A Facile Synthesis of Diaryl Pyrroles Led to the Discovery of Potent Colchicine Site Antimitotic Agents

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Abstract: Three different series of *cis*-restricted analogues of combretastatin A-4 (CA-4), corresponding to thirty-nine molecules that contained a pyrrole nucleus interposed between the two aryl rings, were prepared by a palladium-mediated coupling approach and evaluated for their antiproliferative activity against six human cancer cell lines. In the two series of 1,2-diaryl pyrrole derivatives, results suggested that the presence of the 3',4',5'-trimethoxyphenyl moiety at the *N*-1 position of the pyrrole ring was more favorable for antiproliferative activity. In the series of 3,4-diarylpyrrole analogues, three compounds (**11i-k**) exhibited maximal antiproliferative activity, showing excellent antiproliferative activity against the CA-4 resistant HT-29 cells. Inhibition of tubulin polymerization of selected 1,2 pyrrole derivatives (**9a**, **9c**, **9o** and **10a**) was similar to that observed with CA-4, while the isomeric 3,4-pyrrole analogues **11i-k** were generally from 1.5- to 2-fold more active than CA-4. Compounds **11j** and **11k** were the only compounds that showed activity as inhibitors of colchicine binding comparable to that CA-4. Compound **11j** had biological properties consistent with its intracellular target being tubulin. This compound was able to block the cell cycle in metaphase and to induce significant apoptosis at a concentration of 25 nM, following the mitochondrial pathway, with low toxicity for normal cells. More importantly, compound **11j** exerted activity *in vivo* superior to that of CA-4P, being able to significantly reduce tumor growth in a syngeneic murine tumor model even at the lower dose tested (5.0 mg/kg).

Keywords. Antimitotic agents, structure-activity relationship, 1*H*-pyrrole, antiproliferative activity, tubulin polymerization.

1. Introduction

Amongst diverse cancer therapeutic targets, microtubules represent one of the most important and the target of several effective chemotherapeutic compounds and antibody-drug conjugates [1-4]. Microtubules are polymeric structures composed of αβ-tubulin heterodimers, and microtubules are one of the components of the cytoskeleton of eukaryotic cells. They are involved in multiple cellular functions, including maintenance of cell shape, organization of intracellular architecture, chromosome segregation during cell division and intracellular organelle transport [5-7]. A large number of natural and synthetic chemically distinct compounds, called "tubulin interactors," bind to soluble tubulin and/or directly to tubulin in microtubules, and these compounds interfere with the dynamic equilibrium of polymerization and depolymerization and, consequently, exhibit significant antimitotic properties and inhibit cell proliferation [8-10].

Combretastatin A-4 (CA-4, **1a**; Figure 1), a *cis*-stilbenoid natural product isolated from the South Africa tree *Combretum caffrum* [11], is one of the most prominent microtubule-destabilizing, lead compounds. CA-4 has very high cytotoxicity against a wide range of human cancer cell lines, including multidrug resistant lines [12], and has antivascular effects [13,14]. The mechanism of action of CA-4 is through inhibition of tubulin polymerization by interacting with β-tubulin at the colchicine site [15]. Due to the low water solubility of CA-4, a water-soluble phosphate pro-drug (CA-4P, Oxi2021, **1b**) was synthesized. CA-4P is also a potent vascular disrupting agent (VDA), showing the ability to cause rapid and irreversible collapse of vasculature within solid tumors

[16-18], and **1b** has shown promising results in clinical trials for the treatment of advanced and solid tumors [19,20].

Because of its activity and simple structure, a wide number of CA-4 analogues have been developed and evaluated with structural modifications of both aryl rings (termed A and B) and of the olefinic bridge [20-23]. Structure-activity relationship (SAR) studies of CA-4 established that the *cis*-orientation of the double bond between the two aryl rings and an A-ring with a 3',4',5'-trimethoxyphenyl substitution pattern were fundamental requirements for potent antitubulin activity. B-ring structural modifications suggest that the 4'-methoxy group is optimal for cytotoxicity, while the 3'-hydroxy moiety is not essential for potent activity [24,25]. The *cis*-olefinic bridge of CA-4 easily isomerizes to the thermodynamically more stable but minimally cytotoxic *trans*-form in solution during storage, administration and/or metabolism [26,27]. Among the strategies to overcome *cis*/*trans* double bond isomerization is replacing the double bond with a vicinal diaryl-substituted five-membered aromatic heterocyclic ring. This work has yielded active, non-isomerizable and chemically stable *cis*-restricted bioisosteric analogues of CA-4 [27-31]. Among the designed analogues, a few research groups have reported compounds containing pyrrole as the surrogate of the stilbene core of CA-4, while other structural elements of CA-4, such as the presence of the 3',4',5'-trimethoxyphenyl A-ring, are maintained [32,33]. In contrast, the vicinal substituent, corresponding to the B-ring of CA-4, was extensively modified.

Banwell et al. reported a series of methyl 4,5-diaryl-1*H*-pyrrole-2-carboxylates that target the colchicine site of tubulin, with compounds **2** and **3a** the more promising members of the series [34]. These two derivatives showed potent antiproliferative activity, with IC_{50} values of 29 and 31 nM against the CA46 Burkitt lymphoma cell line. The potencies of **2** and **3a** for inhibition of tubulin assembly were comparable with

that observed with CA-4. In contrast, the same authors found that the isomeric methyl 3,4-diaryl-1*H*-pyrrole-2-carboxylates **4** and **5** were essentially inactive as anti-mitotic agents [35]. This lack of activity may arise because of conformational impediments imposed by the C2-carbomethoxy group associated with the latter compounds.

While the *N*-*p*-methoxybenzyl (PMB) analogue of compound **3a**, corresponding to derivative **3b**, was inactive both as an antiproliferative agent and as an inhibitor of tubulin assembly, the corresponding 4,5-dimethyl derivative **6** synthesized by Barker and co-workers exhibited remarkable activity, with an IC_{50} value of 0.07 and 0.21 μM against the MDA-MB-231 breast cancer and K562 human leukemia cell lines, respectively [36]. Sun *et al.* [37] reported a series of 1,2-diaryl pyrroles with the 3',4',5'-trimethoxyphenyl ring at the 2-position of the pyrrole ring. Among the tested compounds, the most active derivative was **7a**, which effectively inhibited the growth of SGC-7901, HT-1080 and KB cancer cells, with IC_{50} values of 0.390, 0.070 and 0.045 μM, respectively. Moreover, **7a** showed lower toxicity towards normal L929 cells, with an IC_{50} value of 30 μM. Compound **7a** acted by inhibiting tubulin polymerization with an IC_{50} value of 10 μM, significantly less potent than CA-4 (IC_{50}=0.92 μM). Interestingly, antiproliferative activity decreased 9-50-fold when the 3'-NH_2 was changed to 3'-OH (**7b**) [37]. Semenov and co-workers described 3,4-diarylpyrroles prepared in four synthetic steps, which included the Bearton-Zand reaction, starting from derivatives obtained by the Knoevenagel condensation of a variety of benzaldehydes and aryl nitromethane species. Compound **8**, with a 3',4',5'-trimethoxyphenyl ring A and a 4'-methoxyphenyl ring B was the most active compound of the series, displaying strong antimitotic microtubule destabilizing effects, using a sea urchin embryo assay [38]. This derivative was prepared in a four-step sequence, starting

from the compound obtained by condensation of 3,4,5-trimethoxy benzaldehyde and 4-methoxyphenyl nitromethane [38].

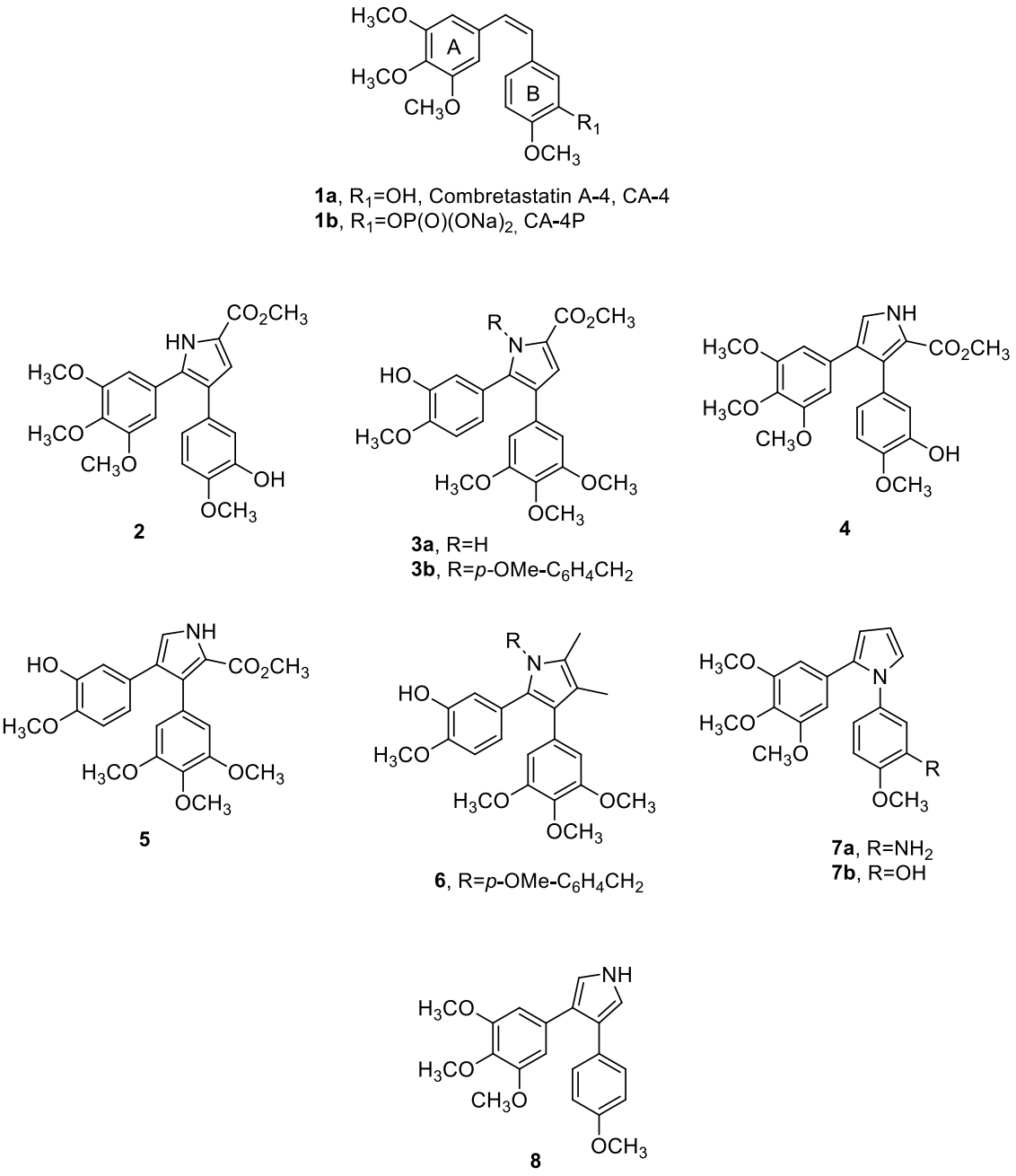


Figure 1. Structures of CA-4 (**1a**), CA-4P (**1b**) and several synthesized vicinal diaryl pyrrole derivatives (**2-8**) reported in the literature.

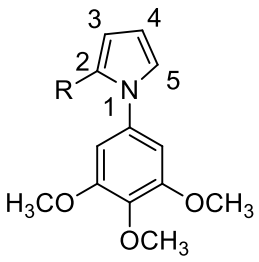
The good antiproliferative and antitubulin activities of **7a** and **8** make 1,2- and 3,4-diaryl pyrrole derivatives worthy of further investigation. We therefore report here the design and synthesis of three different series of compounds characterized by the presence of a pyrrole ring as a suitable mimic for the *cis*-olefin bridge of CA-4. We also focused our attention on examining SARs by varying substituents on the aryl moiety, corresponding to the B-ring of CA-4, which was placed at the C-2, *N*-1 or C-4 position of the pyrrole ring, fixing the vicinal 3',4',5'-trimethoxyphenyl ring, as the A-ring of CA-4, at the *N*-1, C-2 or C-3 position, respectively, of the pyrrole nucleus.

We have synthesized two different regioisomeric series of 1,2-diarylsubstituted pyrrole derivatives with general structures **9** and **10** (Figure 2) obtained by switching the substitution pattern of the two aryl rings. As first aryl ring, we chose the 3',4',5'-trimethoxyphenyl group, corresponding to the A-ring of CA-4, and modifications were mainly focused on variations of electron-releasing substituents on the second phenyl moiety, corresponding to the B-ring of CA-4. Since the presence of a *para*-methoxy or a *para*-ethoxy group on ring B is fundamental for antitubulin activity, an additional substituent (F, Cl or Me) at the *meta*-position of the *para*-methyl/methoxy/ethoxyphenyl ring was introduced. Previous investigators reported that activity can be maintained by replacing the 3'-hydroxy-4'-methoxyphenyl ring B of CA-4 with the lipophilic 2-naphthyl moiety [39,40]. Thus, compounds **9a** and **10a** were synthesized for activity comparison, along with the bioisosteric analogue of 2'-naphthyl derivative **9a**, corresponding to the 2'-benzo[*b*]thienyl compound **9b**. In summary, 1,2-disubstituted pyrroles with general formula **9** incorporated the 3',4',5'-trimethoxyphenyl residue at the *N*-1-position of the pyrrole ring, while varying its C-2 position with a 2'-naphthyl (**9a**), benzo[*b*]thien-2-yl (**9b**) or a phenyl ring with different substituents (compounds **9c**-**p**) [41].

Starting from the 2'-naphthyl (**9a**), 4'-tolyl (**9c**), 3',4'-dimethylphenyl (**9d**), 4'-ethylphenyl (**9f**), 4'-methoxyphenyl (**9h**) and 4'-ethoxyphenyl (**9n**) derivatives, in order to understand whether an interchange of the substitution pattern of rings A and B at the 1- and 2-positions on the pyrrole nucleus can affect biological activity, the corresponding regioisomeric derivatives **10a**, **10c-f** and **10h**, respectively, were synthesized. In addition, the 1'-naphthyl, 3'-methoxyphenyl and 4'-*N*,*N*-dimethylaminophenyl derivatives **10b**, **10g** and **10i**, respectively, were prepared.

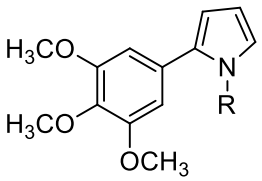
The third isomeric series of 3,4-diarylsubstituted pyrrole derivatives with general structure **11** was characterized by the presence of a common 3',4',5'-trimethoxyphenyl ring at the 3-position of the pyrrole ring, and, as for compounds with general formula **9**, we examined naphth-2-yl (**11a**), its biososteric 2'-benzo[*b*]thienyl (**11b**), and electron-releasing and electron withdrawing substitutions (**11c-o**) on the phenyl at the 4-position of the pyrrole ring. The effect of a *meta*-electron withdrawing group (chlorine or fluorine atom) was also examined in combination with the *para*-methoxy/ethoxy group of compounds **8** and **11i**, respectively.

The 1-(3',4',5'-trimethoxyphenyl)-2-substituted pyrroles **9a-p** and the isomeric 3-(3',4',5'-trimethoxyphenyl)-4-substituted pyrroles **11a-n** were obtained by an efficient and simple convergent method starting from common precursors, 1-(3',4',5'-trimethoxyphenyl)-2-bromopyrrole and 1-tosyl-3-(3',4',5'-trimethoxyphenyl)-4-bromopyrrole derivatives, paving the way for an easy and rapid strategy to develop timely, novel, unsymmetrical pyrrole analogues of CA-4 in only a few synthetic steps and with a wide range of structural diversity.



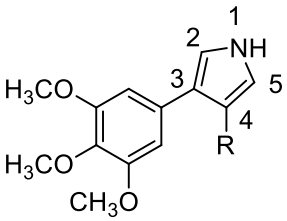
9a-p

9a, R=naphth-2-yl
9b, R=benzo[*b*]thien-2'-yl
9c, R=4'-CH_3-C_6H_4
9d, R=3',4'-$(CH_3)_2$-C_6H_3
9e, R=4'-CH_3, 3'-OCH_3-C_6H_3
9f, R=4'-C_2H_5-C_6H_4
9g, R=4'-*n*-C_3H_7-C_6H_4
9h, R=4'-OCH_3-C_6H_3
9i, R=4'-OCH_3, 3'-F-C_6H_4
9j, R=4'-OCH_3, 3'-Cl-C_6H_4
9k, R=4'-OCH_3, 3'-CH_3-C_6H_3
9l, R=4'-OCH_3, 3',5'-$(CH_3)_2$-C_6H_2
9m, R=4'-SCH_3-C_6H_4
9n, R=4'-OCH_2CH_3-C_6H_4
9o, R=4'-OCH_2CH_3, 3'-Cl-C_6H_3
9p, R=4'-SCH_2CH_3-C_6H_4



10a-i

10a, R=naphth-2-yl
10b, R=naphth-1-yl
10c, R=4'-CH_3-C_6H_4
10d, R=3',4'-$(CH_3)_2$-C_6H_3
10e, R=4'-C_2H_5-C_6H_4
10f, R=4'-OCH_3-C_6H_3
10g, R=3'-OCH_3-C_6H_3
10h, R=4'-OCH_2CH_3-C_6H_4
10i, R=4'-$(CH_3)_2$N-C_6H_4



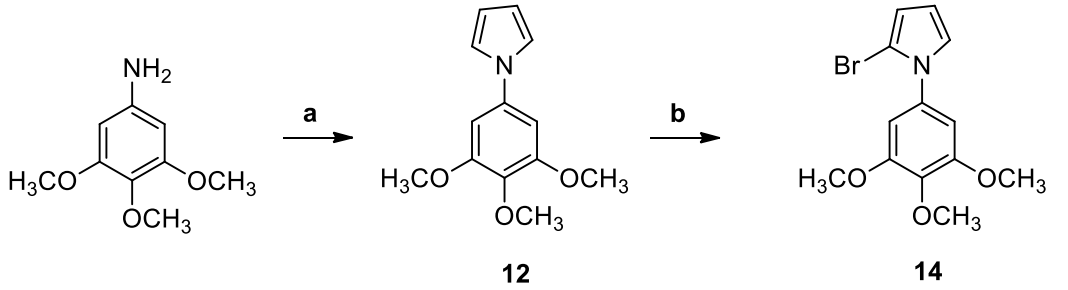
11 a-o

11a, R=naphth-2-yl
11b, R=benzo[*b*]thien-2'-yl
11c, R=4'-CH_3-C_6H_4
11d, R=4'-CF_3-C_6H_4
11e, R=4'-C_2H_5-C_6H_4
11f, R=4'-OCF_3-C_6H_4
11g, R=4'-OCH_3, 3'-F-C_6H_4
11h, R=4'-OCH_3, 3'-Cl-C_6H_4
11i, R=4'-OCH_2CH_3-C_6H_4
11j, R=4'-OCH_2CH_3, 3'-F-C_6H_3
11k, R=4'-OCH_2CH_3, 3'-Cl-C_6H_3
11l, R=4'-SCH_2CH_3-C_6H_4
11m, R=4'-$OCH_2CH_2CH_3$-C_6H_4
11n, R=4'-OCH_2OCH_3-C_6H_4

Figure 2. Structures of 1,2-diaryl pyrroles **9a-p** and **10a-i** and isomeric 3-(3',4',5'-trimethoxyphenyl)-4-substituted-pyrroles **11a-n**.

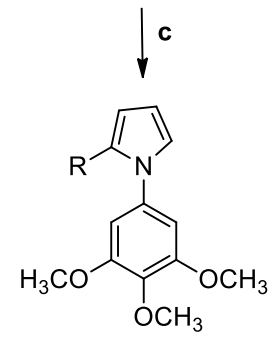
2. Chemistry

1,2-Disubstituted pyrrole derivatives **9a-p** and **10a-i** were prepared by the reaction sequence shown in Scheme 1. 1-(3',4',5'-Trimethoxyphenyl)-1*H*-pyrrole **12** and 1-aryl pyrrole analogues **13a-i** were synthesized in good yields by the classical Clauson-Kass pyrrole synthesis method, which involves the condensation reaction of 3',4',5'-trimethoxyaniline and appropriately substituted anilines with 2,5-dimethoxytetrahydrofuran in refluxing glacial acetic acid. Compounds **12** and **13a-i** in DMF at 0 °C were treated with *N*-bromosuccinimide (NBS) to furnish the corresponding 1-aryl-2-bromo-1*H*-pyrrole analogues **14** and **15a-i**, respectively. Suzuki cross-coupling reaction of these latter brominated products with the appropriate arylboronic acid in the presence of $PdCl_2$(DPPF) and CsF in 1,4-dioxane furnished the desired 1-(3',4',5'-trimethoxyphenyl)-2-aryl-1*H*-pyrroles **9a-p** and the regioisomeric 1-aryl-2-(3',4',5'-trimethoxyphenyl)-1*H*-pyrroles **10a-i**. Compounds **9h** and **9n** were previously synthesized by Sun et al. using a different approach, which involved a three-step procedure starting from the appropriate acetophenone [37].

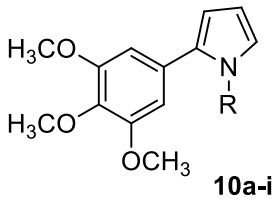


12 **14**

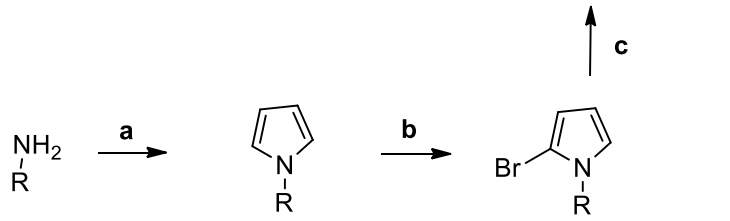
9a, R=naphth-2-yl
9b, R=benzo[*b*]thien-2'-yl
9c, R=4'-CH_3-C_6H_4
9d, R=3',4'-$(CH_3)_2$-C_6H_3
9e, R=4'-CH_3, 3'-OCH_3-C_6H_3
9f, R=4'-C_2H_5-C_6H_4
9g, R=4'-*n*-C_3H_7-C_6H_4
9h, R=4'-OCH_3-C_6H_3
9i, R=4'-OCH_3, 3'-F-C_6H_4
9j, R=4'-OCH_3, 3'-Cl-C_6H_4
9k, R=4'-OCH_3, 3'-CH_3-C_6H_3
9l, R=4'-OCH_3, 3',5'-$(CH_3)_2$-C_6H_2
9m, R=4'-SCH_3-C_6H_4
9n, R=4'-OCH_2CH_3-C_6H_4
9o, R=4'-OCH_2CH_3, 3'-Cl-C_6H_3
9p, R=4'-SCH_2CH_3-C_6H_4



9a-p



10a-i

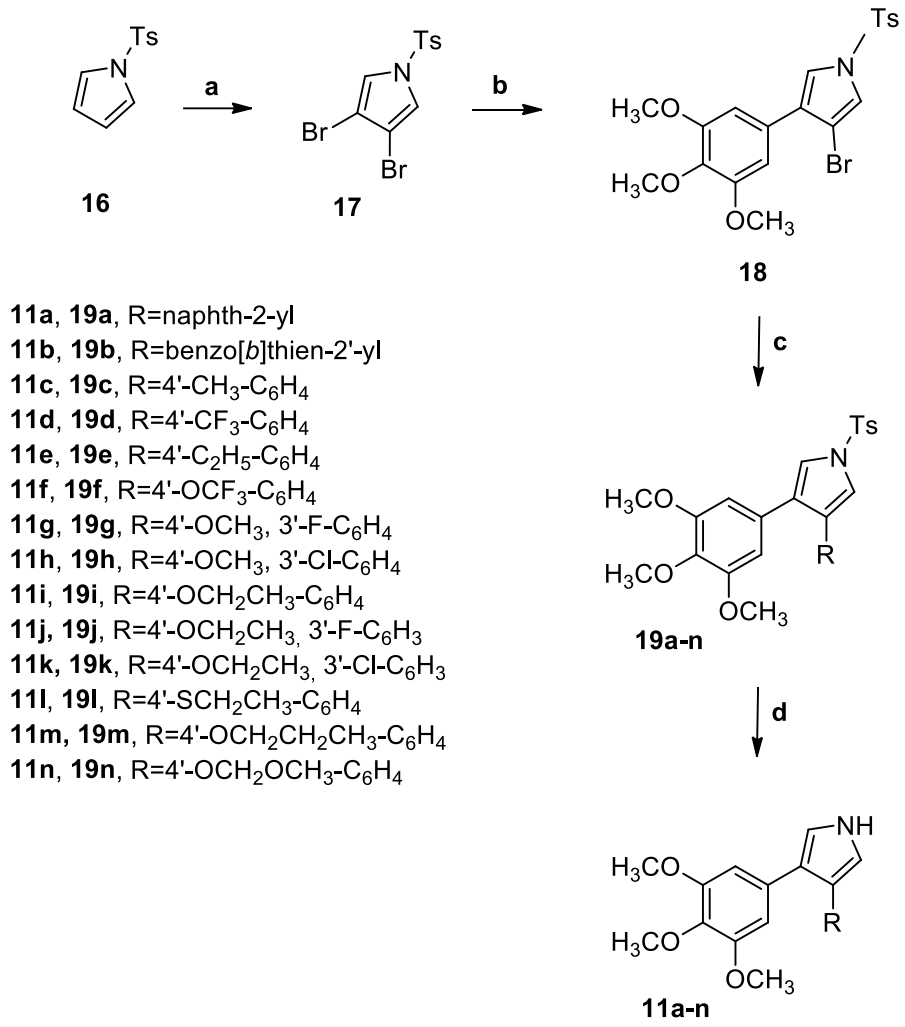


13a-i **15a-i**

10a, **13a**, **15a**, R=naphth-2-yl
10b, **13b**, **15b**, R=naphth-1-yl
10c, **13c**, **15c**, R=4'-CH_3-C_6H_4
10d, **13d**, **15d**, R=3',4'-$(CH_3)_2$-C_6H_3
10e, **13e**, **15e**, R=4'-C_2H_5-C_6H_4
10f, **13f**, **15f**, R=4'-OCH_3-C_6H_3
10g, **13g**, **15g**, R=3'-OCH_3-C_6H_3
10h, **13h**, **15h**, R=4'-OCH_2CH_3-C_6H_4
10i, 13i, **15i**, R=4'-$(CH_3)_2N$-C_6H_4

Scheme 1. Reagents: **a**: 2,5-dimethoxytetrahydrofuran, 1,2-DCE, AcOH, water, reflux; **b**: NBS, DMF, 0 °C; **c**: $PdCl_2$(DPPF), $ArB(OH)_2$, CsF, 1,4-dioxane, 65 °C.

The starting material for the synthesis of 3-(3',4',5'-trimethoxyphenyl)-4-substituted aryl-1*H*-pyrroles **11a-n** was 3,4-dibromo-1-tosyl-1*H*-pyrrole **17**, which can be obtained in moderate yield from commercially available 1-tosyl-1*H*-pyrrole **16** by electrophilic bromination with bromine in glacial acetic acid. Starting from compound **17**, the Suzuki coupling with 3,4,5-trimethoxyphenylboronic acid with $Pd(PPh_3)_4$ and Na_2CO_3 in refluxing THF allowed the insertion of the 3',4',5'-trimethoxyphenyl ring at the 3-position of the pyrrole nucleus, to furnish the key building block 3-(3',4',5'-trimethoxyphenyl)-4-bromo-1-tosylpyrrole **18**. This intermediate was then coupled with the appropriate boronic acid by a second Suzuki reaction with $Pd(PPh_3)_4$ and Na_2CO_3 as catalyst and base, respectively, and using a refluxing mixture of 1,2-dimethoxyethane and water as solvent. This strategy allowed us to insert different aryl moieties at the 4-position of compound **18**, to give the 3-(3',4',5'-trimethoxyphenyl)-4-aryl-1-tosylpyrroles **19a-n**. These latter compounds dissolved in ethanol were subjected to alkaline hydrolysis under basic conditions using 1 M aqueous NaOH to afford the target 3-(3',4',5'-trimethoxyphenyl)-4-substituted-1*H*-pyrrole derivatives **11a-n**.



Scheme 2. Reagents: **a**: Br_2, AcOH, reflux, 90 min; **b**: 3,4,5-trimethoxyphenylboronic acid, $Pd(PPh_3)_4$, aq. Na_2CO_3, THF, reflux; **c**: $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, 1,2-dimethoxyethane/water, reflux; **d**: 1 M aqueous NaOH, EtOH, 50 °C.

3. Biological Results and Discussion

3.1. In vitro antiproliferative activities.

Compounds **9a-p**, **10a-i** and **11a-n** were evaluated for their antiproliferative activities against six different human cancer cell lines and compared with compound **8** [38] and CA-4 (**1a**) (Table 1). The cell lines used were T-cell leukemia (CCRF-CEM), B-cell leukemia (SEM), promyelocytic leukemia (HL-60), non-small cell lung carcinoma (A549), cervix carcinoma (HeLa) and colon adenocarcinoma (HT-29). CA-4 had single-

digit nanomolar activity (IC_{50} 1-5 nM) against four of the six lines, while A549 and HT-29 cells were more resistant, with IC_{50} values of 180 and 3100 nM, respectively. In contrast, while generally not as active as CA-4, compound **8** had good activity against all six lines. As shown in Table 1, the 1- and 2-(3',4',5'-trimethoxyphenyl)-1*H*-pyrroles (**9a-p** and **10a-i**, respectively) had little or no activity against the A549 cells as compared with the other lines. In contrast, most of the 3-(3',4',5'-trimethoxyphenyl)-4-substituted-1*H*-pyrrole analogues **11a-n** had submicromolar activity against the A549 cells, with derivatives **11c** and **11i-k** being the most potent, with IC_{50} values from 28 to 98 nM. These compounds were thus more active than CA-4 and essentially equipotent with **8** in A549 cells. Only a few derivatives in the compound **9** and **10** series (specifically, **9a**, **9h**, **9n**, **9o** and **10a**) showed antiproliferative activity against HT-29 cells superior to that of CA-4, and none approached the potent activity of compound **8**. Again, in contrast, except for **11f** and **11n**, all compounds with general structure **11** showed excellent antiproliferative activity against the CA-4 resistant HT-29 cells, and **11g** and **11i-k** had activity equivalent to that of compound **8**.

Seven of the compound **11** derivatives (**11a-c, 11g** and, especially, **11i-k**), had IC_{50} values < 100 nM against three-five of the six cell lines, as compared with four cell lines with the reference compounds CA-4 (**1a**) and **8**.

In comparing the IC_{50} values, we found that the position of the 3',4',5'-trimethoxyphenyl moiety at either the *N*-1, C-2 or C-3 position of the 1*H*-pyrrole ring had a profound effect on antiproliferative activity, since the potency increased following the order C-3 position>*N*-1 position>C-2 position.

In examining the effect of switching the positions of the two aromatic rings at the *N*-1 and C-2 positions on the 1*H*-pyrrole system (**9a** *vs.* **10a**, **9c** *vs.* **10c**, **9d** *vs.* **10d**, **9f** *vs.* **10e**, **9h** *vs.* **10f** and **9n** *vs.* **10h**), there were relatively few major differences in

cytotoxicity in the six cell lines examined. The major exceptions (> 3-fold difference in IC_{50} values) were in HeLa cells for the **9d**/**10d** pair, in SEM cells for the **9f**/**10e** pair and in HeLa and HT-29 cells for the **9n**/**10h** pair.

The effect of moving the methoxy group in the B ring from the *para* to the *meta* position can be seen by comparing the activities of **10f** and **10g**, respectively. The former compound was more active than the latter in all six cell lines, ranging from a 1.6-fold difference in CCRF-CEM cells to a 7-fold difference in HL-60 and SEM cells.

Comparing the activities of the three isomeric 2'-naphthyl derivatives **9**-**11a**, the latter compound was from 2-to 15-fold more potent than both **9a** and **10a** against each cancer cell line, with the greatest difference being observed in the CCRF-CEM cells. Compound **11a** had the greatest activity (IC_{50}: 32-88 nM) against CCRF-CEM, HL-60 and SEM cells and was less active against HeLa, A549 and HT-29 cells.

The replacement of the 2'-naphthyl by the bioisosteric 2'-benzo[*b*]thienyl ring in the compound **11** series (compounds **11a** and **11b**, respectively) had contrasting effects, with a 1.5- and 3-fold increase in activity against HL-60 and HeLa cells, respectively, but with a 1.5-fold reduction in activity against CCRF-CEM and HT-29 cells, while **11a** and **11b** had similar activity against SEM and A549 cells. An opposite effect was observed in the compound **9** derivatives, with the naphth-2'-yl derivative **9a** being 2-9-fold more active than the benzo[*b*]thien-2'-yl derivative **9b** against all cell lines.

The *para*-tolyl derivative **11c** had IC_{50} values ranging from 17 to 54 nM against four of the six cell lines, but A549 and HT-29 cells were somewhat less sensitive, with IC_{50} values of 0.098 and 0.13 μM, respectively. Replacement of the *para*-methyl of **11c** with the more electron-withdrawing trifluoromethyl moiety (compound **11d**) resulted in a 3-28-fold reduction in antiproliferative activity, most pronounced with the CCRF-CEM cells. Lengthening the alkyl chain from methyl (**11c**) to ethyl (**11e**) at the 4'-position of

the phenyl ring resulted in a loss of activity in all cell lines. A similar effect was also observed in comparing the antiproliferative activities of the three regioisomeric 1-(3',4',5'-trimethoxyphenyl)-2-substituted-1*H*-pyrrole derivatives **9c** (4'-methyl), **9f** (4'-ethyl) and **9g** (4'-*n*-propyl).

Replacement of the methyl group of **11c** with a more electron-releasing methoxy moiety (to furnish **8**) was beneficial for activity against HeLa and HT-29 cells, with a 2- and 4-fold increase of activity, respectively, while a 2-8-fold reduction of potency was observed against CCRF-CEM, HL-60 and SEM cells, but the difference in activity was minimal against A549 cells. A substantial loss in antiproliferative activity was also observed when the electron-releasing methoxy group of compound **8** was replaced with the strong electron-withdrawing and bulkier trifluoromethoxy moiety (**11f**).

Relative to the activity of **8**, the insertion of an additional electron-withdrawing (F or Cl) at the *m*-position of the *p*-methoxyphenyl ring had varying effects on antiproliferative activity. Introduction of a fluorine atom furnished the *p*-OMe, *m*-F derivative **11g**. Relative to **8**, **11g** had similar antiproliferative activity against HeLa cells, 2-, 8- and 7-fold enhanced activity against CCRF-CEM, HL-60 and SEM cells, and 1.5- and 2-fold lower activity against HT29 and A549 cells, respectively. The addition of a *meta*-chlorine atom in **8**, to yield **11h**, produced a 2-10-fold reduction in activity in five cell lines (most pronounced in HeLa and HT29 cells), while **11h** was 1.5-fold more active than **8** in the HL-60 cells.

The *para*-ethoxyphenyl derivative **11i** was about 2-12-fold more potent than its methoxy counterpart **8** in three of the six cancer cell lines and equipotent in A549 cells, but **8** was about 1.5-fold more potent than **11i** against HL-60 and HT-29 cells. Thus, the *p*-ethoxyphenyl ring is a good surrogate for the B-ring of CA-4. The replacement of the ethoxy group with a weak electron releasing thioethyl moiety, resulting in compound

11l, produced a strong drop in potency against all cell lines. The same effect was observed in the series of 1-(3',4',5'-trimethoxyphenyl)-2-aryl 1*H*-pyrrole derivatives for compounds **9n** (4'-ethoxy) and **9p** (4'-thioethyl). The reduction of potency was even more pronounced when the 4'-ethoxy group of **11i** was replaced with the *n*-propoxy homologue **11m** and its methoxymethoxy bioisosteric analogue **11n**.

While the 4'-ethoxy group was favorable for potency, the introduction of an additional electron-withdrawing *meta*-fluorine group in compound **11i**, resulting in compound **11j**, produced a 1.5-3-fold increase in antiproliferative activity against five of the six cell lines, with the two compounds being equipotent against HL-60 cells. In contrast, the compound with an alternative and bulkier EWG, a *m*-Cl group (**11k**), had less antiproliferative activity in CCRF-CEM, SEM and HT-29 cells as compared with both **11i** and **11j**. However, **11k** and **11i** were equipotent against HL-60, HeLa and A549 cells, and **11k** was slightly more active than **11j** in HL60 and slightly less active in HeLa and A549 cells. In the series of 3,4-disubstituted 1*H*-pyrrole derivatives, the methoxy and ethoxy groups at the *para*-position of the phenyl ring (compounds **8** and **11i**, respectively) generally enhanced biological activity, and the introduction of an additional electron-withdrawing fluorine atom at the *meta*-position of the *para*-methoxy/ethoxy phenyl ring (compounds **11g** and **11j**) usually increased antiproliferative activity.

Table 1. *In vitro* inhibitory effects of compounds **8**, **9a-p**, **10a-i**, **11a-n** and CA-4 (**1a**)

Compound	IC_{50} (μM)					
	CCRF-CEM	HL-60	SEM	HeLa	A549	HT-29
9a	1.31±0.3	0.40±0.06	0.15±0.03	0.41±0.03	3.1±1.0	0.65±0.21
9b	2.92±0.7	3.01±0.39	0.29±0.09	0.95±0.19	>10	5.91±2.8
9c	2.43±0.7	0.35±0.06	0.27±0.04	3.22±0.3	9.62±1.4	3.91±0.9
9d	3.20±0.57	4.01±0.7	0.75±0.14	3.28±0.6	>10	7.81±3.7
9e	2.81±0.86	3.62±0.52	2.31±0.4	>10	>10	>10
9f	3.01±0.32	1.13±0.15	0.31±0.06	9.92±1.2	>10	5.44±0.86
9g	4.91±2.2	>10	>10	>10	>10	>10
9h	2.51±0.92	0.47±0.7	0.15±0.03	1.92±0.3	>10	2.12±0.72
9i	1.71±0.44	0.37±0.05	0.2±0.03	6.12±1.5	9.01±1.9	4.12±1.9
9j	2.91±0.90	3.22±0.59	0.55±0.07	9.81±0.80	>10	>10
9k	2.51±0.92	3.93±0.6	1.01±0.12	9.81±1.1	>10	>10
9l	9.11±1.4	>10	7.92±1.5	>10	>10	>10
9m	2.81±0.9	0.81±0.07	0.20±0.03	>10	>10	>10
9n	1.91±0.7	0.36±0.05	0.14±0.02	0.39±0.09	4.52±1.7	0.39±0.15
9o	2.92±1.1	0.39±0.06	0.10±0.02	0.35±0.07	8.40±2.4	0.73±0.27
9p	4.63±1.0	3.72±0.55	1.55±025	3.91±0.76	>10	9.42±2.4
10a	1.41±0.36	0.40±0.05	0.19±0.03	0.43±0.09	5.52±1.3	0.84±0.28
10b	8.61±2.9	>10	7.12±0.7	>10	>10	>10
10c	2.41±1.0	0.63±0.11	0.14±0.04	3.31±0.70	>10	5.22±1.6
10d	3.51±0.99	4.01±0.7	2.01±0.3	>10	>10	>10
10e	4.31±1.6	3.61±0.55	1.33±0.2	>10	>10	8.34±2.2
10f	3.01±0.67	0.43±0.06	0.27±0.05	3.42±0.57	>10	5.52±1.5
10g	5.01±2.1	3.11±0.5	2.01±0.3	>10	>10	>10
10h	3.02±0.67	0.43±0.06	0.27±0.05	3.42±0.57	>10	5.52±1.5
10i	3.91±0.92	2.62±0.6	0.61±0.1	5.61±1.2	>10	7.12±2.3
11a	0.0879±0.031	0.054±0.009	0.032±0.002	0.137±0.019	0.228±0.036	0.255±0.036
11b	0.155±0.004	0.0353±0.004	0.0373±0.002	0.043±0.007	0.228±0.041	0.383±0.045
11c	0.047±0.001	0.0317±0.003	0.0172±0.003	0.054±0.012	0.098±0.008	0.13±0.026
11d	1.34±0.14	0.933±0.039	0.343±0.017	0.445±0.054	0.408±0.026	0.865±0.095

11e	0.226±0.031	0.164±0.015	0.048±0.002	0.297±0.046	0.257±0.036	0.289±0.069
11f	6.26±0.43	5.95±0.33	4.793±0.139	5.23±0.95	4.231±0.789	5.46±0.87
11g	0.053±0.014	0.0336±0.004	0.0053±0.005	0.033±0.009	0.203±0.36	0.048±0.008
11h	0.28±0.021	0.177±0.012	0.0693±0.017	0.297±0.061	0.243±0.031	0.279±0.012
11i	0.0166±0.002	0.149±0.022	0.0028±0.004	0.037±0.008	0.081±0.012	0.042±0.009
11j	0.0075±0.001	0.168±0.017	0.0017±0.005	0.026±0.005	0.028±0.009	0.028±0.006
11k	0.153±0.032	0.144±0.021	0.039±0.003	0.037±0.007	0.079±0.008	0.064±0.005
11l	3.07±0.29	2.66±0.11	0.48±0.026	0.386±0.051	0.336±0.061	0.630±0.087
11m	4.24±0.43	3.62±0.52	0.913±0.086	0.37±0.042	0.683±0.055	0.703±0.092
11n	>10	>10	>10	>10	8.0±1.1	>10
8	0.117±0.019	0.255±0.007	0.0346±0.003	0.028±0.008	0.088±0.011	0.031±0.045
CA-4 (1a)	0.002±0.001	0.001±0.0002	0.005±0.0001	0.004±0.001	0.180±0.050	3.1±0.1

[a]IC_{50}= compound concentration required to inhibit tumor cell proliferation by 50%. Values are the mean ± SE from the dose-response curves of at least three independent experiments carried out in triplicate.

*3.2. Effects of compound **11j** in non-tumor cells.*

To get an initial indication about the cytotoxic potential of these compounds in normal human cells, the most active compound, **11j**, was evaluated *in vitro* against peripheral blood lymphocytes (PBL) from healthy donors. The compound showed an IC_{50} greater than 10 μM, both in quiescent lymphocytes and in lymphocytes in an active phase of proliferation induced by phytohemagglutinin (PHA), a mitogenic stimulus, suggesting low toxicity for normal cells.

3.3. In vitro inhibition of tubulin polymerization and colchicine binding.

The most potent derivatives, **9a**, **9c**, **9i**, **9n-o**, **10a**, **10c, 10h**, **11c** and **11i-k**, and the reference compound CA-4 were evaluated for their inhibition of tubulin assembly and for inhibitory effects on [^{3}H]colchicine binding to tubulin to establish that their antiproliferative activities were associated with an interaction at the colchicine site of tubulin (Table 2).

Compounds **11i-k** exhibited the strongest activity against tubulin assembly, with compounds **11j** and **11k** the most potent of all (IC_{50}, 0.66 and 0.71 μM, respectively), almost twice as active as CA-4 (IC_{50}, 1.2 μM), while derivative **11i** (IC_{50}, 0.81 μM) was 1.5-fold more active than CA-4. Several compounds (**9a**, **9c**, **9o**, **10a** and **11c**) showed activity comparable to that of CA-4, although they were generally less potent than CA-4 as antiproliferative agents. Derivatives **9i**, **10c** and **10h** were 2-fold less potent than CA-4, and **9n** was almost 5-fold less active than CA-4.

In the [^{3}H]colchicine binding assay, compounds **11c** and **11i-k**, when tested at 5 μM, strongly inhibited binding to tubulin, with 83-93% inhibition, and derivatives **11i-k** showed potency comparable to that of CA-4, which in these experiments inhibited colchicine binding by 98%. For the most active compounds **11i-k**, compounds **11i** and **11j**, at a ten-fold reduced concentration (0.5 μM), were almost as potent as CA-4 (80, 83 and 89% inhibition, respectively), while derivative **11k** exhibited moderate activity (68% inhibition).

At 5 μM, all evaluated 1,2-diaryl pyrrole derivatives were less active than CA-4 at inhibiting the binding of [^{3}H]colchicine to tubulin, with 55-74% inhibition.

In summary, these results demonstrated that **11i** and **11j** had activities superior to that of CA-4 as inhibitors of tubulin assembly and similar potency as inhibitors of colchicine binding, even though they were less active as antiproliferative agents than CA-4 in CCRF-CEM, HL-60 and HeLa cells.

Table 2. Inhibition of tubulin polymerization and colchicine binding by compounds **9a**, **9c**, **9i**, **9n-o**, **10a**, **10c, 10h, 11c**, **11i-k** and CA-4 (**1a**)

Compound	Tubulin assembly[a] IC_{50}±S.D (μM)	Colchicine binding[b] % ±S.D	
		5 μM drug	0.5 μM drug
9a	1.6±0.2	74±0.6	n.d
9c	1.6±0.1	60±3	n.d
9i	2.6±0.2	64±3	n.d.
9n	5.6±0.3	63±3	n.d.
9o	1.3±0.06	68±2	n.d.
10a	1.6±0.05	70±2	n.d.
10c	2.4±0.03	55±5	n.d.
10h	2.3± 0.02	57±2	n.d.
11c	1.2±0.07	83±1	n.d.
11i	0.82±0.1	92±1	80±2
11j	0.66±0.004	93±4	83±0.9
11k	0.71±0.07	90±2	68±2
CA-4 (**1a**)	1.2±0.04	98±1	89±2

[a] Inhibition of tubulin polymerization. Tubulin was at 10 μM.
[b] Inhibition of [^{3}H]colchicine binding. Tubulin and colchicine were at 0.5 and 5 μM concentrations, respectively.
n.d.=not determined

3.4. Molecular modeling studies.

The putative binding mode of several of the newly synthesized compounds was explored by molecular docking studies on the colchicine site of tubulin (PDB ID: 4O2B) [42]. The structural rigidity conferred by the central pyrrole ring allowed all the docked molecules to perfectly overlap the co-crystallized colchicine, mimicking its central core (Figure 3 A-D). All the derivatives placed their trimethoxyphenyl ring in proximity of βCys241, potentially forming a key interaction point for tubulin polymerization inhibition, as shown for compound **11c**. The differently substituted

phenyl rings are placed in a hydrophobic sub-pocket formed by the α- and β-tubulin interface, in which potential hydrophobic interactions with the surrounding amino acids, including βMet259, βThr314, αAla180 and αVal181, could further increase the binding affinity of the molecules for the active site.

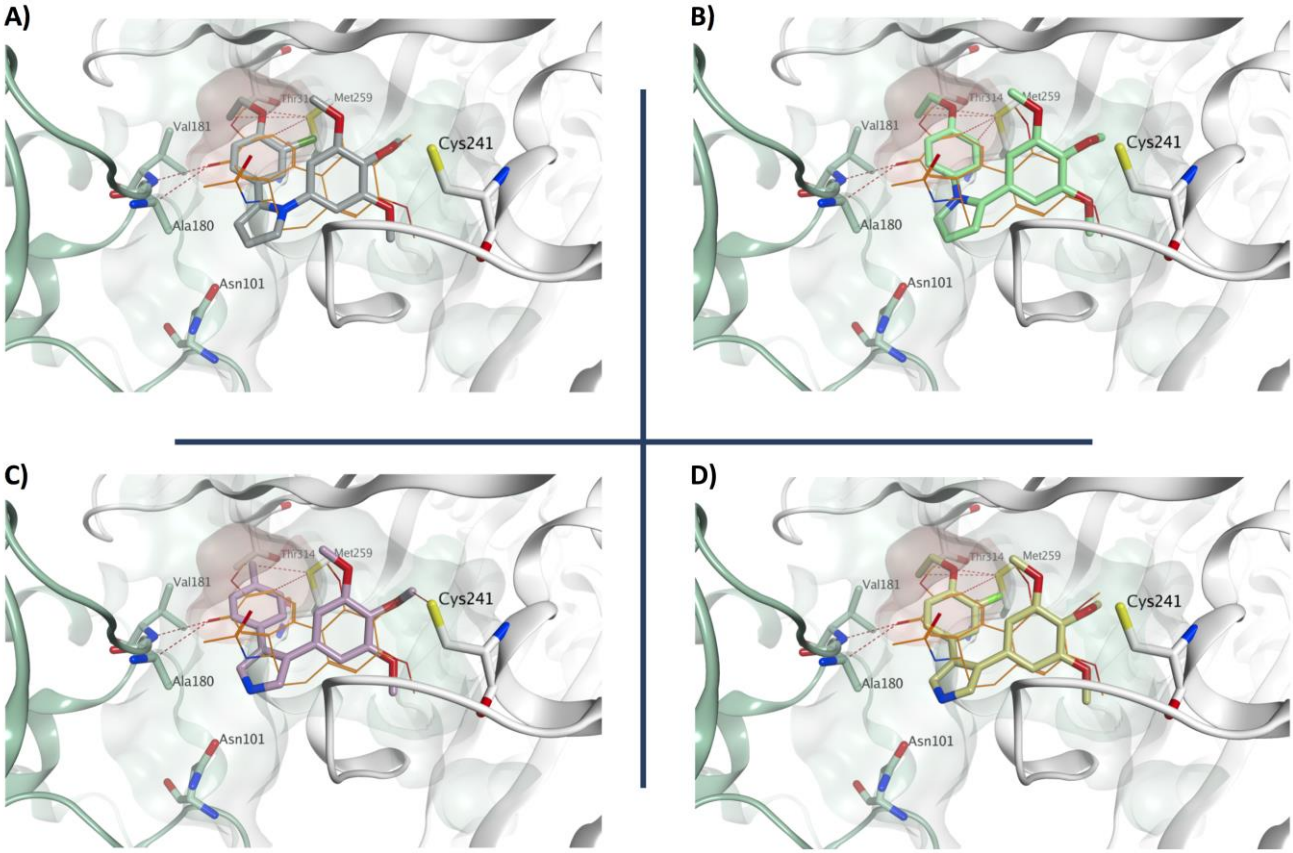


Figure 3. Proposed binding modes for compounds **9o** (**A**), **10h** (**B**), **11c** (**C**) and **11j** (**D**) in the colchicine site. The trimethoxyphenyl ring is in proximity of βCys241, while the differently substituted phenyl rings are placed in a hydrophobic sub-pocket formed near βMet259, βThr314, αAla180 and αVal181. The pyrrole nitrogen of **11c** and **11j** is oriented toward αAsn101. Distance between the nitrogen atom (donor) of 11c and 11j to the oxygen atom (acceptor) of αAsn101 is 3.39 and 3.41 Å respectively and thus in the range of a moderate-weak H-bond. Co-crystallized colchicine is shown in orange. The tubulin α-subunit is shown as a mint green ribbon, while the β-(delete space) subunit is represented as a white ribbon. The hydrophobic sub-pocket is highlighted as a red surface. Hydrogen atoms of compounds are not shown to enhance the clarity of the Figure.

Overall, all the derivatives seemed to occupy the binding site in a favorable conformation, potentially reflecting their similar ability to inhibit tubulin assembly. Interestingly, the pyrrole nitrogen of 3,4-diarylpyrrole derivatives **11c** and **11j** is oriented toward αAsn101, potentially forming an extra hydrogen-bond to further stabilize these molecules in the active site. This extra interaction, not present in the 1,2-diarylpyrrole compounds **9o** and **10h**, could explain the better inhibition of colchicine binding found for the 3,4-diarylpyrrole derivatives. The slightly better inhibition of colchicine binding seen for all the *p*-ethoxy derivatives **11i**, **11j** and **11k** could be a consequence of a better occupation of the hydrophobic sub-pocket by the ethoxy group in comparison with the smaller methyl substituent.

*3.5. Compound **11j** induced mitotic arrest of the cell cycle.*

Typically, tubulin inhibitors cause mitotic arrest in cells. To evaluate the effects of compound **11j** on cell cycle progression, we treated both HeLa and HT-29 cells for 24 h with different compound concentrations and analyzed the treated cells, labeled with propidium iodide (PI), by flow cytometry. Compound **11j**, as shown in Figure 4, induced cell cycle arrest in the G2/M phase in both cell lines in a concentration-dependent manner. The G2/M arrest was accompanied by a reduction of cells in both the G1 and S phases.

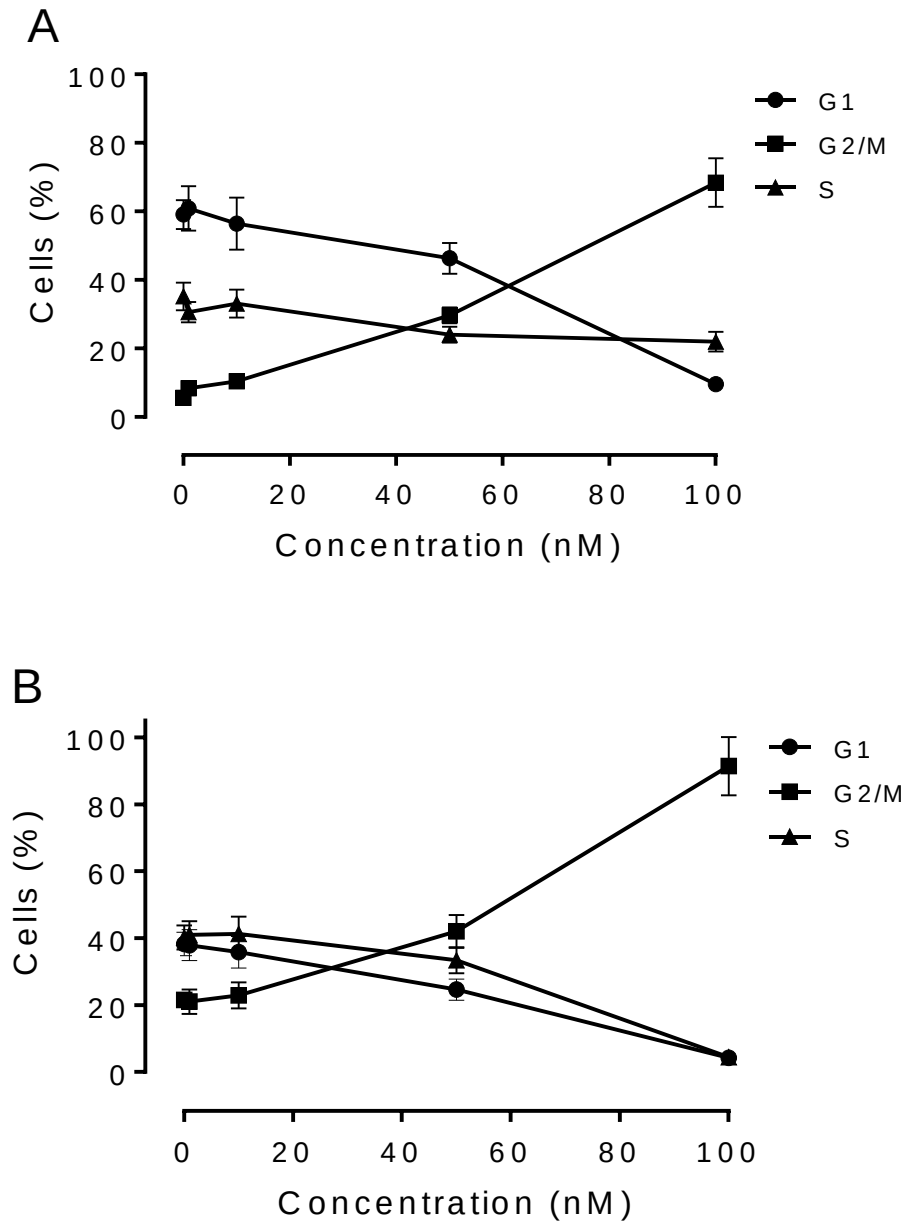


Figure 4. Effect of **11j** on cell cycle distribution in HeLa (Panel A) and HT-29 (Panel B) cells. Cells were treated with **11j** for 24 h, at 1, 10, 50 or 100 nM. Cells were then fixed and labeled with PI and analyzed by flow cytometry as described in the Experimental Section. Data are represented as mean of two separate experiments ± SEM.

*3.6 Compound **11j** induced alteration of cell cycle checkpoint proteins in HeLa cells.*

We studied the effects of **11j** on the expression of selected checkpoint proteins involved in regulation of the cell cycle. Cells that enter mitosis do so through the involvement of

cyclin B1 complexed to cdc2. This complex is activated through the dephosphorylation of phospho-cdc2, which in turn is a cdc25c-dependent process which ultimately leads to the phosphorylation of cyclin B1. The enzyme when phosphorylated triggers cells to enter mitosis [43,44].

As shown in Figure 5, a significant increase of cyclin B1 expression was found after both 24 and 48 h treatments with **11j** at 50 nM. In contrast, the levels of cdc25c were strongly reduced at the same concentration, and, in good agreement, the expression of phosphorylated cdc2 was strongly decreased after both 24 and 48 h treatments. It is important to note that dephosphorylation of this protein is essential to activate the cdc2/cyclin B complex, and this effect is stimulated by cdc25c [43,44]. Thus, our results indicate that cdc2/cyclin B1 complexes cannot be activated following **11j** treatment, and this blocked cells from exiting mitosis and should lead to an apoptotic cell death.

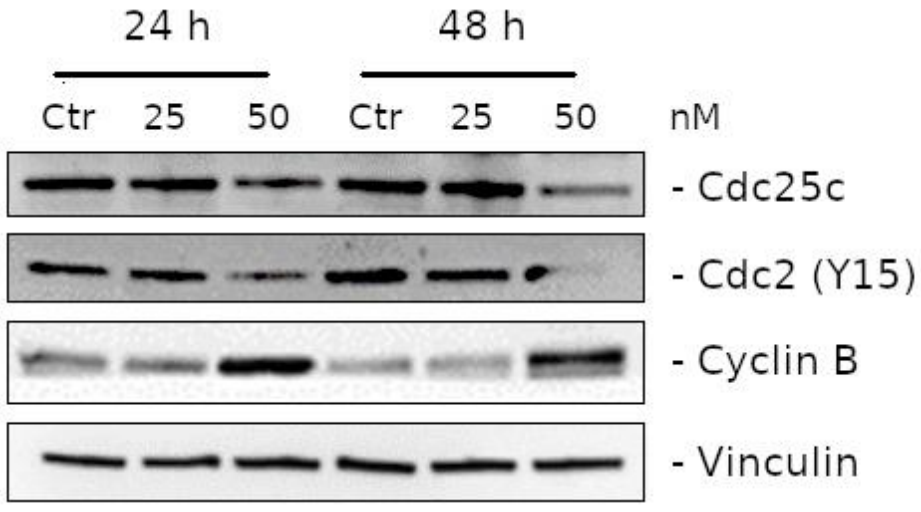


Figure 5. Effect of compound **11j** on cell cycle checkpoint proteins. HeLa cells were treated at the indicated times with 25 or 50 nM **11j**. The cells were harvested and lysed for detection of the expression of the indicated proteins by western blot analysis. An anti-vinculin antibody was used to confirm equal protein loading.

*3.7. Compound **11j** induced apoptosis in HeLa cells.*

To evaluate how HeLa cells died following treatment with **11j**, we double labeled the cells with annexin-V and PI and analyzed them by flow cytometry (Figure 7). This procedure allows the quantitation of four different cell populations: living cells (annexin-V^-/PI^-), early apoptotic cells (annexin-V^+/PI^-), late apoptotic cells (annexin-V^+/PI^+) and necrotic cells (annexin-V^-/PI^+). HeLa cells treated with 50 nM **11j** showed a significant increase of apoptotic cells after 24 h that increased further at 48 h.

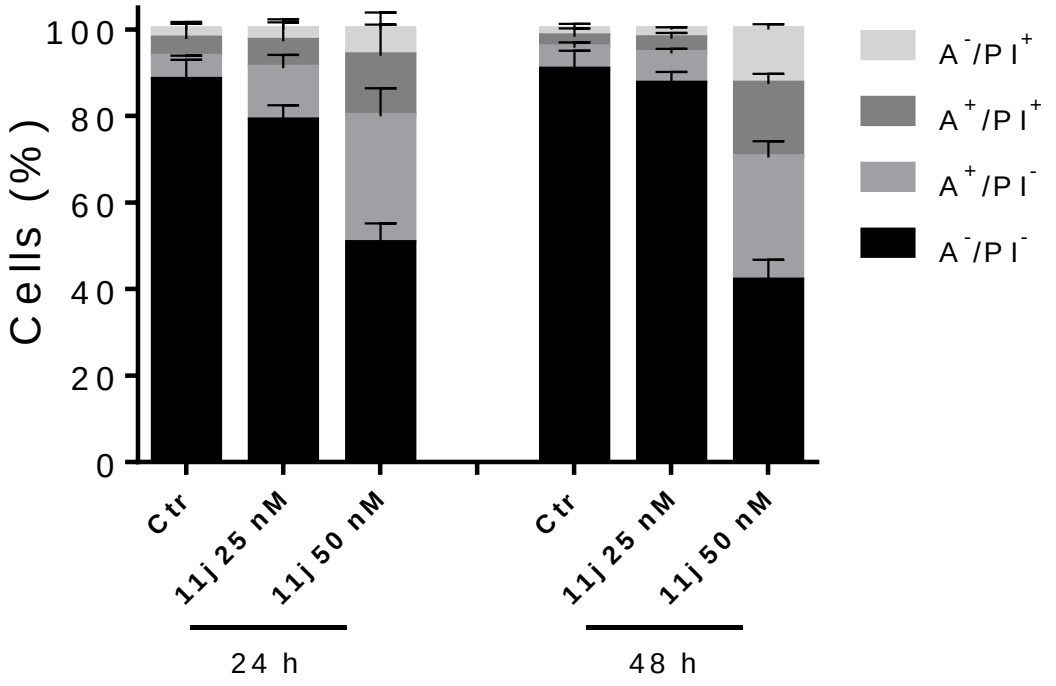


Figure 6. Flow cytometric analysis of HeLa cells with compound **11j** at 25 and 50 nM after incubation for 24 h or 48 h. The cells were harvested and labeled with annexin-V-FITC and PI and analyzed by flow cytometry. Data are represented as mean ± SEM of three independent experiments.

*3.8 Compound **11j** induced apoptosis through the mitochondrial pathway.*

In the initial steps of induction of apoptosis, the mitochondrial transmembrane potential ($\Delta\psi_{mt}$) is reduced and this event is accompanied by release of cytochrome *c* into the cytoplasm [45,46]. As shown in Figure 7A, compound **11j** at both 25 and 50 nM induced, in a time- and concentration-dependent manner, a significant increase in the percentage of cells with depolarized mitochondria, as evidenced by the increase in JC-1 monomers. These results agree with previous studies with other antimitotic agents in several different cell lines [47-50].

One important consequence of mitochondrial depolarization followed by the release of cytochrome *c* into the cytoplasm is an increase in reactive oxygen species (ROS) production [51]. Consequently, we evaluated whether ROS production increased following treatment with compound **11j**. To do this, we used the fluorescent probe 2,7-dichlorodihydrofluorescein diacetate (H_2-DCFDA), which is oxidized to the fluorescent compound dichlorofluorescein (DCF) upon ROS production. Compound **11j** at 50 nM induced the production of ROS in HeLa cells after both 24 and 48 h treatments (Figure 7B), consistent with the reduction of $\Delta\psi_{mt}$ described above.

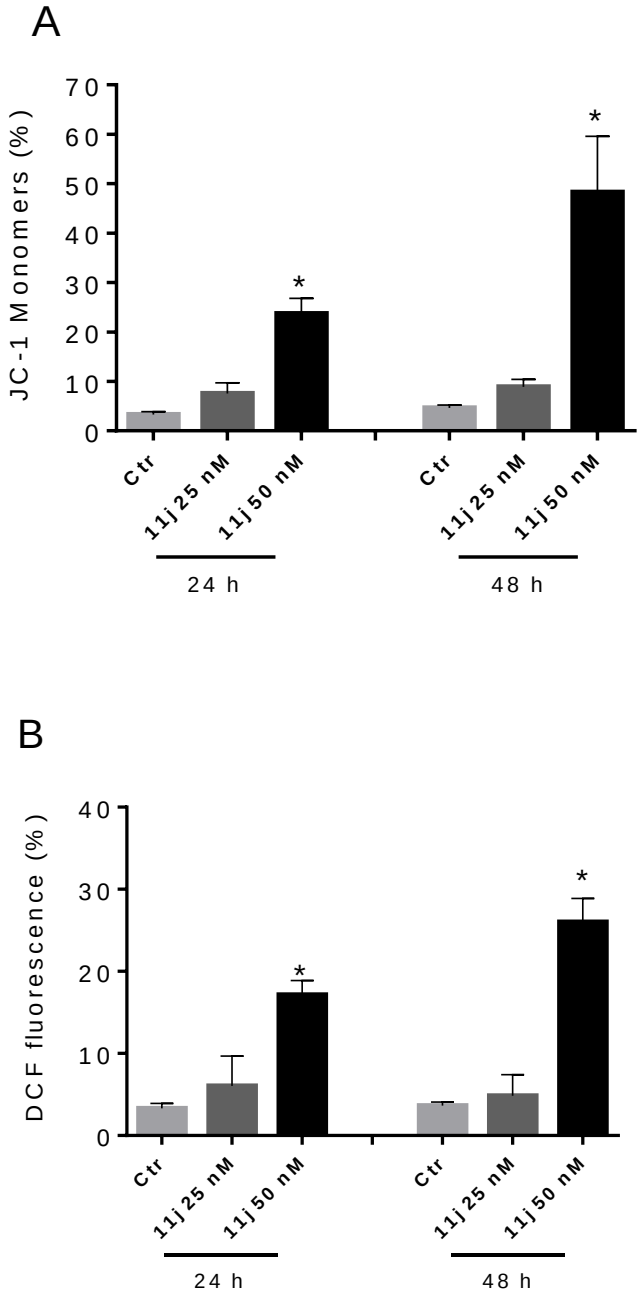


Figure 7. Evaluation of mitochondrial membrane potential ($\Delta\psi_{mt}$) (Panel A) and evaluation of ROS production after treatment of HeLa cells with **11j** (Panel B). Cells were treated with 25 or 50 nM **11j** for 24 or 48 h and then stained with the fluorescent probe JC-1 or H_2-DCFDA. Cells were then analyzed by flow cytometry as described in the Experimental Section. Data are presented as mean ± SEM of three separate experiments. *$p<0.05$ vs respective controls.

*3.9. Compound **11j** induced PARP cleavage and down regulation of Xiap and MCL-1.*

To study further the apoptotic process induced by **11j**, we evaluated by western blot analysis the expression of the cleaved fragment of poly(ADP)ribose polymerase (PARP), a common marker of apoptosis [52]. Hela cells were treated with compound **11j** at 25 or 50 nM for 24 or 48 h, and the appearance of the cleavage fragment of PARP, as shown in Figure 8, occurred as early as 24 h after initiating treatment with 25 nM compound **11j**.

Moreover, we also analyzed the cells for the expression of the anti-apoptotic proteins Bcl-2, Mcl-1 and Xiap. The Bcl-2 protein family controls the mitochondrial pathway of apoptosis [53]. Bcl-2 is located in the outer mitochondrial membrane and protects cells from apoptosis through the control of mitochondrial permeability and release of cytochrome *c*. As shown in Figure 8, treatment of Hela cells with **11j** at 50 nM induced a significant decrease in the expression of this protein. Further, another member of the Bcl-2 family of anti-apoptotic proteins, Mcl-1, was reduced to an even greater extent than Bcl-2 after treatment with 50 nM compound **11j** (Figure 8).

Xiap is a member of the family of IAPs (inhibitors of apoptosis proteins). Due to the direct interactions of this protein with several caspases, such as caspase-3, -7 and -9, Xiap inhibits their enzymatic activity [54]. Our findings (Figure 8) indicate that Xiap expression was reduced after both 24 and 48 h treatments with 50 nM **11j**. Thus, **11j** induced downregulation of Bcl-2, Mcl-1 and Xiap, resulting in impairment of their anti-apoptotic functions.

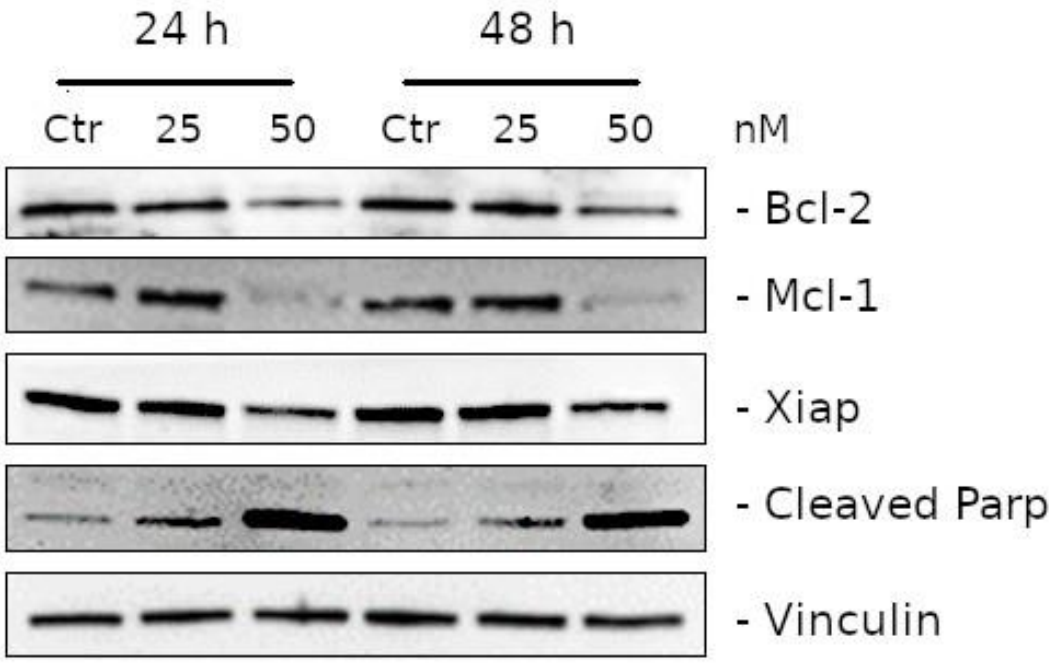


Figure 8. Compound **11j** induced PARP cleavage and down regulation of the anti-apoptotic proteins Bcl-2, Mcl-1 and Xiap. HeLa cells were treated with **11j** for 24 or 48 h at 25 or 50 nM, as indicated. The cells were harvested and lysed for detection of the expression of the indicated proteins by western blot analysis. Anti-vinculin antibody was used to confirm equal protein loading

*3.10. Compound **11j** reduced tumor growth in a murine allograft tumor model.*

The *in vivo* antitumor effect of compound **11j** was evaluated in an allograft tumor model in mice [55]. EO771 murine breast cancer cells were injected orthotopically into the mammary fat pad of C57BL/6 female mice, and, when the tumor had reached a volume of about 100 mm^3, compound **11j** was administered by the intraperitoneal route every other day at two different doses (5.0 and 15 mg/kg). As reference compound, CA-4P (**1b**) was used at 30 mg/kg. As shown in Figure 9, **11j** significantly reduced tumor volume in a dose-dependent manner. Importantly, it was more effective than CA-4P even at the lower dose tested (5.0 mg/kg). The reduced tumor volume was paralleled by a reduction in tumor mass (Figure 9, Panel B). Further, even at the higher dose (15

mg/kg), **11j** did not cause a significant reduction in animal body weight, suggesting low toxicity for this compound (Figure 9, Panel C).

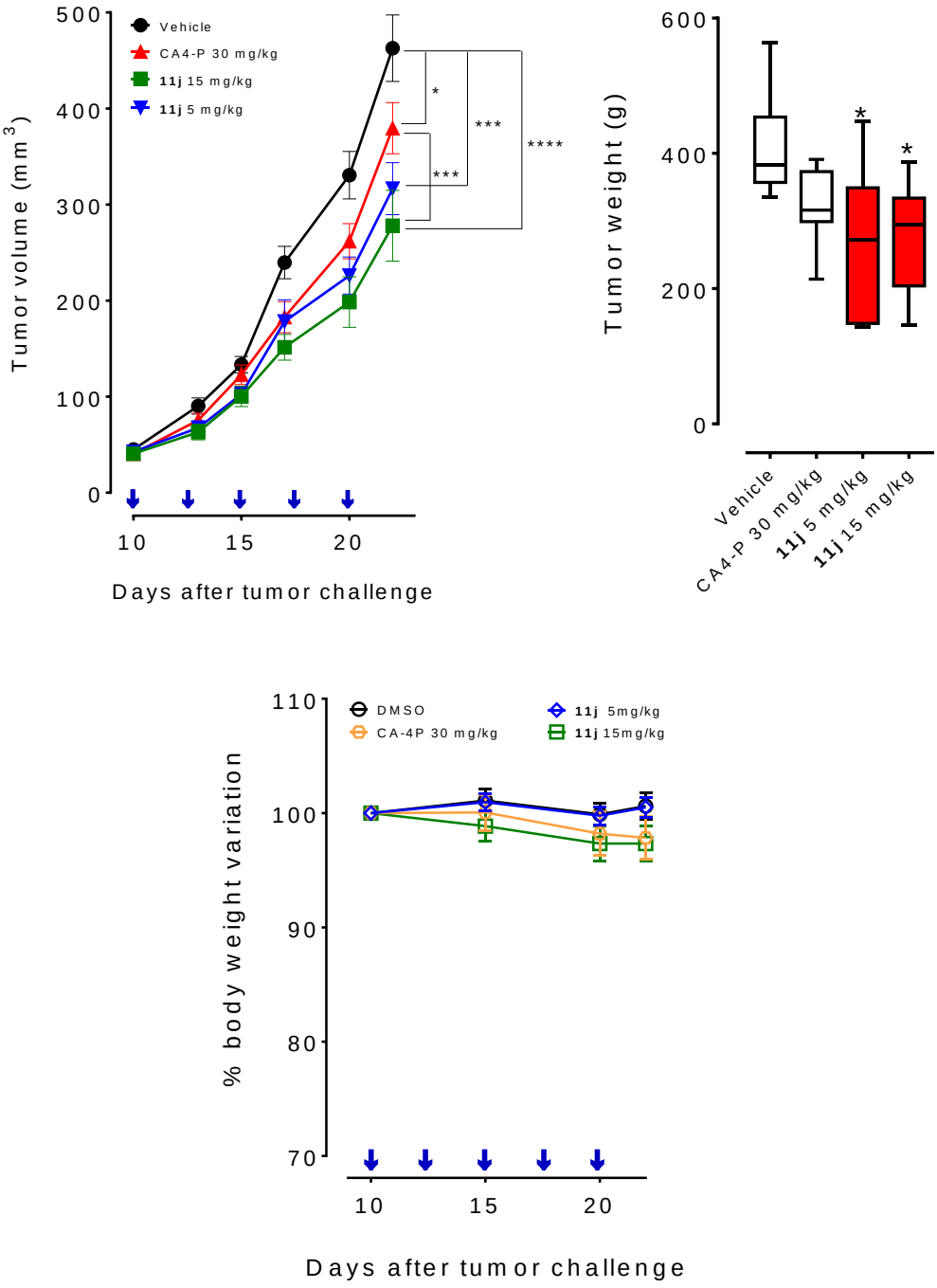


Figure 9. Inhibition of mammary tumor growth by compound **11j** in a syngeneic orthotopic murine model. Female C57BL/6 mice were injected orthotopically with 10^6 EO711 murine breast cancer cells. Tumor-bearing mice were administered the vehicle, as control, 15 or 5.0 mg/kg of **11j** or CA-4P, as reference compound, at 30 mg/kg. The

drugs were given intraperitoneally on the days indicated by the arrows. A. Tumor volume. B. Final tumor weight. C. Body weight variation. as a measure of toxicity. Data are presented as mean ± SEM at each time point for 5 animals per group. Asterisks indicate a significant difference between the treated and the control group. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

4. Conclusions

We described the synthesis and data obtained with almost forty analogues of CA-4. These compounds contained a pyrrole nucleus interposed between the two aryl rings and were prepared by a palladium-mediated coupling approach. For the 1,2-diaryl-1*H*-pyrrole derivatives, with the exception of the two isomeric 2'-naphthyl derivatives **9a** and **10a**, compounds **9c** (4'-tolyl), **9d** (3',4'-xylyl), **9f** (4'-ethylphenyl), **9h** (4'-methoxyphenyl) and **9n** (4'-ethoxyphenyl) were more potent than the isomeric derivatives **10e-f** and **10h**, respectively, suggesting that the presence of the 3',4',5'-trimethoxyphenyl moiety at the *N*-1 position of the pyrrole ring was more favorable for antiproliferative activity.

In the series of 3-(3',4',5'-trimethoxyphenyl)-4-aryl-1*H*-pyrrole analogues **11a-n**, SAR analysis indicated that compounds **11g** (3'-fluoro-4'-methoxyphenyl), **11i** (4'-ethoxyphenyl), **11j** (3'-fluoro-4'-ethoxyphenyl) and **11k** (3'-chloro-4'-ethoxyphenyl) exhibited maximal antiproliferative activity, which correlated with inhibition of tubulin polymerization and of colchicine binding to tubulin. By comparing compounds with small (C_1 or C_2) alkyl or alkoxy groups, the CF_3 and OCF_3 substitution on the phenyl B ring reduced antiproliferative activity against all cancer cell lines. Substituents at the *para*-position of the phenyl B ring showed antiproliferative activity in the order of OEt>Me>OMe>C_2H_5>CF_3>SCH_2CH_3>*n*-OC_3H_7>OCF_3. Comparison of the halogenated compounds (**9i** *vs.* **9j**, **11g** *vs.* **11h**, **11j** *vs.* **11k**), obtained by the introduction of electron-withdrawing substituents such as F or Cl at the *meta*-position of

para-methoxy and *para*-ethoxyphenyl derivatives **9h**, **8** and **11i**, the data indicated that the order of influence of halogen atoms on antiproliferative activity was F>Cl, so that antiproliferative activity decreased with increasing size of the halide substituent. Inhibitor potency on tubulin polymerization of selected 1,2 pyrrole derivatives (**9a**, **9c**, **9o** and **10a**) was similar to that of CA-4, while the isomeric 3,4-pyrrole analogues were generally from 1.5- to 2-fold more active than CA-4 (see compounds **11i-k**), although these derivatives exhibited antiproliferative activity generally lower than that of CA-4 on the CCRF-CEM, HL-60, SEM and HeLa cell lines. Compounds **11j** and **11k** were the only compounds that showed activity as inhibitors of colchicine binding comparable to that CA-4. In this series of compounds, inhibition of tubulin assembly correlated more closely with inhibition of [^{3}H]colchicine binding to tubulin than with antiproliferative activity.

From a pharmacological point of view, compound **11j** was able to block the cell cycle in metaphase and to induce apoptosis at low concentrations (25 nM), following the mitochondrial pathway. More importantly, the compound exerted good activity *in vivo*, being able to significantly reduce tumor growth in a syngeneic murine tumor model with low toxicity.

5. Experimental Procedures

5.1. Chemistry.

5.1.1. Materials and methods.

^{1}H spectra and ^{13}C NMR spectra were recorded on a Bruker AC 200, Bruker Avance III 400 or Varian 400 Mercury Plus spectrometer. Chemical shifts (δ) are given in parts per million (ppm), coupling constants *J* are given in Hertz, and spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). Mass spectra were

obtained using a Waters ZQ 2000 (Waters Instruments, UK) electrospray ionization (ESI) single quadrupole mass spectrometer. Melting points (mp) were determined on a Buchi 510 oil bath and were uncorrected. The purity of tested compounds was determined by combustion elemental analyses conducted by the Microanalytical Laboratory of the Chemistry Department of the University of Ferrara with a Yanagimoto MT-5 CHN recorder elemental analyzer. All tested compounds yielded data consistent with a purity of at least 95% as compared with the theoretical values. Analytical thin layer chromatography (TLC) was carried out using glass plates precoated with silica gel (0.25 mm). Compounds on TLC plates were visualized by exposure to UV light (UV) or aqueous $KMnO_4$. Flash column chromatography was performed using silica gel 60 (230-400 mesh). Petroleum ether used was a mixture of alkanes boiling between 40-60 °C. Unless otherwise indicated, common reagents or materials were obtained from commercial sources and used without further purification.

*5.1.2. General procedure A for the synthesis of compounds **12** and **13a-i**.*

A mixture of the appropriate aniline (10 mmol) and 2,5-dimethoxytetrahydrofuran (1.32 g, 10 mmol) in a mixture of 1,2-dichloroethane (20 mL), acetic acid (0.7 mL) and water (10 mL) was heated under reflux for 24 h. After removal of the solvent, the crude residue was dissolved in a mixture of water (15 mL) and dichloromethane (20 mL). The organic extract was washed with a 10% sodium carbonate solution (10 mL) and brine (10 mL) and dried over Na_2SO_4 and the solvent removed *in vacuo*. The resulting residue was purified by silica gel column chromatography to afford the desired product **12** or **13a-i**.

*5.1.2.1. 1-(3,4,5-Trimethoxyphenyl)-1H-pyrrole (**12**).* Following general procedure A, the crude residue obtained from 3,4,5-trimethoxyaniline was purified by flash-

chromatography, using EtOAc:petroleum ether 2:8 (v/v) as eluent, to furnish compound **12** as a white solid, yield >95%, mp 99-101 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.86 (s, 3H), 3.90 (s, 6H), 6.33-6.34 (m, 2H), 6.60 (s, 2H), 7.01-7.02 (m, 2H). MS (ESI): $[M+1]^+$=234.11.

*5.1.2.2. 1-(Naphthalen-2-yl)-1H-pyrrole (**13a**).* Following general procedure A, the crude residue obtained from naphthalen-2-amine was purified by flash-chromatography, using EtOAc:petroleum ether 0.5:9.5 (v/v) as eluent, to furnish compound **13a** as a white solid. Yield 75%, mp 110-111 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.29 (t, J=2.0 Hz, 2H), 7.29 (t, J=2.0 Hz, 2H), 7.52-7.62 (m, 2H), 7.64 (s, 1H), 7.84 (s, 1H), 7.89-7.95 (m, 3H). MS (ESI): $[M+1]^+$=194.11.

*5.1.2.3. 1-(Naphthalen-1-yl)-1H-pyrrole (**13b**).* Following general procedure A, the crude residue obtained from naphthalen-1-amine was purified by flash-chromatography, using EtOAc:petroleum ether 1:9 (v/v) as eluent, to furnish compound **13b** as a yellow oil. Yield 93%. ^{1}H-NMR ($CDCl_3$) δ: 6.42 (t, J=2.0 Hz, 2H), 7.00 (t, J=2.0 Hz, 2H), 7.48-7.51 (m, 4H), 7.54-7.73 (m, 1H), 7.84-7.94 (m, 2H). MS (ESI): $[M+1]^+$=194.45.

*5.1.2.4. 1-(p-Tolyl)-1H-pyrrole (**13c**).* Following general procedure A, the crude residue obtained from *p*-toluidine was purified by flash-chromatography, using EtOAc:petroleum ether 0.5:9.5 (v/v) as eluent, to furnish compound **13c** as a white solid. Yield 99%, mp 82-84 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.39 (s, 3H), 6.35 (s, 2H), 7.1 (s, 2H), 7.23 (d, J=8.0 Hz, 2H), 7.30 (d, J=8.2 Hz, 2H). MS (ESI): $[M+1]^+$=157.98.

*5.1.2.5. 1-(3,4-Dimethylphenyl)-1H-pyrrole (**13d**).* Following general procedure A, the crude residue obtained from 3,4-dimethylaniline was purified by flash-chromatography, using EtOAc:petroleum ether 0.25:9.75 (v/v) as eluent, to furnish compound **13d** as a

yellow solid. Yield > 95%, mp 53-55 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.29 (s, 3H), 2.32 (s, 3H), 6.31 (m, 2H), 6.92 (t, J=3.0 Hz, 1H), 7.03-7.19 (m, 4H). $[M+1]^+$=172.98.

*5.1.2.6. 1-(4-Ethylphenyl)-1H-pyrrole (**13e**).* Following general procedure A, the crude residue obtained from 4-ethylaniline was purified by flash-chromatography, using EtOAc:petroleum ether 1:9 (v/v) as eluent, to furnish compound **13e** as a yellow solid. Yield 88%, mp 71-74 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.26 (t, J=7.6 Hz, 3H), 2.68 (q, J=7.4 Hz, 2H), 6.32–6.34 (m, 2H), 7.05-7.06 (m, 2H), 7.20 (d, J=8.0 H, 2H), 7.28 (d, J=8.0 Hz, 2H). $[M+1]^+$=172.98.

*5.1.2.7. 1-(4-Methoxyphenyl)-1H-pyrrole (**13f**).* Following general procedure A, the crude residue obtained from 4-anisidine was purified by flash-chromatography, using EtOAc:petroleum ether 1:9 (v/v) as eluent, to furnish compound **13f** as a white solid. Yield 75%, mp 110-111 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.84 (s, 3H), 6.24 (t, J=2.4 Hz, 2H), 6.93-7.01 (m, 4H), 7.20-7.32 (m, 2H). MS (ESI): $[M+1]^+$=174.08.

*5.1.2.8. 1-(3-Methoxyphenyl)-1H-pyrrole (**13g**).* Following general procedure A, the crude residue obtained from 3-anisidine was purified by flash-chromatography, using EtOAc:petroleum ether 1:9 (v/v) as eluent, to furnish compound **13g** as a yellow oil. Yield 68%. ^{1}H-NMR ($CDCl_3$) δ: 3.85 (s, 3H), 6.34 (t, J=2.2 Hz, 2H), 6.76-6.81 (m, 1H), 6.92-7.01 (m, 1H) 7.08 (t, J=2.2 Hz, 2H), 7.28-7.36 (m, 2H). $[M+1]^+$=174.04.

*5.1.2.9. 1-(4-Ethoxyphenyl)-1H-pyrrole (**13h**).* Following general procedure A, the crude residue obtained from 4-ethoxyaniline was purified by flash-chromatography, using EtOAc:petroleum ether 2:8 (v/v) as eluent, to furnish compound **13h** as a white solid. Yield > 95%, mp 82-84 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.41 (t, J=7.2 Hz, 3H), 4.10 (q,

J=6.8 Hz, 2H), 6.37 (m, 2H), 6.93 (d, H=7.2 Hz, 2H), 7.0 (m, 2H), 7.28 (d, H=7.4 Hz, 2H). MS (ESI): $[M+1]^+$=174.08.

*5.1.2.10. N,N-Dimethyl-4-(1H-pyrrol-1-yl)aniline (**13i**).* Following general procedure A, the crude residue obtained from 4-*N*,*N*-dimethylaniline was purified by flash-chromatography, using EtOAc:petroleum ether 1:9 (v/v) as eluent, to furnish compound **13i** as a white solid. Yield 67%, mp 71-74 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.97 (s, 6H), 6.0 (t, J=2.2 Hz, 2H), 6.75-6.79 (m, 2H), 6.98 (t, J=2.0 Hz, 2H), 7.25-7.29 (m, 2H). $[M+1]^+$=187.08.

*5.1.3. General procedure B for the preparation of compounds **14** and **15a-i**.*

To a stirred solution of the appropriate 1-arylpyrrole **12** or **13a-i** (5 mmol) in DMF (20 mL) in an ice water bath was added NBS (0.89 g, 5 mmol, 1 equiv.) in small portions. The reaction mixture was stirred at 0 °C for 6 h, poured into a 30 mL ice-water mixture and extracted with dichloromethane (3 x 15 mL). The combined organic layers were washed with water (3 x 10 mL) and brine (20 mL) and dried over Na_2SO_4. After concentration *in vacuo*, the crude residue was purified by column chromatography on silica gel by elution with the appropriate mobile phase, which was a variable mixture of EtOAc and petroleum ether, to afford the title compound **14** or **15a-i**.

*5.1.3.1. 2-Bromo-1-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**14**).* Following general procedure B, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 1:9 (v/v) for elution, furnished **14** as a white solid. Yield 62%, mp 113-114 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.87 (s, 6H), 3.89 (s, 3H), 6.27-6.31 (m, 2H), 6.57 (s, 2H), 6.89-6.90 (m, 1H). MS (ESI): $[M+1]^+$=313.01.

*5.1.3.2. 2-Bromo-1-(naphthalen-2-yl)-1H-pyrrole (**15a**).* Following general procedure B, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 0.2:9.8 (v/v) for elution, furnished **15a** as a white solid. Yield 72%, mp 81-83 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.39 (d, J=2.2 Hz, 1H), 7.09 (d, J=2.0 Hz, 1H), 7.26-7.52 (m, 4H), 7.74 (s, 1H), 7.82-7.89 (m, 3H). MS (ESI): $[M+1]^+$=273.01.

*5.1.3.3. 2-Bromo-1-(naphthalen-1-yl)-1H-pyrrole (**15b**).* Following general procedure B, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 0.2:9.8 (v/v) for elution, furnished **15b** as a white solid. Yield 57%, mp 49-52 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.42 (t, J=2.0 Hz, 2H), 7.00 (m, 1H), 7.48-7.51 (m, 4H), 7.54-7.73 (m, 1H), 7.84-7.94 (m, 2H). MS (ESI): $[M+1]^+$=273.05.

*5.1.3.4. 2-Bromo-1-(p-tolyl)-1H-pyrrole (**15c**).* Following general procedure B, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 0.15:9.85 (v/v) for elution, furnished **15c** as a yellow oil. Yield 53%. ^{1}H-NMR ($CDCl_3$) δ: 2.37 (s, 3H), 6.22 (m, 2H), 6.85 (m, 1H), 6.97 (d, J=8.8 Hz, 2H), 7.27 (d, J=8.8 Hz, 2H). MS (ESI): $[M+1]^+$=236.98.

*5.1.3.5. 2-Bromo-1-(3,4-dimethylphenyl)-1H-pyrrole (**15d**).* Following general procedure B, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 0.25:9.75 (v/v) for elution, furnished **15d** as a colorless oil. Yield: 67%. ^{1}H-NMR ($CDCl_3$) δ: 2.26 (s, 3H), 2.32 (s, 3H), 6.31 (d, J=8.0 Hz, 2H), 6.92 (t, J=3.0 Hz, 1H), 7.03-7.19 (m, 3H). $[M+1]^+$=250.08.

*5.1.3.6. 2-Bromo-1-(4-ethylphenyl)-1H-pyrrole (**15e**).* Following general procedure B, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 0.2:9.8 (v/v) for elution, furnished **15e** as a colorless oil. Yield 57%. ^{1}H-NMR ($CDCl_3$)

δ: 1.28 (t, J=7.6 Hz, 3H), 2.70 (q, J=7.4 Hz, 2H), 6.26–6.30 (m, 2H), 6.86-6.88 (m, 1H), 7.24-2.29 (m, 4H). $[M+1]^+$=251.98.

*5.1.3.7. 2-Bromo-1-(4-methoxyphenyl)-1H-pyrrole (**15f**).* Following general procedure **B**, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 0.2:9.8 (v/v) for elution, furnished **15f** as a white solid. Yield 75%, mp 110-111 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.85 (s, 3H), 6.27 (m, 2H), 6.85 (bs, 1H), 6.97 (d, J=12 Hz, 2H), 7.27 (d, J=8.8 Hz, 2H). MS (ESI): $[M+1]^+$=253.01.

*5.1.3.8. 2-Bromo-1-(3-methoxyphenyl)-1H-pyrrole (**15g**).* Following general procedure B, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 0.25:9.75 (v/v) for elution, furnished **15g** as a colorless oil. Yield 64%. ^{1}H-NMR ($CDCl_3$) δ: 3.84 (s, 3H), 6.27-6.32 (m, 2H), 6.91-6.98 (m, 4H), 7.35 (t, J=7.8 Hz, 1H). $[M+1]^+$=253.78.

*5.1.3.9. 2-Bromo-1-(4-ethoxyphenyl)-1H-pyrrole (**15h**).* Following general procedure B, the crude residue purified by flash chromatography, using EtOAc: petroleum ether 0.25:9.75 (v/v) for elution, furnished **15h** as a white solid. Yield 58%, mp 74-76 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.41 (t, J=7.2 Hz, 3H), 4.10 (q, J=6.8 Hz, 2H), 6.28 (m, 2H), 6.85 (bs, 1H), 6.95 (d, H=7.2 Hz, 2H), 7.24 (d, H=7.2 Hz, 2H). MS (ESI): $[M+1]^+$=265.98.

*5.1.3.10. 4-(2-Bromo-1H-pyrrol-1-yl)-N,N-dimethylaniline (**15i**).* Following general procedure B, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 0.25:9.75 (v/v) for elution, furnished **15i** as a white solid. Yield 62%, mp 72-74 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.00 (s, 6H), 6.21-6.28 (m, 2H), 6.70-6.75 (m, 2H), 6.82 (q, J=2.0 Hz, 1H), 7.17-7.22 (m, 2H). $[M+1]^+$=265.08.

*5.1.4. General procedure C for the synthesis of final compounds **9a-p** and **10a-i**.*

A stirred suspension of 1-(3',4'.5'-trimethoxyphenyl)-2-bromopyrrole **14** or 1-aryl-2-bromopyrrole **15a-i** (1 mmol) and the appropriate arylboronic acid (1.5 mmol, 1.5 equiv.) in dioxane (10 mL containing 4 drops of water) was flushed with Ar, $PdCl_2$(DPPF) (82 mg, 0.1 mmol) and CsF (380 mg, 2.5 mmol) were added, and the mixture was heated under nitrogen at 45 °C for 30 min, then at 65 °C for 6 h. The reaction mixture was cooled to ambient temperature, diluted with CH_2Cl_2 (20 mL), filtered on a pad of celite and evaporated by rotary evaporation. The residue was dissolved with CH_2Cl_2 (20 mL), and the resultant solution was washed sequentially with water (10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated, and the residue was purified by flash chromatography on silica gel.

*5.1.4.1. 2-(Naphthalen-2-yl)-1-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**9a**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 2:8 (v/v) for elution, furnished **9a** as a yellow solid. Yield 72%, mp 116-118 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.61 (s, 6H), 3.85 (s, 3H), 6.41-6.42 (m, 1H), 6.43 (s, 2H), 6.56-6.57 (m, 1H), 7.00-7.01 (m, 1H), 7.24 (dd, J=8.4 and 1.6 Hz, 1H), 7.28-7.30 (m, 1H), 7.41-7.43 (m, 1H), 7.65 (d, J=8.4 Hz, 1H), 7.68-7.70 (m, 2H), 7.78-7.80 (m, 1H). ^{13}C-NMR ($CDCl_3$) δ: 56.09 (2C), 61.03, 103.44 (2C), 109.38, 110.92, 119.83, 124.41, 125.68, 126.09, 126.47, 126.63, 127.37, 127.54, 127.89, 130.42, 131.90, 133.28, 136.30, 136.73, 153.24 (2C). MS (ESI): $[M+1]^+$=360.16. Anal. calcd for $C_{23}H_{21}NO_3$. C, 76.86; H, 5.89; N, 3.90; found: C, 76.64; H, 5.78; N, 3.82.

*5.1.4.2. 2-(Benzo[b]thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**9b**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 2:8 (v/v) for elution, furnished **9b** as a yellow solid.

Yield: >95%, mp 101-103 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.75 (s, 6H), 3.90 (s, 3H), 6.35 (dd, J=3.6 and 2.4 Hz, 1H), 6.58 (s, 2H), 6.62 (dd, J=4.0 and 1.6 Hz, 1H), 6.68 (s, 1H), 6.92 (dd, J=4.0 and 1.6 Hz, 1H), 7.22-7.27 (m, 2H), 7.58 (d, J=8.4 Hz 1H), 7.65 (d, J=8.4 Hz 1H). ^{13}C-NMR ($CDCl_3$) δ: 56.25 (2C), 61.09, 104.46 (2C), 109.34, 110.17, 111.75, 120.04, 121.78, 123.18, 123.85, 124.24, 125.44, 127.63, 134.91, 135.61, 139.04, 140.06, 153.32 (2C). MS (ESI): $[M+1]^+$=365.96. Anal. calcd for $C_{21}H_{19}NSO_3$. C, 76.86; H, 5.89; N, 3.90; found: C, 76.64; H, 5.78; N, 3.82.

*5.1.4.3. 2-(p-Tolyl)-1-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**9c**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 2:8 (v/v) for elution, furnished **9c** as a white solid. Yield 61%, mp 88-95 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.30 (s, 3H), 3.67 (s, 6H), 3.86 (s, 3H), 6.34-6.40 (m, 4H), 6.93 (bs, 1H), 7.04-7.05 (m, 4H). ^{13}C-NMR ($CDCl_3$) δ: 21.16, 56.12 (2C), 61.08, 103.43 (2C), 109.06, 110.01, 123.78, 128.31 (2C), 128.79 (2C), 130.14, 134.08, 136.12, 136.37 (2C), 153.16 (2C). MS (ESI): $[M+1]^+$=323.15. Anal. calcd for $C_{20}H_{21}NO_3$. C, 74.28; H, 6.55; N, 4.33; found: C, 73.99; H, 6.37; N, 4.02.

*5.1.4.4. 2-(3,4-Dimethylphenyl)-1-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**9d**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 3:7 (v/v) for elution, furnished **9d** as a yellow oil. Yield 61%. ^{1}H-NMR ($CDCl_3$) δ: 2.17 (s, 3H), 2.20 (s, 3H), 3.67 (s, 6H), 3.85 (s, 3H), 6.33-6.34 (m, 1H), 6.37-6.38 (m, 3H), 6.91 (dd, J=8.4 and 1.6 Hz, 1H), 6.93-6.94 (m, 1H), 6.96 (d, J=8.4 Hz, 1H), 7.02 (d, J=1.6 Hz, 1H). ^{13}C-NMR ($CDCl_3$) δ: 19.38, 19.74, 56.09 (2C), 61.02, 103.48 (2C), 108.92, 109.81, 123.59, 125.80, 129.22, 129.55, 130.46, 134.12, 134.73, 136.07, 136.37, 136.55, 153.05 (2C). MS (ESI): $[M+1]^+$=337.91. Anal. calcd for $C_{21}H_{23}NO_3$. C, 74.75; H, 6.87; N, 4.15; found: C, 74.54; H, 6.37; N, 4.02.

*5.1.4.5. 2-(3-Methoxy-4-methylphenyl)-1-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**9e**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 2:8 (v/v) for elution, furnished **9e** as a yellow oil. Yield 70%. ^{1}H-NMR ($CDCl_3$) δ: 2.17 (s, 3H), 3.59 (s, 3H), 3.68 (s, 6H), 3.86 (s, 3H), 6.32-6.34 (m, 1H), 6.36 (s, 2H), 6.37-6.40 (m, 1H), 6.58 (d, J=1.6 Hz, 1H), 6.74 (dd, J=8.4 and 1.6 Hz, 1H), 6.90-6.93 (m 1H), 7.01 (d, J=8.4 Hz, 1H). ^{13}C-NMR ($CDCl_3$) δ: 15.84, 55.05, 56.14 (2C), 61.00, 103.60 (2C), 108.96, 109.79, 110.24, 120.30, 123.75, 124.77, 130.20, 131.54, 134.17, 136.39, 136.66, 153.14, 157.11 (2C). MS (ESI): $[M+1]^+$=354.10. Anal. calcd for $C_{21}H_{23}NO_4$. C, 71.37; H, 6.56; N, 3.96; found: C, 71.17; H, 6.33; N, 3.54.

*5.1.4.6. 2-(4-Ethylphenyl)-1-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**9f**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 1:9 (v/v) for elution, furnished **9f** as a yellow oil. Yield 46%. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (t, J=7.0 Hz, 3H), 2.60 (q, J=6.8 Hz, 2H), 3.65 (s, 6H), 3.89 (s, 3H), 6.34-6.39 (m, 4H), 6.93 (m, 1H), 7.06 (m, 4H). ^{13}C-NMR ($CDCl_3$) δ: 14.88, 56.13 (2C), 61.08, 63.46, 103.40 (2C), 108.99, 109.59, 123.44, 127.32 (2C), 129.67 (2C), 131.86, 136.36, 136.52, 136,73, 136.80, 153.82 (2C). MS (ESI): $[M+1]^+$=338.14. Anal. calcd for $C_{21}H_{23}NO_3$. C, 74.75; H, 6.87; N, 4.15; found: C, 73.51; H, 6.22; N, 3.87.

*5.1.4.7. 2-(4-n-Propylphenyl)-1-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**9g**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 4:6 (v/v) for elution, furnished **9g** as a yellow oil. Yield 40%. ^{1}H-NMR ($CDCl_3$) δ: 0.91 (t, J=8.0 Hz, 3H), 1.58 (m, 2H), 2.53 (m, 2H), 3.65 (s, 6H), 3.85 (s, 3H), 6.34-6.36 (m, 3H), 7.04-7.05 (m, 4H), 7.40 (d, J=7.8 Hz, 2H). ^{13}C-NMR

($CDCl_3$) δ: 13.79, 24.58, 37.71, 56.09 (2C), 61.11, 103.34 (2C), 109.10, 110.05, 115.07, 123.68, 128.23 (2C), 128.41 (2C), 129.57, 134.13, 136.36, 141.02, 153.15 (2C). MS (ESI): $[M+1]^+$=352.21. Anal. calcd for $C_{22}H_{25}NO_3$. C, 74.75; H, 6.87; N, 4.15; found: C, 73.51; H, 6.22; N, 3.87.

*5.1.4.8. 2-(4-Methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**9h**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 2:8 (v/v) for elution, furnished **9h** as a white solid. Yield 47%, mp 118-120 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.67 (s, 6H), 3.78 (s, 3H), 3.85 (s, 3H), 6.33-6.34 (m, 2H), 6.37 (s, 2H), 6.77 (d, J=8.8 Hz, 2H), 6.91-6.92 (m, 1H), 7.07 (d, J=8.8 Hz, 2H). ^{13}C-NMR ($CDCl_3$) δ: 55.24, 56.07 (2C), 61.00, 103.35 (2C), 108.92, 109.57, 113.50 (2C), 123.42, 125.64, 129.60 (2C), 133.73, 136.28, 136.49, 153.11 (2C), 158.28. MS (ESI): $[M+1]^+$=340.15. Anal. calcd for $C_{20}H_{21}NO_4$. C, 70.78; H, 6.24; N, 4.13; found: C, 70.61; H, 6.13; N, 3.89.

*5.1.4.9. 2-(3-Fluoro-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**9i**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 1.5:8.5 (v/v) for elution, furnished **9i** as a white solid. Yield 68%, mp 125-127 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.16 (s, 6H), 3.70 (s, 3H), 3.86 (s, 3H), 6.35-6.37 (m, 2H), 6.38 (s, 2H), 6.75 (d, J=8.4, 1H), 6.88 (d, J=2.0 Hz, 1H), 6.90-6.92 (m, 1H), 7.24 (s, 1H). ^{13}C-NMR ($CDCl_3$) δ: 56.23 (2C), 61.13, 67.17, 81.33, 103.52 (2C), 109.15, 109.76, 110.23, 111.82, 112.21, 113.03, 115.95, 116.00, 124.18 (2C), 125.03, 153.3 (2C). MS (ESI): $[M+1]^+$=357.92. Anal. calcd for $C_{20}H_{20}FNO4$. C, 67.22; H, 5.64; N, 3.92; found: C, 67.06; H, 5.37; N, 3.69.

*5.1.4.10. 2-(3-Chloro-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**9j**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 1.5:8.5 (v/v) for elution, furnished **9j** as a white solid. Yield 53%, mp 123-125 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.70 (s, 6H), 3.86 (s, 6H), 6.30-6.33 (m, 1H), 6.36-6.38 (m, 3H), 6.75 (d, J=8.4 Hz, 1H), 6.90-6.92 (m, 2H), 7.27 (d, J=2.0 Hz, 1H). ^{13}C-NMR ($CDCl_3$) δ: 56.18 (2C), 61.04, 103.53 (2C), 109.08, 110.11, 111.54, 121.92, 124.04 (2C), 126.53, 127.57, 129.90, 132.27, 135.99, 136.86, 153.24 (2C), 153.53. MS (ESI): $[M+1]^+$=373.90. Anal. calcd for $C_{20}H_{20}ClNO_4$. C, 64.26; H, 5.39; N, 3.75; found: C, 64.01; H, 5.12; N, 3.44.

*5.1.4.11. 2-(4-Methoxy-3-methylphenyl)-1-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**9k**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 2:8 (v/v) for elution, furnished **9k** as a yellow oil. Yield 62%. ^{1}H-NMR ($CDCl_3$) δ: 2.14 (s, 3H), 3.68 (s, 6H), 3.77 (s, 3H), 3.85 (s, 3H), 6.32-6.34 (m, 2H), 6.38 (s, 2H), 6.66 (d, J=8.4 Hz, 1H), 6.86-6.91 (m, 2H), 7.00-7.01 (m, 1H). ^{13}C-NMR ($CDCl_3$) δ: 16.16, 55.32, 56.10 (2C), 61.00, 98.95, 103.44 (2C), 108.85, 109.47, 123.24, 125.13, 126.09, 126.93, 130.85, 133.97, 136.39, 136.50, 153.04 (2C), 156.54. MS (ESI): $[M+1]^+$=354.10. Anal. calcd for $C_{21}H_{23}NO_4$. C, 71.37; H, 6.56; N, 3.96; found: C, 71.17; H, 6.39; N, 3.57.

*5.1.4.12. 2-(4-Methoxy-3,5-dimethylphenyl)-1-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**9l**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 2:8 (v/v) for elution, furnished **9l** as a colorless oil. Yield 54%. ^{1}H-NMR ($CDCl_3$) δ: 2.17 (s, 6H), 3.66 (s, 3H), 3.67 (s, 6H), 3.84 (s, 3H), 6.32-6.36 (m, 2H), 6.37 (s, 2H), 6.80 (s, 2H), 6.92 (t, J=3.8 Hz, 1H). ^{13}C-NMR ($CDCl_3$) δ: 16.13 (2C), 56.20 (2C), 59.81, 61.11, 103.51 (2C), 109.01, 109.92,

115.06, 119.92, 123.51, 128.63, 129.04 (2C), 130.35, 133.82, 136.36, 153.11 (2C), 155.81. MS (ESI): $[M+1]^+$=368.00. Anal. calcd for $C_{22}H_{25}NO_4$. C, 71.91; H, 6.86; N, 3.81; found: C, 71.77; H, 6.57; N, 3.54.

*5.1.4.13. 2-(4-(Methylthio)phenyl)-1-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**9m**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 2:8 (v/v) for elution, furnished **9m** as a colorless oil. Yield 68%. ^{1}H-NMR ($CDCl_3$) δ: 2.43 (s, 3H), 3.68 (s, 3H), 3.70 (s, 3H), 3.85 (s, 3H), 6.34-6.40 (m, 4H), 6.92 (t, J=4.0 Hz, 1H), 7.08 (d, J=7.2 Hz, 2H), 7.10 (d, J=7.2 Hz, 2H). ^{13}C-NMR ($CDCl_3$) δ: 15.92, 56.16 (2C), 61.09, 103.48 (2C), 109.23, 110.27, 124.23, 126.31 (2C), 128.63 (2C), 129.89, 133.45, 136.26, 133.48, 136.75, 153.29 (2C). MS (ESI): $[M+1]^+$=355.91. Anal. calcd for $C_{20}H_{21}NSO_3$. C, 67.58; H, 5.96; N, 3.94; found: C, 67.28; H, 5.81; N, 3.77.

*5.1.4.14. 2-(4-Ethoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**9n**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 2:8 (v/v) for elution, furnished **9n** as a yellow solid. Yield 71%, mp 93-95 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.39 (t, J=7.2 Hz, 3H), 3.67 (s, 6H), 3.86 (s, 3H), 3.99 (q, J=7.2 Hz, 2H), 6.33-6.34 (m, 2H), 6.37 (s, 2H), 6.75 (d, J=8.8 Hz, 2H), 6.91-6.92 (m, 1H), 7.07 (d, J=8.8 Hz, 2H). ^{13}C-NMR ($CDCl_3$) δ: 14.81, 56.07 (2C), 61.00, 63.40, 103.34 (2C), 108.92, 109.53, 114.08 (2C), 123.36, 125.51, 129.59 (2C), 133.80, 136.30, 142.13, 153.10 (2C), 157.63. MS (ESI): $[M+1]^+$=354.41. Anal. calcd for $C_{21}H_{23}NO_4$. C, 71.37; H, 6.56; N, 3.96; found: C, 71.09; H, 6.22; N, 3.68.

*5.1.4.15. 2-(3-Chloro-4-ethoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**9o**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 2:8 (v/v) for elution, furnished **9o** as a white solid. Yield

72%, mp 124-127 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (t, J=7.2 Hz, 3H), 3.70 (s, 6H), 3.79 (s, 3H), 4.07 (q, J=6.8 Hz, 2H), 6.33-6.33 (m, 2H), 6.38 (s, 2H), 6.75 (d, J=8.4, 1H), 6.88 (d, J=2.2 Hz, 1H), 6.90-6.92 (m, 1H), 7.24 (s, 1H). ^{13}C-NMR ($CDCl_3$) δ: 14.75, 56.24 (2C), 61.09, 64.80, 103.56 (2C), 109.15, 110.15, 112.87, 122.40, 124.05, 126.47, 127.59, 129.98, 132.42, 136.08, 136.89, 153.04, 153.29 (2C). MS (ESI): $[M+1]^+$=389.12. Anal. calcd for $C_{21}H_{22}ClNO_4$. C, 65.03; H, 5.72; N, 3.61; found: C, 64.85; H, 5.65; N, 3.39.

*5.1.4.16. 2-(4-(Ethylthio)phenyl)-1-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**9p**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 3:7 (v/v) for elution, furnished **9p** as an oil. Yield 70%. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (t, J=7.4 Hz, 3H), 2.90 (q, J=7.2 Hz, 2H), 3.67 (s, 6H), 3.90 (s, 3H), 6.35 (t, J=3.6 Hz, 1H), 6.35 (s, 2H), 6.93-6.94 (m, 2H), 7.07 (d, J=8.8 Hz, 2H), 7.18 (d, J=9.0 Hz, 2H). ^{13}C-NMR ($CDCl_3$) δ: 14.31, 27.61, 56.07 (2C), 61.03, 103.31 (2C), 109.17, 110.33, 124.18, 128.56 (2C), 128.65 (2C), 130.52, 133.31, 134.48, 136.14, 136.62, 153.19 (2C). MS (ESI): $[M+1]^+$=369.91. Anal. calcd for $C_{21}H_{23}NSO_3$. C, 67.58; H, 5.96; N, 3.94; found: C, 67.22; H, 5.76; N, 3.77.

*5.1.4.17. 1-(Naphthalen-2-yl)-2-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**10a**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 2:8 (v/v) for elution, furnished **10a** as a white solid. Yield 56%, mp 128-136 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.50 (s, 6H), 3.79 (s, 3 H), 6.36 (s, 2H), 6.40 (d, J=2.2 Hz, 1H), 6.47 (m, 1H), 7.04 (t, 1H), 7.24 (d, J=2.2 Hz, 1H) 7.47-7.52 (m, 2H), 7.34-7.79 (m, 4H). ^{13}C-NMR ($CDCl_3$) δ: 55.75 (2C), 60.89, 105.62 (2C), 109.26, 110.19, 123.31, 124.45, 124.88, 126.24, 126.76, 127.68, 127.76, 128.41, 128.66,

131.77, 133.33, 133.89, 136.64, 138.12, 152.76 (2C). MS (ESI): $[M+1]^+$=360.15. Anal. calcd for $C_{23}H_{21}NO_3$. C, 76.86; H, 5.89; N, 3.90; found: C, 76.69; H, 5.63; N, 3.71.

*5.1.4.18. 1-(Naphthalen-1-yl)-2-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**10b**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 2:8 (v/v) for elution, furnished **10b** as a yellow oil. Yield 83%. ^{1}H-NMR ($CDCl_3$) δ: 3.37 (s, 6H), 3.70 (s, 3H), 6.24 (s, 2H), 6.44 (t, J=3.2 Hz, 1H), 6.57 (dd, J=3.6 and 1.6 Hz, 1H), 6.95 (dd, J=2.8 and 2.0 Hz, 1H), 7.36 (dd, J=7.6 and 1.2 Hz, 1H), 7.43-7.48 (m, 3H), 7.50 (dd, J=7.6 and 1.2 Hz, 1H), 7.86-7.90 (m, 2H). ^{13}C-NMR ($CDCl_3$) δ: 55.51 (2C), 60.75, 104.45 (2C), 108.60, 108.81, 123.30, 125.29, 125.69, 125.74, 126.60, 127.23, 127.96, 128.31 (2C), 131.29, 134.00, 135.50, 136.33, 137.58, 152.56 (2C). MS (ESI): $[M+1]^+$=360.01. Anal. calcd for $C_{23}H_{21}NO_3$. C, 76.86; H, 5.89; N, 3.90; found: C, 76.71; H, 5.60; N, 3.69.

*5.1.4.19. 1-(p-Tolyl)-2-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**10c**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 2:8 (v/v) for elution, furnished **10c** as a white solid. Yield 82%, mp 66-68 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.35 (s, 3H), 3.62 (s, 6H), 3.82 (s, 3H), 6.33-6.35 (m, 3H), 6.41 (dd, J=3.4 and 1.6 Hz, 1H), 6.88-6.90 (m, 1H), 7.07 (d, J=8.4 Hz, 2H), 7.13 (d, J=8.4 Hz, 2H). ^{13}C-NMR ($CDCl_3$) δ: 20.95, 55.75 (2C), 60.89, 105.56 (2C), 108.81, 109.69, 124.20, 125.84 (2C), 127.09, 128.47, 129.50 (2C), 133.76, 136.57, 138.10, 152.66 (2C). MS (ESI): $[M+1]^+$=324.01. Anal. calcd for $C_{20}H_{21}NO_3$. C, 74.28; H, 6.55; N, 4.33; found: C, 73.99; H, 6.26; N, 4.02.

*5.1.4.20. 1-(3,4-Dimethylphenyl)-2-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**10d**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 2:8 (v/v) for elution, furnished **10d** as a yellow oil. Yield

57%. ^{1}H-NMR ($CDCl_3$) δ: 2.21 (s, 3H), 2.25 (s, 3H), 3.61 (s, 6H), 3.81 (s, 3H), 6.30-6.33 (m, 3H), 6.39-6.42 (m, 1H), 6.56 (d, J=8,2 Hz, 1H), 6.89-6.91 (m, 1H), 6.99 (s, 1H), 7.06 (d, J=8.2 Hz, 1H). ^{13}C-NMR ($CDCl_3$) δ: 19.36, 19.82, 55.85 (2C), 60.99, 105.31, 105.63 (2C), 108.77, 109.61, 123.58, 124.36, 127.15, 128.66, 128.82, 130.02, 135.35, 137.42, 138.45, 152.70 (2C). MS (ESI): $[M+1]^+$=337.91. Anal. calcd for $C_{21}H_{23}NO_3$. C, 74.75; H, 6.87; N, 4.15; found: C, 74.54; H, 6.37; N, 4.02.

*5.1.4.21. 1-(4-Ethylphenyl)-2-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**10e**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 2:8 (v/v) for elution, furnished **10e** as a yellow oil. Yield 77%. ^{1}H-NMR ($CDCl_3$) δ: 1.22 (t, J=7.8 Hz, 3H), 2.81 (q, J=6.8 Hz, 2H), 3.60 (s, 6H), 3.81 (s, 3H), 6.31 (s, 2H), 6.34-6.39 (m, 1H), 6.93-6.95 (m, 2H), 7.06 (m, 4H). ^{13}C-NMR ($CDCl_3$) δ: 17.01, 28.51, 55.81 (2C), 60.98, 97.36, 105.58 (2C), 108.90, 109.70, 121.53, 124.29, 126.12 (2C), 128.45 (2C), 133.84, 138.36, 143.18, 152.75 (2C). MS (ESI): $[M+1]^+$=338.14. Anal. calcd for $C_{21}H_{23}NO_3$. C, 74.75; H, 6.87; N, 4.15; found: C, 73.51; H, 6.22; N, 3.87.

*5.1.4.22. 1-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**10f**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 2:8 (v/v) for elution, furnished **10f** as a yellow solid. Yield 71%, mp 112-114 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.61 (s, 6H), 3.80 (s, 6H), 6.23 (m, 1H), 6.24 (s, 2H), 6.81-6.83 (m, 2H), 7.15 (dd, J=2.4 and 8.0 Hz, 2H), 7.07 (d, J=8.8 Hz, 2H). ^{13}C-NMR ($CDCl_3$) δ: 55.3 (2C), 55.63, 60.12, 106.32 (2C), 108.23, 110.45, 113.56 (2C), 122.48, 125.50 (2C), 126.34, 129.59, 134.12, 141.64, 153.01 (2C), 157.52. MS (ESI): $[M+1]^+$=340.41. Anal. calcd for $C_{20}H_{21}NO_4$. C, 76.86; H, 5.89; N, 3.90; found: C, 76.63; H, 5.77; N, 3.68.

*5.1.4.23. 1-(3-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**10g**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 2:8 (v/v) for elution, furnished **10g** as a white solid. Yield 57%, mp 111-112 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.63 (s, 6H), 3.70 (s, 3H), 3.82 (s, 3H), 6.33-6.34 (m, 2H), 6.39 (s, 2H), 6.75-6.77 (m, 2H), 6.91-6.92 (m, 1H), 7.05-7.07 (m, 2H). ^{13}C-NMR ($CDCl_3$) δ: 55.40, 55.84 (2C), 60.90, 93.99, 105.57 (2C), 109.05, 109.99, 111.65, 112.59, 118.26 (2C), 123.24, 124.11, 128.40, 129.67, 152.72 (2C), 159.96. MS (ESI): $[M+1]^+$=340.15. Anal. calcd for $C_{20}H_{21}NO_4$. C, 76.86; H, 5.89; N, 3.90; found: C, 76.59; H, 5.68; N, 3.71.

*5.1.4.24. 1-(4-Ethoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**10h**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 2:8 (v/v) for elution, furnished **10h** as a yellow solid. Yield 54%, mp 96-99 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (t, J=7.2 Hz, 3H), 3.62 (s, 6H), 3.99 (s, 3H), 4.07 (q, J=6.8 Hz, 2H), 6.26-6.28 (m, 3H), 6.40-6.43 (m, 1H), 6.81 (d, J=7.2 Hz, 2H), 6.83-6.86 (m, 1H), 7.17 (d, J=7.2 Hz, 2H). ^{13}C-NMR ($CDCl_3$) δ: 14.75, 55.79 (2C), 60.89, 63.77, 105.50 (2C), 108.65, 109.42, 114.68 (2C), 124.35, 127.16 (2C), 128.49, 133.57, 133.84, 136.46, 152.67 (2C), 157.69. MS (ESI): $[M+1]^+$=354.12. Anal. calcd for $C_{21}H_{23}NO_4$. C, 71.37; H, 6.56; N, 3.96; found: C, 71.01; H, 6.23; N, 3.47.

*5.1.4.25. N,N-Dimethyl-4-(2-(3,4,5-trimethoxyphenyl)-1H-pyrrol-1-yl)aniline (**10i**).* Following general procedure C, the crude residue purified by flash chromatography, using EtOAc:petroleum ether 3:7 (v/v) for elution, furnished **10i** as a yellow oil. Yield 59%. ^{1}H-NMR ($CDCl_3$) δ: 2.94 (s, 6H), 3.63 (s, 6H), 3.81 (s, 3H), 6.30-6.39 (m, 4H), 6.65 (d, J=9.2 Hz, 2H), 6.85-6.87 (m, 1H), 7.06 (d, J=8.8 Hz, 2H). ^{13}C-NMR ($CDCl_3$) δ: 40.82 (2C), 55.88 (2C), 60.98, 105.51 (2C), 108.37, 109.05, 112.55 (2C), 124.55,

127.03 (2C), 128.82, 130.15, 133.90, 136.38, 149.62, 152.69 (2C). MS (ESI): $[M+1]^+$=353.15. Anal. calcd for $C_{21}H_{24}N_2O_3$. C, 71.57; H, 6.86; N, 7.95; found: C, 71.21; H, 6.73; N, 7.68.

*5.1.5. 3,4-Dibromo-1-tosyl-1H-pyrrole (**17**).* A solution of bromine (1.5 mL, 28 mmol) in glacial AcOH (15 mL) was added dropwise over 30 min to a solution of 1-tosyl-1*H*-pyrrole (2.7 g, 12 mmol) in glacial AcOH (30 ml), and the mixture was maintained at reflux for 90 min. The resulting dark solution was concentrated under reduced pressure, and the crude dark residue was dissolved in dichloromethane, filtered over a celite pad that was washed with dichloromethane, and the combined filtrates were concentrated at reduced pressure. The resulting residue was purified by reverse phase flash chromatography (silica c18 60 g, water+0.1% formic acid/MeCN+0.1% formic acid, from 98:2 to 10:90), to afford the product **17** as yellow crystals. Yield: 25%, mp 135-137 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.44 (s, 3H), 7.18 (s, 2H), 7.36 (d, J=8.8 Hz, 2H), 7.77 (d, J=8.8 Hz, 2H). MS (ESI): $[M+1]^+$=379.4.

*5.1.6. 3-Bromo-4-(3',4',5'-trimethoxyphenyl)-1-tosyl-1H-pyrrole (**18**).* A solution of 3,4-dibromo-1-tosyl-1*H*-pyrrole **17** (192 mg, 0.50 mmol), 3,4,5-trimethoxybenzene boronic acid (119 mg, 0.58 mmol, 1.15 equiv.) and a 2 M aqueous solution of Na_2CO_3 (0.5 mL, 1 mmol) in THF (5 mL) was purged with Ar for 10 min. Tetrakis(triphenylphosphine)palladium [$Pd(Ph_3)_4$] (54 mg, 0.05 mmol) was added, and the mixture was degassed for 10 min and heated with stirring at 65 °C for 24 h. The suspension was cooled to ambient temperature, diluted with EtOAc (10 mL), filtered through a pad of celite and the filtrate evaporated *in vacuo*. Volatiles were removed and the crude residue purified by flash chromatography on silica gel, using a mixture of EtOAc-petroleum ether 3:7 (v/v) as eluent to afford **18** as a yellow oil. Yield 43%. ^{1}H-

NMR ($CDCl_3$) δ: 2.42 (s, 3H), 3.71 (s, 3H), 3.83 (s, 6H), 6.82 (s, 2H), 7.52 (d, J=8.0 Hz, 2H), 7.72 (m, 2H), 7.75 (d, J=8.0 Hz, 2H). MS (ESI): $[M+1]^+$=468.2.

*5.1.5. General procedure D for the synthesis of compounds **19a-n**.*

A stirred suspension of 3-bromo-1-tosyl-4-(3,4,5-trimethoxyphenyl)pyrrole **18** (182.0 mg, 0.40 mmol), the appropriate arylboronic acid (0.40 mmol) and sodium carbonate (124 mg, 1.18 mmol) in a mixture of 1,2-dimethoxyethane (4 mL) and water (0.60 mL) was degassed under a stream of nitrogen over 10 min. $Pd(Ph_3)_4$ (46 mg, 0.040 mmol) was added and the mixture degassed again for 10 min, then stirred at 85 °C for 24 h. The reaction mixture was cooled to ambient temperature, diluted with EtOAc, filtered and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel using a mixture of EtOAc:petroleum ether 2:8 (v/v) as eluent to afford the title compound.

*5.1.5.1. 3-(Naphthalen-2-yl)-1-tosyl-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**19a**).* Following general procedure D, compound **19a** was obtained as a yellow oil. Yield; 60%. ^{1}H-NMR (DMSO-d_6) δ: 2.41 (s, 3H), 2.84 (s, 6H), 2.95 (s, 3H), 6.82 (s, 2H), 7.32 (dd, J=8.0 Hz, 2 H), 7.47-7.52 (m, 4H), 7.67 (d, J=7.2 Hz, 2H), 7.71-7.73 (m, 2 H), 7.82-7-89 (m, 2H), 8.32-8.37 (m, 1H). MS (ESI): 514.3.

*5.1.5.2. 3-(Benzo[b]thiophen-2-yl)-1-tosyl-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**19b**).* Following general procedure D, compound **19b** was obtained as a yellow oil, yield 69%. ^{1}H-NMR (DMSO-d_6) δ: 2.41 (s, 3H), 3.62 (s, 6H), 3.65 (s, 3H), 6.87 (s, 2H), 7.28-2-33 (m, 2H), 7.49-7-52 (m, 2H), 7.68-7.76 (m, 4H), 7.89-8.05 (m, 3H). MS (ESI): 520.3.

*5.1.5.3. 3-(p-Tolyl)-1-tosyl-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**19c**).* Following general procedure D, compound **19c** was obtained as a yellow oil. Yield: 69%. ^{1}H-NMR (DMSO-d_6) δ: 1.51 (s, 3H), 2.42 (s, 3H), 2.84 (s, 6H), 2.95 (s, 3H), 5.57 (s, 2H), 6.43 (s, 2 H), 6.90-7.12 (m, 4H), 7.48-7.53 (m, 2H), 7.63 (d, J=2.2 Hz, 1H), 8.01 (d, J=2.2 Hz, 1H). MS (ESI): 478.3.

*5.1.5.4. 1-Tosyl-3-(4-(trifluoromethyl)phenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**19d**).* Following general procedure D, compound **19d** was obtained as a white foam. Yield: 74%. ^{1}H-NMR (DMSO-d_6) δ: 2.41 (s, 3H), 3.58 (s, 6H), 3.63 (s, 3H), 6.47 (s, 2H), 7.41-7.53 (m, 2H), 7.67-7.72 (m, 4H), 7.99-8.04 (m, 4H). MS (ESI): 532.4.

*5.1.5.5. 3-(4-Ethylphenyl)-1-tosyl-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**19e**).* Following general procedure D, compound **19e** was obtained as a colorless oil. Yield: 92%. ^{1}H-NMR (DMSO-d_6) δ: 1.15 (t, J=7.9 Hz, 3H), 2.41 (s, 3H), 2.60 (q, J=6.9 Hz, 2H), 3.28 (s, 6H), 3.62 (s, 3H), 6.47 (s, 2H), 6.86-6.89 (m, 2H), 7.10-7.14 (m, 2H), 7.42 (d, J=3.0 Hz, 1H), 7.49 (m, 2H), 7.61 (d, J=3.0 Hz, 1H), 7.99-8.04 (m, 2H). MS (ESI): 492.4.

*5.1.5.6. 1-Tosyl-3-(4-(trifluoromethoxy)phenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**19f**).* Following general procedure D, compound **19f** was obtained as a white foam. Yield: 72%. ^{1}H-NMR (DMSO-d_6) δ: 2.41 (s, 3H), 3.58 (s, 6H), 3.67 (s, 3H), 6.41 (s, 2H), 7.34 (bs, 3H), 7.50 (d, J=7.0 Hz, 1H), 7.62-7.68 (m, 3H), 7.61 (d, J=3.0 Hz, 1H), 7.99-8.02 (m, 2H). MS (ESI): 548.4.

*5.1.5.7. 3-(3-Fluoro-4-methoxyphenyl)-1-tosyl-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**19g**).* Following general procedure D, compound **19g** was obtained as a colorless oil. Yield: 68%. ^{1}H-NMR (DMSO-d_6) δ: 2.41 (s, 3H), 3.28 (s, 6H), 3.62 (s, 3H), 3.81 (s,

3H), 6.47 (s, 2H), 6.86-6.89 (m, 1H), 7.10-7.14 (m, 2H), 7.42 (d, J=3.0 Hz, 1H), 7.49 (m, 2H), 7.61 (d, J=3.0 Hz, 1H), 7.99-8.04 (m, 2H). MS (ESI): 512.4.

*5.1.5.8. 3-(3-chloro-4-methoxyphenyl)-1-tosyl-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole **(19h)***. Following general procedure D, compound **19h** was obtained as a white foam. Yield: 64%. ^{1}H-NMR (DMSO-d_6) δ: 2.41 (s, 3H), 3.28 (s, 3H), 3.60 (s, 6H), 3.65 (s, 3H), 7.09-7.12 (m, 2H), 7.34 (d, J=3.0 Hz, 2H), 7.49 (d, 9.0 J=9.0 Hz, 2H), 7.59-7.61 (m, 3H), 7.99-8.04 (m, 2H). MS (ESI): 528.4.

*5.1.5.9. 3-(4-Ethoxyphenyl)-1-tosyl-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole **(19i)***. Following general procedure D, compound **19i** was obtained as a colorless oil. Yield: 82%. ^{1}H-NMR (DMSO-d_6) δ: 1.30 (t, J=7.9 Hz, 3H), 2.41 (s, 3H), 3.28 (s, 6H), 3.62 (s, 3H), 4.0 (q, J=6.9 Hz, 2H), 6.47 (s, 2H), 6.86-6.89 (m, 2H), 7.10-7.14 (m, 2H), 7.42 (d, J=3.0 Hz, 1H), 7.49 (m, 2H), 7.61 (d, J=3.0 Hz, 1H), 7.99-8.04 (m, 2H). MS (ESI): 508.17.

*5.1.5.10. 3-(4-Ethoxy-3-fluorophenyl)-1-tosyl-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole **(19j)***. Following general procedure D, compound **19j** was obtained as a colorless oil. Yield 58%. ^{1}H-NMR (DMSO-d_6) δ: 1.30 (t, J=7.9 Hz, 3H), 2.41 (s, 3H), 3.28 (s, 6H), 3.62 (s, 3H), 4.0 (q, J=6.9 Hz, 2H), 6.47 (s, 2H), 6.86-6.89 (m, 1H), 7.10-7.14 (m, 2H), 7.41-7.47 (m, 2H), 7.49 (d, J=3.0 Hz, 1H) 7.61 (d, J=3.0 Hz, 1H), 7.99-8.04 (m, 2H). MS (ESI): 526.4.

*5.1.5.11. 3-(3-Chloro-4-ethoxyphenyl)-1-tosyl-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole **(19k)***. Following general procedure D, compound **19k** was obtained as a colorless oil. Yield: 80%. ^{1}H-NMR (DMSO-d_6) δ: 1.33 (t, J=7.9 Hz, 3H), 2.41 (s, 3H), 3.25 (s, 6H), 3.62 (s, 3H), 4.01 (q, J=6.9 Hz, 2H), 6.45 (s, 2H), 6.86-6.87 (m, 1H), 7.08-7.14 (m, 2H),

7.41-7.47 (m, 2H), 7.49 (d, J=3.0 Hz, 1H) 7.63 (d, J=3.0 Hz, 1H), 7.99-8.04 (m, 2H). MS (ESI): 543.3.

*5.1.5.12. 3-(4-(Ethylthio)phenyl)-1-tosyl-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**19l**).* Following general procedure D, compound **19l** was obtained as a white foam. Yield: 70%. ^{1}H-NMR (DMSO-d_6) δ: 1.20 (t, J=7.9 Hz, 3H), 2.41 (s, 3H), 2.96 (q, J=6.9 Hz, 2H), 3.28 (s, 6H), 3.62 (s, 3H), 6.47 (s, 2H), 6.86-6.89 (m, 2H), 7.10-7.14 (m, 2H), 7.42 (d, J=3.0 Hz, 1H), 7.49 (m, 2H), 7.61 (d, J=3.0 Hz, 1H), 7.99-8.04 (m, 2H). MS (ESI): 524.4.

*5.1.5.13. 3-(4-n-Propoxyphenyl)-1-tosyl-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**19m**).* Following general procedure D, compound **19m** was obtained as a colorless oil. Yield: 25%. ^{1}H-NMR (DMSO-d_6) δ: 0.99 (t, J=7.9 Hz, 3H), 2.32 (m, 2H), 2.41 (s, 3H), 3.28 (s, 6H), 3.62 (s, 3H), 3.91 (t, J=6.9 Hz, 2H), 6.52 (s, 2H), 6.82-6.86 (m, 2H), 7.13-7.19 (m, 2H), 7.39 (d, J=3.0 Hz, 1H), 7.49 (m, 2H), 7.63 (d, J=3.0 Hz, 1H), 7.99-8.04 (m, 2H). MS (ESI): 522.3.

*5.1.5.14. 3-(4-(Methoxymethoxy)phenyl)-1-tosyl-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**19n**).* Following general procedure D, compound **19n** was obtained as a yellow oil. Yield: 55%. ^{1}H-NMR (DMSO-d_6) δ: 2.41 (s, 3H), 3.18 (s, 2H), 3.56 (s, 6H), 3.62 (s, 3H), 3.65 (s, 3H), 6.44 (s, 2H), 6.97-6.99 (m, 2H), 7.12-7.64 (m, 2H), 7.46-7.51 (m, 3H), 7.64 (d, J=3.0 Hz, 1H), 7.82-8.10 (m, 2H). MS (ESI): 524.3.

*5.1.6. General procedure E for the synthesis of compounds **11a-n**.*

The appropriate 1-tosyl-3-(3,4,5-trimethoxyphenyl)-4-aryl-1*H*-pyrrole **19a-n** (0.5 mmol) was stirred at 50 °C for 24 h in a mixture of EtOH (20 mL) and aqueous 1 N NaOH (9 mL, 9 mmol, 18 equiv.). After cooling on an ice bath, the mixture was

adjusted to pH 1 with aqueous 1 N HCl and concentrated under vacuum. The aqueous, concentrated mixture was diluted with a saturated aqueous solution of $NaHCO_3$, extracted with CH_2Cl_2 (2x10 mL) and the organic phase washed sequentially with water (5 mL) and brine (5 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash chromatography using a gradient from 95:5 to 6:4 (v/v) of EtOAc and petroleum ether for elution to provide the title compound.

*5.1.6.1. 3-(Naphthalen-2-yl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**11a**).* Following general procedure E, the crude residue purified by flash chromatography furnished **11a** as a white solid. Yield 55%, mp 88-90 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.69 (s, 6H), 3.87 (s, 3H), 6.50 (s, 2H), 7.06-7.10 (m, 2H), 7.38 (dd, J=8.4, 2.0 Hz, 1H), 7.41-7.48 (m, 2H), 7.75-7.82 (m, 3H), 7.85 (d, J=7.7 Hz, 1H), 11.01 (bs, 1H). MS (ESI): $[M+1]^+$=360.3. Anal. calcd for $C_{23}H_{21}NO_3$. C, 76.86; H, 5.89; N, 3.90; found: C, 76.53; H, 5.61; N, 3.73.

*5.1.6.2. 3-(Benzo[b]thiophen-2-yl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**11b**).* Following general procedure E, the crude residue purified by flash chromatography furnished **11b** as a yellow solid. Yield 24%, mp 88-90 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.63 (s, 6H), 3.67 (s, 3H), 6.64 (s, 2H), 7.02-7.05 (m, 1H), 7.14-7.17 (m, 2H), 7.21-7.32 (m, 2H), 7.69 (d, J=7.3 Hz, 1H), 7.84 (d, J=7.7 Hz, 1H), 11.29 (bs, 1H). MS (ESI): $[M+1]^+$=366.3. Anal. calcd for $C_{21}H_{19}NO_3S$. C, 69.02; H, 5.24; N, 3.83; found: C, 68.87; H, 4.98; N, 3.42.

*5.1.6.3. 3-(p-Tolyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**11c**).* Following general procedure E, the crude residue purified by flash chromatography furnished **11c** as a white solid. Yield 68%, mp 98-101 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.35 (s, 3H), 3.69 (s, 6H), 3.87 (s, 3H), 6.50 (s, 2H), 6.90-6.95 (m, 2H), 7.10 (d, J=8.0 Hz, 2H), 7.21 (d, J=8.0 Hz,

2H), 8.31 (bs, 1H). ^{13}C-NMR ($CDCl_3$) δ: 21.17, 55.93 (2C), 61.02, 105.69 (2C), 116.91, 117.26, 123.68, 128.81 (2C), 128.86 (2C), 131.48, 132.75, 135.45 (2C), 136.23, 152.92 (2C). MS (ESI): $[M+1]^+$=324.3. Anal. calcd for $C_{20}H_{21}NO_3$. C, 74.28; H, 6.55; N, 4.33; found: C, 73.99; H, 6.21; N, 4.01.

*5.1.6.4. 3-(4-(Trifluoromethyl)phenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**11d**).* Following general procedure E, the crude residue purified by flash chromatography furnished **11d** as a white solid. Yield 58%, mp 138-140 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.70 (s, 6H), 3.88 (s, 3H), 6.45 (s, 2H), 6.94-6.99 (m, 2H), 7.42 (d, J=8.0 Hz, 2H), 7.53 (d, J=8.0 Hz, 2H), 8.41 (bs, 1H). ^{13}C-NMR ($CDCl_3$) δ: 55.98 (2C), 61.05, 105.87 (2C), 117.58, 117.74, 117.93, 122.45, 125.887 ($^1J_{CF}$=269.9 Hz), 123.97, 125.09, 127.694 ($^2J_{CF}$=32.4 Hz), 128.33, 128.75, 130.89, 136.60, 139.55, 153.12 (2C). MS (ESI): $[M+1]^+$=378.3. Anal. calcd for $C_{20}H_{18}F_3NO_3$. C, 63.66; H, 4.81; N, 3.71; found: C, 63.31; H, 4.64; N, 3.38.

*5.1.6.5. 3-(4-Ethylphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**11e**).* Following general procedure E, the crude residue purified by flash chromatography furnished **11e** as a white solid. Yield 51%, mp 96-98°C. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (t, J=7.6 Hz, 3H), 2.64 (q, J=7.6 Hz, 2H), 3.68 (s, 6H), 3.86 (s, 3H), 6.50 (s, 2H), 6.91-6.95 (m, 2H), 7.13 (d, J=8.0 Hz, 2H), 7.23 (d, J=7.0 Hz, 2H), 8.25 (bs, 1H). ^{13}C-NMR ($CDCl_3$) δ: 15.89, 28.63, 55.89 (2C), 61.01, 105.63 (2C), 116.79 (2C), 117.26 (2C), 123.77, 127.66 (2C), 129.00 (2C), 131.44, 133.05, 142.01, 152.91 (2C). MS (ESI): $[M+1]^+$=338.3. Anal. calcd for $C_{21}H_{23}NO_4$. C, 74.75; H, 6.87; N, 4.15; found: C, 74.63; H, 6.32; N, 3.89.

*5.1.6.6. 3-(4-(Trifluoromethoxy)phenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**11f**).* Following general procedure E, the crude residue purified by flash chromatography

furnished **11f** as a white solid. Yield 56%, mp 88-95 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.69 (s, 6H), 3.87 (s, 3H), 6.44 (s, 2H), 6.93-6.96 (m, 2H), 7.14 (d, J=8.0 Hz, 2H), 7.31-7.33 (m, 2H), 8.39 (bs, 1H). ^{13}C-NMR ($CDCl_3$) δ: 55.88 (2C), 61.04, 105.67 (2C), 117.19, 117.53 (2C), 120.86 (2C), 122.45, 123.79, 130.14 (2C), 130.94, 134.75, 136.44, 147.55, 153.07 (2C). ^{19}F-NMR ($CDCl_3$) δ:-57.798. MS (ESI): $[M+1]^+$=394.3. Anal. calcd for $C_{20}H_{18}F_3NO_4$. C, 61.07; H, 4.61; N, 3.56; found: C, 60.84; H, 4.29; N, 3.09.

*5.1.6.7. 3-(3-Fluoro-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**11g**).* Following general procedure E, the crude residue purified by flash chromatography furnished **11g** as a yellow solid. Yield 67%, mp 106-108 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.73 (s, 6H), 3.88 (s, 3H), 3.89 (s, 3H), 6.49 (s, 2H), 6.87-6.91 (m, 3H), 9.99-7.02 (m, 1H), 7.09-7.11 (m, 1H), 8.27 (bs, 1H). ^{13}C-NMR ($CDCl_3$) δ: 56.03 (2C), 56.45, 61.04, 105.80 (2C), 113.28, 116.31 and 116.49 ($^{2}J_{CF}$=18 Hz), 117.16 and 117.24 ($^{3}J_{CF}$=8.4 Hz), 122.38, 123.63, 124.50, 124.53, 129.13, 131.15, 136.46, 145.80 and 145.90 ($^{2}J_{CF}$=20.0 Hz), 153.04 (2C), 150.97 and 153.395 ($^{1}J_{CF}$=242 Hz). MS (ESI): $[M+1]^+$=358.3. Anal. calcd for $C_{20}H_{20}FNO_4$. C, 67.22; H, 5.64; N, 3.92; found: C, 67.01; H, 5.33; N, 3.55.

*5.1.6.8. 3-(3-Chloro-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**11h**).* Following general procedure E, the crude residue purified by flash chromatography furnished **11e** as a white solid. Yield 15%, mp 112-114 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.62 (s, 6H), 3.65 (s, 3H), 3.83 (s, 3H), 6.47 (s, 2H), 6.95-6.97 (m, 1H), 7.00 (t, J=2.2 Hz, 1H), 7.06-7.09 (m, 1H), 7.16 (dd, J=8.6, 2.2 Hz, 1H), 7.26 (d, J=2.2 Hz, 1H), 11.09 (bs, 1H). MS (ESI): $[M+1]^+$=374.2. Anal. calcd for $C_{20}H_{20}ClNO_4$. C, 64.26; H, 5.39; N, 3.75; found: C, 63.98; H, 5.01; N, 3.47.

*5.1.6.9. 3-(4-Ethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**11i**).* Following general procedure E, the crude residue purified by flash chromatography furnished **11i** as a white solid. Yield 51%, mp 118-120 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (t, J=6.8 Hz, 3H), 3.70 (s, 6H), 3.87 (s, 3H), 4.04 (q, J=6.8 Hz, 2H), 6.50 (s, 2H), 6.84 (d, J=8.8 Hz, 2H), 6.86-6.88 (m, 1H), 6.94 (d, J=2.4 Hz, 1H), 7.21 (d, J=8.8 Hz, 2H), 8.29 (bs, 1H). ^{13}C-NMR ($CDCl_3$) δ: 14.96, 55.96 (2C), 61.01, 63.52, 105.63 (2C), 114.29 (2C), 116.84, 117.04, 123.39, 123.56, 128.12, 129.99 (2C), 131.51, 136.19, 152.95 (2C), 157.41. MS (ESI): $[M+1]^+$=354.3. Anal. calcd for $C_{21}H_{23}NO_4$. C, 71.37; H, 6.56; N, 3.96; found: C, 71.02; H, 6.36; N, 3.58.

*5.1.6.10. 3-(4-Ethoxy-3-fluorophenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**11j**).* Following general procedure E, the crude residue purified by flash chromatography furnished **11j** as a yellow solid. Yield 93%, mp 132-134 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (t, J=6.8 Hz, 3H), 3.72 (s, 6H), 3.87 (s, 3H), 4.11 (q, J=6.8 Hz, 2H), 6.49 (s, 2H), 6.88-6.92 (m, 3H), 6.94-6.98 (m, 1H), 7.07 (dd, J=8.2 and 2.0 Hz, 1H), 8.22 (bs, 1H). ^{13}C-NMR ($CDCl_3$) δ: 14.92, 56.02, 56.20, 61.04, 65.13, 105.77 (2C), 114.77, 116.37 and 116.56 ($^{2}J_{CF}$=19 Hz), 117.13 and 117.24 ($^{3}J_{CF}$=11 Hz), 122.41, 123.61, 124.50, 129.09, 129.16, 131.16, 136.43, 145.01, 151.26 and 153.70 ($^{1}J_{C\text{-}F}$=243 Hz), 153.03 (2C). MS (ESI): $[M+1]^+$=372.3. Anal. calcd for $C_{21}H_{22}FNO_4$. C, 67.91; H, 5.97; N, 3.77; found: C, 67.62; H, 5.69; N, 3.43.

*5.1.6.11. 3-(3-Chloro-4-ethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**11k**).* Following general procedure E, the crude residue purified by flash chromatography furnished **11k** as a yellow solid. Yield 41%, mp 123-125 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (t, J=6.8 Hz, 3H), 3.72 (s, 6H), 3.87 (s, 3H), 4.10 (q, J=6.8 Hz, 2H), 6.50 (s, 2H), 6.85 (d, J=8.4 Hz, 1H), 6.89-6.92 (m, 2H), 7.08-7.10 (m, 1H), 7.39 (d, J=2.0 Hz, 1H), 8.23

(bs, 1H). ^{13}C-NMR ($CDCl_3$) δ: 14.82, 56.05 (2C), 61.04, 64.92, 105.74 (2C), 113.31, 117.10, 117.25, 122.16, 122.47, 123.57, 128.17 (2C), 129.23, 130.24, 131.16, 136.45, 152.72, 153.05. MS (ESI): $[M+1]^+$=388.3. Anal. calcd for $C_{21}H_{22}ClNO_4$. C, 65.03; H, 5.72; N, 3.61; found: C, 64.62; H, 5.43; N, 3.28.

*5.1.6.12. 3-(4-(Ethylthio)phenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**11l**).* Following general procedure E, the crude residue purified by flash chromatography furnished **11l** as a white solid. Yield 60%, mp 103-105 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (t, J=7.6 Hz, 3H), 2.93 (q, J=7.2 Hz, 2H), 3.70 (s, 6H), 3.87 (s, 3H), 6.48 (s, 2H), 6.91-6.93 (m, 4H), 7.25-7.26 (m, 4H), 8.36 (bs, 1H). ^{13}C-NMR ($CDCl_3$) δ: 14.51, 28.14, 55.95 (2C), 61.03, 105.71 (2C), 117.14 (2C), 117.39 (2C), 123.10, 123.71, 129.28 (2C), 129.45 (2C), 131.26, 133.51 (2C), 133.64, 152.99 (2C). MS (ESI): $[M+1]^+$=370.3. Anal. calcd for $C_{21}H_{23}NO_3S$. C, 68.27; H, 6.27; N, 3.79; found: C, 67.98; H, 6.02; N, 3.56.

*5.1.6.13. 3-(4-Propoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**11m**).* Following general procedure E, the crude residue purified by flash chromatography furnished **11m** as a white solid. Yield 81%, mp 118-120 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.05 (t, J=7.2 Hz, 3H), 1.79-1.82 (m, 2H), 3.70 (s, 6H), 3.87 (s, 3H), 3.92 (t, J=6.8 Hz, 2H), 6.50 (s, 2H), 6.83-6.94 (m, 4H), 7.21-7.23 (m, 2H), 8.23 (bs, 1H). ^{13}C-NMR ($CDCl_3$) δ: 10.60, 22.71, 55.97 (2C), 61.02, 69.67, 105.64 (2C), 114.34 (2C), 116.82, 117.03 (2C), 123.44, 123.58, 128.04, 129.98 (2C), 131.51, 152.94 (2C), 157.63. MS (ESI): $[M+1]^+$=368.3. Anal. calcd for $C_{22}H_{25}NO_4$. C, 71.91; H, 6.86; N, 3.81; found: C, 71.57; H, 6.45; N, 3.54.

*5.1.6.14. 3-(4-(Methoxymethoxy)phenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (**11n**).* Following general procedure E, the crude residue purified by flash chromatography

furnished **11n** as a white solid. Yield 20%, mp 126-128 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.36 (s, 3H), 3.58 (s, 6H), 3.63 (s, 3H), 3.82 (s, 2H), 6.45 (s, 2H), 6.86 (t, J=2.3 Hz, 1H), 6.93-6.98 (m, 2H), 7.00 (t, J=2.4 Hz, 1H), 7.13-7.17 (m, 2H), 11.04 (bs, 1H). MS (ESI): $[M+1]^+$=370.3. Anal. calcd for $C_{21}H_{23}NO_5$. C, 68.28; H, 6.28; N, 3.79; found: C, 68.02; H, 5.97; N, 3.47.

5.2. Biological assays and computational studies.

All experimental procedures utilized in the biological and computational studies described here were performed as previously reported: antiproliferative assay [56], tubulin polymerization and colchicine binding to tubulin [57-59], molecular modeling [60, 61], cell cycle analysis [56], annexin V-FITC apoptosis assay [56], mitochondrial membrane potential and ROS assays [56], cellular protein expression and anti-tumor activity *in vivo* [56]. Additional details are provided in the Supplementary data.

5.2.1. Statistical analysis.

Graphs and statistical analyses were performed using GraphPad Prism software (v. 7.0, GraphPad, La Jolla, CA, USA). All data in graphs represented the mean of at least three independent experiments ± SEM. Statistical significance was determined using Student's t-test or ANOVA (one- or two-way) depending on the type of data. Asterisks indicate a significant difference between the treated and the control group, unless otherwise specified * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Disclaimer

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Supplementary data. Protocols for biological assays and computational studies: antiproliferative assay, tubulin polymerization and colchicine binding to tubulin, molecular modeling, cell cycle analysis, annexin V-FITC apoptosis assay, mitochondrial membrane potential and ROS assays, cellular protein expression and anti-tumor activity *in vivo*. ^{1}H-NMR and ^{13}C-NMR spectra of selected compounds **8**, **9a-e**, **9g-h**. **9j-l**, **9n-p**, **10a-c**, **10e**, **10g-h**, **11c-g** and **11i-m.** Supplementary data associated with this article can be found in the online version.

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