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2-Amino-4-methyl-5-phenylethyl substituted-7-*N*-benzyl-pyrrolo[2,3-*d*]pyrimidines as novel antitumor antimitotic agents that also reverse tumor resistance ☆

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ABSTRACT

Gangjee et al. recently reported a novel series of 2-amino-4-methyl-5-phenylethyl substituted-7-benzyl-pyrrolo[2,3-*d*]pyrimidines, some of which exhibited two digit nanomolar antitumor and antimitotic activity and were not subject to P-glycoprotein (Pgp) or multidrug resistance protein 1 (MRP1) mediated tumor resistance (unlike the Vinca alkaloids and taxanes). Some of these compounds, in addition to their antitumor activity, had the ability to reverse the Pgp-mediated resistance to clinically used antimitotic agents. This report consists of an attempt to optimize the various activities of the parent compounds by synthetic variations of the phenyl ring of the 5-phenylethyl side chain. The target compounds were synthesized via a nine-step synthesis involving a Sonogashira reaction. The substituted phenylacetylenes as coupling partners were in turn synthesized from unactivated aryl bromides or iodides. The target compounds exhibited moderate cytotoxicity against MCF-7 tumor cells. However, most of these compounds showed improved cytotoxicity against the resistant NCI/ADR and MCF-7/VP. This study afforded an analog which reversed both Pgp-mediated as well as MRP1-mediated resistance to clinically used antimitotic agents, along with its own antimitotic mediated antitumor activity. In addition, in the NCI-60 cell line panel one of the compounds inhibited the growth of MDA-MD-435 breast cancer cell line at submicromolar concentration.

1. Introduction

Microtubules are long, protein polymers that are formed by α-tubulin and β-tubulin heterodimers.[2] These heterodimers first form a short microtubule nucleus followed by elongation and arrangement in the form of tubes. The polymerization of microtubules occurs via complex polymerization dynamics that involves GTP hydrolysis to supply energy at the time that tubulin, with bound GTP, adds to the microtubule ends. In mitosis, the duplicated chromosomes of cells are divided into two identical sets prior to division into two daughter cells and the polymerization dynamics of microtubules plays a pivotal role in this process as part of cell replication.[2,3] The crucial involvement of microtubules in mitosis makes them a target for antitumor agents.

Three distinct classes of antimitotic agents have been identified. The *Vinca* alkaloids (Fig. 1) exemplified by vincristine, vinblastine, vindesine, and vinorelbine. These are β-tubulin binding agents that interfere with proper mitotic spindle formation by preventing the normal dynamics and polymerization of spindle microtubule formation. The *Vinca* alkaloids are important in the treatment of leukemias, lymphomas, small cell lung cancer, and other cancers.[4,5] These agents are also known as microtubule-destabilizing agents or microtubule polymerization inhibitors or depolymerizers. The second group comprises of the taxanes (Fig. 1) exemplified by paclitaxel (Taxol) and docetaxel (Taxotere). Paclitaxel increases microtubule polymerization and thus interferes with spindle microtubule dynamics and prevents the dividing cell from progression. These are microtubule-stabilizing agents or polymerizing agents. The taxanes are clinically useful in the treatment of breast, lung, ovarian, head and neck, and bladder carcinomas among others. The third class typified by colchicine is comprised of a diverse collection of small molecules that bind to the colchicine binding site. These compounds are microtubule polymerization inhibitors similar to the *Vinca* alkaloids. However they bind to a different site and their depolymerization mechanism of action is also different. Combretastatins (Fig. 1) are a class of drugs that are in clinical trials as antitumor agents that bind to the colchicine binding site.[2] There are no clinically approved antitumor agents that bind to the colchicine site. However, several of these agents are currently in clinical trials.[6,7]

Abbreviations: Pgp, P-glycoprotein; MDR, multiple drug resistance; MRP, multidrug resistance protein; GTP, guanosine triphosphate; MCF-7, Michigan Cancer Foundation-7; NCI, National Cancer Institute; PCC, Pearson correlation coefficient; TLC, thin layer chromatography; NMR, nuclear magnetic resonance.

☆ See Ref. 1.

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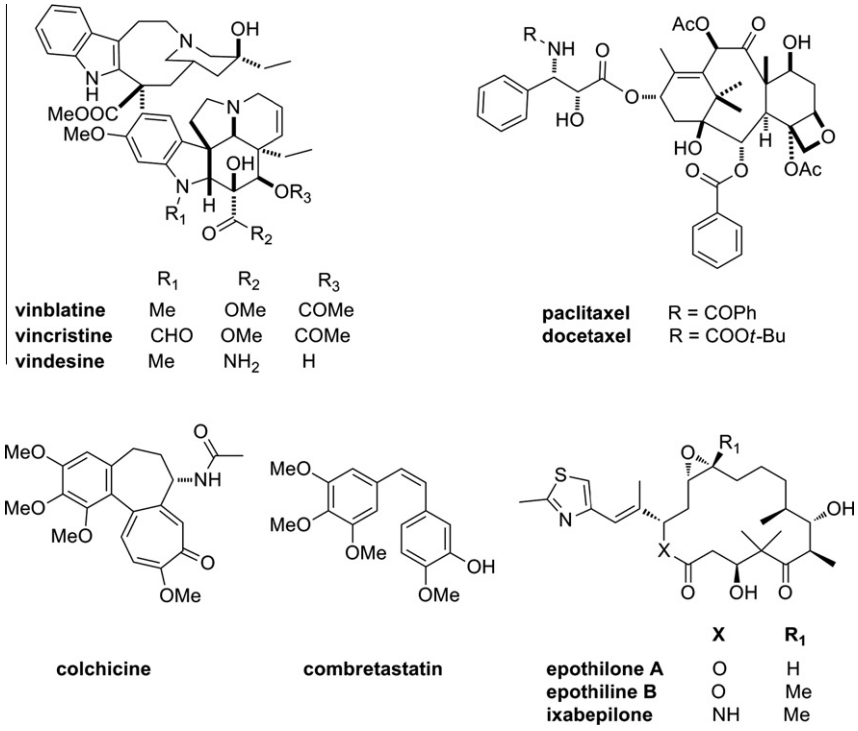


Figure 1. Examples of antimitotic agents.

The clinical success of the taxanes has encouraged efforts to identify and develop other classes of microtubule-targeted anticancer agents that might overcome the limitations of existing drugs.

Epothilones are a newer class of antimitotic agents and were found to bind at the same site as the taxanes. Ixabepilone was the first member from the epothilones family to be approved by the USFDA for the treatment of metastatic breast cancer in October 2007.[8,9]

Though antimitotic agents have shown to be some of the most successful agents against malignancies, resistance both intrinsic and acquired, is of major concern, and results in treatment failures. Multidrug or multiple drug resistance (MDR) is a major drawback of cancer chemotherapy including the clinically used antimitotic agents. These arise from intrinsic or acquired mechanisms of resistance. A major mechanism of MDR occurs via an overexpression of energy dependent (adenosine triphosphate; ATP), unidirectional transmembrane efflux pumps. Ultimate failure of chemotherapy with antimitotic drugs often results due to MDR.[10] P-Glycoprotein (Pgp) is a 170 kDa protein that belongs to the ATP-binding cassette superfamily of transporters.[11–13] A series of homologous proteins termed multidrug-resistance-proteins (MRPs) have also been reported.[14,15] The first MRP termed MRP1 was identified in a drug resistant lung cancer cell line that does not express Pgp.[16] All these transporters bind drugs within the cell and release them to the extracellular space using ATP.[16] Tumor cells preexposed to cytotoxic compounds often overexpress these pumps which allow the cells to manifest resistance in the presence of the cytotoxic drug. MDR affects cancer patients with leukemia as well as solid tumors. Overexpression of Pgp has been reported in a number of tumor types, particularly after the patient has received chemotherapy indicating the clinical importance of Pgp in MDR.[13] In addition, it has also been reported that Pgp expression may be a prognostic indicator in certain cancers and is associated with poor response to chemotherapy.[17] MRP1 overexpression is not consistently found in tumors even though it is expressed in a high percentage of leukemia and solid tumors; however, its prognostic relevance is not clear.[18] The simultaneous expression and function of both proteins has been shown to be correlated with poor overall survival.

The clinical significance of Pgp along with its limited expression in normal tissues makes Pgp a viable target for inhibition to reverse MDR. Several Pgp inhibitors or modulators have been developed to reverse MDR that occur for chemotherapeutic agents in general and include antimitotic inhibitors.[17]

The other clinical mechanism of resistance to microtubule targeted agents involves the expression of specific isotypes of β-tubulin, of which the βIII-tubulin isotype is of paramount concern. There is unequivocal clinical evidence that βIII-tubulin expression is involved in resistance to taxoids and vinca alkaloids in multiple tumor types including non-small cell lung,[19–21] breast,[22] and ovarian cancers.[23,24]

Thus new agents that possess antimitotic and antitumor activities without substrate activity for Pgp are highly coveted and would be useful antitumor agents as single agents or in combination with chemotherapeutic agents including antimitotic agents.

2. Design of antimitotic compounds

The initial series of compounds (**1–5**, Fig. 2) showed excellent to moderate (10^{-8}–10^{-5} M) tumor cell inhibitory activity in the NCI 60 tumor cell line panel.[25] The debenzylated compounds showed poor antitumor activity indicating the importance of the benzyl moiety. In COMPARE analysis, the Pearson correlation coefficients (PCC) of this series of compounds matched with microtubule targeting agents (vinblastine, paclitaxel, rhizoxine, maystansine and vincristine). These compounds were also compared in human tumor cell lines (T-24 and MCF-7) with cell lines resistant to anticancer drugs due to overexpression of Pgp or MRP-1 (NCI/ADR, MCF-7/VP). It was found that **1** and **4** exhibited potency against all the cell lines (**1**: submicromolar inhibitor and **4**: micromolar inhibitor). Additionally, compounds **2–5** caused dose-dependent sensitization of NCI/ADR cells to vinblastine indicating inhibition of Pgp. However, they were ineffective in blocking the actions of MRP1.

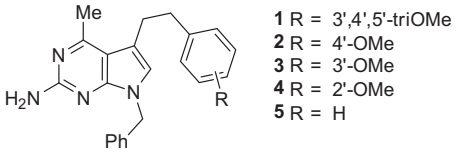


Figure 2. Lead compounds.

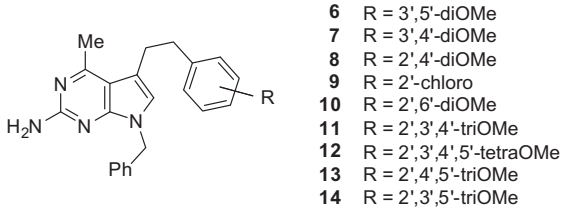


Figure 3. Target compounds **6**–**14**.

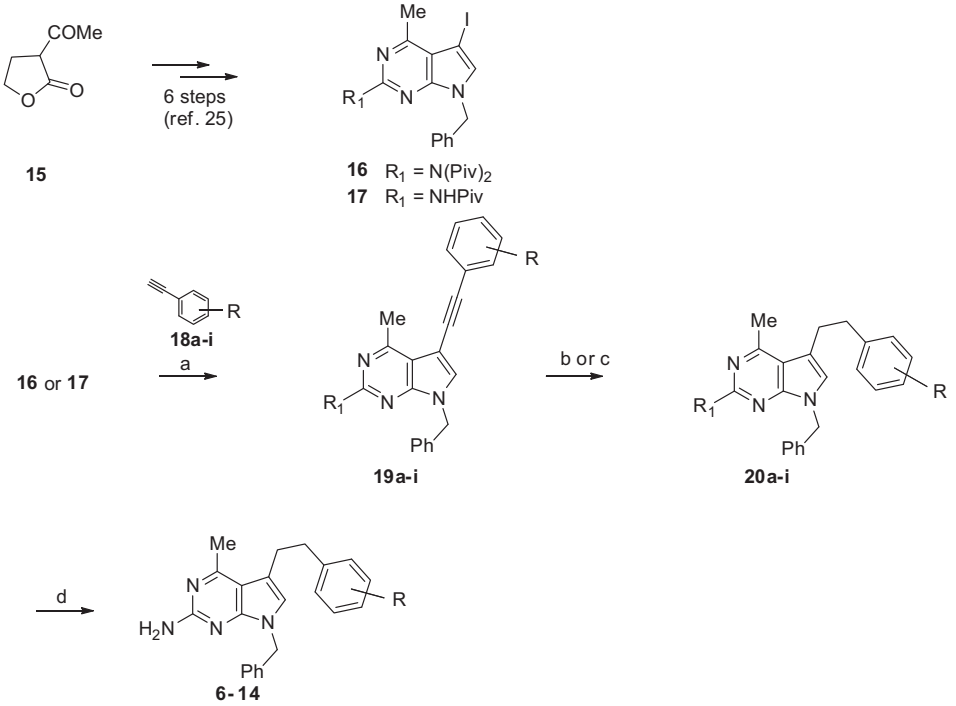
In an attempt to optimize the antitubulin, antitumor, and resistance reversal activities of the parent compounds by variations of the substitutions on the phenyl ring of the 5-phenylethyl side chain, compounds **6–14** (Fig. 3) were designed and synthesized. Since the most potent compounds of the parent series were **1** (3,4,5-trimethoxyphenylethyl side chain) and **4** (2-methoxyphenylethyl side chain), various combinations of methoxy substitutions were included on the phenyl ring of the 5-phenylethyl side chain in order to probe for optimum cytotoxicity with resistance-reversal activity.

3. Chemistry

The synthesis of the target compounds **6–14** were accomplished as indicated in Scheme 1. Compounds **16** and **17** were obtained from 2-acetylbutyrolactone **15** as described previously.[25] Sonogashira coupling of either **16** or **17** with various substituted phenylacetylenes (either commercially available or synthesized as shown in Schemes 2 and 3) using catalytic amount of tetrakistriphenylphosphine palladium (0) {$Pd(PPh_3)_4$}, copper iodide (CuI) and triethylamine afforded **19a–i** in 75–95% yields. Subsequent catalytic hydrogenation of **19a–i** with 5% palladium-on-charcoal, afforded **20a–i** in 85–96% yield. Hydrolysis with 1 N NaOH afforded analytically pure **6–14** in 70–80% yield. The structure of **6** was unequivocally confirmed from its X-ray crystal structure; which also proved the regiospecific iodination at the 5-position.[26]

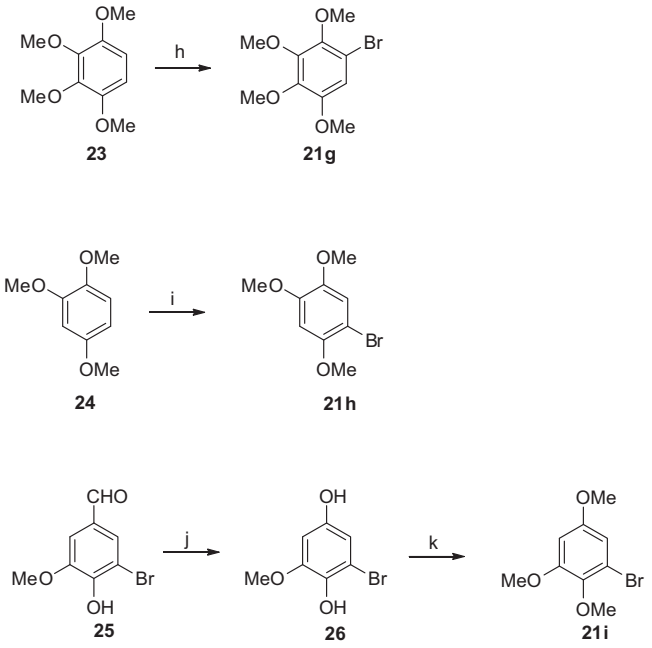
Scheme 2 shows the synthesis of substituted acetylenes **18c**, **18e** and **18f–i** (other acetylenes were commercially available) as coupling partners in the Sonogashira coupling reactions with **16** or **17** (Scheme 1). Substituted iodobenzenes **21c** and **21e** were reacted with trimethylsilylacetylene by microwave irradiation at 120 °C for 5 min using catalytic palladium on charcoal as a palladium-source in the Sonogashira coupling reaction.[27] Microwave irradiation was necessary to force the reaction of these non-activated acetylenes. Subsequent desilylation using *tert*-butylammonium fluoride (*t*-BAF) lead to acetylenes **18c** and **18e**. Substituted bromobenzenes **21f–i** did not react with trimethylsilylacetylene using the above conditions. Hence Sonogashira coupling conditions developed by Fu et al.[28] for unactivated bromobenzenes were used. Thus, substituted bromobenzenes were reacted with trimethylsilylacetylene using catalytic amount of dichlorobis(benzonitrile)palladium(II) as a palladium source and tri-*tert*-butylphoshine ($P(t\text{-}Bu)_3$), a bulky, electron-rich ligand. The reaction proceeded smoothly at ambient temperature with yields in the 90% range. A tetrafluoroborate salt of this ligand (P(t-Bu)3).HBF4) worked equally efficiently for this reaction. Subsequent desilylation lead to desired acetylenes **18f–i**.

Substituted bromobenzenes, which were not available commercially, were synthesized using literature methods.[29–31] Thus, compounds **21g** and **21h** were synthesized by regioselective bromin ation (Scheme 3).[29,30] While **21i** was synthesized from **25** by a Dakin reaction[31] followed by methylation.



Scheme 1. Synthesis of target compounds. Reagents and conditions: (a) 10 mol % $Pd(PPh_3)_4$, NEt_3, 20 mol % CuI, dichloroethane, rt, 20–24 h; (b) 5% Pd/C, DCM/MeOH (5:1), H_2 50 psi; (c) 5% Pd/C, DCM/MeOH (1:1), H_2 50 psi; (d) 1 N NaOH, MeOH, 80 °C, 24 h.

Scheme 2. Synthesis of substituted aryl alkynes. Reagents and conditions: (e) trimethylsilylacetylene, 10% Pd/C (4 mol % Pd), 16 mol % PPh_3, 16 mol % CuI, NEt_3, CH_3CN, M.W. 120 °C, 5 min; (f) 3 mol % $Pd(PhCN)_2Cl_2$, 7 mol % P(*t*-Bu)$_3$, 2 mol % CuI, HN(*i*-Pr)$_2$, dioxane, rt 20 h; (g) *t*-BAF, THF, rt, 2 h.



Scheme 3. Synthesis of substituted aryl bromides. Reagents and conditions: (h) Br_2, DCM, −10 °C, 1 h; (i) Br_2, glacial HOAc, 0 °C, 1 h; (j) 1 N KOH, 3% H_2O_2, 30 min; (k) dimethyl sulfate, acetone, rt, 14 h.

4. Results and discussion

The cytotoxicity, resistance factors [resistance factor = IC_{50} value against drug-resistant cancer cell line (NCI/ADR or MCF-7/VP)/IC_{50} value against drug-sensitive cancer cell line (MCF-7)] and Pgp/MDR-reversal activities of compounds **1**, **4** and **6–14** are shown in Table 1. Compounds **6–14** exhibited two-digit micromolar IC_{50} values against MCF-7 cells. Compound **13** with 2,4,5-trimethoxyphenyl side chain was the most potent compound with an IC_{50} of 15 μM against MCF-7 cells. Interestingly, all the compounds were equipotent for the drug-resistant tumor cells than the parental MCF-7 cells. This suggests that these compounds were not substrates for either Pgp or MRP1. In fact, several compounds were somewhat more cytotoxic against NCI/ADR and/or MCF/VP cells than that against MCF-7 cells (e.g., **6–10** and **12**). For comparison, paclitaxel, vinblastine, and vincristine inhibited MCF-7 cells at nanomolar concentration. However, they exhibited diminished inhibitory activity against drug-resistant NCI/ADR cells. Cisplatin exhibited similar inhibitory activity in the micromolar range against all the cell lines.

Four (**8**, **10**, **12** and **14**) of the nine compounds inhibited Pgp activity, making NCI/ADR cells more sensitive to vinblastine (indicated as 'Yes' in the Pgp reversal column). Three (**10**, **11** and **12**) of the nine compounds inhibited MRP1, making MCF7/VP cells more sensitive to vincristine (indicated as 'Yes' in MRP reversal column). It is also possible that the increased sensitivity of vincristine in the presence of **8, 10, 12** and **14** toward the resistant cell lines could be due to a synergistic effect as well.

Compound **14** was the most unusual compound and remarkably afforded sensitization of resistant tumor cells where the resistance was due to both Pgp and MRP1. To our knowledge, this is the only known pyrrolo[2,3-*d*]pyrimidine analog that has antitumor activity of its own and restores sensitivity of antitumor agents to tumor cells resistant to these agents due to both Pgp and MRP1. Though **14** has a modest antitumor activity it serves as a lead compound for optimization of antitumor activity and ability to restore sensitivity to antimitotic agents in tumor cells resistant to these agents due to Pgp and/or MRP1. Such agents could be used alone or in

Table 1
Cytotoxicity data (IC_{50} values in μM), MDR reversal activities and effect on microtubule depolymerization of compounds **6–14**

Compd #	Cytotoxicity[a]					Reversal		Microtubule depolymerization
	MCF-7 (Pgp-/MRP1−)	NCI/ADR (Pgp+/MRP1−)	Resistance factor[c]	MCF-7/VP (Pgp-/MRP1+)	Resistance factor[c]	Pgp	MRP	
1[b]	0.3 ± 0.16	0.25 ± 0.12	0.83	0.25 ± 0.16	0.83	No	No	Yes
4[b]	2.3 ± 0.6	2.7 ± 0.4	1.17	2.6 ± 0.1	1.13	Yes	No	ND
6	25 ± 6	12 ± 5	0.48	17 ± 6	0.68	No	Yes	No
7	17 ± 6	5 ± 3	0.29	7 ± 2	0.41	No	No	No
8	17 ± 4	6 ± 2	0.35	12 ± 2	0.71	Yes	No	No
9	20 ± 4	11 ± 5	0.55	15 ± 4	0.75	No	No	No
10	22 ± 4	12 ± 5	0.54	20 ± 4	0.91	Yes	No	Yes
11	18 ± 3	11 ± 5	0.61	20 ± 6	1.11	No	Yes	Yes
12	24 ± 6	7 ± 4	0.29	9 ± 5	0.37	Yes	No	Yes
13	15 ± 3	10 ± 4	0.66	15 ± 7	1	No	No	No
14	50 ± 11	33 ± 11	0.66	63 ± 13	1.26	Yes	Yes	No
paclitaxel	0.0025 ± 0.0006	2.7 ± 0.5	1080	0.0028 ± 0.0006	1.1			No
vinblastine	0.0028 ± 0.0005	>375	>134	0.003 ± 0.0006	1.1			Yes
vincristine	0.0006 ± 0.0001	100	167	0.0093 ± 0.0014	15.5			Yes
cisplatin	11.5 ± 4.1	12.2 ± 4.2	1.1	7.0 ± 2.9	0.6			NA

[a] Values are expressed as μM and represent the mean ± SEM for four experiments.
[b] From Ref. 25.
[c] Resistance factor = IC_{50} value against drug-resistant cancer cell line (NCI/ADR or MCF-7/VP)/IC_{50} value against drug-sensitive cancer cell line (MCF-7).

Table 2
Tumor cell inhibitory activity GI_{50} (μM) of compound **12** (NCI)

Leukemia		Colon cancer (ctd.)		Melanoma (ctd.)		Renal cancer (ctd.)	
Panel/cell line	GI_{50}	Panel/cell line	GI_{50}	Panel/cell line	GI_{50}	Panel/cell line	GI_{50}
HL-60 (TB)	2.06	HCT-116	3.68	SK-MEL-5	1.46	TK-10	7.62
K-562	2.48	HCT-15	3.2	UACC-257	10.4	UO-31	5.37
MOL T-4	3.96	HT29	3.6	UACC-62	2.13	Prostate cancer	
RPMI-8226	1.69	KM12	3.33			PC-3	3.08
SR	2.35	SW-620	3.19			DU-145	2.5
NSCLC		CNS cancer		Ovarian cancer		Breast cancer	
A549/ATCC	5.33	SF-268	4.4	OVCAR-3	1.2	MCF7	1.05
EKVX	7.73	SF-295	1.39	OVCAR-4	8.83	NCI/ADR-RES	2.02
HOP-62	3.96	SF-539	1.86	OVCAR-5	11.9	MDA-MB-231/ATCC	3.69
HOP-92	1.39	SNB-19	4.68	OVCAR-8	4.71	HS 578T	2.46
NCI-H226	4.27	SNB-75	2.25	SK-OV-3	2.98	MDA-MB-435	0.34
NCI-H23	3.61	U251	3.93			BT-549	9.75
NCI-H322M	6.92	Melanoma		Renal cancer		T-47D	1.69
NCI-H460	2.81	LOX IMVI	5.29	786-0	4.5	MDA-MB-468	2.82
NCI-H522	6.05	MALME-3M	10.4	A498	2.25		
Colon cancer		M14	3.33	ACHN	9.36		
COLO 205	2.8	SK-MEL-2	5.09	CAKI-1	2.5		
HCC-2998	5.89	SK-MEL-28	4.05	SN12C	6.86		

combination with existing antimitotic agents against resistant tumors. Additionally, compounds **10–12** caused microtubule depolymerization similar to **1** and vinca alkaloids.

Compound **12**, the 2,3,4,5-tetraOMe analog was also tested in the NCI 60[32] cell line panel. It inhibited the growth of most of the cell lines at GI_{50} values that ranged from submicromolar to two digit micromolar (Table 2). Compound **12** had a GI_{50} value of 0.34 μM against MDA-MD-435 breast cancer cell line. Additionally it exhibited single digit micromolar GI_{50} values against almost 53 cell lines.

5. Conclusion

Nine pyrrolo[2,3-*d*]pyrimidine analogues are reported as antitumor antimitotic agents. All the compounds were found to be one to two-digit micromolar inhibitors of the proliferation of MCF-7 cells in culture. Interestingly, all of these compounds (except **11** and **14**) were cytotoxic against sensitive and resistant MCF-7/VP (which overexpresses MRP1) and NCI/ADR (which overexpresses pgp) cell lines. Five (**6**, **8** ,**10**, **11**, **12**) out of the nine compounds were found to restore sensitivity of vincristine or vinblastine to either Pgp or MRP1 activity, respectively; while compound **14** restores sensitivity to both. This raises the possibility of further development of these compounds as adjuvents to paclitaxel or such agents which are not active against resistant cell lines. The advantage of this lies in these compounds having their own antimicrotubule activity; probably binding at a novel binding site on tubulin (other than that of the paclitaxel domain, vinca domain or colchicine domain) and also potentiating the activity of antimicrotubule agents used in combination with these agents.

6. Experimental

6.1. General methods for biological evaluations: materials

MCF-7 breast carcinoma cells and NCI/ADR cells, an MDR line that overexpresses Pgp, were obtained from the Division of Cancer Treatment of the National Cancer Institute. MCF-7/VP cells that express MRP1 but not Pgp were provided by Drs. Schneider and Cowan.[33] Human bladder carcinoma T24 cells were from the American Type Culture Collection. Sulforhodamine B, antibodies against β-tubulin (T-4026), proteinase K (P2308), RNase A (R-5503), and actomyosin (A-6394) were obtained from Sigma Chemical Company (St. Louis, MO). RPMI-1640 and α-MEM culture media and fetal bovine serum were from GibcoBRL (Grand Island, NY).

6.1.1. Assay of cytotoxicity and reversals of MDR

To test for reversal of Pgp-mediated MDR, NCI/ADR cells were placed into 96-well tissue culture plates at approximately 15% confluency and allowed to attach and recover for 24 h. The cells were then treated with varying concentrations (as allowed by solubility) of a test compound in the presence of 0 or 50 nM vinblastine for 48 h according to previously described procedures.[34–40] After 48 h, cell survival was assayed using the sulforhodamine B (SRB) binding assay.[41] The percentage of cells killed is calculated as the percentage decrease in SRB binding as compared with control cultures and is taken from the mean of the absorbance measurements of three equally treated wells. Reversal of MDR is indicated if the compound enhances the toxicity of vinblastine toward the NCI/ADR cells. The reversal index (Pgp Antagonism Score) is calculated as the percentage of surviving NCI/ADR cells in the absence of vinblastine/the percentage of surviving NCI/ADR cells in the presence of vinblastine. Control cultures included equivalent amounts of ethanol (as the solvent control), which does not modulate the growth or drug-sensitivity of these cells at the doses used in these studies. To assess the toxicity of the compounds toward drug-sensitive cells, the effects of the test modulators on the growth of drug-sensitive MCF-7 cells were determined by the same methods. To test for reversal of MRP1-mediated MDR, MCF-7/VP cells were placed into 96-well tissue culture plates at approximately 15% confluency and were allowed to attach and recover for 24 h. The cells were then treated with varying concentrations (as allowed by solubility) of a test compound in the presence of 0 or 1 nM vincristine for 48 h as above.

After 48 h, cell survival was assayed using the SRB binding assay. Reversal of MDR is indicated if the compound enhances the toxicity of vincristine toward the MCF-7/VP cells. The reversal index (MRP1 Antagonism Score) is calculated as the percentage of surviving MCF-7/VP cells in the absence of vincristine/the percentage of surviving MCF-7/VP cells in the presence of vincristine.

6.1.2. Effect on tubulin assembly in vitro

Microtubule protein (MTP), comprising tubulin and MAPs, was isolated from fresh bovine brain by three cycles of assembly and disassembly, and pure tubulin was isolated by subsequent ion-ex-

change chromatography, as described by Vallee.[42] The effects of compounds **6–14** on tubulin assembly were determined following the procedures of Bai et al.[43] Briefly, 0.25 mL samples containing 2.5 mg of tubulin/mL (67 μM tubulin) in 1 M glutamate, pH 6.6, containing 4% DMSO were incubated with ethanol, compounds **6–14**, paclitaxel, vinblastine or vincristine at 0 °C for 15 min. GTP (0.5 mM) was then added to the samples followed by rapid warming to 37 °C, and the absorbance at 340 nm was continuously monitored for approximately 60 min. Effect on microtubule assembly was recorded as change in A340. A decreased A340 indicates microtubule depolymerization.

6.1.3. NCI-60 cancer cell line screening[44]

In these tests, the human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 μL at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line are fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (T_z). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/mL gentamicin. Additional four, 10-fold or 1/2 log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 μl of these different drug dilutions are added to the appropriate microtiter wells already containing 100 μl of medium, resulting in the required final drug concentrations. Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 μl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μl) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (T_z), control growth, (C), and test growth in the presence of drug at the five concentration levels (T_i)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

$$[(T_i - T_z)/(C - T_z)] \times 100 \text{ for concentrations for which } T_i > / = T_z$$

$$[(T_i - T_z)/T_z] \times 100 \text{ for concentrations for which } T_i < T_z.$$

Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50% (GI_{50}) is calculated from $[(T_i - T_z)/(C - T_z)] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from $T_i = T_z$. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from $[(T_i - T_z)/T_z] \times 100 = -50$. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested.

6.2. General methods for synthesis

All evaporations were carried out in vacuo using a rotary evaporator. Analytical samples were dried in vacuo (0.2 mm Hg) in a CHEM-DRY drying apparatus over P_2O_5 at 50–70 °C. Melting points were determined on a MEL-TEMP II melting point apparatus with FLUKE 51 K/J electronic thermometer and are uncorrected. Nuclear magnetic resonance spectra for proton (^{1}H NMR) were recorded on a Bruker WH-300 (300 MHz) spectrometer. The chemical shift values are expressed in ppm (parts per million) relative to tetramethylsilane as an internal standard: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad singlet. The relative integrals of peak areas agreed with those expected for the assigned structures. Thin-layer chromatography (TLC) was performed on WHATMAN UV254 silica gel plates with a fluorescent indicator, and the spots were visualized under 254 and/or 365 nm illumination. Proportions of solvents used for TLC are by volume. Column chromatography was performed on a 230–400 mesh silica gel purchased from Fisher Scietific. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Element compositions are within 0.4% of the calculated values. All solvents and chemicals were purchased from Aldrich Chemical Co. or Fisher Scientific and were used as received.

6.2.1. General procedure for the synthesis of 19a–i

To a 50 mL round bottom flask equipped with a stir bar and covered with an aluminum foil was added **16** or **17** (0.5 mmol), substituted phenylacetylene (1.5 mmol), copper(I)iodide (20 mol %), tetrakis(triphenylphosphine)palladium(0) (10 mol %) and flask was sealed with a rubber septum. The flask was evacuated and back filled with nitrogen (repeated three times). Anhydrous triethylamine (0.15 mL) and anhydrous dichloroethane (10 mL) were then added with the help of a syringe. The yellow-brown solution was stirred at room temperature under nitrogen atmosphere in dark for 24 h (until disappearance of starting material; monitored by TLC). At the end of the reaction, silica gel (1 g) was added and solvents evaporated to make a silica gel plug which was loaded on top of a silica gel column (3 cm × 15 cm) pre-treated with triethylamine/ethyl acetate/hexanes (1:1:25) and continued with a gradient elution, collecting 10 mL fractions. For **19a, 19c, 19e** and **19g–i** (dipivalated compounds), the product eluted with triethylamine/ethyl acetate/hexanes (1:1:15); while for **19b, 19d** and **19f** (monopivalated compounds), the product eluted with triethylamine/ethyl acetate/hexanes (1:1:10). The fractions containing product spots (TLC) were pooled and evaporated under reduced pressure. The residual solvents were further removed using high vacuum oil pump.

6.2.1.1. *N*-{7-Benzyl-4-methyl-5-[(3,5-dimethoxyphenyl)ethynyl]-7H-pyrrolo[2,3-d]pyrimidin-2-yl}-N-(2,2-dimethylpropanoyl)-2,2-dimethylpropanamide (19a).

Compound **19a** was synthesized from **16** (266 mg, 0.5 mmol), 3,5-dimethoxyphenylacetylene **18a** (243.3 mg, 1.5 mmol) using the general procedure described above to afford after purification 272 mg (96%) as a colorless semisolid: TLC R_f 0.24 (ethyl acetate/triethylamine/hexanes, 1:1:6); ^{1}H NMR (DMSO-d_6): 1.18 (s, 18H, C(CH_3)$_3$), 2.89 (s, 3H, 4-

CH_3), 3.78 (s, 6H, OCH_3), 5.42 (s, 2H, $CH_2C_6H_5$), 6.57–6.58 (d, 1H, C_6H_3), 6.69 (s, 2H, C_6H_3), 7.21–7.32 (m, 5H, C_6H_5), 8.16 (s, 1H, C6–*H*); HRMS (EI): calcd for $C_{34}H_{38}N_4O_4$: 566.2893; found: 566.2841.

6.2.1.2. N-{7-Benzyl-4-methyl-5-[(3,4-dimethoxyphenyl)ethynyl]-7H-pyrrolo[2,3-d]pyrimidin-2-yl}-N-2,2-dimethylpropanamide (19b). Compound **19b** was synthesized from **17** (225 mg, 0.5 mmol), 3,4-dimethoxyphenylacetylene **18b** (243.3 mg, 1.5 mmol) using the general procedure described above to afford after purification 237 mg (98%) as a lustrous off-white solid: TLC R_f 0.21 (ethyl acetate/triethylamine/hexanes, 1:1:3); mp 158–161 °C; ^{1}H NMR (DMSO-d_6): 1.26 (s, 9H, $C(CH_3)_3$), 2.87 (s, 3H, 4-CH_3), 3.80 (s, 6H, OCH_3), 5.41 (s, 2H, $CH_2C_6H_5$), 7.0–7.36 (m, 8H, C_6H_3 and C_6H_5), 7.89 (s, 1H, C6–*H*), 9.92 (br, 1H, N*H*Piv, exch); HRMS (EI): calcd for $C_{29}H_{30}N_4O_3$: 482.2318; found: 482.2303.

6.2.1.3. N-{7-Benzyl-4-methyl-5-[(2,4-dimethoxyphenyl)ethynyl]-7H-pyrrolo[2,3-d]pyrimidin-2-yl}-N-(2,2-dimethylpropanoyl)-2,2-dimethylpropanamide (19c). Compound **19c** was synthesized from **16** (266 mg, 0.5 mmol), 2,4-dimethoxyphenylacetylene **18c** (243.3 mg, 1.5 mmol) using the general procedure described above to afford after purification 266 mg (94%) as a off-white solid: TLC R_f 0.2 (ethyl acetate/triethylamine/hexanes, 1:1:6); ^{1}H NMR (DMSO-d_6): 1.15 (s, 18H, $C(CH_3)_3$), 2.89 (s, 3H, 4-CH_3), 3.79 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 5.38 (s, 2H, $CH_2C_6H_5$), 6.62 (m, 1H, C_6H_3), 7.18–7.35 (m, 6H, C_6H_3 and C_6H_5), 8.04 (s, 1H, C6–*H*); HRMS (EI) calcd for $C_{34}H_{38}N_4O_4$: 566.2893; found: 566.2921.

6.2.1.4. N-{7-Benzyl-4-methyl-5-[(2-chlorophenyl)ethynyl]-7H-pyrrolo[2,3-d]pyrimidin-2-yl}-N-2,2-dimethylpropanamide (19d). Compound **19d** was synthesized from **17** (225 mg, 0.5 mmol), 2-chlorophenylacetylene **18d** (205 mg, 1.5 mmol) using the general procedure described above to afford after purification 218 mg (93%) as a white solid: TLC R_f 0.524 (ethyl acetate/triethylamine/hexanes, 1:1:3); mp 134–136 °C; ^{1}H NMR (DMSO-d_6): 1.24 (s, 9H, $C(CH_3)_3$), 2.87 (s, 3H, 4-CH_3), 5.40 (s, 2H, $CH_2C_6H_5$), 7.30–7.64 (m, 9H, C_6H_3 and C_6H_5), 8.01 (s, 1H, C6–*H*), 9.92 (br, 1H, N*H*Piv, exch); Anal. Calcd for $C_{27}H_{25}ClN_4O$: C, 70.96; H, 5.51; N, 12.26; Cl, 7.75. Found: C, 70.82; H, 5.52; N, 12.12; Cl, 7.85.

6.2.1.5. N-{7-Benzyl-4-methyl-5-[(2,6-dimethoxyphenyl)ethynyl]-7H-pyrrolo[2,3-d]pyrimidin-2-yl}-N-(2,2-dimethylpropanoyl)-2,2-dimethylpropanamide (19e). Compound **19e** was synthesized from **16** (243 mg, 0.45 mmol), 2,6-dimethoxyphenylacetylene **18e** (230 mg, 1.35 mmol) using the general procedure described above to afford after purification 250 mg (96.5%) as a light green solid : TLC R_f 0.2 (ethyl acetate/triethylamine/hexanes, 1:1:6); mp 158–159.5 °C ^{1}H NMR (DMSO-d_6): 1.17–1.18 (s, 18H, $C(CH_3)_3$), 2.91 (s, 3H, 4-CH_3), 3.83 (s, 6H, OCH_3), 5.40 (s, 2H, $CH_2C_6H_5$), 6.72–6.81 (m, 3H, C_6H_3), 7.18–7.35 (m, 5H, C_6H_5), 8.15 (s, 1H, C6–*H*); HRMS (EI): calcd for $C_{34}H_{38}N_4O_4$: 566.2893; found: 566.2871.

6.2.1.6. N-{7-Benzyl-4-methyl-5-[(2,3,4-trimethoxyphenyl)ethynyl]-7H-pyrrolo[2,3-d]pyrimidin-2-yl}-N-2,2-dimethylpropanamide (19f). Compound **19f** was synthesized from **17** (296 mg, 0.66 mmol), 2,3,4-trimethoxyacetylene **18f** (384 mg, 2 mmol), copper(I)iodide (25 mg, 0.13 mmol), tetrakis(triphenylphosphine)palladium(0) (76.2 mg, 0.066 mmol), using the general procedure described above to afford after purification 329 mg (97%) as a sticky brown semisolid : TLC R_f 0.27 (ethyl acetate/triethylamine/hexanes, 1:1:3); ^{1}H NMR (DMSO-d_6): 1.25 (s, 9H, $C(CH_3)_3$), 2.87 (s, 3H, 4-CH_3), 3.76–3.89 (m, 9H, OCH_3), 5.39 (s, 2H, $CH_2C_6H_5$), 6.82–6.85 (d, 1H, C_6H_2, J = 8.7 Hz), 7.17–7.20 (d, 1H, C_6H_2, J = 8.7 Hz), 7.33–7.62 (m, 5H, C_6H_5), 7.88 (s, 1H, C6–*H*), 9.88 (br, 1H, N*H*Piv, exch); HRMS (EI): calcd for $C_{30}H_{32}N_4O_4$: 512.2424; found: 512.2456.

6.2.1.7. N-{7-Benzyl-4-methyl-5-[(2,3,4,5-tetramethoxyphenyl)ethynyl]-7H-pyrrolo[2,3-d]pyrimidin-2-yl}-N-(2,2-dimethylpropanoyl)-2,2-dimethylpropanamide (19g). Compound **19g** was synthesized from **16** (191.5 mg, 0.36 mmol), 2,3,4,5-tetramethoxyphenylacetylene **18g** (240 mg, 1.08 mmol) copper(I) iodide (13 mg, 0.072 mmol), tetrakis(triphenylphosphine)palladium (0) (41 mg, 0.036 mmol), using the general procedure described above to afford after purification 202 mg (89.5%) as a light brown solid : TLC R_f 0.22 (ethyl acetate/triethylamine/hexanes, 1:1:6); ^{1}H NMR (DMSO-d_6): 1.18 (s, 18H, $C(CH_3)_3$), 2.96 (s, 3H, 4-CH_3), 3.82–3.93 (t, 12H, OCH_3), 5.40 (s, 2H, $CH_2C_6H_5$), 6.71 (s, 1H, C_6H), 7.21–7.34 (m, 5H, C_6H_5), 8.11 (s, 1H, C6–*H*); HRMS (EI): calcd for $C_{36}H_{42}N_4O_6$: 626.3104; found: 626.3188.

6.2.1.8. N-{7-Benzyl-4-methyl-5-[(2,4,5-trimethoxyphenyl)ethynyl]-7H-pyrrolo[2,3-d]pyrimidin-2-yl}-N-(2,2-dimethylpropanoyl)-2,2-dimethylpropanamide (19h). Compound **19h** was synthesized from **16** (213 mg, 0.4 mmol), 2,4,5-trimethoxyphenylacetylene **18h** (230 mg, 1.2 mmol) copper(I)iodide (15 mg, 0.08 mmol), tetrakis(triphenylphosphine)palladium(0) (45 mg, 0.04 mmol), using the general procedure described above to afford after purification 225 mg (94%) as a light brown solid: TLC R_f 0.19 (ethyl acetate/triethylamine/hexanes, 1:1:6); mp 145–147 °C; ^{1}H NMR (DMSO-d_6): 1.16–1.168 (d, 18H, $C(CH_3)_3$), 2.91 (s, 3H, 4-CH_3), 3.71–3.72 (br, 3H, OCH_3), 3.83–3.84 (br, 6H, OCH_3), 5.39 (s, 2H, $CH_2C_6H_5$), 6.73–6.74 (d, 1H, C_6H_2, J = 2.4 Hz), 6.98–6.99 (d, 1H, C_6H_2, J = 2.4 Hz), 7.20–7.31 (m, 5H, C_6H_5), 8.05 (s, 1H, C6–*H*); HRMS (EI): calcd for $C_{35}H_{40}N_4O_5$: 596.2998; found: 596.2959.

6.2.1.9. N-{7-Benzyl-4-methyl-5-[(2,3,5-trimethoxyphenyl)ethynyl]-7H-pyrrolo[2,3-d]pyrimidin-2-yl}-N-(2,2-dimethylpropanoyl)-2,2-dimethylpropanamide (19i). Compound **19i** was synthesized from **16** (186 mg, 0.35 mmol), 2,3,5-trimethoxyphenylacetylene **18i** (201 mg, 1.05 mmol) copper(I)iodide (13 mg, 0.07 mmol), tetrakis(triphenylphosphine)palladium(0) (39 mg, 0.035 mmol), using the general procedure described above to afford after purification 200 mg (96%) as a light brown semisolid : TLC R_f 0.2 (ethyl acetate/triethylamine/hexanes, 1:1:6); ^{1}H NMR (DMSO-d_6): 1.15–1.19 (m, 18H, $C(CH_3)_3$), 2.94 (s, 3H, 4-CH_3), 3.73–3.83 (m, 9H, OCH_3), 5.43 (s, 2H, $CH_2C_6H_5$), 6.59–7.32 (m, 7H, C_6H_2 and C_6H_5), 8.18 (s, 1H, C6–*H*); HRMS (EI): calcd for $C_{35}H_{40}N_4O_5$: 596.2998; found: 596.3001.

6.2.2. General procedure for the synthesis of compounds 20a, 20c, 20e, 20g–20i

To a Parr hydrogenation bottle was added 5% Pd/C followed by a solution of **19a/19c/19e/19g–19i** dissolved in dichloromethane (20 mL) and methanol (5 mL). The mixture was hydrogenated at 50 psi at room temperature for 3.5 h. After filtration, the catalyst was thoroughly washed with hot methanol–methylene chloride mixture (1:1, 50 mL). The filtrate was concentrated in vacuo and silica gel (1 g) was added to the residue. The solvent was evaporated to afford a plug. The silica gel plug obtained was loaded onto a silica gel column and eluted with 1:1:10 ethyl acetate/triethylamine/hexanes. Fractions containing the product (TLC) were pooled, and the solvent was evaporated to afford analytically pure compound.

6.2.2.1. N-{7-Benzyl-4-methyl-5-[(3,5-dimethoxyphenyl)ethyl]-7H-pyrrolo[2,3-d]pyrimidin-2-yl}-N-(2,2-dimethylpropanoyl)-2,2-dimethylpropanamide (20a). Compound **20a** was synthesized from **19** (152 mg, 0.27 mmol) and 5% Pd/C (150 mg) using

the general procedure described above to afford after purification 140 mg (91%) as a white solid: TLC R_f 0.238 (ethyl acetate/triethylamine/hexanes, 1:1:6); ^{1}H NMR (DMSO-d_6): 1.10 (s, 18H, C(CH_3)$_3$), 2.71 (s, 3H, 4-CH_3), 2.87 (t, 2H, –CH_2–), 3.13 (t, 2H, –CH_2–), 3.67 (s, 6H, OCH_3), 5.33 (s, 2H, $CH_2C_6H_5$), 6.31 (s, 1H, C6–*H*), 6.46–7.48 (m, 8H, C_6H_3 and C_6H_5); HRMS (EI): calcd for $C_{34}H_{42}N_4O_4$ 570.3206; found: 570.3235.

6.2.2.2. N-{7-Benzyl-4-methyl-5-[(2,4-dimethoxyphenyl)ethyl]-7H-pyrrolo[2,3-d]pyrimidin-2-yl}-N-(2,2-dimethylpropanoyl)-2,2-dimethylpropanamide (20c). Compound **20c** was synthesized from **19c** (150 mg, 0.265 mmol), 5% Pd/C (150 mg) using the general procedure described above to afford after purification 140 mg (92%) as a white semisolid : TLC R_f 0.21 (ethyl acetate/triethylamine/hexanes, 1:1:6); ^{1}H NMR (DMSO-d_6): 1.14 (s, 18H, C(CH_3)$_3$), 2.72 (s, 3H, 4-CH_3), 2.75 (t, 2H, –CH_2–), 2.95 (t, 2H, –CH_2–), 3.69 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 5.31 (s, 2H, $CH_2C_6H_5$), 6.49 (s, 1H, C6–*H*), 6.99–7.39 (m, 8H, C_6H_3 and C_6H_5); HRMS (ESI): calcd for $C_{34}H_{43}N_4O_4$ (M+H)$^+$: 571.3284; found: 571.3311.

6.2.2.3. N-{7-Benzyl-4-methyl-5-[(2,6-dimethoxyphenyl)ethyl]-7H-pyrrolo[2,3-d]pyrimidin-2-yl}-N-(2,2-dimethylpropanoyl)-2,2-dimethylpropanamide (20e). Compound **20e** was synthesized from **19e** (150 mg, 0.26 mmol) and 5% Pd/C (150 mg) using the general procedure described above to afford after purification 139 mg (92%) as a white liquid : TLC R_f 0.22 (ethyl acetate/triethylamine/hexanes, 1:1:6); ^{1}H NMR (300 MHz) (DMSO-d_6): 1.16 (s, 18H, C(CH_3)$_3$), 2.72 (s, 3H, 4-CH_3), 2.79–2.83 (m, 4H, CH_2CH_2), (s, 6H, OCH_3), 5.37 (s, 2H, $CH_2C_6H_5$), 6.65–6.71 (m, 3H, C_6H_3), 7.12–7.38 (m, 6H, C_6H_5 and C6–*H*); HRMS (ESI): calcd for $C_{34}H_{43}N_4O_4$ (M+H)$^+$: 571.3284; found: 571.3286.

6.2.2.4. N-{7-Benzyl-4-methyl-5-[(2,3,4,5-tetramethoxyphenyl)ethyl]-7H-pyrrolo[2,3-d]pyrimidin-2-yl}-N-(2,2-dimethylpropanoyl)-2,2-dimethylpropanamide (20g). Compound **20g** was synthesized from **19g** (150 mg, 0.24 mmol) and 5% Pd/C (150 mg) using the general procedure described above to afford after purification 136 mg (86%) as a colorless, sticky oil: TLC R_f 0.24 (ethyl acetate/triethylamine/hexanes, 1:1:6); ^{1}H NMR (DMSO-d_6): 1.09 (s, 18H, C(CH_3)$_3$), 2.72 (s, 3H, 4-CH_3), 2.86–2.87 (t, 2H, –CH_2–), 3.02–3.04 (t, 2H, –CH_2–), 3.62–3.75 (m, 12H, OCH_3), 5.31–5.33 (br, 2H, $CH_2C_6H_5$), 6.55 (s, 1H, C6–*H*), 7.07–7.43 (m, 6H, C_6H_1 and C_6H_5); HRMS (EI): calcd for $C_{36}H_{46}N_4O_6$: 630.3417; found: 630.3403.

6.2.2.5. N-{7-Benzyl-4-methyl-5-[(2,4,5-trimethoxyphenyl)ethyl]-7H-pyrrolo[2,3-d]pyrimidin-2-yl}-N-(2,2-dimethylpropanoyl)-2,2-dimethylpropanamide (20h). Compound **20h** was synthesized from **19h** (150 mg, 0.25 mmol) and 5% Pd/C (150 mg) using the general procedure described above to afford after purification 135 mg (89%) as a off-white semisolid : TLC R_f 0.35 (ethyl acetate/triethylamine/hexanes, 1:1:3); ^{1}H NMR (DMSO-d_6): 1.14–1.23 (m, 18H, C(CH_3)$_3$), 2.73 (s, 3H, 4-CH_3), 2.79–3.02 (m, 4H, –CH_2–CH_2–), 3.58–3.75 (t, 3H, OCH_3), 5.32 (s, 2H, $CH_2C_6H_5$), 6.63–7.38 (m, 8H, C6–*H*, C_6H_2 and C_6H_5); HRMS (EI): calcd for $C_{35}H_{44}N_4O_5$: 600.3311; found: 600.3333.

6.2.2.6. N-{7-Benzyl-4-methyl-5-[(2,3,5-trimethoxyphenyl)ethyl]-7H-pyrrolo[2,3-d]pyrimidin-2-yl}-N-(2,2-dimethylpropanoyl)-2,2-dimethylpropanamide (20i). Compound **20i** was synthesized from **19i** (150 mg, 0.25 mmol) and 5% Pd/C (150 mg) using the general procedure described above to afford after purification 135 mg (89%) as a white solid : TLC R_f 0.489 (ethyl acetate/triethylamine/hexanes, 1:1:3); mp 94.5–96 °C ^{1}H NMR (DMSO-d_6): 1.14–1.19 (d, 18H, C(CH_3)$_3$), 2.75 (s, 3H, 4-CH_3), 2.87–2.88 (t, 2H, –CH_2), 3.03–3.04 (t, 2H, –CH_2–), 3.58–3.76 (t, 9H, OCH_3), 5.32 (s, 2H, $CH_2C_6H_5$), 6.32–6.45 (m, 2H, C_6H_2), 7.10–7.44 (m, 6H, C6–*H* and C_6H_5); HRMS (EI): calcd for $C_{35}H_{44}N_4O_5$: 600.3311; found: 600.3285.

6.2.3. General procedure for the synthesis of compounds 20b, 20d, 20f

To a Parr hydrogenation bottle was added 5% Pd/C followed by a solution of **19b/19d/19f** dissolved in dichloromethane (10 mL) and methanol (10 mL). The mixture was hydrogenated at 50 psi at room temperature for 24 h. After filtration, the catalyst was thoroughly washed with hot methanol–methylene chloride mixture (1:1, 50 mL). The filtrate was concentrated in vacuo and silica gel (1 g) was added to the residue. The solvent was evaporated to afford a plug. The silica gel plug obtained was loaded onto a silica gel column and eluted with 1:1:6 ethyl acetate/triethylamine/hexanes. Fractions containing the product (TLC) were pooled, and the solvent was evaporated to afford analytically pure compound.

6.2.3.1. N-{7-Benzyl-4-methyl-5-[(3,4-dimethoxyphenyl)ethyl]-7H-pyrrolo[2,3-d]pyrimidin-2-yl}-N-2,2-dimethylpropanamide (20b). Compound **20b** was synthesized from **19b** (130 mg, 0.27 mmol), 5% Pd/C (130 mg) using the general procedure described above to afford after purification 117 mg (89%) as a colorless semisolid: TLC R_f 0.21 (ethyl acetate/triethylamine/hexanes, 1:1:3); ^{1}H NMR (DMSO-d_6): 1.22 (s, 9H, C(CH_3)$_3$), 2.68 (s, 3H, 4-CH_3), 2.86 (t, 2H, –CH_2–), 3.04 (t, 2H, –CH_2–), 3.66–3.69 (d, 6H, OCH_3), 5.31 (s, 2H, $CH_2C_6H_5$), 6.75 (s, 1H, C6–*H*), 6.79–7.29 (m, 8H, C_6H_3 and C_6H_5), 9.68 (s, 1H, N*H*Piv, exch); HRMS (EI): calcd for $C_{29}H_{34}N_4O_3$: 486.2631; found: 486.2667.

6.2.3.2. N-{7-Benzyl-4-methyl-5-[(2-chlorophenyl)ethyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl}-*N*-2,2-dimethylpropanamide (20d). Compound **20d** was synthesized from **19d** (150 mg, 0.33 mmol), 5% Pd/C (150 mg) using the general procedure described above to afford after purification 140 mg (92%) as a white semisolid: TLC R_f 0.69 (ethyl acetate/triethylamine/hexanes, 1:1:3); ^{1}H NMR (DMSO-d_6): 1.22 (s, 9H, C(CH_3)$_3$), 2.69 (s, 3H, 4-CH_3), 3.04 (t, 4H, –CH_2–CH_2–), 5.31 (s, 2H, $CH_2C_6H_5$), 7.21–7.30 (m, 10H, C6–*H*, C_6H_3 and C_6H_5), 9.71 (br, 1H, N*H*Piv, exch). HRMS (EI): calcd for $C_{27}H_{30}N_4OCl$: 416.2108; found: 416.2107.

6.2.3.3. N-{7-Benzyl-4-methyl-5-[(2,3,4-trimethoxyphenyl)ethyl]-7H-pyrrolo[2,3-d]pyrimidin-2-yl}-N-2,2-dimethylpropanamide (20f). Compound **20f** was synthesized from **19** (150 mg, 0.29 mmol) and 5% Pd/C (150 mg) using the general procedure described above to afford after purification 104 mg (69%) as a white semisolid: TLC R_f 0.265 (ethyl acetate/triethylamine/hexanes, 1:1:3); ^{1}H NMR (DMSO-d_6): 1.23 (s, 9H, C(CH_3)$_3$), 2.72 (s, 3H, 4-CH_3), 2.80 (t, 2H, –CH_2–), 2.97 (t, 2H, –CH_2–), 3.72–3.74 (m, 9H, OCH_3), 5.32 (s, 2H, $CH_2C_6H_5$), 6.69–7.31 (m, 9H, C6–*H* , C_6H_2, C_6H_5), 9.71 (br, 1H, N*H*Piv, exch); HRMS (EI): calcd for $C_{30}H_{36}N_4O_4$: 516.2737; found: 516.3350.

6.2.4. General procedure for the synthesis of compounds 6–14

To a round bottom flask were added **20a–i** followed by methanol (10 mL) and 1 N sodium hydroxide (2 mL). The reaction mixture was heated at reflux for 24 h. The reaction was then cooled and methanol evaporated under vacuum. The precipitated solid obtained was filtered, washed with cold water and air-dried. This solid was then recrystallized from a mixture of dichloromethane and hexanes (1:2).

6.2.4.1. 7-Benzyl-4-methyl-5-[2-(3,5-dimethoxyphenyl)ethyl]-7Hpyrrolo[2,3-d]pyrimidin-2-amine (6). Compound **6** was synthesized from **20a** (70 mg, 0.12 mmol) using the general procedure described above to afford 41 mg (83%) as a white crystalline solid:

TLC R_f 0.45 (ethyl acetate/triethylamine/hexanes, 5:1:3); mp 144.2–145.8 °C; ^{1}H NMR (DMSO-d_6): 2.52 (s, 3H, 4-CH_3), 2.82 (t, 2H, –CH_2–), 2.94 (t, 2H, –CH_2–), 3.70 (s, 6H, OCH_3), 5.16 (s, 2H, CH_2C$_6$H$_5$), 6.05 (br, 2H, 2-NH_2, exch), 6.30–6.31 (d, 1H, C$_6H_3$), 6.39–6.40 (d, 2H, C$_6H_3$), 6.78 (s, 1H, C6–*H*), 7.09–7.31 (m, 5H, C$_6H_5$). Anal. Calcd for $C_{24}H_{26}N_4O_2$: C, 71.62; H, 6.51; N, 13.92. Found: C, 71.28; H, 6.32; N, 13.92.

6.2.4.2. 7-Benzyl-4-methyl-5-[2-(3,4-dimethoxyphenyl)ethyl]-7Hpyrrolo[2,3-d]pyrimidin-2-amine (7). Compound **7** was synthesized from **20b** (70 mg, 0.1145 mmol) using the general procedure described above to afford 51 mg (87%) as a white fluffy solid: TLC R_f 0.44 (ethyl acetate/triethylamine/hexanes, 5:1:3); mp 109.0–110.0 °C; ^{1}H NMR (DMSO-d_6): 2.51 (s, 3H, 4-CH_3), 2.82 (t, 2H, –CH_2–), 2.94 (t, 2H, –CH_2–), 3.67–3.69 (d, 6H, OCH_3), 5.15 (s, 2H, CH_2C$_6$H$_5$), 6.12 (br, 2H, 2-NH_2, exch), 6.72–7.29 (m, 9H, C$_6H_3$, C6–*H* and C$_6H_5$). Anal. Calcd for $C_{24}H_{26}N_4O_2{\cdot}0.5H_2O$: C, 70.05; H, 6.61; N, 13.62. Found: C, 69.70; H, 6.61; N, 13.45.

6.2.4.3. 7-Benzyl-4-methyl-5-[2-(2,4-dimethoxyphenyl)ethyl]-7Hpyrrolo[2,3-d]pyrimidin-2-amine (8). Compound **8** was synthesized from **20c** (100 mg, 0.175 mmol) using the general procedure described above to afford 61 mg (70.5%) as faint green lustrous crystals: TLC R_f 0.49 (ethyl acetate/triethylamine/hexanes, 5:1:3); mp 132–133.3 °C; ^{1}H NMR (DMSO-d_6): 2.54 (s, 3H, 4-CH_3), 2.74 (t, 2H, –CH_2–), 2.76 (t, 2H, –CH_2–), 3.72–3.74 (m, 6H, OCH_3), 5.16 (s, 2H, CH_2C$_6$H$_5$), 6.06 (br, 2H, 2-NH_2, exch), 6.39–7.30 (m, 9H, C$_6H_3$, C6–*H* and C$_6H_5$). Anal. Calcd for $C_{24}H_{26}N_4O_2$: C, 71.62; H, 6.51; N, 13.92. Found: C, 71.34; H, 6.49; N, 13.76.

6.2.4.4. 7-Benzyl-4-methyl-5-[2-(2-chlorophenyl)ethyl]-7Hpyrrolo[2,3-d]pyrimidin-2-amine (9). Compound **9** was synthesized from **20d** (65 mg, 0.14 mmol) using the general procedure described above to afford 44 mg (90%) as yellow needles: TLC R_f 0.46 (ethyl acetate/triethylamine/hexanes, 5:1:3); mp 144.5–146.5 °C; ^{1}H NMR (DMSO-d_6): ^{1}H NMR (DMSO-d_6): 2.54 (s, 3H, 4-CH_3), 2.98 (br, 4H, –CH_2–CH_2–), 5.16 (s, 2H, CH_2C$_6$H$_5$), 6.07 (br, 2H, 2-NH_2, exch), 6.76 (s, 1H, C6–*H*), 7.08–7.39 (m, 8H, C$_6H_4$ and C$_6H_5$). Anal. Calcd for $C_{22}H_{21}N_4Cl$: C, 70.11; H, 5.62; N, 14.87; Cl, 9.41. Found: C, 70.40; H, 5.72; N, 14.97; Cl, 9.16.

6.2.4.5. 7-Benzyl-4-methyl-5-[2-(2,6-dimethoxyphenyl)ethyl]-7Hpyrrolo[2,3-d]pyrimidin-2-amine (10). Compound **10** was synthesized from **20e** (135 mg, 0.236 mmol) using the general procedure described above to afford 85 mg (89%) as white fluffy crystals: TLC R_f 0.45 (ethyl acetate/triethylamine/hexanes, 5:1:3); mp 179.1–180.6 °C; ^{1}H NMR (DMSO-d_6): 2.56 (s, 3H, 4-CH_3), 2.76 (t, 2H, –CH_2–), 2.81 (t, 2H, –CH_2–), 3.72 (m, 6H, OCH_3), 5.17 (s, 2H, CH_2C$_6$H$_5$), 6.06 (br, 2H, 2-NH_2, exch), 6.60–7.15 (m, 9H, C$_6H_3$, C6–*H* and C$_6H_5$). Anal. Calcd for $C_{24}H_{26}N_4O_2$: C, 71.62; H, 6.51; N, 13.92. Found: C, 71.45; H, 6.50; N, 13.83.

6.2.4.6. 7-Benzyl-4-methyl-5-[2-(2,3,4-trimethoxyphenyl)ethyl]-7Hpyrrolo[2,3-d]pyrimidin-2-amine (11). Compound **11** was synthesized from **20f** (82 mg, 0.16 mmol) using the general procedure described above to afford 60 mg (87%) as a lustrous off-white solid: TLC R_f 0.47 (ethyl acetate/triethylamine/hexanes, 5:1:3); mp 115–116.5 °C; ^{1}H NMR (DMSO-d_6): 2.55 (s, 3H, 4-CH_3), 2.76–2.84 (m, 4H, –CH_2–CH_2–), 3.72–3.74 (m, 9H, OCH_3), 5.16 (s, 2H, CH_2C$_6$H$_5$), 6.07 (br, 2H, 2-NH_2, exch), 6.67–7.29 (m, 8H, C$_6H_2$, C6–*H* and C$_6H_5$). Anal. Calcd for: $C_{25}H_{28}N_4O_3$: C, 69.42; H, 6.53; N, 12.95. Found: C, 69.25; H, 6.58; N, 12.90.

6.2.4.7. 7-Benzyl-4-methyl-5-[2-(2,3,4,5-tetramethoxyphenyl)ethyl]-7Hpyrrolo[2,3-d]pyrimidin-2-amine (12). Compound **12** was synthesized from **20f** (63 mg, 0.1 mmol) using the general procedure described above to afford 34 mg (73.5%) as a lustrous white crystals: TLC R_f 0.27 (ethyl acetate/triethylamine/hexanes, 5:1:3); mp 120.2–121.2 °C; ^{1}H NMR (DMSO-d_6): 2.54 (s, 3H, 4-CH_3), 2.78–2.79 (t, 2H, –CH_2–), 2.80–2.81 (t, 2H, –CH_2–), 3.66–3.78 (m, 12H, OCH_3), 5.17 (s, 2H, CH_2C$_6$H$_5$), 6.07 (br, 2H, 2-NH_2, exch), 6.60 (s, 1H, C$_6H_3$), 6.81 (s, 1H, C6–*H*), 7.11–7.32 (m, 5H, C$_6H_5$). Anal. Calcd for $C_{26}H_{30}N_4O_4$: C, 67.51; H, 6.54; N, 12.11. Found: C, 67.14; H, 6.65; N, 11.99.

6.2.4.8. 7-Benzyl-4-methyl-5-[2-(2,4,5-trimethoxyphenyl)ethyl]-7Hpyrrolo[2,3-d]pyrimidin-2-amine (13). Compound **13** was synthesized from **20g** (80 mg, 0.133 mmol) using the general procedure described above to afford 51.5 mg (88%) as fluffy white crystals: TLC R_f 0.30 (ethyl acetate/triethylamine/hexanes, 5:1:3); mp 142–144 °C; ^{1}H NMR (DMSO-d_6): 2.52 (s, 3H, 4-CH_3), 2.75–2.76 (t, 2H, –CH_2–), 2.82–2.85 (t, 2H, –CH_2–), 3.60–3.74 (t, 9H, OCH_3), 5.15 (s, 2H, CH_2C$_6$H$_5$), 6.05 (br, 2H, 2-NH_2, exch), 6.63–7.28 (m, 8H, C$_6H_2$, C6–*H* and C$_6H_5$). Anal. Calcd for: $C_{25}H_{28}N_4O_3$: C, 69.42; H, 6.53; N, 12.95. Found: C, 69.31; H, 6.45; N, 12.81.

6.2.4.9. 7-Benzyl-4-methyl-5-[2-(2,3,5-trimethoxyphenyl)ethyl]-7Hpyrrolo[2,3-d]pyrimidin-2-amine (14). Compound **14** was synthesized from **20h** (80 mg, 0.133 mmol) using the general procedure described above to afford 45 mg (78%) as a white powder: TLC R_f 0.33 (ethyl acetate/triethylamine/hexanes, 5:1:3); mp 111–111.6 °C; ^{1}H NMR (DMSO-d_6): 2.54 (s, 3H, 4-CH_3), 2.80–2.90 (m, 4H, –CH_2–CH_2–), 3.60–3.76 (t, 9H, OCH_3), 5.17 (s, 2H, CH_2C$_6$H$_5$), 6.08 (br, 2H, 2-NH_2, exch), 6.34–6.35 (d, 1H, C$_6H_2$, J = 2.7 Hz), 6.45–6.45 (d, 1H, C$_6H_2$, J = 2.7 Hz), 6.82 (s, 1H, C6–*H*), 7.11–7.297 (m, 5H, C$_6H_5$). Anal. Calcd for $C_{25}H_{28}N_4O_3$: C, 69.42; H, 6.53; N, 12.95. Found: C, 69.47; H, 6.45; N, 12.84.

6.2.5. General procedure for the synthesis of compounds 22c and 22e

To a 5 mL microwave reaction tube (Biotage Inc.) equipped with a stir bar was added **21c** or **21e** (1 mmol), 10% Pd/C (0.04 mmol), triphenylphosphine (0.16 mmol), copper (I) iodide (0.16 mmol), trimethylsilylacetylene (1.05 mmol), triethylamine (3 mL) and acetonitrile (2 mL). The microwave tube was sealed with a crimp-cap and subjected to microwave heating in a microwave reactor (Initiator, Biotage Inc.) set at 120 °C with 'fixed hold time' for 5 min. At the end of the reaction, the grey-black suspension was passed through Celite pad, washing thoroughly with hot ethyl acetate (20 mL). The filtrate concentrated in reduced pressure and silica gel (1 g) was added. Further evaporation lead to a silica gel plug which was loaded on top of a silica gel column (2 cm × 20 cm) and eluted with 5% ethyl acetate in hexanes. Fractions corresponding to the product spot were evaporated under reduced pressure to obtain an analytically pure product.

6.2.5.1. (2,4-Dimethoxyphenylethynyl)trimethylsilane (22c). Compound **22c** was synthesized from **21c** (264 mg, 1.0 mmol) using the general procedure described above to afford 170 mg (73%) as a off-white solid: TLC R_f 0.63 (ethyl acetate/hexanes, 1:3); mp 46.1–47.2 °C (lit.[45] mp 45.0–47.0 °C); ^{1}H NMR (CDCl$_3$): 0.26 (s, 9H, Si(CH_3)$_3$), 3.95–3.99 (m, 6H, OCH_3), 6.43 (d, 2H, C$_6H_3$), 7.42 (d, 1H, C$_6H_3$); HRMS (EI): calcd for $C_{13}H_{18}O_2Si$: 234.1076; found: 234.1067. This compound matched in all aspects (^{1}H NMR) with that reported in the literature.[45]

6.2.5.2. (2,6-Dimethoxyphenylethynyl)trimethylsilane (22e). Compound **22e** was synthesized from **21e** (264 mg, 1.0 mmol) using the general procedure described above to afford 176 mg (75%) as a off-white solid: TLC R_f 0.63 (ethyl acetate/hexanes, 1:3); mp 105–106 °C; ^{1}H NMR (CDCl$_3$): 0.17 (s, 9H, Si(CH_3)$_3$), 3.75 (s, 6H, OCH_3), 6.37–6.40 (d, 2H, C$_6H_3$), 7.15 (d, 1H, C$_6H_3$);

HRMS (EI): calcd for $C_{13}H_{18}O_2Si$: 234.1076; found: 234.1106. The ^1H NMR spectrum of this compound matched with that reported in the literature.[46]

6.2.6. General procedure for the synthesis of compounds 22f–i

Aryl bromide (1.00 mmol; in case it is solid), $Pd(PhCN)_2Cl_2$ (11.5 mg, 0.030 mmol) and CuI (3.8 mg, 0.020 mmol) are added to a dry, 10-mL round bottom flask, which is then sparged with argon and charged with dioxane (1.0 mL; Aldrich Sure/Seal anhydrous/99.8%). P(*t*-Bu)$_3$ (70 μL of a 1 M solution in dioxane; 0.07 mmol; made from 1gm P(*t*-Bu)$_3$ and 4.95 mL anhydrous dioxane; preserved under argon), HN(*i*-Pr)$_2$ (170 μL, 1.20 mol; freshly distilled over CaH_2), aryl bromide (1.00 mmol, in case it is liquid), and the trimethylsilylacetylene (1.20 mmol) are added via syringe to the stirred reaction mixture. After the aryl bromide has been consumed, the reaction mixture is diluted with EtOAc (5 mL), filtered through a small pad of Celite (with EtOAc rinsings), concentrated in reduced pressure and silica gel (1 g) was added. Further evaporation lead to a silica gel plug which was loaded on top of a silica gel column (2 cm × 20 cm) and eluted with 5% ethyl acetate in hexanes. Fractions corresponding to the product spot were evaporated under reduced pressure to obtain analytically pure product.

6.2.6.1. (2,3,4-Trimethoxyphenylethynyl)trimethylsilane (22f). Compound **22f** was synthesized from **21f** (247.1 mg, 1.0 mmol) using the general procedure described above to afford 218 mg (82.5%) as an yellow oil: TLC R_f 0.47 (ethyl acetate/hexanes, 1:3); ^1H NMR ($CDCl_3$): 0.25 (s, 9H, Si(CH_3)$_3$), 3.85 (s, 6H, OCH_3), 3.97 (s, 3H, OCH_3), 6.57–6.60 (d, 1H, C_6H_2, J = 8.7 Hz), 7.12–7.14 (d, 1H, C_6H_2, J = 8.7 Hz); HRMS (EI): calcd for $C_{14}H_{20}O_3Si$: 264.1181; found: 264.1235.

6.2.6.2. (2,3,4,5-Tetramethoxyphenylethynyl)trimethylsilane (22g). Compound **22g** was synthesized from **21g** (247.1 mg, 1.0 mmol) using the general procedure described above to afford 218 mg (82.5%) as an orange oil: TLC R_f 0.576 (ethyl acetate/hexanes, 1:3); ^1H NMR ($CDCl_3$): 0.27 (s, 9H, Si(CH_3)$_3$), 3.83–3.94 (m, 12H, OCH_3), 6.27 (s, 1H, C_6H); HRMS (EI): calcd for $C_{15}H_{22}O_4Si$: 294.1287; found: 294.1298.

6.2.6.3. (2,4,5-Trimethoxyphenylethynyl)trimethylsilane (22h). Compound **22h** was synthesized from **21h** (247 mg, 1.0 mmol) using the general procedure described above to afford 185 mg (70%) as an yellow-orange oil: TLC R_f 0.475 (ethyl acetate/hexanes, 1:3); ^1H NMR ($CDCl_3$): 0.26 (s, 9H, Si(CH_3)$_3$), 3.84–3.89 (m, 9H, OCH_3), 6.57 (s, 1H, C_6H_2), 7.04 (s, 1H, C_6H_2); HRMS (EI): calcd for $C_{14}H_{20}O_3Si$: 264.1181; found: 264.1153.

6.2.6.4. (2,3,5-Trimethoxyphenylethynyl)trimethylsilane (22i). Compound **22i** was synthesized from **21i** (618 mg, 2.5 mmol), $Pd(PhCN)_2Cl_2$ (28.75 mg, 0.075 mmol), CuI (9.5 mg, 0.050 mmol), P(*t*-Bu)$_3$ (175 μL of a 1 M solution; 0.175 mmol), HN(*i*-Pr)$_2$ (425 μL, 1.20 mol), and trimethylsilylacetylene (390 μL, 3 mmol) using the general procedure described above to afford 519.5 mg (84%) as a colorless oil: TLC R_f 0.56 (ethyl acetate/hexanes, 1:3); ^1H NMR ($CDCl_3$): 0.27 (s, 9H, Si(CH_3)$_3$), 3.77–3.88 (m, 9H, OCH_3), 6.44–6.64 (m, 2H, C_6H_2); HRMS (EI): calcd for $C_{14}H_{20}O_3Si$: 264.1181; found: 264.1199.

6.2.7. General procedure for the synthesis of compounds 18c, 18e and 18f–i

To a 25 mL round bottom flask was added **22c**/**22e**/**22f–i** (1 mmol) followed by the addition of 5 mL anhydrous tetrahydrofuran. To this solution was added 1 M *tert*-butylammonium fluoride solution in THF (0.5 mmol). The colorless solution becomes light to dark brown. The solution was stirred for 2 h at room temperature under nitrogen. At the end of the reaction, silica gel (500 mg) was added to the reaction mixture and solvent evaporated in reduced pressure to get a silica gel plug, which was loaded on top of a silica gel column (2 cm × 5 cm) and eluted with 5% ethyl acetate in hexanes. Fractions corresponding to the product spots were evaporated under reduced pressure to get substituted aryl alkynes.

6.2.7.1. 1-Ethynyl-2,4-dimethoxybenzene (18c). Compound **18c** was synthesized from **22c** (540 mg, 2.3 mmol) and *tert*-butylammonium fluoride solution (1.15 mL, 1.15 mmol) using the general procedure described above to afford 240 mg (70%) as an yellow-brown oil: TLC R_f 0.48 (ethyl acetate/hexanes, 1:3); ^1H NMR ($CDCl_3$): 3.25 (s, 1H, C_2H), 3.79–3.94 (m, 6H, OCH_3), 6.47 (d, 2H, C_6H_3), 7.41 (d, 1H, C_6H_3). The ^1H NMR spectrum of this compound matched with that reported in the literature.[45]

6.2.7.2. 1-Ethynyl-2,6-dimethoxybenzene (18e). Compound **18e** was synthesized from **22e** (150 mg, 0.64 mmol) and *tert*-butylammonium fluoride solution (0.3 mL, 0.3 mmol) using the general procedure described above to afford 99 mg (90%) as off-white solid: TLC R_f 0.46 (ethyl acetate/hexanes, 1:3); ^1H NMR ($CDCl_3$): 3.35 (s, 1H, C_2H), 3.90 (s, 6H, OCH_3), 6.53–6.56 (d, 3H, C_6H_3); HRMS (EI): calcd for $C_{10}H_{10}O_2$ 162.0680; found 162.0681. The ^1H NMR spectrum of this compound matched with that reported in the literature.[46]

6.2.7.3. 1-Ethynyl-2,3,4,5-tetramethoxybenzene (18g). Compound **18g** was synthesized from **22g** (150 mg, 0.64 mmol) and *tert*-butylammonium fluoride solution (0.3 mL, 0.3 mmol) using the general procedure described above to afford 115 mg (84%) as an orange-red oil: TLC R_f 0.47 (ethyl acetate/hexanes, 1:3); ^1H NMR ($CDCl_3$): 3.25 (s, 1H, C_2H), 3.83–3.94 (m, 12H, OCH_3), 6.82 (s, 1H, C_6H); HRMS (EI): calcd for $C_{12}H_{14}O_4$: 222.0892; found: 222.0861.

6.2.7.4. 1-Ethynyl-2,4,5-trimethoxybenzene (18h). Compound **18h** was synthesized from **22h** (300 mg, 1.135 mmol) and *tert*-butylammonium fluoride solution (0.6 mL, 0.6 mmol) using the general procedure described above to afford 170 mg (78.5%) as a white solid: TLC R_f 0.239 (ethyl acetate/hexanes, 1:3); mp 113.8–114.2 °C; ^1H NMR ($CDCl_3$): 3.14 (s, 1H, C_2H), 3.72–3.81 (m, 9H, OCH_3), 6.38–6.39 (d, 1H, C_6H_2, J = 4.2 Hz); 6.84–6.85 (d, 1H, C_6H_2, J = 4.2 Hz); HRMS (EI): calcd for $C_{11}H_{12}O_3$: 192.0786; found: 192.0786.

6.2.7.5. 1-Ethynyl-2,3,5-trimethoxybenzene (18i). Compound **18i** was synthesized from **22i** (519 mg, 1.96 mmol) and *tert*-butylammonium fluoride solution (1 mL, 1.0 mmol) using the general procedure described above to afford 315 mg (83%) as an colorless oil: TLC R_f 0.43 (ethyl acetate/hexanes, 1:3); ^1H NMR ($CDCl_3$): 3.25 (s, 1H, C_2H), 3.76–3.86 (m, 9H, OCH_3), 6.51–6.53 (m, 2H, C_6H_2); HRMS (EI): calcd for $C_{11}H_{12}O_3$: 192.0786; found: 192.0759.

6.2.8. Synthesis of 1-bromo-2,3,4,5-tetramethoxybenzene (21g)

1,2,3,4-Tetramethoxybenzene **23** (80 mg, 0.40 mmol) wag dissolved in dichloromethane (0.5 ml) and cooled down to −10 °C. Bromine (32 mg, 0.80 mmol) in methylene chloride (0.2 ml) was added dropwise within 1 min while the mixture was stirred. Reaction was continued for 1 h at −10 °C. At the end of the reaction, silica gel (500 mg) was added and solvent evaporated under reduced pressure to obtain a plug. The product was purified by column chromatography using 10% EtOAc in hexanes to obtain 89.2 mg (80%) of **21g**, The compound solidified on refrigeration, mp 36.6–

38.2 °C (lit.[29] 36.0–38.0 °C); ^{1}H NMR ($CDCl_3$): 3.8–3.9 (m, 12H, OCH_3), 6.8 (s, 1H, C_6H_1). HRMS (EI): calcd for $C_{10}H_{13}O_4Br$: 275.9997; found: 275.9945.

6.2.9. Synthesis of 1-bromo-2,4,5-trimethoxybenzene (21h)

Bromine (79 mg, 1 mmol) in glacial acetic acid (1 mL) was added dropwise within 1 min to a vigorously stirred solution of 1,2,4-trimethoxybenzene **24** (168 mg, 1 mmol) in glacial acetic acid (1 mL) at 0 °C. The reaction was completed in 10 min as monitored by TLC. To the resulting mixture was added water (25 mL) and was extracted with methylene chloride (10 mL × 2). The organic layer was washed with satd Na_2CO_3 (20 mL), water (10 mL) and brine (10 mL). Organic extracts were dried over anhyd $MgSO_4$. Silica gel (500 mg) was added and the solvent was evaporated under reduced pressure to afford a silica plug which was loaded on a silica column (2 cm × 10 cm) and eluted with 10% EtOAc in hexanes. Fractions containing the product spot (TLC) were pooled and evaporated under reduced pressure to afford **21h** (156 mg, 63%) as off-white lustrous solid, TLC R_f 0.2 (ethyl acetate/hexanes, 1:5); mp 51.6–52.2 °C (lit.[30] mp 54.0–55.5 °C); ^{1}H NMR ($CDCl_3$): 3.92–4.0 (m, 9H, OCH_3), 6.6 (s, 1H, C_6H_2), 7.1 (s, 1H, C_6H_2).

6.2.10. Synthesis of 1-bromo-2,3,5-trimethoxybenzene (21i)

To a mixture of 5-Bromovanillin **25** (2.3 g, 10 mmol) in 1 N KOH (10.5 mL) was added 3% aqueous hydrogen peroxide solution (22.5 mL). The dark purple solution thus obtained was stirred at 40 °C for 30 min and then cooled to room temperature, acidified with dil HCl and extracted with ether (100 mL × 2). Organic extracts dried over anhyd sodium sulfate, evaporated to obtain a crude product, which was recrystallized from water to obtain **26** (1.95 g, 90%) as a buff colored solid; mp 141.5 (lit.[31] mp 141 °C). ^{1}H NMR ($CDCl_3$): 3.86 (s, 3H, OCH_3), 4.62 (br, 1H, OH, exch.), 5.48 (br, 1H, OH, exch.), 6.41–6.42 (d, 1H, C_6H_2, J = 2.7 Hz), 6.58 (d, 1H, C_6H_2, J = 2.7 Hz).

To a round bottom flask was added **26** (766.5 mg, 3.5 mmol), anhydrous K_2CO_3 (1.93 g, 14 mmol) and acetone (20 mL; freshly distilled over CaH_2), followed by dimethyl sulphate (1.33 mL, 14 mmol) under nitrogen. The reaction was continued at room temperature for 14 h and then quenched by the addition of cold water (20 mL) and stirred for additional 2 h. Acetone was then evaporated under reduced pressure and aqueous layer was extracted with EtOAc (25 mL × 3). Organic extracts washed with water and dried over anhyd sodium sulfate. Silica gel (2 g) was added and the solvent evaporated to afford a plug which was loaded on a silica column (3 cm × 15 cm) and eluted with 5% EtOAc in hexanes. Fractions containing the product spot (TLC) were pooled and evaporated under reduced pressure to afford **21i** (799 mg, 92%) as a colorless oil which solidified on refrigeration, mp 36–38 °C (lit[31] mp 37–38 °C); ^{1}H NMR ($CDCl_3$): 3.76–3.83 (t, 9H, OCH_3), 6.43–6.44 (d, 1H, C_6H_2, J = 2.7 Hz), 6.63 (d, 1H, C_6H_2, J = 2.7 Hz).

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Supplementary data

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