Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2875–2878

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design and Synthesis of Pfmrk Inhibitors as Potential Antimalarial Agents

Zili Xiao,[a] Norman C. Waters,[b] Cassandra L. Woodard,[b] Zhiyu Li[b] and Pui-Kai Li[a,*]

[a]*Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA*
[b]*Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Silver Spring, MD 20910, USA*

Received 18 May 2001; accepted 14 August 2001

Abstract—The synthesis and inhibitory activities of 10 potential inhibitors of Pfmrk, a *Plasmodium falciparum* cyclin-dependent protein kinase, are described. The most potent inhibitor is a 3-phenyl-quinolinone compound with an IC_{50} value of 18 μM. It is the first compound reported to inhibit Pfmrk at the micro molar range.

Malaria is one of the most serious tropical diseases and it affects 400 million people with 1–2 million deaths every year.[1,2] Over the last several decades, drug resistance in malaria parasites has been a major health problem. There has been a widespread resistance to chloroquine,[3] an antimalarial agent that has been used for many years. In addition, resistance to suphadoxine/pyrimethamine, the first line therapy of malaria, is now emerging in several African countries.[4] Therefore, development of new antimalarial agents has become one of the highest priorities. Of the four known human malaria parasites, *Plasmodium falciparum* is the most lethal form and the research into the basic biology of the parasite has begun to search for new molecular targets for the development of new antimalarial agents.

Recently, a family of protein kinases [cyclin-dependent kinases (CDKs)] that control cell cycle progression has been the potential targets for drug development.[5,6] CDKs are conserved among eukaryotic species and several CDKs have been isolated from *Plasmodium falciparum*. The best characterized Plasmodial CDK is a homologue of human CDK1 called PfPK5.[7] Human CDK1 inhibitors such as hymenialdisine, indirubin-3′-monooxime and purvalanol show inhibitory activities against PfPK5.[7] Pfmrk is another well characterized Plasmodial CDK that shows significant homology with human CDK7.[8,9] In humans, CDK7 associates with TFIIH transcription factor and regulates transcription and DNA repairs.[10] Both PfPK5 and Pfmrk are identified as potential targets for the development of antimalarial agents.

Although inhibitors of PfPK5 have been identified,[7] Pfmrk inhibitors have never been reported. Most of the CDK inhibitors are designed to compete with the ATP binding site of the kinases. Known ATP-competitive human CDK2 inhibitors Olomoucine and Roscovitine (Fig. 1) failed to inhibit Pfmrk.[8] Both inhibitors contain the purine ring system. Recently, kinase inhibitors containing lactam moiety such as Oxindole and Kenpaullone are reported (Fig. 1).[11,12] Kenpaullone is a potent inhibitor of CDK1, CDK2, and CDK5[12] while oxindole is a selective inhibitor of CDK4.[11] It appears that the unsubstituted lactam moiety is critical in forming H-bonding with the peptide backbone at the ATP binding site. In Kenpaullone, its CDK1 inhibitory activity decreases 50-fold when the nitrogen in the lactam moiety is methylated.[12]

In an attempt to identify new lead compound(s) to inhibit Pfmrk, we design and synthesize 10 compounds (**1**–**10**) containing lactam moiety with five different structural classes as potential Pfmrk inhibitors (Fig. 2). The structural classes are: (1) 3-phenyl-quinolinone (**1** and **2**), (2) dihydro-indolo quinolinone (**3**), (3) benzofuro (3,2-**c**) quinolinone (**4**–**6**), (4) benzopyrano (4,3-**c**) quinolinone (**7**), and (5) benzofuro (2,3-**b**) quinolinone (**8**–**10**).

The synthesis of compounds **3** was carried out according to the literature procedure.[13] The syntheses of compounds **1** and **2** are shown in Scheme 1. Reacting aldehyde **11**

*Corresponding author. Fax: +1-614-688-8556; e-mail: li27@osu.edu

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00578-9

with 3-methoxyphenyl acetic acid **12** yielded the *E* and *Z* (2:1) mixture of *o*-nitro-α-(3-methoxyphenyl) cinnamic acid **13**. Hydrogenation of **13** followed by intramolecular cyclization afforded the dihydroquinolinone **14**. Oxidation of **14** with DDQ followed to demethylation yielded **16**. Compound **1** was obtained by reacting **16** with methylbromoacetate followed by ester hydrolysis. Reacting **16** with 2-chloroethanol yielded **2**.

Compounds **4–6** and **8–10** were synthesized from the same synthetic pathway (Scheme 2). Stirring phenylacetate **18** with diethyl oxalate at 60 °C yielded **19**, which underwent thermal decarbonylation at 175 °C to form **20**. Refluxing **20** with aniline or *p*-substituted aniline afforded **21a–c**. Compounds **21a–c** are the intermediates for the syntheses of compounds **4–6** and **8–10**. For example, refluxing **21a** in anhydrous pyridine hydrochloride yielded a mixture of **4** and **8** in a ratio of 2:5.

Compound **7** was synthesized as shown in Scheme 3. Heating isatin **22**, 2-methoxyphenyl acetic acid **23** with sodium acetate at 200–230 °C for 50 min yielded compound **24**. Intramolecular esterification of **24** in anhydrous pyridine hydrochloride at reflux afforded **7**.

We have developed a microtiter plate drug screen to identify inhibitors of Pfmrk. Pfmrk activity was assayed with Pfcyc1, a plasmodial cyclin that stimulates Pfmrk activity. The carboxy terminal domain of RNA polymerase II (CTD) was used as substrate since it has been shown to be more favorable substrate than Histone H1 (unpublished results). In brief, Pfmrk and Pfcyc1 were expressed and purified from *Escherichia coli* as pre-

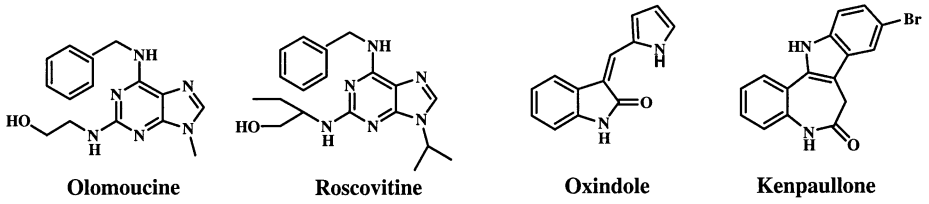


Figure 1. Known CDK inhibitors.

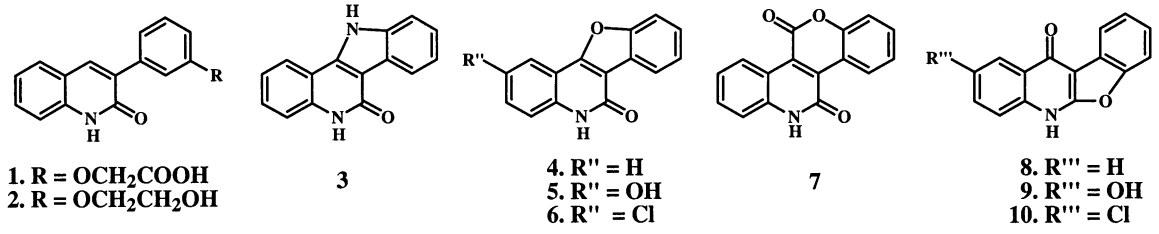
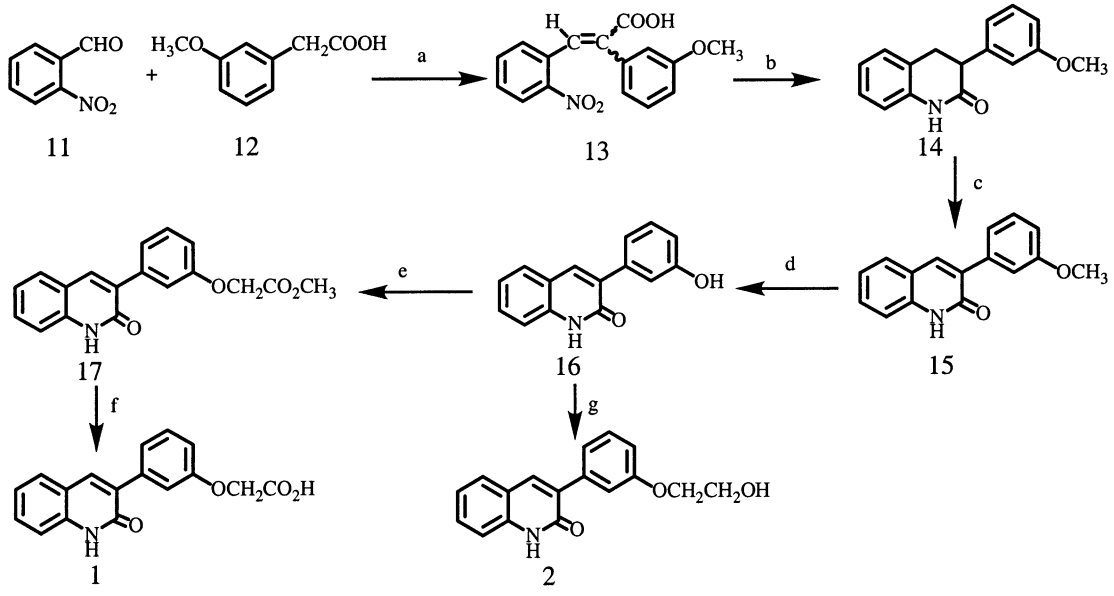
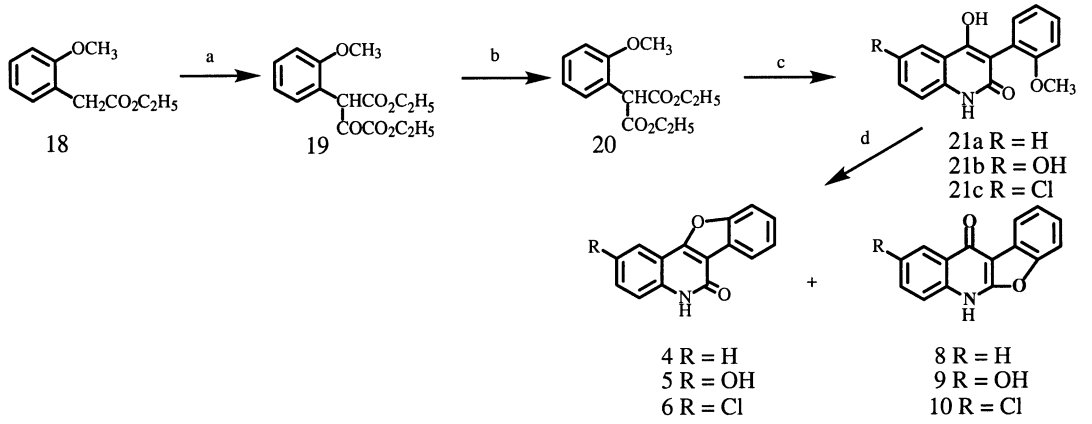


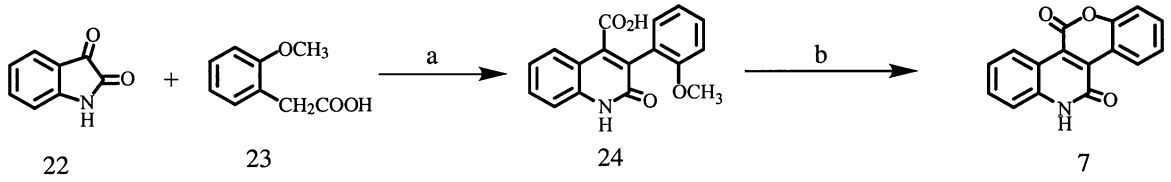
Figure 2. Proposed Pfmrk inhibitors.



Scheme 1. Synthesis of compounds **1** and **2**. Reagents: (a) Ac_2O, TEA, reflux 2.5 h; (b) 5% Pd–C/H_2, CH_3OH, rt, 18 h; (c) DDQ, $ClCH_2CH_2Cl$, reflux 48 h; (d) BBr_3, CH_2Cl_2, rt, 2.5 h; (e) $BrCH_2CO_2CH_3$, NaH, DMF, rt 6 h; (f) (1) KOH/CH_3OH; (2) HCl; (g) $ClCH_2CH_2OH$, NaH, DMF, rt, 24 h.



Scheme 2. Synthesis of compounds **4–6** and **8–10**. Reagents: (a) diethyl oxalate, EtOK, benzene, 60 °C, 30 min; (b) 175 °C, –CO; (c) *p*-substituted aniline, Ph_2O, reflux 3.5 h; (d) anhyd pyridine hydrochloride, reflux 1.5 h.



Scheme 3. Synthesis of compound **7**. Reagents: (a) AcONa, 200–230 °C, 50 min; (b) Pyr–HCl, reflux 1 h.

viously reported.[7] The kinase drug screen was performed in a 96-well filter plate assay. Assay began by incubating Pfmrk (4 μg) and drug in kinase buffer (50 mM Tris–HCl pH 7.5, 10 mM $MgCl_2$, 1 mM DTT), 3 μg Pfcyc1 and 10 μg CTD for 5 min at 30 °C to facilitate drug binding and followed by the addition of [γ-^{32}P] ATP (5 μCi, 3000 Ci mmol/mL). The mixture was incubated at 30 °C for 30 min. Following incubation, plates were washed on a vacuum manifold with six consecutive washes of 200 μL of 5% phosphoric acid per well. Following the last wash, scintillation fluid was added and the plate analyzed in a Topcount microtiter plate scintillation counter (Packard). Each reaction was performed in triplicate and controls were included on each plate in order to subtract background from the reactions. Counts per min for each set of reactions were averaged with a variation less than 5%. Each inhibitor was tested over a range of concentration for determination of dose-responsiveness and for calculation of IC_{50} values. The IC_{50} values of the inhibitors are shown in Table 1.

The IC_{50} values range from 18 to 539 μM. The IC_{50} values of inhibitors **6**, **7** and **10** could not be determined due to solubility problems. Compounds containing the structural classes dihydro-indolo quinolinone (**3**) and benzofuro (3,2-**c**) quinolinone (**4–6**) are not tolerated by the enzyme. Both inhibitors **3** and **4** did not exhibit any inhibitory activities. Substitution of H with OH group at the 2-position of **4** resulted in inhibitor (**5**) with very weak inhibitory activity (539 μM). Benzofuro (2,3-**b**) quinolinones (**8** and **9**) exhibited marginal inhibitory activities (< 371 and 187 μM). Increasing the rotational degree of freedom on the inhibitors (**1** and **2**) increase the inhibitory activities. The most potent inhibitor (**2**) has an IC_{50} value of 18 μM. Inhibitor **2** is the first compound reported to inhibit Pfmrk and at the μM range. It has been identified as the lead compound for Pfmrk inhibition and detail structure–activity relationships studies of the inhibitors are in progress.

Table 1. Pfmrk inhibitory activities (IC_{50} in μM) of compounds **1–10**

Compd	IC_{50} (μM)	Compd	IC_{50} (μM)
1	102	**6**	Not tested
2	18	**7**	Not tested
3	No inhibition	**8**	< 371
4	No inhibition	**9**	187
5	539	**10**	Not tested

Purified Pfmrk was assayed with varying concentrations of inhibitors in the presence of Pfcyc1 and CTD.

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