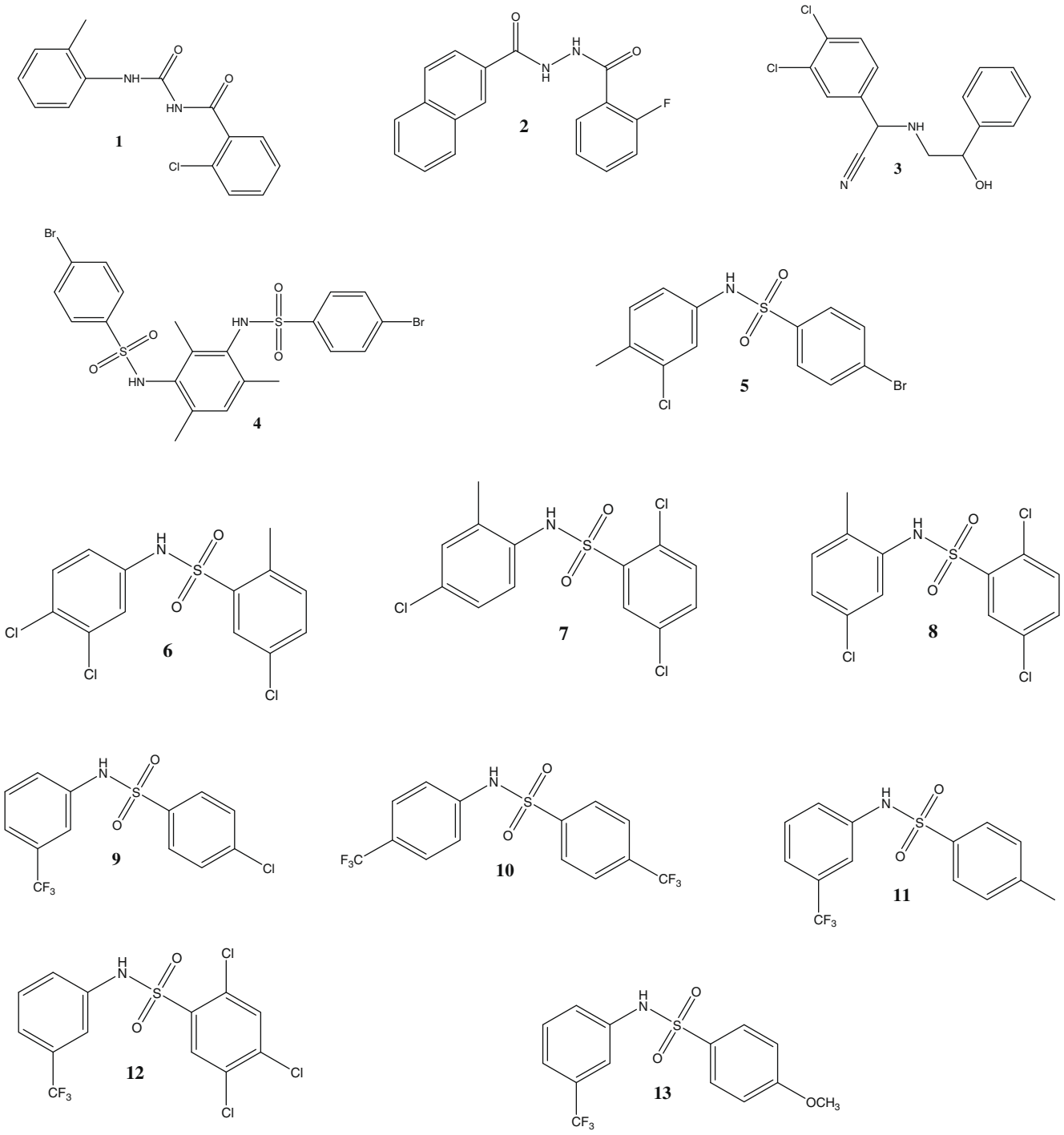

Figure 1. Structures of benzyl-sulfonamides.

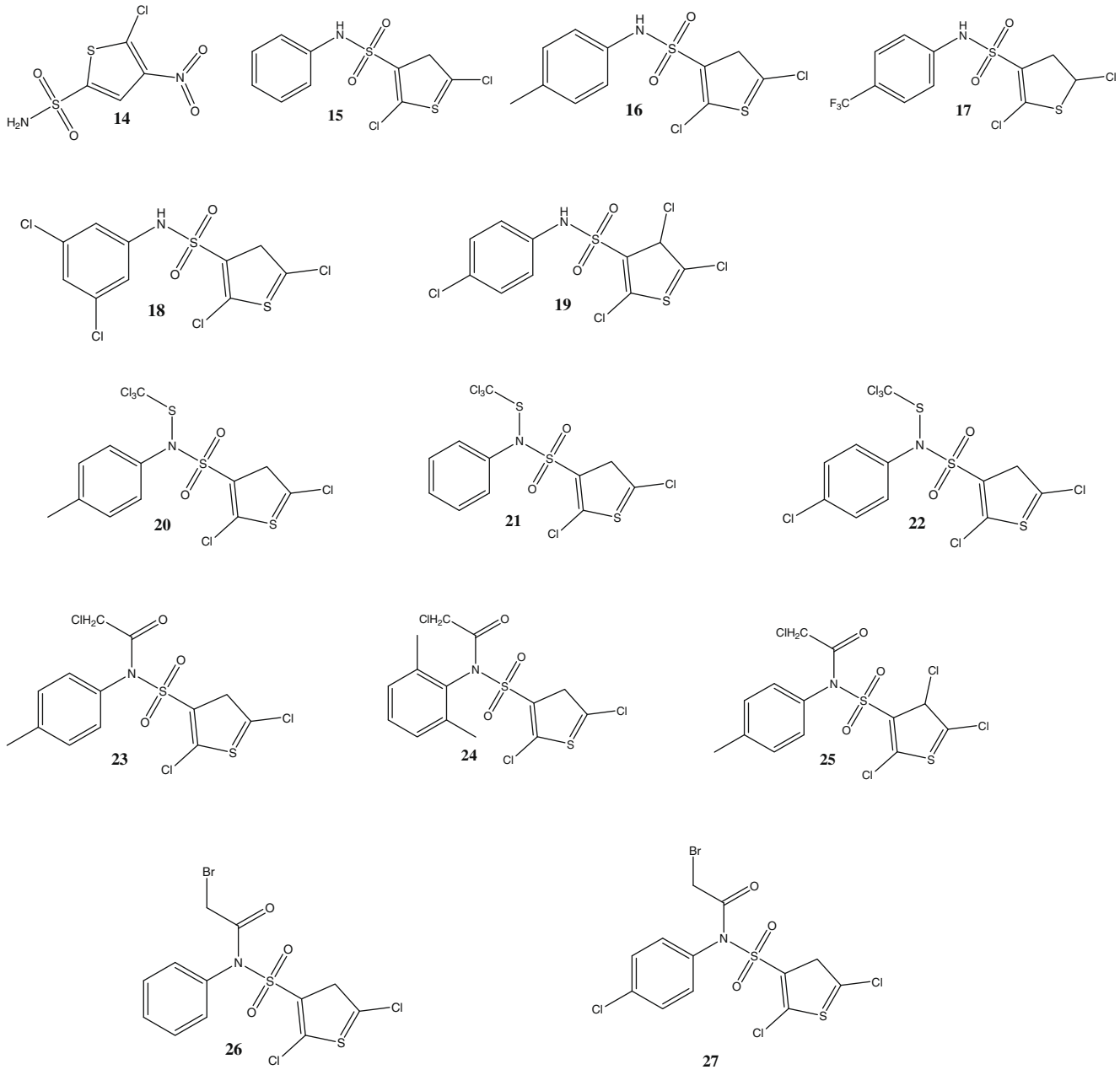

Figure 2. Structures of thiophene sulfonamides.

Table 1
Inhibitory activity of compounds on Pfmrk activity (IC_{50} values, μM)

Compound	Pfmrk	Compound	Pfmrk
1	0.2	**14**	17.4
2	0.4	**15**	14.9
3	0.6	**16**	0.6
4	50.2	**17**	0.7
5	15.9	**18**	332.8
6	15.1	**19**	31.5
7	11.0	**20**	0.3
8	45.4	**21**	29.7
9	13.1	**22**	19.7
10	16.5	**23**	127.2
11	0.7	**24**	20.7
12	0.6	**25**	40.1
13	48.4	**26**	0.6
		27	0.8

(**3**). These linkers provide multiple opportunities to form hydrogen bonds with the active site residues of Pfmrk. Previous Pfmrk inhibitors have demonstrated a correlation between the number of hydrogen bonds and the increase in inhibition.[21] Compound **4** was a poor Pfmrk inhibitor even though hydrogen bonding properties exist; this may be due to the introduction of a third aromatic ring system into the compound. Several large residues line the Pfmrk active site that excludes binding of large compounds; it is believed that these unique residues may be exploited for inhibitory specificity over the human CDKs (discussed below).

There were little differences in potency between benzene sulfonamides and thiophene sulfonamides. Both classes contained several compounds with sub-micromolar IC_{50} values. Three separate subclasses of thiophene containing compounds that have variations in the sulfonamide linker were evaluated. This included trichloromethiosulfonamides (**20**, **21**, and **22**), chlorohydrosulfonlyacetamides

Table 2
Selected compounds tested against human CDKs: IC_{50} (μM) values

Compound	CDK7	CDK6	CDK1	CDK2
6	0.4	110.4	0.8	10.5
11	0.4	208.8	25.1	12.5
12	0.2	58.8	0.1	10.0
16	0.8	51.3	49.3	31.4
20	0.7	18.0	10.5	11.5
26	33.4	34.9	21.5	28.5

(**23**, **24**, and **25**), and bromohydrosulfonylacetamides (**26** and **27**). The bromohydrosulfonylacetamides were the most potent subclass of Pfmrk inhibitors and chlorohydrosulfonlyacetamides demonstrated poor Pfmrk inhibition as evident from IC_{50} values. The linker region between the thiophene and benzene ring system obviously plays a critical role in binding to Pfmrk and accounts for variability in the inhibitor potency, compare compounds **16**, **20**, **23**, and **22** with **27**. These compounds have identical benzene and thiophene moieties and only the linker regions are dissimilar. Substitutions within the aromatic ring systems are likely contributors to inhibition, however only a few compounds with limited aromatic substitution patterns were tested and therefore it is difficult to draw specific conclusions.

The structural conservation of the CDK active site creates challenges in designing selective CDK inhibitors. Several compounds are broad-spectrum CDK inhibitors however recent reports have demonstrated that sensitivity can be achieved by exploiting minor amino acid differences within the active site.[25,26] Furthermore, inhibitor sensitivity profile differences exist between plasmodial and human CDKs. In fact, many of the human CDK inhibitors fail to inhibit plasmodial CDKs at the same level of potency.[18] To assess the cross-reactivity of this compound class, we selected several of the most potent Pfmrk inhibitors for testing against human CDKs. We observed that most of the compounds are weak inhibitors of CDK1, CDK2, and CDK6, with the only exception of compounds **6** and **12** which inhibit CDK1 in the sub-micromolar range (Table 2). Except for compound **26**, all compounds tested were potent inhibitors of CDK7 with IC_{50} values in the sub-micromolar range. This is not too surprising as Pfmrk shares the greatest sequence homology with human CDK7 (46% identity). Many CDK inhibitors target either the hinge region between the N-terminal and C-terminal domains and/or a small hydrophobic pocket within the active site. In CDK7 and Pfmrk, amino acids within the hydrophobic pocket (Tyr-96, Ser-99, Lys-100, residue numbers in Pfmrk) are different than those found in most CDKs.[27] Although the crystal structure of Pfmrk has not been solved, molecular modeling and docking studies suggest that these amino acid differences may be responsible for the observed selectivity for several CDK inhibitors. Furthermore, several unique amino acids are predicted to restrict the size of the Pfmrk active site. For example, Phe-15, Tyr-96, Phe-143 in Pfmrk reduces the overall size of the active site by approximately 20% of that of most CDKs, thus excluding many broad-spectrum CDK inhibitors. To our knowledge, this is the first report of these chemical classes as CDK inhibitors.

The same compounds selected for cross-reactivity studies against human CDKs, were also tested for antimalarial activity and cytotoxicity against mammalian cell lines. Using an ex vivo growth inhibition assay, eight compounds tested against the *P. falciparum* drug resistance W2 strain demonstrated moderate growth inhibition (Table 3). There was little difference in antimalarial activity between benzene sulfonamides (**6**, **11**, and **12**) or thiophene sulfonamides (**16**, **20**, and **26**). Since most of these compounds possess Pfmrk IC_{50} values in the sub-micromolar range, it is not surprising that they all have similar antimalarial activity. At this point, we do not know if these compounds kill the malaria parasite through inhibition of Pfmrk. In fact, compound **6** is a poor inhibitor of Pfmrk but possesses moderate antimalarial activity. Since the extent of the parasite's tolerability towards Pfmrk inhibition is not yet known, definitive correlation between Pfmrk inhibition and antimalarial activity of these compounds cannot be drawn. Recent studies with kinase inhibitors have demonstrated that week inhibition of particular cell cycle kinases may be just as effective as potent inhibitors because some cells cannot tolerate any modulation in kinase activity. Future studies using these compounds, alongside a Pfmrk functional assay, may shed light on the biological role of Pfmrk and provide a better understanding of the correlation between Pfmrk inhibition and antimalarial activity.

Several studies describe different series of sulfonamides in general and benzene sulfonamides in particular as being strong antitumor agents.[28,29] These compounds were shown to block mitosis through inhibition of tubulin polymerization[30] or induce G1 cell cycle arrest through an unknown mechanism.[31] Because these compounds were potent inhibitors of human CDK7, it was prudent that the most potent compounds were tested against mammalian cells lines for in vitro antiproliferative activity. Compounds were tested against a rat macrophage cell line and against several cancer cell lines. If the WRAIR compounds of interest induced cell death mainly via CDK inhibition, we would expect these compounds to show enhanced cytotoxic activity against cancer cell lines compared to the normal ones. Both of our controls, alsterpaullone and indirubin, are potent human CDK inhibitors and possess anti-tumor activities.[32,33] They exhibit low to sub-micromolar IC_{50} values against all cell lines. Faidallah et al. tested several new benzene sulfonamides for anticancer properties against a panel of 60 different tumor cell lines in NCI's in vitro-disease-oriented human tumor cell screen and obtained IC_{50}s comparable to values reported here.[29] All of the compounds demonstrated low toxicity profiles as compared to the CDK inhibitors alsterpaullone and indirubin, suggesting that inhibition of CDK7 may be a poor indicator of cytotoxicity (Table 3). Although these compounds were more potent against the malaria parasite than mammalian cells lines, the therapeutic index (TI), compared to the antimalarial

Table 3
Cytotoxicity of selected compounds against *P. falciparum* and various cell lines (IC_{50}: μM)[a]

Compound	*P. falciparum* W2 strain	RAW-264.7 (TIB-71) rat macrophage	DU-145 (HTB-81) prostate cancer	LOVO (CCL-229) human colorectal carcinoma	Hs 578 (HTB-126) ER^- human breast carcinoma	MCF-7 (HTB-22) ER^+ human breast carcinoma
6	17.0	76.6	45.4	55.0	54.0	57.3
11	31.7	98.7	50.2	96.5	55.9	68.3
12	24.7	90.4	44.2	65.1	55.1	63.8
16	31.0	74.7	81.1	69.3	40.2	66.1
20	21.2	39.3	53.0	70.3	24.9	30.1
26	23.3	89.5	91.0	71.6	51.1	62.0
Alsterpaullone	4.3	2.2	1.1	<1	1.9	2.7
Indirubin	1.1	6.1	6.8	2.2	6.9	6.7
Mefloquine	0.1	6.5	14.4	7.9	12.1	10.6

[a] Each data point represents the mean of at least two independent experiments with two independent measurements per experiment.

mefloquine, is not promising. (For example, TI against rat macrophage cell for **11** is 3.1 vs 65 for mefloquine.) Future testing of these compounds in malaria animal models may be a better assessment of efficacy and toxicity. Nevertheless, these potent Pfmrk inhibitors demonstrated moderate antimalarial activity and low mammalian cell toxicity.

In summary, we have demonstrated that substituted thiophene and benzene sulfonamides are potent inhibitors of Pfmrk with submicromolar IC_{50} values. These chemical classes are the most potent Pfmrk inhibitors reported to date. Although these compounds failed to inhibit several human CDKs, they were potent against human CDK7. This result may reflect structural similarities between Pfmrk and CDK7. Nevertheless, one sub-class among the compounds tested, bromohydrosulfonylacetamides, demonstrates specific potency for only Pfmrk and has demonstrated limited toxicity to mammalian cell lines. Future efforts should focus on this chemotype and testing of derivatives to establish structure–activity relationships that may lead to more potent and selective Pfmrk inhibitors. Exploring various bromohydrosulfonylacetamides containing substitutions within the thiophene and benzene ring systems may yield more promising compounds.

Acknowledgments

The opinions or assertions contained herein are the private views of the authors, and are not to be construed as official, or as reflecting true views of the United States Department of the Army or the Department of Defense. The authors thank Erin Milner for critical review of the manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.039.

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