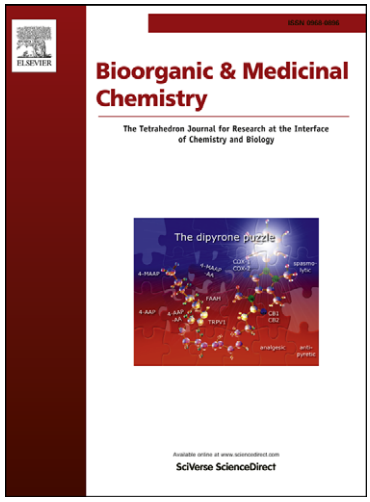
Accepted Manuscript



Synthesis of 5,7-disubstituted-4-methyl-7*H*-pyrrolo[2, 3-*d*]pyrimidin-2-amines as microtubule inhibitors

Aleem Gangjee, Sonali Kurup, Charles D. Smith

PII: S0968-0896(12)01002-4
DOI: http://dx.doi.org/10.1016/j.bmc.2012.12.029
Reference: BMC 10495

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 17 September 2012
Revised Date: 13 December 2012
Accepted Date: 22 December 2012

Please cite this article as: Gangjee, A., Kurup, S., Smith, C.D., Synthesis of 5,7-disubstituted-4-methyl-7*H*-pyrrolo[2, 3-*d*]pyrimidin-2-amines as microtubule inhibitors, *Bioorganic & Medicinal Chemistry* (2013), doi: http://dx.doi.org/10.1016/j.bmc.2012.12.029

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Synthesis of 5,7-disubstituted-4-methyl-7*H*-pyrrolo[2, 3-*d*]pyrimidin-2-amines as microtubule inhibitors

Aleem Gangjee,[•, †] Sonali Kurup, [†, §] Charles D. Smith [‡]

[†] *Division of Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, PA 15282*

[‡] *Department of Pharmaceutical Sciences, Medical University of South Carolina, Charleston, SC 29425*

[§] Current address: *Department of Biopharmaceutical Sciences, College of Pharmacy, Roosevelt University, Schaumburg, IL 60173*

* To whom correspondence should be addressed. Phone: 412-396-6070. Fax: 412-396-5593. Email: gangjee@duq.edu

† Duquesne University

‡ Medical University of South Carolina

§ Roosevelt University

[a] Abbreviations: MT, microtubule; MDR, multiple drug resistance; Pgp, P-glycoprotein; MRP, multidrug-resistance proteins; TLC, thin layer chromatography; NMR, nuclear magnetic resonance; HRMS, high resolution mass spectra.

ACCEPTED MANUSCRIPT

Abstract

Compounds **1-4** were previously reported as potent antimitotic and antitumor agents with Pgp modulatory effects. Compounds **5-18** have been synthesized in an attempt to optimize the various activities of **1-4**. Compounds **5-10** explored the influence of methoxy substitutions on the 7-benzyl moiety in **1**, while **11-18** investigated the influence of incorporation of a sulfur linker at C5 compared to **1-3**. Compounds **5-10** demonstrated potent single-digit micromolar tumor cell cytotoxicity, Pgp modulation and microtubule inhibition. Compound **7** of this series was the most potent and showed GI_{50} values in the nanomolar range against several human tumor cell lines in the standard NCI preclinical in vitro screen. Antitumor activity and Pgp modulatory effects were found to decrease for the 5-phenylthio compounds **11-14** compared to their 5-phenylethyl analogs **2-4** and the standard compound Taxol. Incorporation of methoxy substitutions on the 7-benzyl moiety improved antitumor activity for the 5-phenylthio compounds **16** and **17**. Compounds **16** and **17** demonstrated single to two-digit micromolar inhibition of tumor cells.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Keywords:

Pyrrolo[2,3-*d*]pyrimidines, Microtubule inhibitors, Microwave assisted organic synthesis

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

1. Introduction

The clinical prognosis of cancer remains relatively poor, suggesting a need for new therapeutic strategies for the improvement of drugs in clinical use.[1] Microtubules (MTs) represent an excellent target and several inhibitors have a prominent role in cancer chemotherapy. Microtubule inhibitors disrupt or suppress both microtubule structure and normal functions by inhibition or promotion of microtubule assembly, resulting in cell cycle arrest in the mitotic phase and induction of apoptosis.[1,2] Microtubule inhibitors or antimitotic agents have been classified on the basis of their mechanism of action and effects on polymerization dynamics, and subsequently, on the basis of their binding site on tubulin.[3] MT-destabilizing agents inhibit MT polymerization at high concentrations while MT-stabilizing agents promote microtubule assembly and prevent depolymerization. Three main sites of binding have been identified for antimitotic agents on microtubules or tubulin, namely the Vinca binding site, Taxol binding site and the Colchicine binding site.[4] The Vinca alkaloids bind to the β-subunit of soluble tubulin dimers and induce a conformational change in tubulin which decreases tubulin self-association.[5] Taxol and its derivatives bind poorly to soluble tubulin, but with high affinity to tubulin along the length of the microtubule. Taxol binding to the β-subunit stabilizes the microtubule by inducing a conformational change in the tubulin that increases its affinity for neighboring tubulin molecules. Ixabepilone, a recently approved antimitotic agent for the treatment of metastatic breast cancer also binds to the Taxol binding domain.[6] Colchicine has been well studied for its antimitotic effects, however it is not used clinically in cancer treatment due to its toxicity.[7] It initially binds to soluble

ACCEPTED MANUSCRIPT

tubulin, forming a tubulin–colchicine complexes that binds more tightly to their tubulin neighbours than tubulin itself, so that the dissociation of tubulin is reduced.

Multidrug or multiple drug resistance (MDR) is a major drawback of the clinically used antimitotic agents and has resulted in treatment failures.[8] A major mechanism of MDR occurs via the overexpression of the P-glycoprotein (Pgp) efflux pump. Pgp is a membrane transport protein that has been associated with MDR. Pgp binds drugs within the cell and releases them to the extracellular space using ATP, thereby decreasing the intracellular drug concentration. Tumor cells pre-exposed to cytotoxic compounds often overexpress these pumps, inducing resistance in the presence of the cytotoxic drug. Due to the lack of detailed structural information on binding sites for Pgp binders, the rational design of antimitotic agents with Pgp inhibitory effects has been limited, although a few examples have also been reported. [9-11]

Figure 1

Gangjee et. al.[11] previously reported the 7-benzyl-4-methyl-5-substituted phenylethyl-7*H*-pyrrolo-[2,3-*d*]pyrimidin-2-amines **1-4** (Figure 1) as antitumor agents with submicromolar to micromolar tumor cell inhibition and Pgp inhibitory effects. The antitumor effects were attributed to microtubule inhibition. Compounds **1-4** were reported to bind to a novel/unknown site on microtubules that is distinct from the Taxol, Vinca and Colchicine binding sites. Novel small molecules that bind to microtubules at novel sites and are not substrates for drug efflux pumps (Pgp) would show significant potential as therapeutic agents in cancer chemotherapy. Such agents could be used in combination regimens with other antimitotic agents to afford a synergistic/additive effect, overcome resistance and lower toxicity. Thus, it was of interest to further delineate the

ACCEPTED MANUSCRIPT

structure-activity relationship (SAR) for **1-4**. Compounds **5-18** were designed as analogs of **1-4** to optimize antitumor potential and Pgp modulatory effects.

Removal of the 7-benzyl moiety in **1** was previously reported to decrease activity by 100 to 10,000-fold against tumor cell lines, indicating the importance of the N7-benzyl moiety on the inhibitory potency of **1**.[11] Compounds **5-10** (Figure 1) were synthesized to determine the effects of variation in the substitutions on the 7-benzyl moiety of **1** on antitumor activities and Pgp modulation. Methoxy substitutions were incorporated at varied positions of the 7-benzyl moiety while maintaining the 3,4,5-triOMephenylethyl substitution at C5. Compounds **11-14** (Figure 1) were synthesized as 5-thio analogs of **2-4** to determine the impact of variation in the C5-phenylethyl linker on antitumor activity and Pgp modulation. The sulfur atom incorporated at C5 was anticipated to mimic the two-atom bridge of **2-4** due to a larger atomic size of the sulfur atom compared to a carbon or nitrogen atom. This modification would allow a side chain phenyl distance somewhere between a one- and two-carbon-atom bridge and also cause a decrease in the C-S-C angle (98°) compared to a C-C-C angle (109°), consequently altering the orientation of the C5 phenyl ring. Compounds **11-14** incorporated a small set of electron donating and withdrawing groups on the C5-thiophenyl moiety while maintaining the 7-benzyl substitution. Additionally, compounds **15-18** were synthesized to determine the influence on biological activity of varied substitutions on the 7-benzyl moiety in combination with a 5-thiophenyl moiety.

2. Chemistry

ACCEPTED MANUSCRIPT

Gangjee et. al.[11] synthesized **1-4** in a ten-step sequence starting from 2-acetylbutyrolactone. A convergent synthesis of the proposed target compounds **5-10** could be envisioned to proceed via removal of the 7-benzyl followed by alkylation at *N*7 with the appropriately substituted benzyl chlorides. Debenzylation and alkylation at *N*7 proceeded in poor yields, hence **5-10** were synthesized following a modification of the previously reported procedures as shown in Schemes **1** and **2**.

Scheme 1

Reaction of **19a-f**[12] with pivaloyl chloride in the presence of triethylamine and DMAP for 12 h afforded a mixture of the dipivaloylated **22a-d** and/or monopivaloylated compounds **23a-f** (Scheme 1). Decreasing the electron density of the pyrrole ring promotes regioselective halogenation at C5. Pivaloylation of the 2-amino group in **20a-d**[12] and **21a-f** directs the iodination regiospecifically to C5 yielding compounds **22a-f**. Based on the success of reported microwave assisted Sonogashira reactions,[13,14] the Sonogashira reaction of **22a-f** with 3,4,5-trimethoxyphenyl acetylene was attempted using dichloroethane as solvent, triethylamine as base, $Pd(PPh_3)_4$ as catalyst and CuI as an additive under microwave irradiation at 100 ^oC for 10 min. Compounds **23a-f** were synthesized in 71-80% yields. Microwave irradiation significantly shortened the duration of reaction (10 min at 100 ^oC *vs* 12 h at room temperature). Reduction with hydrogen and 5% palladium-on-charcoal in a mixture of methanol and methylene chloride, followed by removal of the pivaloyl protecting group on reflux with 1 N NaOH and methanol afforded target compounds **5-10** in 55-66 % yield.

Scheme 2

ACCEPTED MANUSCRIPT

The synthesis of target compounds **11-18** was envisioned to proceed via substitution of the 5-iodo in N-[5-iodo-7-benzylsubstituted-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethyl propanamide, **24a**,[11] **24b**, **22d** and **24c** with the appropriate thiol (Scheme 2). Among heterocyclic halides, there are few reports on C-S bond formation via copper mediated cross coupling.[15-18] We elected to utilize a microwave assisted, copper catalyzed reaction under ligand-free conditions for the synthesis of target compounds **11-18**. It was anticipated that microwave irradiation and the appropriate choice of solvent would lower reaction duration. Attempts were made to couple **24a** with the appropriate thiol (1.2 eq) using stoichiometric amount of CuI (1.3 eq), K_2CO_3 as base (1.5 eq) and DMF as the solvent under time and temperature variations. Optimal results were obtained at 100 ^oC for 4 h. Compounds **25a-h** were synthesized in 68-81% yields. The phenyl thiols were utilized in slight excess (1.2 eq) due to the formation of the corresponding phenyl disulfides as a byproduct in this reaction. This is the first report on microwave assisted, copper-catalyzed reaction of 5-iodo substituted pyrrolo[2,3-*d*]pyrimidines with substituted phenyl thiols at a lower reaction temperature of 100 ^oC under ligand free conditions. Higher reaction temperatures resulted in lower yields and increased disulfide formation. The microwave assisted reaction of **24a** with the appropriate thiol, K_2CO_3 in DMF, in the absence of CuI from 60-150 ^oC was unsuccessful. No reaction occurred and the starting material was recovered, confirming that the reaction proceeds via a copper-catalyzed, Ullman-type coupling. Removal of the pivaloyl protecting group in **25a-h** was achieved on reflux with 1 N NaOH to afford target compounds **11-18** in 50-65 % yield.

ACCEPTED MANUSCRIPT

3. Biological Evaluation and Discussion

Table 1

Target compounds **5-18** were evaluated for cytotoxicity toward JC mammary adenocarcinoma cells and for their impact on Pgp activity according to the reported protocols (Table 1).[19] JC mouse adenocarcinoma cells express high levels of Pgp and can be used for the evaluation of Pgp substrate activity and/or inhibition. The lowest concentration of the target compounds displaying a maximal increase in the intracellular accumulation of [^{3}H] Taxol, a known substrate of Pgp was determined. A known microtubule inhibitor and antitumor agent, Taxol was included for comparison.

Compounds **5-10** with varied *N*7-benzyl substitutions demonstrated IC_{50} values ranging from 0.6 μM to 3 μM in the cytotoxicity assay. Compound **7** was the most potent compound in this series being only 4-fold less potent than the standard compound Taxol, while compounds **5** and **10** were 6.6-fold less potent than Taxol. Among the mono-OMe benzyl substituted compounds, the 4-OMe was slightly better than the 2- and 3-OMe substituted compounds. In the di- and tri-OMe benzyl substituted compounds, the presence of a 4-OMe slightly improved potency, with the 3,4-diOMe and 3,4,5-triOMe both being better than 3,5-diOMe substituted compound, although less potent than **7**. In the drug accumulation assay for determination of Pgp activity, all of the compounds with the exception of **10**, demonstrated a significant increase in the intracellular accumulation of [^{3}H] Taxol at values close to their IC_{50} ranging from 0.2 μM to 2 μM. Compound **7** demonstrated an increase in the intracellular accumulation of [^{3}H] Taxol at the lowest concentration for this series of compounds, followed by compounds **6**, **8** and **9** suggesting Pgp modulatory effects for compound **7** >**6** >**8** >**9**.

ACCEPTED MANUSCRIPT

The 5-thiosubstituted compounds **11-14** demonstrated micromolar cytotoxicity in JC cells. Of compounds **11-14**, compound **11** demonstrated IC50 values (cytotoxicity) at the lowest concentration, and was approximately 2-fold more cytotoxic in JC cells than **12-14**. Against Pgp, compound **12** demonstrated an increase in the intracellular accumulation of [^{3}H] Taxol below its IC_{50}, while **11**, **13** and **14** did not show any increase in the accumulation of [^{3}H] Taxol at the evaluated concentrations. Based on the cytotoxicity and Pgp modulation for **12**, a combination of the 5-(2-methoxy)phenylthio substitution was attempted with a variety of 7-benzyl substitutions for compounds **15-17**. Antitumor activity improved for the 4-OMe and 3,4,5-triOMe benzyl substitutions in **16** and **17** respectively. Compounds **16** and **17** demonstrated single-digit micromolar cytotoxicity (IC_{50} values) in JC cells and were approximately 4- fold better than **11**. Pgp modulation however, was abolished with substitution at the 7-benzyl moiety. A combination of the 3,4,5-triOMe phenylthio substitution at C5 with the 3,4,5-triOMe benzyl substitution at *N*7 was attempted in compound **18** in an effort to improve antitumor activity. Compound **18** showed a dramatic decrease in antitumor activity compared to its phenylethyl analog, **10**.

Figure 2

Based on the promising results for JC cell inhibition and Pgp modulation, compound **7** was evaluated specifically for its effect on microtubules at varied doses (Figure 2). Compound **7** produced dose-dependent microtubule depolymerization, confirming inhibition of microtubule polymerization as the mechanism for tumor cell inhibition.

Table 2

ACCEPTED MANUSCRIPT

Compound **7** was further evaluated in the NCI 60 tumor cell line panel.[20] Compound **7** inhibited most of the cell lines with GI_{50} values that ranged from two-digit nanomolar to submicromolar concentrations (Table 2). In addition, compound **7** also demonstrated submicromolar GI_{50} values toward resistant tumor cell lines NCI/ADR-RES (202 nM), HCT-15 colon (242 nM) and renal cancer cell lines, TK-10 (686 nM) and UO-31 (397 nM).

4. Conclusions

We synthesized fourteen novel microtubule inhibitors **5-18** to determine the effect of the substitution pattern of the 5,7-disubstituted pyrrolo[2,3-*d*]pyrimidines on antitumor activity and Pgp modulation. Compounds **5-10** and **16** demonstrated single digit micromolar effects in JC tumor cells. The ethyl linker was found to be preferred over the thio linker at C5 for antitumor activity. Methoxy substitutions on the *N*7-benzyl ting improved antitumor activity over the unsubstituted benzyl moiety for both the C5-ethyl and C5-thio linked compounds. Compounds **5-9** induced significant Pgp modulation as demonstrated in the *in vitro* drug accumulation assay. The ethyl linker was also found to be preferred over the thio linker at C5 for Pgp modulation. Although substitutions at the *N*7-benzyl improved antitumor activity over the unsubstituted benzyl moiety for both the C5-ethyl and C5-thio linked compounds, Pgp modulation was not observed for C5-thio linked compounds with substitutions at the *N*7-benzyl moiety. Our results indicate that the potency of antitumor activity and Pgp modulation does indeed vary with different substitutions and that an optimal combination of the substitutions in the 7-benzyl moiety, the C5-linker and the C5-phenyl moiety is essential for inhibition of microtubules, tumor

ACCEPTED MANUSCRIPT

cells and Pgp. Compound **7** emerged as the most viable candidate for future evaluation with its potent microtubule and Pgp modulatory effects and remarkable nanomolar cytotoxic effects in the NCI 60 cell line panel.

5. Experimental Section

5.1 Synthesis All evaporations were carried out *in vacuo* with a rotary evaporator. Thin-layer chromatography (TLC) was performed on silica gel plates (Whatman 250 μM PE SilG/UV) with fluorescent indicator. Spots were visualized by UV (254 and 366 nm) illumination. Column chromatography was performed using Merck silica gel (200-400 mesh). Proton nuclear magnetic resonance (^{1}H) spectra were recorded on a Bruker WH-300 (300 MHz) or Bruker WH-400 (400 MHz) spectrometer. The chemical shift values were expressed in ppm (parts per million) relative to tetramethylsilane as internal standard; s = singlet, d = double, t = triplet, q = quartet, m = multiplet, br = broad singlet. The relative integrals of peak areas agreed with those expected for the assigned structures. Melting points were determined on a Mel-Temp II melting point apparatus with FLUKE 51 K/J electronic thermometer and were uncorrected. Analytical samples were dried *in vacuo* (0.2mm Hg) in a CHEM DRY drying apparatus over P_2O_5. Elemental analysis was performed by Altlantic Microlabs, Norcross, GA, USA. Element compositions are within ±0.4% of the calculated values. Fractional moles of water or organic solvents frequently found in some analytical samples could not be prevented despite 24–48 h of drying *in vacuo* and were confirmed where possible by their presence in the ^{1}H NMR spectra. High-resolution mass spectra (HRMS), using Electron Impact

ACCEPTED MANUSCRIPT

(EI), were recorded on a VG Autospec (Fisons Instruments) micromass (EBE Geometry) double-focusing mass spectrometer. All solvents and chemicals were purchased from Aldrich Chemical Co. and Fisher Scientific and were used as received.

5.1.1 *N*-(2-methoxybenzyl)-*N*-(2,2-dimethylpropanoyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)-2,2-dimethylpropanamide (20a) and *N*-[7-(2-methoxybenzyl)-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (21a)

To a 100 mL round-bottom flask, under nitrogen, was added **19a** (0.8 g, 2.43 mmol), PivCl (12 mL), DMAP (100 mg, 0.5 mmol), NEt_3 (15 ml) and dichloroethane (55 mL). The mixture was stirred at 50-55 ^{o} C for 12 h. The reaction mixture was diluted with CH_2Cl_2 (120 mL) and washed sequentially with H_2O (30 mL) and brine (30 mL). The organic layer was separated and dried over Na_2SO_4, filtered, and dried *in vacuo* to afford brown oil. The crude product was purified by flash chromatography on silica gel (gradient, 1:1:15 EtOAc/NEt_3/hexane to 1:1:5 EtOAc/NEt_3/hexane) and washed with diethyl ether or methanol to afford 0.8 g (62 %) of **20a** as an off-white solid (R_f, ^{1}H NMR as that reported)[12] and 0.39 g (39 %) of **21a** as a yellow solid; TLC R_f 0.54 (EtOAc/NEt_3/Hexane, 5:1:3); mp 119-120 ^{o}C; ^{1}H NMR (DMSO-d_6): δ 1.21 (s, 9 H, $C(CH_3)_3$), 2.61 (s, 3 H, CH_3), 3.86 (s, 3 H, OCH_3), 5.33 (s, 2 H, CH_2), 6.65 (d, 1 H, CH), 6.71 (d, 2 H, C_6H_4), 6.82 (t, 1 H, C_6H_4), 7.02 (d, 2 H, C_6H_4), 7.25 (t, 2 H, C_6H_4), 7.36 (d, 1 H, CH), 9.70 (s, 1 H, NH); HRMS (EI) Calcd for $C_{20}H_{24}N_4O_2$ *m/z* = 352.1899, found *m/z* = 352.1888.

ACCEPTED MANUSCRIPT

5.1.2 *N*-(3-methoxybenzyl)-*N*-(2,2-dimethylpropanoyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)-2,2-dimethylpropanamide (20b) and *N*-[7-(3-methoxybenzyl)-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (21b)

Compounds **20b** and **21b** were synthesized as described for **20a** and **21a** with **19b**. The crude mixture was purified by flash chromatography on silica gel to afford 0.36 g of **20b** (25%) as a yellow solid (R_f, ^{1}H NMR as that reported) [12] and **21b** as a yellow semisolid (0.9 g, 70 %); TLC R_f 0.55 (EtOAc/NEt_3/Hexane, 5:1:3); ^{1}H NMR (DMSO-d_6): δ 1.22 (s, 9 H, $C(CH_3)_3$), 2.59 (s, 3 H, CH_3), 3.70 (s, 3 H, OCH_3), 5.34 (s, 2 H, CH_2), 6.63 (d, 1 H, CH), 6.81-7.20 (m, 4 H, C_6H_4), 7.46 (d, 1 H, CH), 9.95 (s, 1 H, NH). HRMS (EI) Calcd for $C_{20}H_{24}N_4O_2$ *m/z* = 352.1899, found *m/z* = 352.1909.

5.1.3 *N*-(4-methoxybenzyl)-*N*-(2,2-dimethylpropanoyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)-2,2-dimethylpropanamide (20c) and *N*-[7-(4-methoxybenzyl)-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (21c)

Compounds **20c** and **21c** were synthesized as described for **20a** and **21a** with **19c**. The crude mixture was purified by flash chromatography on silica gel to afford **20c** (25%) as a yellow semisolid (R_f, ^{1}H NMR as that reported) [12] and **21c** as a yellow semisolid (65 %); TLC R_f 0.54 (EtOAc/NEt_3/Hexane, 5:1:3); ^{1}H NMR (DMSO-d_6): δ 1.24 (s, 3 H, $C(CH_3)_3$), 2.60 (s, 3 H, CH_3), 3.69 (s, 3 H, OCH_3), 5.29 (s, 2 H, CH_2), 6.66 (d, 1 H, CH), 6.84 (d, 2 H, C_6H_4), 7.28 (d, 2 H, C_6H_4), 7.42 (s, 1 H, CH), 9.81 (s, 1 H, NH).

5.1.4 *N*-[7-(3,4-Dimethoxybenzyl)-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (21d)

ACCEPTED MANUSCRIPT

Compound **21d** was synthesized as described for **21a** with **19d** and was obtained as a yellow foam; TLC R_f 0.52 (EtOAc/NEt_3/Hexane, 5:1:3); ^{1}H NMR (DMSO-d_6): δ 1.23 (s, 9 H, $C(CH_3)_3$), 2.58 (s, 3 H, CH_3), 3.68 (s, 3 H, OCH_3), 3.71 (s, 3 H, OCH_3), 5.26 (s, 2 H, CH_2), 6.58 (d, 1 H, CH), 6.82-7.19 (m, 3 H, C_6H_3), 7.45 (d, 1 H, CH), 9.76 (s, 1 H, NH); HRMS (EI) $[M]^+$: Calcd for $C_{21}H_{26}N_4O_3$ *m/z* = 382.2004, found *m/z* = 382.1998.

5.1.5 *N*-[7-(3,5-Dimethoxybenzyl)-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (21e)

Compound **21e** was synthesized as described for **21a** with **19e** and was obtained as a light brown semisolid (0.82 g, 68 %); TLC R_f 0.53 (EtOAc/NEt_3/Hexane, 5:1:3); ^{1}H NMR (DMSO-d_6): δ 1.22 (s, 9 H, $C(CH_3)_3$), 2.60 (s, 3 H, CH_3), 3.68 (s, 6 H, $(OCH_3)_2$), 5.28 (s, 2 H, CH_2), 6.37 (s, 1 H, C_6H_3), 6.48 (s, 2 H, C_6H_3), 6.62 (d, 1 H, CH), 7.47 (d, 1 H, CH), 9.79 (s, 1 H, NH); HRMS (EI) $[M]^+$: Calcd for $C_{21}H_{26}N_4O_3$ *m/z* = 382.2004, found *m/z* = 382.2003.

5.1.6 *N*-(3,4,5-Trimethoxybenzyl)-*N*-(2,2-dimethylpropanoyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)-2,2-dimethylpropanamide (20d) and 5.1.20 *N*-[7-(3,4,5-Trimethoxybenzyl)-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2 dimethylpropanamide (21f)

Compounds **20d** and **21f** were synthesized as described for **20a** and **21a** with **19f**. The crude mixture was purified by flash chromatography on silica gel to afford **20d** (62%) as a yellow solid (R_f, ^{1}H NMR as that reported) [12] and **21f** as a yellow foam (26 %); TLC R_f 0.50 (EtOAc/NEt_3/Hexane, 5:1:3); ^{1}H NMR (DMSO-d_6): δ 1.20 (s, 9 H, $C(CH_3)_3$), 2.57

ACCEPTED MANUSCRIPT

(s, 3 H, CH_3), 3.55 (s, 3 H, OCH_3), 3.71 (s, 6 H, $(OCH_3)_2$), 5.23 (s, 2 H, CH_2), 6.57 (d, 1 H, CH), 6.85 (s, 1 H, C_6H_2), 7.52 (d, 1 H, CH), 9.79 (s, 1 H, NH); HRMS (EI) $[M]^+$: Calcd for $C_{22}H_{28}N_4O_4$ *m/z* = 412.2110, found *m/z* = 412.2122.

5.1.7 *N*-[5-Iodo-7-(2-methoxybenzyl)-*N*-(2,2-dimethylpropanoyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)-2,2-dimethylpropanamide (22a)

To a 100 mL round-bottom flask, protected from light, under nitrogen, was added compound **20a** (350 mg, 0.7 mmol), N-iodo succinimide (NIS) (350 mg, 1.5 mmol) and dry DMF (10 mL). The mixture was stirred at room temperature for 12 h. The reaction mixture was dried *in vacuo*, purified by flash chromatography on silica gel (isocratic, 1:1:15 EtOAc/NEt_3/hexane) and washed with diethyl ether or methanol to afford 360 mg (82%) of **22a** as a white solid (R_f, ^{1}H NMR as that reported). [12]

5.1.8 *N*-[5-Iodo-7-(3,4,5-Trimethoxybenzyl)-*N*-(2,2-dimethylpropanoyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)-2,2-dimethylpropanamide (22b)

Compound **22b** was synthesized as described for **22a** with **20d**(1 g, 2.29 mmol) using the general procedure described above to afford a yellow solid (0.92 g, 78%) (R_f, ^{1}H NMR as that reported) [12]

5.1.9 *N*-[5-Iodo-7-(3-methoxybenzyl)-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (22c)

Compound **22c** was synthesized as described for **22a** with **20b** and was obtained as a yellow foam (0.6 g, 60 %); TLC R_f 0.64 (EtOAc/NEt_3/Hexane, 5:1:3); ^{1}H NMR (DMSO-

ACCEPTED MANUSCRIPT

d_6): δ 1.23 (s, 9 H, $C(CH_3)_3$), 2.79 (s, 3 H, CH_3), 3.71 (s, 3 H, OCH_3), 5.29 (s, 2 H, CH_2), 6.82-7.20 (m, 4 H, C_6H_4), 7.73 (s, 1 H, CH), 9.89 (s, 1 H, NH); HRMS (ESI) $[M + H]^+$: Calcd for $C_{20}H_{24}N_4O_2I$ *m/z* = 479.0944, found *m/z* = 479.0938.

5.1.10 *N*-[5-Iodo-7-(4-methoxybenzyl)-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (22d)

Compound **22d** was synthesized was synthesized as described for **22a** with **21c** and was obtained as a yellow foam (820 mg, 86 %); TLC R_f 0.63 ($EtOAc/NEt_3$/Hexane, 5:1:3); ^1H NMR (DMSO-d_6): δ 1.24 (s, 9 H, $C(CH_3)_3$), 2.80 (s, 3 H, CH_3), 3.70 (s, 3 H, OCH_3), 5.26 (s, 2 H, CH_2), 6.88 (s, 2 H, C_6H_4), 7.33 (d, 2 H, C_6H_4), 7.77 (s, 1 H, CH), 9.86 (s, 1 H, NH); HRMS (EI): Calcd for $C_{20}H_{23}N_4O_2I$ *m/z* = 478.0865, found *m/z* = 478.0856.

5.1.11 *N*-[5-Iodo-7-(3,4-dimethoxybenzyl)-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (22e)

Compound **22e** was synthesized as described for **22a** with **21d** and was obtained as a yellow semisolid (0.42 g, 70 %); TLC R_f 0.61 ($EtOAc/NEt_3$/Hexane, 5:1:3); ^1H NMR (DMSO-d_6): δ 1.24 (s, 9 H, $C(CH_3)_3$), 2.78 (s, 3 H, CH_3), 3.67 (s, 3 H, OCH_3), 3.71 (s, 3 H, OCH_3), 5.23 (s, 2 H, CH_2), 6.85-7.25 (m, 3 H, C_6H_3), 7.74 (s, 1 H, CH), 9.89 (s, 1 H, NH); HRMS (EI) : Calcd for $C_{21}H_{25}IN_4O_3$ *m/z* = 508.0971, found *m/z* = 508.0973.

5.1.12 *N*-[5-Iodo-7-(3,5-dimethoxybenzyl)-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (22f)

ACCEPTED MANUSCRIPT

Compound **22f** was synthesized as described for **22a** with **21e** and was obtained as a yellow semisolid (0.74 g, 75 %); TLC R_f 0.62 (EtOAc/NEt_3/Hexane, 5:1:3); ^{1}H NMR (DMSO-d_6): δ 1.23 (s, 9 H, $C(CH_3)_3$), 2.81 (s, 3 H, CH_3), 3.69 (s, 6 H, $(OCH_3)_2$), 5.24 (s, 2 H, CH_2), 6.38 (s, 1 H, C_6H_3), 6.58 (s, 2 H, C_6H_3), 7.76 (s, 1 H, CH), 9.91 (s, 1 H, CH); HRMS (ESI) $[M + H]^+$: Calcd for $C_{21}H_{26}IN_4O_3$ *m/z* = 509.1041, found *m/z* = 509.1050.

5.1.13 *N*-[7-(2-Methoxybenzyl)-4-methyl-5-(3,4,5-trimethoxyphenylethynyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-*N*-(2,2-dimethylpropanoyl)-2,2-dimethylpropanamide (23a)

To a 5 mL microwave reaction vial was added **22a** (500 mg, 0.80 mmol), 2-methoxyphenyl acetylene (2.26 eq), CuI (0.16 eq), $Pd(Ph_3)_4$ (0.16 eq), NEt_3 (2 mL) and dry dichloroethane (3 mL). The mixture was evacuated and back-filled with nitrogen (three cycles). The reaction mixture was irradiated in a microwave apparatus at 100 °C for 10 min. After the reaction mixture was cooled to ambient temperature, the mixture was filtered. The filtrate was dried *in vacuo* and the crude product was purified by flash chromatography on silica gel (gradient, 1:1:15 EtOAc/NEt_3/hexane to 1:1:5 EtOAc/NEt_3/hexane) and washed with diethyl ether or methanol to afford 415 mg (75%) of **23a** as a yellow semisolid; TLC R_f 0.52 (EtOAc/NEt_3/Hexane, 5:1:10); ^{1}H NMR (DMSO-d_6): δ 1.15 (s, 18 H, $C(CH_3)_6$), 2.89 (s, 3 H, CH_3), 3.69 (s, 3 H, OCH_3), 3.81 (s, 9 H, $(OCH_3)_3$), 5.35 (s, 2 H, CH_2), 6.84 (s, 2 H, C_6H_4), 6.92-7.29 (m, 4 H, C_6H_4), 7.92 (s, 1 H, CH); Anal.($C_{36}H_{42}N_4O_6$ • 0.22 H_2O): C, 68.53; H, 6.78; N, 8.88. Found C, 68.57; H, 6.71; N, 8.59.

ACCEPTED MANUSCRIPT

5.1.14 *N*-[7-(3,4,5-Trimethoxybenzyl)-4-methyl-5-(3,4,5-trimethoxyphenylethynyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-*N*-(2,2-dimethylpropanoyl)-2,2-dimethylpropanamide (23b)

Compound **23b** was synthesized as described for **23a** with **22b** and was obtained as a yellow semisolid (73 %); TLC R_f 0.48 (5:1:10 EtOAc/NEt_3); ^{1}H NMR (DMSO-d_6): δ 1.18 (s, 18 H, $C(CH_3)_6$), 2.89 (s, 3 H, CH_3), 3.60 (s, 3 H, OCH_3), 3.68 (s, 3 H, OCH_3), 3.70 (s, 6 H, $(OCH_3)_2$), 3.80 (s, 6 H, $(OCH_3)_2$), 5.28 (s, 2 H, CH_2), 6.62 (s, 2 H, C_6H_2), 6.82 (s, 2 H, C_6H_2), 8.13 (s, 1 H, CH); HRMS (ESI) $[M + Na]^+$: Calcd for $C_{38}H_{46}N_4O_8Na$ *m/z* = 709.3213, found *m/z* = 709.3157.

5.1.15 *N*-7-(3-methoxybenzyl)-5-[(3,4,5-trimethoxyphenyl)ethynyl]-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (23c)

Compound **23c** was synthesized as described for **23a** with **22c** and was obtained as a yellow semisolid (273 mg, 80 %); TLC R_f 0.52 (EtOAc/NEt_3/Hexane, 5:1:3); ^{1}H NMR (DMSO-d_6): δ 1.22 (s, 9 H, $C(CH_3)_3$), 2.85 (s, 3 H, CH_3), 3.69 (s, 3 H, OCH_3), 3.79 (s, 6 H, $(OCH_3)_2$), 3.86 (s, 3 H, OCH_3), 5.35 (s, 2 H, CH_2), 6.83 (s, 2 H, C_6H_2), 6.83 (m, 2 H, C_6H_4), 7.06 (d, 1 H, C_6H_4), 7.29 (t, 1 H, C_6H_4), 7.75 (s, 1 H, CH), 9.88 (s, 1 H, NH); Anal.($C_{31}H_{34}N_4O_5$ • 0.3 CH_3OH): C, 68.12; H, 6.41; N, 10..16. Found C, 68.20; H, 6.29; N, 9.83.

5.1.16 *N*-7-(4-methoxybenzyl)-5-[(3,4,5-trimethoxyphenyl)ethynyl]-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (23d)

ACCEPTED MANUSCRIPT

Compound **23d** was synthesized as described for **23a** with **22d** and was obtained as a yellow foam (250 mg, 74 %); TLC R_f 0.52 (EtOAc/NEt_3/Hexane, 5:1:3); ^{1}H NMR (DMSO-d_6): δ 1.25 (s, 3 H, $C(CH_3)_3$), 2.84 (s, 3 H, CH_3), 3.68 (s, 3 H, OCH_3), 3.71 (s, 3 H, OCH_3), 3.80 (s, 6 H, $(OCH_3)_2$), 5.30 (s, 2 H, CH_2), 6.81 (s, 2 H, C_6H_2), 6.88 (d, 2 H, C_6H_4), 7.35 (d, 2 H, C_6H_4), 7.87 (s, 1 H, CH), 9.93 (s, 1 H, NH).

5.1.17 *N*-7-(3,4-Dimethoxybenzyl)-5-[(3,4,5-trimethoxyphenyl)ethynyl]-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (23e)

Compound **23e** was synthesized as described for **23a** with **22e** and was obtained as a yellow semisolid (240 mg, 71 %); TLC R_f 0.50 (EtOAc/NEt_3/Hexane, 5:1:3); ^{1}H NMR (DMSO-d_6): δ 1.27 (s, 9 H, $C(CH_3)_3$), 2.55 (s, 3 H, CH_3), 3.67 (s, 3 H, OCH_3), 3.79 (s, 6 H, $(OCH_3)_2$), 3.68-3.70 (s, 6 H, $(OCH_3)_2$), 5.28 (s, 2 H, CH_2), 6.81 (s, 2 H, CH_2), 6.89 (d, 2 H, C_6H_3), 6.96 (s, 1 H, C_6H_3), 7.26 (d, 1 H, C_6H_3), 7.91 (s, 1 H, CH), 9.92 (s, 1 H, CH).

5.1.18 *N*-7-(3,5-Dimethoxybenzyl)-5-[(3,4,5-trimethoxyphenyl)ethynyl]-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (23f)

Compound **23f** was synthesized as described for **23a** with **22f** and was obtained as a yellow semisolid (246 mg, 73 %); TLC R_f 0.48 (EtOAc/NEt_3/Hexane, 5:1:3); ^{1}H NMR (DMSO-d_6): δ 1.23 (s, 9 H, $C(CH_3)_3$), 2.84 (s, 3 H, CH_3), 3.69 (s, 3 H, OCH_3), 3.70 (s, 6 H, $(OCH_3)_2$), 3.79 (s, 6 H, $(OCH_3)_2$), 5.27 (s, 2 H, CH_2), 6.39 (s, 1 H, C_6H_3), 6.58 (s, 2 H, C_6H_3), 6.81 (s, 2 H, C_6H_2), 7.94 (s, 1 H, CH), 9.93 (s, 1 H, NH); HRMS (ESI) $[M + H]^+$: Calcd for $C_{32}H_{37}N_4O_6$ *m/z* = 573.2713, found *m/z* = 573.2697.

ACCEPTED MANUSCRIPT

5.1.19 7-(2-Methoxybenzyl)-5-[(3,4,5-trimethoxyphenyl)ethyl]-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (5)

To a Parr hydrogenation bottle was added **23a** (200 mg, 0.36 mmol), 5% Pd/C (1:3 by weight) 4 mL MeOH and 10 mL CH_2Cl_2. The mixture was shaken under 50 p.s.i. hydrogen for 24 h. The reaction mixture was filtered, dried *in vacuo*. To the resulting off-white semisolid (160 mg, 0.23 mmol) was added aqueous 1 N NaOH (4 mL) and MeOH (20 mL). The reaction mixture was refluxed for 12 h, and concentrated. The slurry obtained was dissolved in water (5 mL) and extracted with CH_2Cl_2 (30 mL). The organic layer was dried over Na_2SO_4 and concentrated to afford a colourless oil. The crude product was purified by flash chromatography on silica gel (gradient, 5:1:10 EtOAc/NEt_3/hexane to 5:1:3 EtOAc/NEt_3/hexane) and washed with diethyl ether or methanol to afford 78 mg (66%) of **5** as a white solid; TLC R_f 0.26 (EtOAc/NEt_3/Hexane, 5:1:3); mp 147-148 ^oC; ^1H NMR (DMSO-d_6): δ 2.53 (s, 3 H, CH_3), 2.82 (t, 2 H, CH_2), 2.99 (t, 2 H, CH_2), 3.60 (s, 3 H, OCH_3), 3.71 (s, 6 H, $(OCH_3)_2$), 3.83 (s, 3 H, OCH_3), 5.12 (s, 2 H, CH_2), 6.06 (s, 2 H, NH_2), 6.45 (d, 1 H, C_6H_4), 6.48 (s, 2 H, C_6H_2), 6.74 (s, 1 H, CH), 6.79 (t, 1 H, C_6H_4), 6.99 (d, 1 H, C_6H_4), 7.23 (t, 1 H, C_6H_4); Anal.($C_{26}H_{30}N_4O_4$): C, 66.90; H, 6.57; N, 12.00. Found C, 66.97; H, 6.56; N, 11.81.

5.1.20 7-(3-Methoxybenzyl)-5-[(3,4,5-trimethoxyphenyl)ethyl]-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (6)

Compound **6** was synthesized as described for **5** with **23c** and was obtained as a white solid (70 mg, 61 %); TLC R_f 0.27 (EtOAc/NEt_3/Hexane, 5:1:3); mp 144-146 ^oC; ^1H NMR (DMSO-d_6): δ 2.48 (s, 3 H, CH_3), 2.80 (t, 2 H, CH_2), 2.96 (t, 2 H, CH_2), 3.59 (s, 3

ACCEPTED MANUSCRIPT

H, OCH_3), 3.68 (s, 9 H, $(OCH_3)_3$), 5.12 (s, 2 H, CH_2), 6.08 (s, 2 H, NH_2), 6.64 (s, 2 H, CH_2), 6.73-6.74 (m, 3 H, C_6H_4), 6.80 (s, 1 H, CH), 7.18 (t, 1 H, C_6H_4); HRMS (ESI) $[M + H]^+$: Calcd for $C_{26}H_{31}N_4O_4$ *m/z* = 463.2345, found *m/z* = 463.2325.

5.1.21 7-(4-Methoxybenzyl)-5-[(3,4,5-trimethoxyphenyl)ethyl]-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (7)

Compound **7** was synthesized as described for **5** with **23d** and was obtained as a white solid (60 mg, 57 %); TLC R_f 0.26 ($EtOAc/NEt_3$/Hexane, 5:1:3); mp 137-138 ^oC; ^1H NMR (DMSO-d_6): δ 2.50 (s, 3 H, CH_3), 2.78 (t, 2 H, CH_2), 2.94 (t, 2 H, CH_2), 3.58 (s, 3 H, OCH_3), 3.69 (s, 9 H, $(OCH_3)_3$), 5.06 (s, 2 H, CH_2), 6.06 (s, 2 H, NH_2), 6.52 (s, 2 H, CH_2), 6.74 (s, 1 H, CH), 6.83 (d, 2 H, C_6H_4), 7.09 (d, 2 H, C_6H_4); HRMS (ESI) $[M + H]^+$: Calcd for $C_{26}H_{31}N_4O_4$ *m/z* = 463.2345, found *m/z* = 463.2331.

5.1.22 7-(3,4-Dimethoxybenzyl)-5-[(3,4,5-trimethoxyphenyl)ethyl]-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (8)

Compound **8** was synthesized as described for **5** with **23e** and was obtained as a light yellow solid (65 mg, 55 %); TLC R_f 0.24 ($EtOAc/NEt_3$/Hexane, 5:1:3); mp 135-137 ^oC; ^1H NMR (DMSO-d_6): δ 2.51 (s, 3 H, CH_3), 2.79 (t, 2 H, CH_2), 2.94 (t, 2 H, CH_2), 3.59 (s, 3 H, OCH_3), 3.69 (s, 6 H, $(OCH_3)_2$), 3.69 (s, 6 H, $(OCH_3)_2$), 5.06 (s, 2 H, CH_2), 6.08 (s, 2 H, NH_2), 6.51 (s, 2 H, C_6H_3), 6.57 (d, 2 H, C_6H_3), 6.76 (s, 1 H, CH), 6.84 (s, 1 H, CH), 6.96 (s, 1 H, CH). HRMS (ESI) $[M + H]^+$: Calcd for $C_{27}H_{33}N_4O_5$ *m/z* = 493.2451, found *m/z* = 493.2430.

ACCEPTED MANUSCRIPT

5.1.23 7-(3,5-Dimethoxybenzyl)-5-[(3,4,5-trimethoxyphenyl)ethyl]-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (9)

Compound **9** was synthesized as described for **5** with **23f** and was obtained as a light yellow solid (70 mg, 57 %); TLC R_f 0.22 (EtOAc/NEt_3/Hexane, 5:1:3); mp 133-135 ^oC; ^1H NMR (DMSO-d_6): δ 2.49 (s, 3 H, CH_3), 2.80 (t, 2 H, CH_2), 2.95 (t, 2 H, CH_2), 3.60 (s, 3 H, OCH_3), 3.67 (s, 6 H, $(OCH_3)_2$), 3.71 (s, 6 H, $(OCH_3)_2$), 5.09 (s, 2 H, CH_2), 6.09 (s, 2 H, NH_2), 6.29 (s, 2 H, C_6H_3), 6.38 (s, 1 H, C_6H_3), 6.54 (s, 2 H, C_6H_2), 6.82 (s, 1 H, CH); HRMS (ESI) $[M + H]^+$: Calcd for $C_{27}H_{33}N_4O_5$ *m/z* = 493.2451, found *m/z* = 493.2433.

5.1.24 7-(3,4,5-Trimethoxybenzyl)-5-[(3,4,5-trimethoxyphenyl)ethyl]-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (10)

Compound **10** was synthesized as described for **5** with **23b** and was obtained as a white solid (78 mg, 66%); TLC R_f 0.20 (EtOAc/NEt_3/Hexane, 5:1:3); mp 136-137 ^oC; ^1H NMR (DMSO-d_6): δ 2.52 (s, 3 H, CH_3), 2.81 (t, 2 H, CH_2), 2.94 (t, 2 H, CH_2), 3.61 (s, 6 H, OCH_3), 3.69-3.71 (s, 12 H, $(OCH_3)_4$), 5.07 (s, 2 H, CH_2), 6.12 (s, 2 H, NH_2), 6.54 (s, 2 H, C_6H_2), 6.60 (s, 1 H, C_6H_2), 6.84(s, 1 H, CH); Anal.($C_{38}H_{50}N_4O_8 \cdot 0.38\ (C_2H_5)_2O$): Calcd C, 66.01; H, 7.54; N, 7.78. Found C, 66.32; H, 7.29; N, 7.51.

5.1.25 *N*-[5-Iodo-7-(2-methoxybenzyl)-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (24b)

Compound **24b** was synthesized as described for **22a** with **21a** and was obtained as a yellow foam (0.86 g, 66 %); TLC R_f 0.65 (EtOAc/NEt_3/Hexane, 5:1:3); ^1H NMR (DMSO-d_6): δ 1.22 (s, 9 H, $C(CH_3)_3$), 2.82 (s, 3 H, CH_3), 3.86 (s, 3 H, OCH_3), 5.32 (s, 2

ACCEPTED MANUSCRIPT

H, CH_2), 6.84-7.30 (m, 4 H, C_6H_4), 7.57 (s, 1 H, CH), 9.82 (s, 1 H, NH); Anal.($C_{20}H_{23}IN_4O_2 \cdot 1.02\ (C_4H_8)O$): Calcd C, 52.41; H, 5.69; N, 10.14; I, 22.97. Found C, 52.81; H, 5.78; N, 10.10; I, 23.25.

5.1.26 *N*-[5-Iodo-7-(3,4,5-Trimethoxybenzyl)-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (24c)

Compound **24c** was synthesized as described for **22a** with **21f** and was obtained as a yellow solid (0.73 g, 73 %); TLC R_f 0.59 (EtOAc/NEt_3/Hexane, 5:1:3); ^{1}H NMR (DMSO-d_6): δ 1.23 (s, 9 H, $C(CH_3)_3$), 2.79 (s, 3 H, CH_3), 3.57 (s, 3 H, OCH_3), 3.75 (s, 6 H, $(OCH_3)_2$), 5.20 (s, 2 H, CH_2), 6.99 (s, 2 H, C_6H_2), 7.82 (s, 1 H, CH), 9.95 (s, 1 H, NH).

5.1.27 *N*-[7-benzyl-5-(4-phenyl)sulfanyl-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (25a)

To a 5 mL microwave reaction vial was added **24a** (300 mg, 0.48 mmol), phenylthiol (1.2 eq), CuI (1.3 eq), K_2CO_3 (1.5 eq) and 4 mL of dry DMF. The mixture was evacuated and back-filled with nitrogen (three cycles). The reaction mixture was irradiated in a microwave apparatus at 100 °C for 4 h. After the reaction mixture was cooled to ambient temperature, the mixture was filtered. The filtrate was dried *in vacuo* and the crude product was purified by flash chromatography on silica gel (gradient, 1:8 to 1:2, EtOAc: hexane) and washed with diethyl ether/methanol to afford 230 mg (78%) of **25a** as a yellow semisolid; R_f 0.50 (5:1:3, EtOAc/NEt_3/Hexane); ^{1}H NMR (DMSO-d_6): δ 1.23 (s,

ACCEPTED MANUSCRIPT

9 H, $C(CH_3)_3$), 2.54 (s, 3 H, CH_3), 5.44 (s, 2 H, CH_2), 7.04-7.36 (m, 10 H, C_6H_5 and C_6H_5), 7.95 (s, 1 H, CH), 9.93 (s, 1 H, NH).

5.1.28 *N*-[7-benzyl-5-(2-methoxyphenyl)sulfanyl-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (25b)

Compound **25b** was synthesized as described for **25a** with 2-methoxyphenylthiol and was obtained as a white foam (76%); R_f 0.45 (5:1:3, EtOAc/NEt_3/Hexane); ^{1}H NMR (DMSO-d_6): δ 1.20 (s, 9 H, $C(CH_3)_3$), 2.39 (s, 3 H, 4-CH_3), 3.83 (s, 3 H, OCH_3), 5.4 (s, 2 H, CH_2), 6.42-7.33 (m, 9 H, C_6H_5 and C_6H_4), 7.84 (s, 1 H, CH), 9.85 (s, 1 H, NH). HRMS(ESI) $[M+H]^+$ *m/z* Calcd for ($C_{26}H_{29}N_4O_2S$) 461.2011 Found 461.1987.

5.1.29 *N*-[7-benzyl-5-(4-methoxyphenyl)sulfanyl-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (25c)

Compound **25c** was synthesized as described for **25a** with 4-methoxyphenylthiol and was obtained as a yellow foam (78%); R_f 0.48 (5:1:3, EtOAc/NEt_3/Hexane); ^{1}H NMR (DMSO-d_6): δ 1.22 (s, 9 H, $C(CH_3)_3$), 2.58 (s, 3 H, CH_3), 3.68 (s, 3 H, OCH_3), 5.41 (s, 2 H, CH_2), 6.86 (d, 2 H, C_6H_4), 7.02 (d, 2 H, C_6H_4), 7.22-7.34 (m, 5 H, C_6H_5), 7.89 (s, 1 H, CH), 9.87 (s, 1 H, NH). HRMS (ESI) $[M + H]^+$: *m/z* Calcd for ($C_{26}H_{29}N_4O_2S$) 461.2011 Found 461.1982.

5.1.30 *N*-[7-benzyl-5-(4-chlorophenyl)sulfanyl-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (25d)

ACCEPTED MANUSCRIPT

Compound **25d** was synthesized as described for **25a** with 4-chlorophenylthiol and was obtained as a yellow semisolid (83%); R_f 0.53 (5:1:3, EtOAc/NEt_3/Hexane); ^{1}H NMR (DMSO-d_6): δ 1.23 (s, 9 H, $C(CH_3)_3$), 2.54 (s, 3 H, CH_3), 5.32 (s, 2 H, CH_2), 7.04-7.73 (m, 9 H, C_6H_5 and C_6H_4), 7.98 (s, 1 H, CH), 9.92 (s, 1 H, NH). HRMS (ESI) $[M + H]^+$: *m/z* Calcd for ($C_{25}H_{26}N_4O_2SCl$) 465.1481 Found 465.1516.

5.1.31 *N*-(2-Methoxybenzyl)-4-methyl-5-[(2-methoxyphenyl)sulfanyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (25e)

Compound **25e** was synthesized as described for **25a** with **24** and was obtained as a white foam (77%); TLC R_f 0.47 (5:1:3, EtOAc/NEt_3/Hexane); ^{1}H NMR (DMSO-d_6): δ 1.20 (s, 3 H, $C(CH_3)_3$), 2.52 (s, 3 H, CH_3), 3.83 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), 5.37 (s, 2 H, CH_2), 6.47 (d, 1 H, C_6H_4), 6.84-7.27 (m, 7 H, C_6H_4 and C_6H_4), 7.69 (s, 1 H, CH), 9.82 (s, 1 H, NH); Anal.($C_{27}H_{30}N_4O_3S$ • 0.533 $(C_2H_5)_2O$): C, 66.00; H, 6.71; N, 10.56; S, 6.04. Found C, 65.95; H, 6.48; N, 10.30; S, 6.23.

5.1.32 *N*-(4-Methoxybenzyl)-4-methyl-5-[(2-methoxyphenyl)sulfanyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (25)

Compound **25** was synthesized as described for **25a** with **22d** and was obtained as a yellow semisolid (72%); TLC R_f 0.46 (5:1:3, EtOAc/NEt_3/Hexane); ^{1}H NMR (DMSO-d_6): δ 1.27 (s, 3 H, $C(CH_3)_3$), 2.50 (s, 3 H, CH_3), 3.73 (s, 3 H, OCH_3), 3.89 (s, 3 H, OCH_3), 5.37 (s, 2 H, CH_2), 6.48 (d, 1 H, C_6H_4), 6.77 (t, 1 H, C_6H_4), 6.89 (d, 2 H, C_6H_4), 6.92 (d, 1 H, C_6H_4), 7.03 (t, 1 H, C_6H_4), 7.38 (d, 2 H, C_6H_4), 7.87 (s, 1 H, CH), 9.95 (s, 1

ACCEPTED MANUSCRIPT

H, NH); HRMS (ESI) $[M + H]^+$: Calcd for $C_{27}H_{31}N_4O_3S$ *m/z* = 491.2117, found *m/z* = 491.2091.

5.1.33 *N*-(2-Methoxybenzyl)-4-methyl-5-[(3,4,5-trimethoxyphenyl)sulfanyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (25g)

Compound **25g** was synthesized as described for **25a** with **24c** and 3,4,5-trimethoxyphenyl thiol and was obtained as a yellow semisolid (72%); TLC R_f 0.47 (5:1:3 Hexane/ TEA /EtOAc); ^{1}H NMR (DMSO-d_6): δ 1.21 (s, 3 H, $C(CH_3)_3$), 2.63 (s, 3 H, CH_3), 3.59 (s, 6 H, $(OCH_3)_2$), 3.86 (s, 3 H, OCH_3), 5.41 (s, 2 H, CH_2), 6.37 (s, 2 H, C_6H_2), 6.77-6.81 (m, 2 H, C_6H_4), 7.04 (d, 1 H, C_6H_4), 7.28 (t, 1 H, C_6H_4), 7.80 (s, 1 H, CH), 9.85 (s, 1 H, NH); HRMS (ESI) $[M + H]^+$: Calcd for $C_{29}H_{35}N_4O_5S$ *m/z* = 551.2328, found *m/z* = 551.2304.

5.1.34 *N*-(3,4,5-Trimethoxybenzyl)-4-methyl-5-[(3,4,5-trimethoxyphenyl)sulfanyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl]-2,2-dimethylpropanamide (25h)

Compound **25h** was synthesized as described for **25a** with **24c** and was obtained as a yellow semisolid (76%); TLC R_f 0.41 (5:1:3 EtOAc/NEt_3); ^{1}H NMR (DMSO-d_6): δ 1.23 (s, 3 H, $C(CH_3)_3$), 2.65 (s, 3 H, CH_3), 3.53 (s, 6 H, $(OCH_3)_2$), 3.60 (s, 3 H, OCH_3), 3.68 (s, 6 H, $(OCH_3)_2$), 3.74 (s, 6 H, $(OCH_3)_2$), 5.33 (s, 2 H, CH_2), 6.30 (s, 2 H, C_6H_2), 6.61 (s, 2 H, C_6H_4), 6.97 (s, 1 H, CH), 8.19 (s, 1 H, NH).

5.1.35 7-Benzyl-5-phenylsulfanyl-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (11)

ACCEPTED MANUSCRIPT

To a 50 mL round-bottom flask was added **25a** (200 mg, 0.33 mmol), aqueous 1 N NaOH (4 mL) and MeOH (20 mL). The reaction mixture was refluxed for 12 h, and concentrated. The slurry obtained was dissolved in water (5 mL) and extracted with CH_2Cl_2 (30 mL). The organic layer was dried over Na_2SO_4 and concentrated to afford a colourless oil. The crude product was purified by flash chromatography on silica gel (gradient, 5:1:10 EtOAc/NEt_3/hexane to 5:1:3 EtOAc/NEt_3/hexane) and washed with diethyl ether to afford **11** (56%) as a white solid; TLC R_f 0.30 (5:1:3, EtOAc/NEt_3/Hexane); mp 142-143 ^oC; ^1H NMR (DMSO-d_6) δ 2.37 (s, 3 H, CH_3), 5.30 (s, 2 H, CH_2), 5.71 (s, 2 H, NH_2), 7.04-7.34 (m, 10 H, C_6H_5 and C_6H_5), 7.54 (s, 1 H, CH); Anal. [$C_{20}H_{18}N_4S\cdot$ 0.2 $(C_2H_5)_2O$]: C, 69.16; H, 5.35; N, 15.64; S, 9.16. Found C, 69.22; H, 5.45; N, 15.75; S, 9.01.

5.1.36 7-Benzyl-5-[(2-methoxyphenyl)sulfanyl]-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (12)

Compound **12** was synthesized as described for **11** with **25b** and was obtained as a white solid (58%); R_f 0.26 (5:1:3, EtOAc/NEt_3/Hexane); mp 164–165 °C; ^1H NMR (DMSO-d_6) δ 2.31 (s, 3 H, CH_3), 3.84 (s, 3 H, OCH_3), 5.29 (s, 2 H, CH_2), 6.35 (s, 2 H, NH_2), 6.35 (d, 1 H, C_6H_4), 6.54 (t, 1 H, C_6H_4), 6.78 (d, 1 H, C_6H_4), 6.99 (d, 1 H, C_6H_4), 7.08 (t, 1 H, C_6H_4), 7.20-7.33 (m, 6 H, C_6H_5), 7.46 (s, 1 H, CH); HRMS (ESI) $[M + H]^+$: *m/z* Calcd for ($C_{21}H_{21}N_4OS$) 377.1436 Found 377.1399.

5.1.37 7-Benzyl-5-[(4-methoxyphenyl)sulfanyl]-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (13)

ACCEPTED MANUSCRIPT

Compound **13** was synthesized as described for **11** with **25c** and was obtained as a white solid (52%); TLC R_f 0.28 (5:1:3 EtOAc/NEt_3/Hexane); mp 158-160 ^oC; ^1H NMR (DMSO-d_6) δ 2.43 (s, 3 H, CH_3), 3.56 (s, 3 H, OCH_3), 3.57 (s, 6 H, $(OCH_3))_2$, 5.30 (s, 2 H, CH_2), 6.34 (s, 2 H, NH_2), 6.32 (s, 2 H, C_6H_2), 7.17-7.30 (m, 5 H, C_6H_5), 7.53 (s, 1 H, CH); HRMS (ESI) $[M + H]^+$: *m/z* Calcd for ($C_{21}H_{21}N_4OS$) 377.1436 Found 377.1454.

5.1.38 7-Benzyl-5-[(4-chlorophenyl)sulfanyl]-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (14)

Compound **14** was synthesized as described for **11** with **25d** and was obtained as a yellow solid (51%); TLC R_f 0.31 (5:1:3, EtOAc/NEt_3/Hexane); mp 134-135 ^oC; ^1H NMR (DMSO-d_6) δ 2.31 (s, 3 H, CH_3), 5.28 (s, 2 H, CH_2), 6.32 (s, 2 H, NH_2), 6.87 (d, 2 H, C_6H_4), 7.02-7.33 (m, 7 H, C_6H_5 and C_6H_4), 7.51 (s, 1 H, CH); HRMS (ESI) $[M + H]^+$: *m/z* Calcd for ($C_{20}H_{18}N_4SCl$) 381.0941 Found 381.0916.

5.1.39 7-(2-Methoxybenzyl)-5-[(2-methoxyphenyl)sulfanyl]-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (15)

Compound **15** was synthesized as described for **11** with **25e** and was obtained as a white solid (105 mg, 53%); TLC R_f 0.20 (5:1:3, EtOAc/NEt_3/Hexane); mp 171-172 ^oC; ^1H NMR (DMSO-d_6): δ 2.33 (s, 3 H, CH_3), 3.84 (s, 3 H, OCH_3), 3.86 (s, 3 H, OCH_3), 5.24 (s, 2 H, CH_2), 6.34 (s, 2 H, NH_2), 6.55-7.27 (m, 8 H, C_6H_4 and C_6H_4), 7.35 (s, 1 H, CH); Anal.($C_{22}H_{22}N_4O_2S$): C, 65.00; H, 5.45; N, 13.78; S, 7.88. Found C, 64.78; H, 5.37; N, 13.68; S, 7.86.

ACCEPTED MANUSCRIPT

5.1.40 7-(4-Methoxybenzyl)-5-[(2-methoxyphenyl)sulfanyl]-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (16)

Compound **16** was synthesized as described for **11** with **25f** and was obtained as a yellow solid (98 mg, 54%); TLC R_f 0.20 (5:1:3, EtOAc/NEt_3/Hexane); mp 165-166 ^oC; ^1H NMR (DMSO-d_6): δ 2.30 (s, 3 H, CH_3), 3.70 (s, 3 H, OCH_3), 3.84 (s, 3 H, OCH_3), 5.19 (s, 2 H, CH_2), 6.30 (s, 2 H, NH_2), 6.47 (d, 1 H, C_6H_4), 6.77 (t, 1 H, C_6H_4), 6.89 (d, 2 H, C_6H_4), 6.98 (d, 1 H, C_6H_4), 7.07 (t, 1 H, C_6H_4), 7.19 (d, 2 H, C_6H_4), 7.42 (s, 1 H, CH); HRMS (ESI) $[M + H]^+$: Calcd for $C_{22}H_{23}N_4O_2S$ *m/z* = 407.1542, found *m/z* = 407.1575.

5.1.41 7-(3,4,5-Trimethoxybenzyl)-5-[(2-methoxyphenyl)sulfanyl]-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (17)

Compound **17** was synthesized as described for **11** with **25g** and was obtained as a white solid (95 mg, 56 %); TLC R_f 0.18 (5:1:3, EtOAc/NEt_3/Hexane); mp 162-163 ^oC; ^1H NMR (DMSO-d_6): δ 2.32 (s, 3 H, CH_3), 3.60 (s, 3 H, OCH_3), 3.70 (s, 6 H, $(OCH_3)_2$), 3.85 (s, 3 H, OCH_3), 5.19 (s, 2 H, CH_2), 6.36 (s, 2 H, NH_2), 6.53 (d, 1 H, C_6H_4), 6.61 (s, 2 H, C_6H_2), 6.74 (t, 1 H, C_6H_4), 6.97 (d, 1 H, C_6H_4), 7.06 (t, 1 H, C_6H_4), 7.47 (s, 1 H, CH); HRMS (ESI) $[M + H]^+$: Calcd for $C_{24}H_{27}N_4O_4S$ *m/z* = 467.1753, found *m/z* = 467.1734.

5.1.42 7-(3,4,5-Trimethoxybenzyl)-5-[(3,4,5-trimethoxyphenyl)sulfanyl]-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (18)

Compound **17** was synthesized as described for **11** with **25h** and was obtained as a white solid (95 mg, 56 %); TLC R_f 0.15 (5:1:3, EtOAc/NEt_3/Hexane); mp 164-165 ^oC; ^1H NMR (DMSO-d_6): δ 2.42 (s, 3 H, CH_3), 3.53 (s, 6 H, $(OCH_3)_2$), 3.56 (s, 3 H, OCH_3), 3.59 (s, 3

ACCEPTED MANUSCRIPT

H, OCH_3), 3.68 (s, 6 H, $(OCH_3)_2$), 5.16 (s, 2 H, CH_2), 6.31 (s, 2 H, C_6H_2), 6.39 (s, 2 H, NH_2), 6.66 (s, 1 H, C_6H_2), 7.52(s, 1 H, CH); HRMS (ESI) $[M + H]^+$: Calcd for $C_{26}H_{31}N_4O_6S$ *m/z* = 527.1964, found *m/z* = 527.1914.

5.2 General methods for biological evaluations:

5.2.1 Cell Culture and Cell lines

Cell line CRL-2116 (ATCC designation : JC) was obtained from the American Type Culture Collection (Manassas, VA) and cultured as recommended. The cells were maintained in RPMI-1640 (Life Technologies, Inc., Rockville, MD) with L-glutamine containing 10% FBS and 50 μg/ml gentamicin. Cells were maintained at 37 ^{0} C and 5% CO_2 in 100 X 20-mm polystyrene tissue culture dishes.

5.2.2 *In Vitro* Cytotoxicity Assay [19]

JC Cells were seeded in 96-well tissue cluture dishes at approximately 20% confluency and allowed to recover and attach for 24h. Cells were then treated with varying concentrations of target compounds **5-18** or Taxol for 48 h. The number of surviving cells remaining in each well was quantitated with the sulfonamide B (SRB) colorimetric assay. Briefly, cells were washed with phosphate-buffered saline (PBS) and fixed to the plate with 10% tricholoracetic acid. The cells were then washed with water and stained with 0.4% SRB in 1% acetic acid. Cells were then rinsed with 1% acetic acid and 10mM tris base buffer was added to dissolve the SRB. The degree of absorbance was determined with a Perkin Elmer HTS 7000 Plus BioAssay plate reader at a wavelength of 570 nm.

ACCEPTED MANUSCRIPT

5.2.3 *In Vitro* Drug Accumulation Assay [19]

JC Cells were seeded in 24-well tissue culture dishes at approximately 25% confluency and allowed to recover and grow to near confluency, approximately 3-4 days. Medium was aspirated and replaced with serum-free medium. Compounds **5-18** were incubated for 30 min at 37^0 C. Approximately 0.1 μCi of [^{3}H]Taxol (75Ci/mmol) (Moravek Biochemicals, Brea , CA) was then added per well and the cultures were incubated for 60 min at 37^0 C. Radioactive medium was aspirated and cells were rapidly washed twice with ice-cold PBS. Intracellular [^{3}H]Taxol was solubilized with 1% sodium dodecyl sulfate (SDS) in water and quantified by liquid scintillation counting using UniverSol (ICN, Costa Mesa, CA).

5.2.4 Cytoskeleton Staining: JC rat mammary adenocarcinoma cells were grown to near confluency on glass cover slips and treated for 24 h with EtOH (control) and varying concentrations 1.25 μM -100 μM of compound **7**. Microtubules were stained with monoclonal anti-β-tubulin and visualized with fluorescein-conjugated anti-mouse IgG. For staining of microfilaments, cells were fixed with 3% p-formaldehyde, permeabilized with 0.2% Triton X-100, and reduced with sodium borohydride (1 mg/mL). Microfilaments were visualized by incubation with 100 nM of TRITC-phalloidin for 45 min at 37 °C. Microtubules and microfilaments were imaged using a Nikon Optiphot-2 microscope with a Bio-Rad MRC 600 Confocal Laser Scanning system. The images were reconstructed using VoxelView Ultra Software and were printed on a Kodak Model XL 7700 digital continuous tone printer.

ACCEPTED MANUSCRIPT

6. Acknowledgements

This work was supported by the National Institutes of Health, National Cancer Institute grant CA 114021 (AG). We thank the National Cancer Institute for performing the in vitro antitumor evaluation in their 60 tumor preclinical screening program.[20]

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

7. References

1) Jordan, M. A.; Wilson, L. *Nat. Rev. Cancer.* **2004**, *4*, 253-65.

2) Jordan, M. A.; Kamath, K. *Curr. Can. Drug Tar.* **2007**, *7*, 730-742.

3) Jordan, A.; Hadfield, J. A.; Lawrence, N. J.; McGown, A.T. *Med. Res. Rev.* **1998**, *18*, 259-296.

4) Nogales, E.; Wolf, S. G.; Khan, I. A.; Luduena, R. F.; Downing, K. H. *Nature* **1995**, *375*, 424-427.

5) Singer, W. D.; Jordan, M. A.; Wilson, L.; Himes, R. H. *Mol. Pharmacol.* **1989**, *36*, 366-370.

6) Conlin, A.; Fornier, M.; Hudis, C.; Kar, S.; Kirkpatrick, P. *Nat. Rev. Drug Discov.* **2007**, *6*, 953-954.

7) Hastie, S. B. *Pharmacol. Ther.* **1991**, *512*, 377-401.

8) Safa, A. R. *Curr. Med. Chem. Anti-Canc. Agents* **2004**, *4*, 1-17.

9) Chen, J.; Ahn, S.; Wang, J.; Lu, Y. *J. Med. Chem.* **2012,** *55*, 7285-7289.

10) Gangjee, A.; Zhao, Y.; Lu, L.; Raghavan, S.; Roberts, R.; Risinger, A.; Hamel, E.; Moobery, S. *J. Med. Chem.* **2010,** *53*, 8116-8128.

11) Gangjee, A.; Yu, J.; Copper, J. E.; Smith, C. D. *J. Med. Chem.* **2007,** *50*, 3290-3301.

12) Gangjee, A.; Namjoshi, O.; Zhao, Y.; Smith, C. D. Abstracts of the 6th Asian Federation for Medicinal Chemistry (AFMC) International Medicinal Chemistry Symposium, Istanbul, Turkey, July 8-11, 2007.

13) Chinchilla, R.; Najera, C. O. *Chem. Rev.* **2007**, *107*, 874-922.

14) Petricci, E.; Radi. M.; Corelli, F.; Botta, M. *Tet. Lett.* **2003**, *44*, 9181-9184.

ACCEPTED MANUSCRIPT

15) Evano, G.; Blanchard, N.; Toumi, M. *Chem. Rev.* **2008**, *108*, 3054-3131.

16) Sperotto, E.; Klink, G. P.; de Vries, J. G.; van Koten, G. *J. Org. Chem.* **2008,** *73*, 5625-5628.

17) Gangjee, A.; Qiu, Y.; Li, W.; Kisliuk, R. L. *J. Org. Chem.* **2008,** 51, 5789-5797.

18) Bagley, M. C.; Davis, T.; Dix, M. C.; Fusillo,V. ; Pigeaux, M.; Rokicki, M.; Kipling, D. *J. Org. Chem.* **2009**, *74*, 8336–8342.

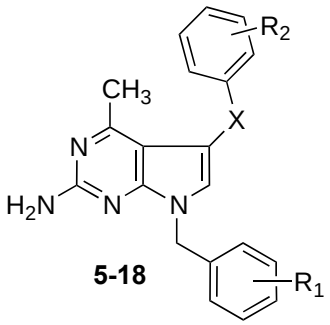
19) Lee, B. D.; French, K. J.; Zhuang, Y.; Smith, C. D. *Oncology Res.* **2003**, *14*, 49-60.

20) http://dtp.nci.nih.gov/branches/btb/ivclsp.html.

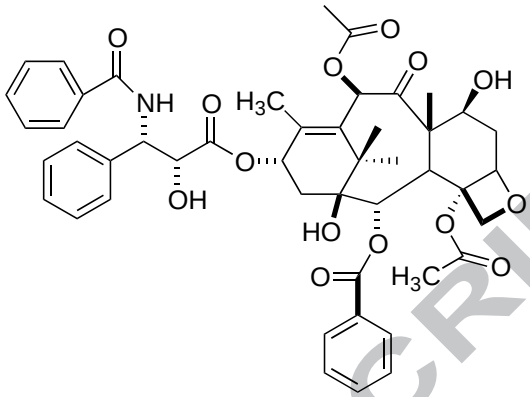
ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 1: Cytotoxicity and Pgp modulatory effects for target compounds **5-18**



5-18



Paclitaxel (PTX)

Cpd	R_1	R_2	X	Cytotoxicity IC_{50} (μM)	Lowest concentration for *in vitro* drug accumulation (μM)
5	2-OMe	3,4,5-triOMe	CH_2CH_2	1 ± 0	2
6	3-OMe	3,4,5-triOMe	CH_2CH_2	1.4 ± 0.4	0.5
7	4-OMe	3,4,5-triOMe	CH_2CH_2	0.6 ± 0.3	0.2
8	3,4-diOMe	3,4,5-triOMe	CH_2CH_2	1.5 ± 0.4	1.2
9	3,5-diOMe	3,4,5-triOMe	CH_2CH_2	3 ± 0.7	1.5
10	3,4,5-triOMe	3,4,5-triOMe	CH_2CH_2	1.0 ± 0	none
11	H	H	S	4 ± 0.7	none
12	H	2-OMe	S	11 ± 0.7	1.5
13	H	4-OMe	S	10 ± 1.2	none
14	H	4-Cl	S	7.5 ± 3.2	none
15	2-OMe	2-OMe	S	9 ± 0.7	none
16	4-OMe	2-OMe	S	2.5 ± 0.4	none
17	3,4,5-triOMe	2-OMe	S	3 ± 0	none
18	3,4,5-triOMe	3,4,5-triOMe	S	>100	none
PTX				0.15	

ACCEPTED MANUSCRIPT

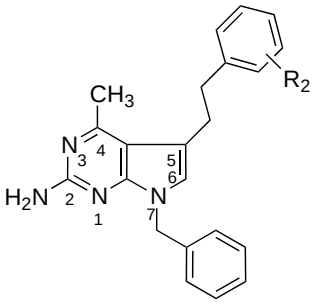
ACCEPTED MANUSCRIPT

Table 2. Tumor cell inhibitory activity GI_{50} (nM) of compound **7** (NCI)

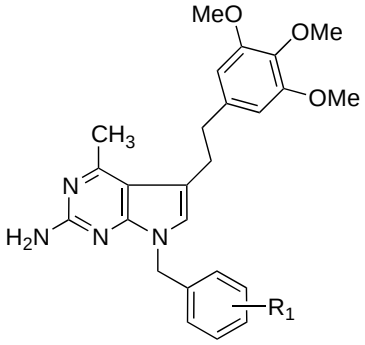
Panel/cell line	**7** GI_{50}	Panel/cell line	**7** GI_{50}	Panel/cell line	**7** GI_{50}	Panel/cell line	**7** GI_{50}
Leukemia		**Ovarian**		**Breast**		RXF-393	**44.1**
CCRF-CEM	>1000	IGROV1	154	HOP-62	362	SN12C	499
HL-60	220	OVCAR-3	164	HOP-92	>1000	TK-10	686
K-562	116	OVCAR-4	338	NCI-H226	927	UO-31	397
RPMI-8226	713	OVCAR-5	596	NCI-H23	428		
SR	120	OVCAR-8	334	NCI-H322M	712	**CNS**	
		NCI/ADR-RES	202	NCI-H460	251	SF-268	379
NSCLC		SK-OV-3	251	NCI-H522	**31.6**	SF-295	**83.9**
A549/ATCC	329			MCF7	**46.4**	SF-539	**96.4**
EKVX	348	**Melanoma**		MDA-MB-231/ATCC	361	SNB-19	234
HOP-62	362	LOXIMVI	364	HS578T	139	SNB-75	172
HOP-92	>1000	MALME-3M	284	BT-549	440	U251	311
NCI-H226	927	M14	204	T-47D	443		
NCI-H23	428	MDA-MB-435	**32.1**	MDA-MB-468	**52.4**	**Colon**	
NCI-H322M	712	SK-MEL-2	446			COLO 205	204
NCI-H460	251	SK-MEL-28	417	**Renal**		HCC-2998	191
NCI-H522	**31.6**	SK-MEL-5	110	786-0	575	HCT-116	265
		UACC-257	>1000	A498	115	HCT-15	242
Prostate		UACC-62	**61.5**	ACHN	216	HT29	247
PC-3	301			CAKI-1	206	KM12	264
DU-145	337					SW-620	208

ACCEPTED MANUSCRIPT

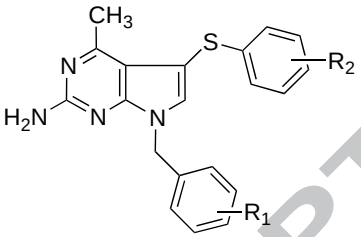
ACCEPTED MANUSCRIPT



	R_2
1	3,4,5- triOMe
2	4- OMe
3	2- OMe
4	H



	R_1
5	2-OMe
6	3-OMe
7	4-OMe
8	3,5-diOMe
9	3,4- diOMe
10	3,4,5- triOMe



	R_1	R_2
11	H	H
12	H	2-OMe
13	H	4-OMe
14	H	4-Cl
15	2-OMe	2-OMe
16	4-OMe	2-OMe
17	3,4,5-triOMe	2-OMe
18	3,4,5-triOMe	3,4,5-triOMe

Figure 1: Target compounds 5-18

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

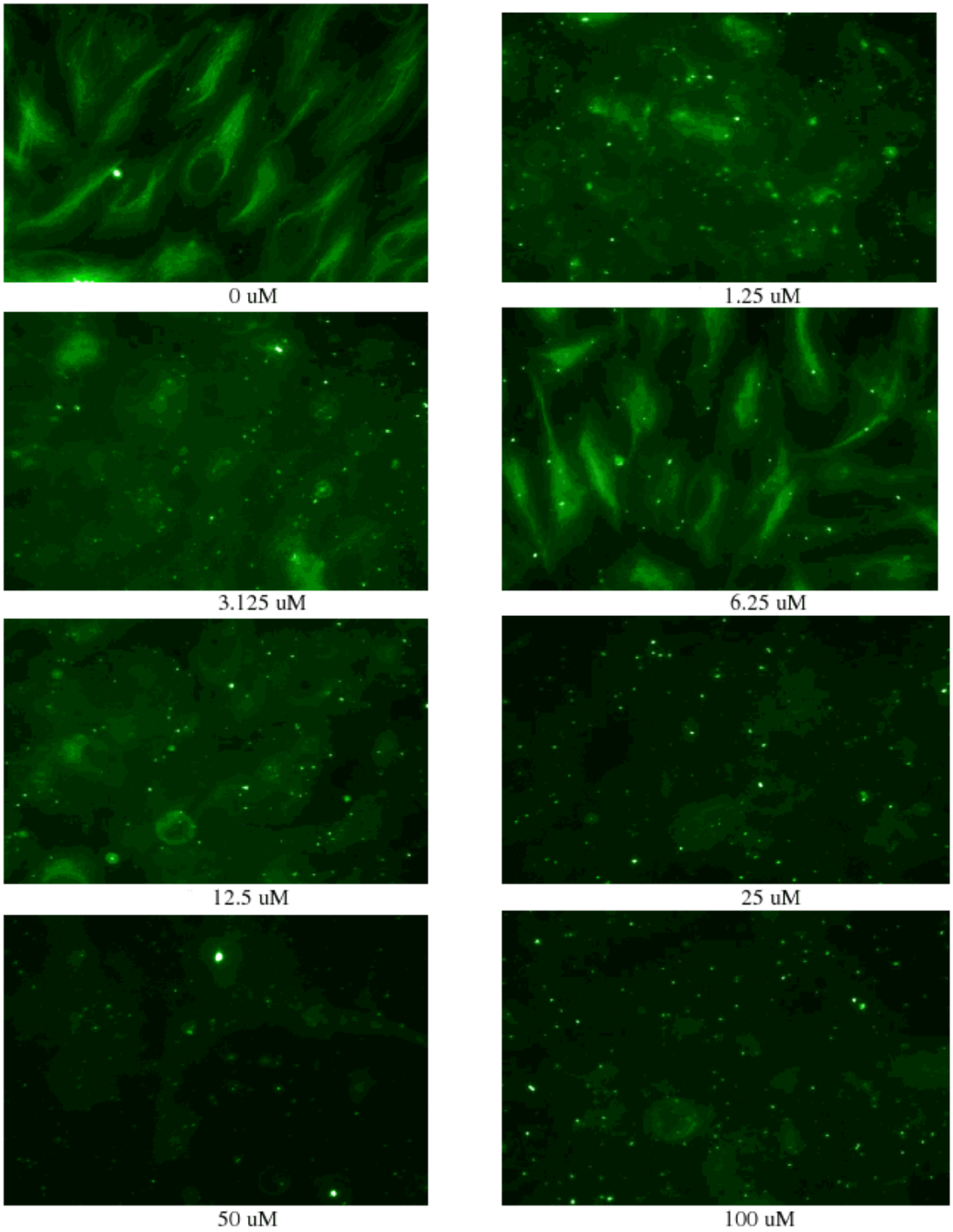
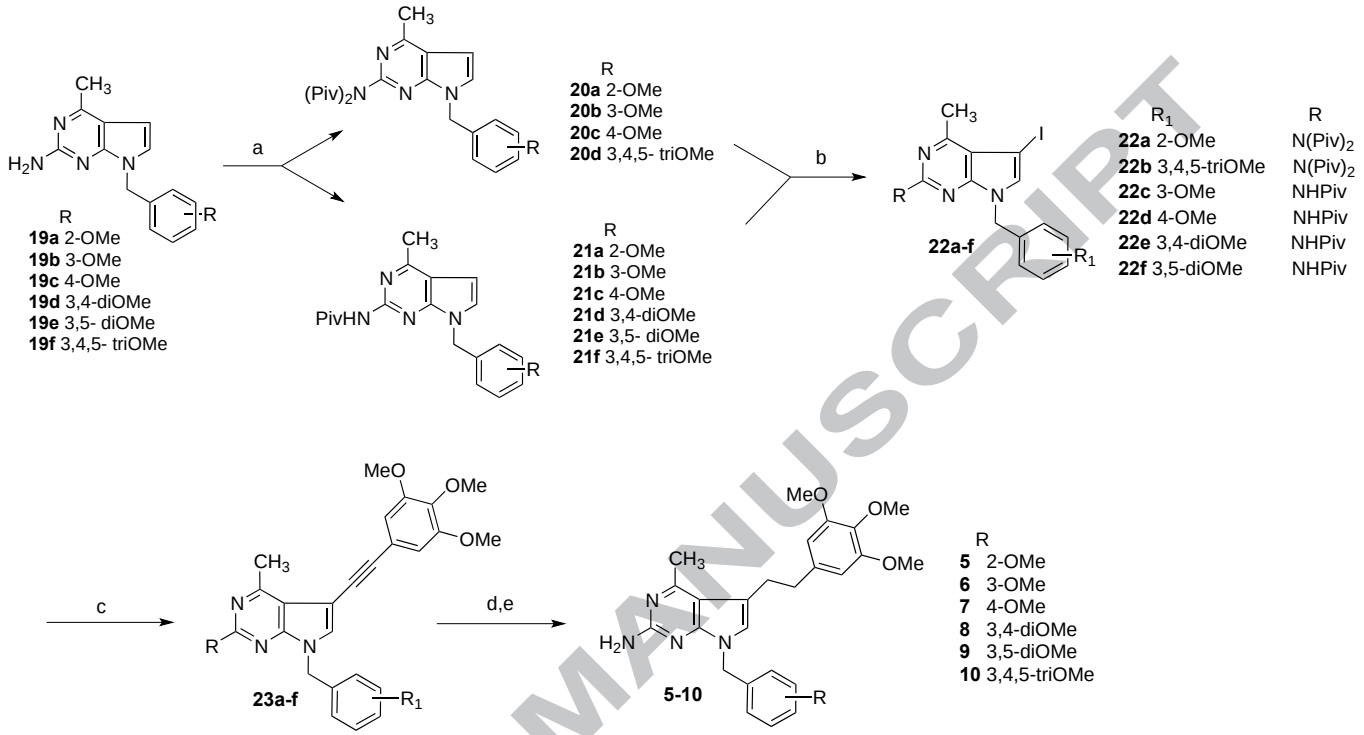


Figure 2: Effects of compound 7 on microtubule structure. JC cells were treated for 24 h with EtOH (control) and varying concentrations 1.25μM-100μM of compound **7**. Microtubules were then visualized by indirect immunofluoresence staining with β-tubulin antibodies.

ACCEPTED MANUSCRIPT

Scheme 1

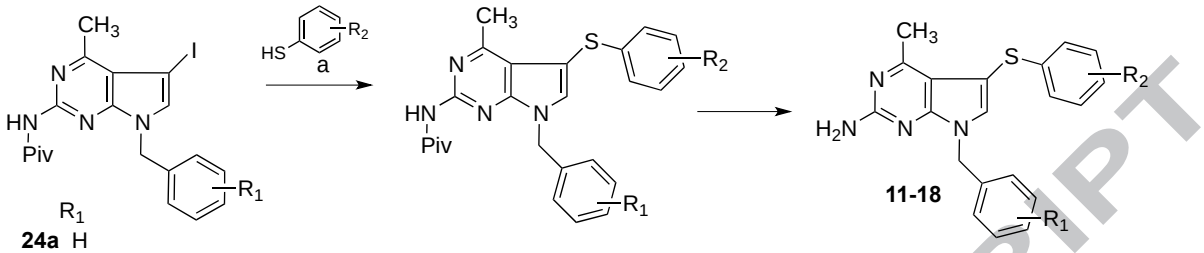


(a) PivCl, DMAP, NEt_3, Dichloroethane, 50-55 ^{0}C.; (b) NIS, DMF, Dark, R.T., 8 h.; (c) 3,4,5-trimethoxyacetylene,$Pd(PPh_3)_4$, CuI,NEt_3, DCE, MW 100 ^{o}C, 10 min.; (d) 5% Pd/C,H_2, 50psi, 3-24 h.; (e) 1N NaOH, reflux, 12 h

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Scheme 2



	R_1
24a	H
24b	2-OMe
22d	4-OMe
24c	3,4,5-triOMe

	R_1	R_2
25a	H	H
25b	H	2-OMe
25c	H	4-OMe
25d	H	4-Cl
25e	2-OMe	2-OMe
25f	4-OMe	2-OMe
25g	3,4,5-triOMe	2-OMe
25h	3,4,5-triOMe	3,4,5-triOMe

(a) CuI, K_2CO_3, DMF, MW, 100 ^oC, 4h.; (b) 1N NaOH, MeOH, reflux, 12h

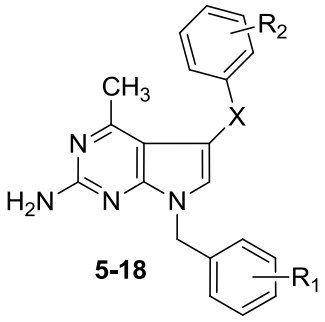
ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Synthesis of 5,7-disubstituted-4-methyl-7*H*-pyrrolo[2, 3-*d*]pyrimidin-2-amines as microtubule inhibitors

Aleem Gangjee,[•, †] Sonali Kurup, [†, §] Charles D. Smith [‡]

Graphical Abstract



5-18

ACCEPTED MANUSCRIPT