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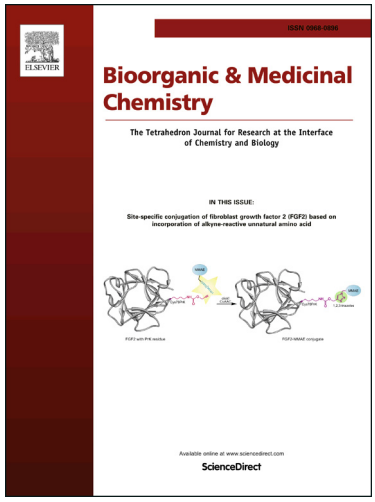


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# Constructing novel dihydrofuran and dihydroisoxazole analogues of *iso*combretastatin-4 as tubulin polymerization inhibitors through [3+2] reactions

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ABSTRACT

[3+2] reactions play a key role in constructing various pharmaceutical moleculars. In this study, using $Mn(OAc)_3$ mediated and 1,3-dipolar [3+2] cyclization reactions, 38 novel dihydrofuran and dihydroisoxazole analogues of *iso*CA-4 were synthesized as inhibitors of tubulin polymerization. Among them, compound **6g** was found to be the most potent cytotoxic agents against PC-3 cells with $IC_{50}$ value of 0.47 μM, and compound **5p** exhibted highest activity on HeLa cells with $IC_{50}$ vaule of 2.32 μM. Tubulin polymerization assay revealed that **6g** was a dose-dependent and effective inhibitor of tubulin assembly. Immunohistochemistry studies and cell cycle distribution analysis indicated that **6g** severely disrupted microtubule network and significantly arrested most cells in the G2/M phase of the cell cycle in PC-3 cells. In addition, molecular docking studies showed that two chiral isomers of **6g** can bind efficiently and similarly at colchicine binding site of tubulin.



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## 1. Introduction

Due to privileged structures and mechanisms, natural products always attract chemists much attention to discovering and developing the final drug entity[1]. It is statisticed that almost 50% commercial drugs in the area of cancer are natural products or their direct derivatives since 1940s[2]. Combretastatin A-4 (CA-4, **Figure 1**), isolated from the stem bark of the African bush willow *Combretum caffrum* in 1989[3, 4], has a strong depolymerization effect on tubulin by combining with the colchicine binding site[5]. As the extremely significant status of tubulin in cell mitosis and search of anticancer drugs[6], CA-4 with a small molecular weight and briefly structure has fascinated a vast amount of further research. Additionally, several clinical used anticancer prodrugs have been carried out, such as CA-4P (fosbretabulin, zybrestat)[7], a water-soluble prodrug synthesized with the 3-*O*-phosphate form and attained the orphan drug status in both USA and Europe for neuroendocrine neoplasm and glioblastoma multiforme[8], and AVE8062 (ombrabulin), which can induce tumour blood flow, acquired from stasis serine amino acid modifying CA-4[9].

The structure-activity relationship (SAR) studies of CA-4 have made clear the indispensability of ring-A (3,4,5-trimethoxy groups) and *cis*-configuration for inhibiting tubulin assembly[10]. Nevertheless, when suffering from heat, light and protic media, the *Z*-restricted configuration can convert rapidly into 100-fold less active *trans*-isomerization[11]. Meanwhile, the poor metabolic stability caused by the olefinic bridge and the weak water solubility of CA-4 resulted in restrictions for its exploitation[12]. Thus, it is clearly that CA-4 and those prodrugs still have a huge development space, and for this reason, an impressive number of analogues have been synthesized. Most derivatization of CA-4 laid their emphasis on the modifications for firming the unstable *Z*-isomerization with various linkers, including silicon[13], butadiene[14], cyclopropane[15], cyclopropyl[16], chiral- lactam[17], furan[18], furazan[19], imidazole[20], 2,3-diarylthiophene[21], *iso*xazole and terphenyl[22], triazoles[23, 24], thiazole[25, 26] and 1,2,4-triazolo-4,3-*b*-pyridazines[27].

Besides, an unexpectedly obtained substance phenstatin[28], and *iso*CA-4, one isomer of CA-4 found by M. Alami[29], share a very interesting structure of the 1,1-diaryl double bond. What's more, both of them have a very strong inhibition on cancer cell growth. The synthetic methods of them are simpler than CA-4 without the control of *cis*-configuration[30, 31]. Hence, M. Alami and co-workers have completed plentiful studies on the modification of 1,1-diaryl double bond scaffold using naphthenic[32], naphthalene[33], arylchromenes[34], azaisoerianin[35], arylbenzoxepins[36] *etc*. Besides, ring-A and ring-B were also transformed to find more active compounds by introductions of fluorine[37], quinolines[38], quinazolines[39], benzofurans and indoles[40]. Of course, there are also some researches worked out by other medicinal chemists, such as oxindole[41], arylcoumarin[42, 43], benzosuberene[44, 45] analogues of *iso*CA-4.

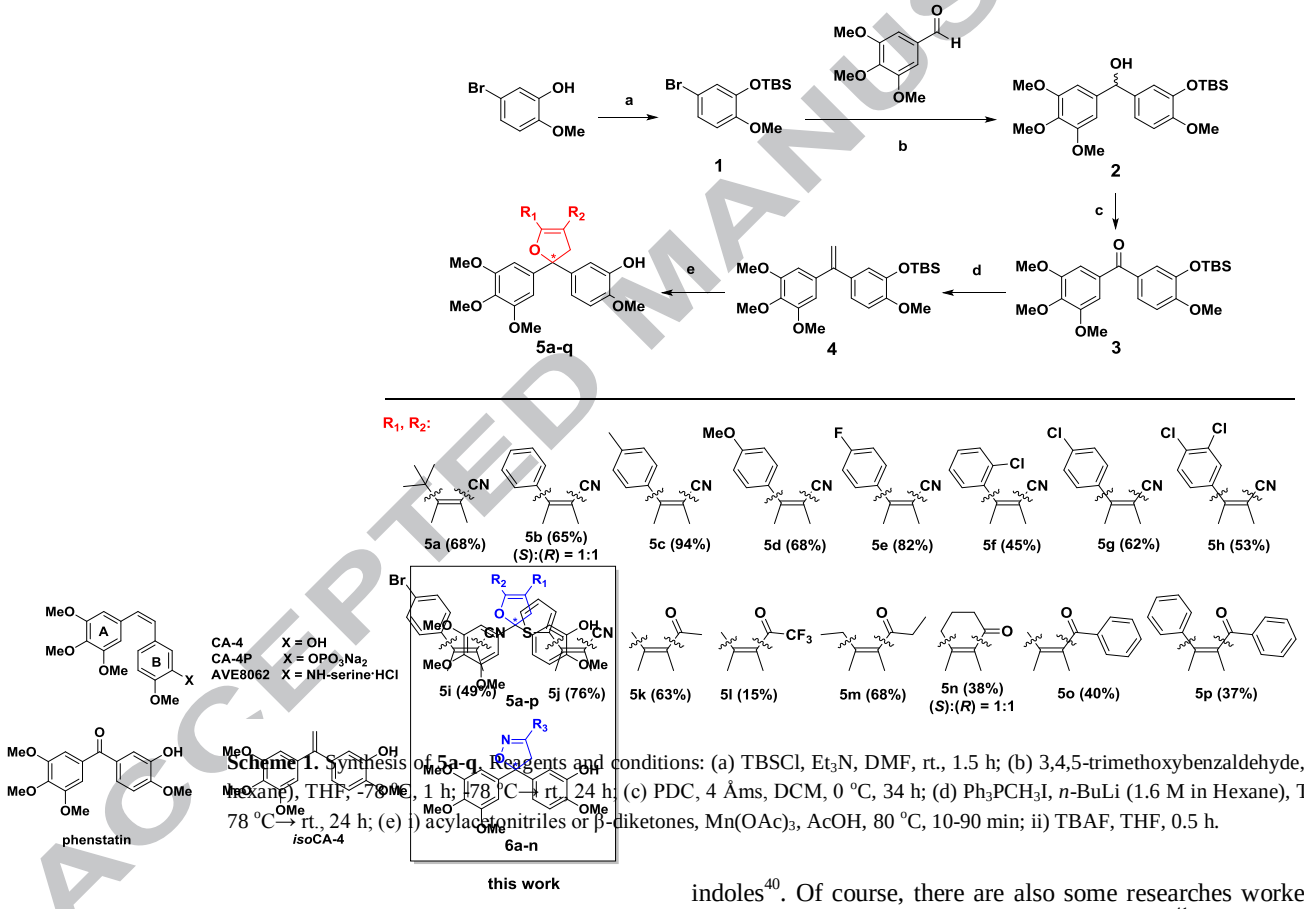



**Figure 1.** The structures of CA-4 and synthetic prodrugs, phenstation, *iso*CA-4, and dihydrofuran, dihydroisoxazole analogues

Dihydrofuran, as a pharmacophore, has gained prominence due to exhibiting excellent and various biological activities. A number of pharmaceuticals (such as aflatoxin B1 and clerodin[46, 47]) and natural products (1-*O*-acetylbritannilactone[48], fraxinellone[49] and azadirachtin[50]) have been explored for clinical applications and further lucubration, respectively. In addition, dihydroisoxazole also has been studied as a vital pharmacophore for inhibitors of HIV[51] and transglutaminase 2[52, 53]. Heretofore, there were few studies for the synthesis of anticancer compounds via introducing dihydrofuran and dihydroisoxazole to *iso*CA-4.

[3+2] reactions including $Mn(OAc)_3$ mediated radical cyclization and 1,3-dipolar cyclization are considered as feasible

**Scheme 1.** Synthesis of **5a-q**. Reagents and conditions: (a) TBSCl, $Et_3N$, DMF, rt., 1.5 h; (b) 3,4,5-trimethoxybenzaldehyde, *n*-BuLi (1.6 M in Hexane), THF, -78 °C, 1 h; -78 °C→ rt., 24 h; (c) PDC, 4 Åms, DCM, 0 °C, 34 h; (d) $Ph_3PCH_3I$, *n*-BuLi (1.6 M in Hexane), THF, -78 °C, 1 h; -78 °C→ rt., 24 h; (e) i) acylacetonitriles or β-diketones, $Mn(OAc)_3$, AcOH, 80 °C, 10-90 min; ii) TBAF, THF, 0.5 h.

measures for the construction of dihydrofuran and dihydroisoxazole compounds. $Mn(OAc)_3$ has drew a great deal of attention in the field of oxidative free-radical chemistry as a single-electron-transfer (SET) reagent with 1.51 V of oxidative potential[54]. Varieties of unique molecules, such as triptolide[55], indolo[1,2-alpha]quinolines[56], polysubstituted pyrroles[57, 58], have been constructed by using its higher selectivity and moderate reactivity. Similarly, 1,3-dipolar cycloaddition reactions also fascinate us due to their superiority of synthetic ability on five-membered heterocycles[59, 60]. That encourages us to adopt these strategies for the constructions of dihydrofuran and dihydroisoxazole analogues of *iso*CA-4.

Therefore, using $Mn(OAc)_3$ mediated and 1,3-dipolar [3+2] cyclization reactions, we herein present the synthesis of two series of novel *iso*CA-4 analogues through the modification of olefin scaffold with dihydrofuran and dihydroisoxazole. Subsequently, their cytotoxic activities were evaluated on PC-3 and HeLa cells. The most potent cytotoxic compound **6g** was selected for tubulin polymerization assay and further probes in mechanism studies, such as immunohistochemistry studies, cell cycle analysis and molecular docking studies.

## 2. Results and discussion

### *2.1. Chemistry*

The synthetic route of target compounds **5a-q** was outlined in **Scheme 1**. As a starting material, 5-bromo-2-methoxyphenol was protected with *tert*-butyldimethylsilyl chloride (TBSCl) in *N*,*N*-dimethylformamide (DMF) and then was reacted with 3,4,5-trimethoxybenzaldehyde to afford the desired alcohol **2**. Subsequently, **2** was oxidized by pyridinium dichromate (PDC) to yield **3**, which produced the key intermediate **4** in 91% yield via methyltriphenylphosphonium iodide ($Ph_3PCH_3I$) Wittig methylenation. Afterwards, **4** was treated with acylacetonitriles or β-diketones by $Mn(OAc)_3$ mediated radical [3+2] cyclization under argon (Ar) atmosphere in glacial acetic acid**,** followed upon tetrabutylammonium fluoride (TBAF) deprotection to target compounds **5a-q**. In this case, the majority of these compounds were generated in modest to good yields (37-94%), except **5l** in 15% yield.

Worsely, we failed in isolating any desired products with some substrates such as 1-(cyanoacetyl)pyrrolidine and methyl acetoacetate. Nonetheless, it was interesting to find that there is no difference in this reaction regardless of the existence or absence of Ar in short time (< 1.5 h). Notably, if the reaction was not terminated in time in the absence of Ar, the main products would convert back into exceedingly oxidic accessory substance (**3**). It is perhaps due to the higher single electron oxidative potential of Mn(III) with 1.51 V in AcOH.

It should be noted that all compounds **5a-q** were synthesized with a chiral carbon atom. The chiral HPLC analysis of **5b** and **5n** revealed that the ratio of chiral isomers is about 1:1. To explore different absolute configurations for biological activities,

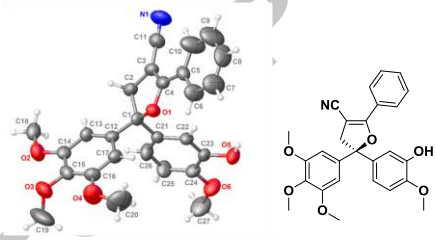
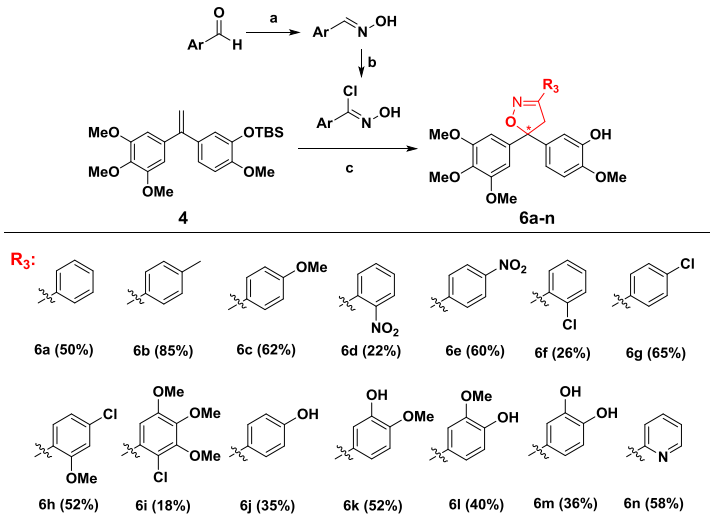


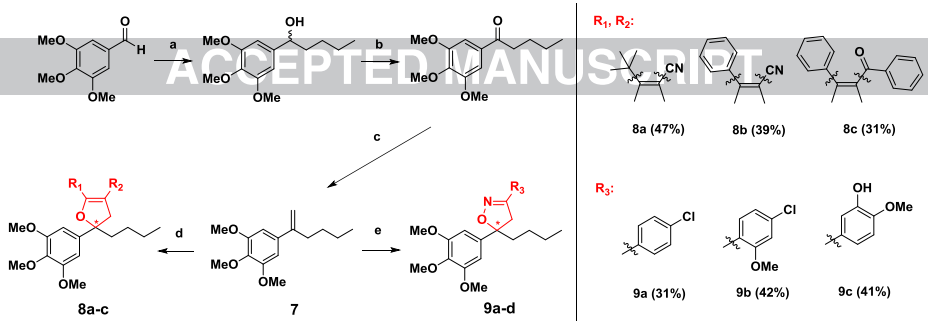
**Figure 2.** The X-ray crystal structure of **(*S*)-5b** (CCDC: 1548952).

**5b** and **5n** were separated to two couples of isomers as the representatives. Fortunately, X-ray crystal structure of **(*S*)-5b**, depicted in **Figure 2**, was acquired to verify this geometric structure. Whereafter, the absolute configurations of other components were confirmed with the help of specific rotations (shown in Table S1).

Subsequently, the dihydroisoxazole analogues **6a-n** were carried out by the [3+2] cycloaddition of intermediate **4** with excess new-made 1,3-dipole congeners and further deprotection (**Scheme 2**). As an intention of enhancing the target compounds competence, 3,4,5-trimethoxybenzene and 3-hydroxy-4-methoxybenzene were retained in ring-A or ring-B. Moreover, 4-hydroxybenzene and 3,4-dihydroxybenzene were also brought in to enlarge the polar in modest yields (35-36%). In this case, compounds **6d**, **6f** and **6i** whose $R_3$ fragment bearing an electron-withdrawing group of *ortho*-$NO_2$ or *ortho*-Cl benzene were synthesized with low yields (18-26%). Yet, **6h** was carried out with a much better yield (52%) with an electron-donor group of methoxyl on *ortho*-position of benzene belonging to $R_3$, suggesting that the conjugation effect of *ortho*-OMe on $R_3$ may dominate the yield in this reaction.




**Scheme 2.** Synthesis of **6a-n.** Reagents and conditions: (a) 50% $NH_2OH$ in $H_2O$, $Et_2O$, 10 min; (b) NCS, DMF, rt. 0.5 h; (c) 1,3-dipole congeners, $Et_3N$, DCM, 0 °C, 1 h; TBAF, THF, 0.5 h.




**Scheme 3.** Synthesis of **8a-c** and **9a-c**. Reagents and conditions: (a) *n*-BuLi (1.6 M in hexane), THF, -78 °C, 1 h; -78 °C→ rt., 24 h; (b) PDC, 4 Åms, DCM, 0 °C, 34 h; (c) $Ph_3PCH_3I$, *n*-BuLi (1.6 M in hexane), THF, -78 °C, 1 h; -78 °C→ rt., 24 h; (d) acylacetonitriles or β-diketones, $Mn(OAc)_3$, AcOH, 80 °C, 0.5 h; (e) 1,3-dipole congeners, $Et_3N$, DCM, 0 °C, 1 h; for **9c**, then TBAF in THF, 0.5 h.

Accidentally, compound **7** is a byproduct of **4** resulted from a previous competing reaction between 3,4,5-trimethoxybenzaldehyde and excess *n*-BuLi with **1**. Next, several products replacing ring-B with butyl were synthesized in **Scheme 3**. Except the sole distinction of butyl, compound **9c** is extremely similar to a compound produced by Daniele Simoni *et al.*[22], which exhibited antiproliferative activity against HeLa cells.

*2.2. Biological evaluation*

2.2.1. Cytotoxic activity

New synthesized compounds **5a-p**, **6a-n, 8a-c** and **9a-c** were screened for anticancer activity against PC-3 (human prostate cancer cell line) and HeLa ( human cervical cancer cell line) with *iso*CA-4 and CA-4 as reference compounds using the sulforhodamine B (SRB) assay[61]. The results of *in vitro* cytotoxic activities ($IC_{50}$) were summarized in **Table 1.**

For dihydrofuran analogues **5a-p**, four compounds **5e** ($R_1$=4-$FC_6H_5$, $R_2$=CN), **5f** ($R_1$=2-$ClC_6H_5$, $R_2$=CN), **5g** ($R_1$=4-$ClC_6H_5$, $R_2$=CN) and **5p** ($R_1$=$C_6H_5$, $R_2$=$COC_6H_5$) exhibited a good cytotoxic activity ($IC_{50}$=0.78-0.86 μM). Compound **5p** displayed best activity against HeLa with $IC_{50}$ vaule of 2.32 μM, fourfold less than CA-4. However, compared with PC-3 cells, HeLa cells showed a much less sensitivity to the dihydrofuran analogues and CA-4, with $IC_{50}$ values of over 10 μM for majority analogues. Notably, the $IC_{50}$ results of **(*R*/*S*)-5b** (9.47 μM for ***R*-5b**, 10.2 μM for ***S*-5b**) and **(*R*/*S*)-5n** (17.0 μM for ***R*-5n**, 15.1 μM for ***S*-5n**) on PC-3 cells indicated no obvious gap in cytotoxic activity between enantiomers.

For dihydroisoxazole analogues **6a-n**, compound **6g** ($R_3$=4-$ClC_6H_5$) exhibited the best ability to inhibit PC-3 cells growth with $IC_{50}$ value of 0.47 μM and a better cytotoxicity on HeLa with $IC_{50}$ value of 3.29 μM. In addition, **6b** and **6f** also displayed cytotoxic activity on PC-3 cells with $IC_{50}$ value of 0.85 and 0.81 μM, respectively, and on HeLa with $IC_{50}$ value of 4.45 and 3.27 μM, respectively. Moreover, **6d, 6i** and **6m** displayed modest cytotoxic activity on PC-3 below 10 μM ($IC_{50}$=8.21–9.56 μm). Interestingly, **6j** ($R_3$=4-$HOC_6H_5$) exhibited potential cytotoxicity with $IC_{50}$ values of 7.57 μM against HeLa, while with $IC_{50}$ value more than 10 μM against PC-3, implying the HeLa cells may be more sensitive to **6j** which is different from other analogues.

For dihydrofuran and dihydroisoxazole analogues **8a-c** and **9a-c** with *n*-butyl-substituted B-ring, all of them displayed unpromising cytotoxic activity over 10 μM on PC-3 cells. The results suggested that B-ring with benzene ring might partly be responsible for cytotoxic activity of these compounds.

2.2.2. Tubulin polymerization assay

To investigate whether there is such an interaction of these novel compounds with tubulin responsible for the cytotoxic activity, we evaluated the effects of the most cytotoxic compound **6g** on tubulin polymerization *in vitro* for 80 minutes at 37 °C. In this assay, paclitaxel was employed as a positive control while *iso*CA-4 and CA-4 as negative controls. The fluorescence increase was monitored every minute for characterization of tubulin polymerization and the results were shown in **Figure 3**. A blank assay of only **6g** (40 μM) was also performed to exclude the jeopardie of a high concentration of **6g** on the results (**Figure S1**). Paclitaxel enhanced the assembly of tubulin, while *iso*CA-4 and CA-4 inhibited it well. The results indicated that compound **6g** displayed a dose-dependent manner on inhibiton of tubulin polymerization with an $IC_{50}$ value of 30.7 μM. From the tubulin polymerization assay, we can conclude that **6g** could inhibit the assembly of tubulin and the cytotoxic activity might be generated from this interaction with tubulin.

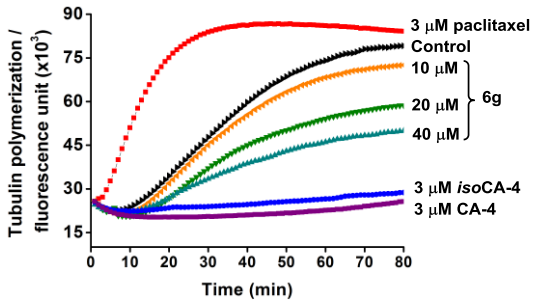



**Figure 3.** Effects of compound **6g**, paclitaxel, CA-4 and *iso*CA-4 on tubulin polymerization *in vitro*. Tubulin (10 mg/mL) was incubated with **6g** (10, 20 and 40 μM), paclitaxel (3 μM), CA-4 (3 μM), *iso*CA-4 (3 μM) and DMSO for 80 minutes at 37 °C. The fluorescence increase was monitored every minute for characterization of tubulin polymerization at 360 nm excitation wavelength and 460 nm emission wavelength.

**Table 1**.
Cytotoxic activities ($IC_{50}$) of **5a-q**, **6a-n**, **8a-c** and **9a-c**.

| Compd. | $IC_{50}$[a] (μM) | | Compd. | $IC_{50}$[a] (μM) | |
|---|---|---|---|---|---|
| | PC-3 | HeLa | | PC-3 | HeLa |
| **5a** | >10 | >10 | **6c** | >10 | >10 |
| **(*R*)-5b** | 9.47 ± 0.11 | >10 | **6d** | 8.21± 1.05 | >10 |

| | | | | | |
|---|---|---|---|---|---|
| **(*S*)-5b** | 10.2 ± 0.07 | >10 | **6e** | >10 | >10 |
| **5c** | 6.99 ± 0.38 | >10 | **6f** | 0.81 ± 0.22 | 3.27 ± 0.65 |
| **5d** | >10 | >10 | **6g** | 0.47 ± 0.11 | 3.29 ± 0.44 |
| **5e** | 0.81 ± 0.06 | 7.21 ± 0.28 | **6h** | >10 | >10 |
| **5f** | 0.79 ± 0.03 | >10 | **6i** | 8.63 ± 0.08 | >10 |
| **5g** | 0.86 ± 0.10 | >10 | **6j** | >10 | 7.57 ± 1.75 |
| **5h** | >10 | >10 | **6k** | >10 | >10 |
| **5i** | >10 | >10 | **6l** | >10 | >10 |
| **5j** | 4.80 ± 0.17 | >10 | **6m** | 9.56 ± 0.86 | >10 |
| **5k** | 6.37 ± 0.26 | >10 | **6n** | >10 | >10 |
| **5l** | 9.45 ± 0.50 | >10 | **8a** | >10 | >10 |
| **5m** | 8.47 ± 0.10 | >10 | **8b** | >10 | >10 |
| **(*R*)-5n** | 17.0 ± 0.08 | >10 | **8c** | >10 | >10 |
| **(*S*)-5n** | 15.1 ± 0.06 | >10 | **9a** | >10 | >10 |
| **5o** | >10 | >10 | **9b** | >10 | >10 |
| **5p** | 0.78 ± 0.09 | 2.32 ± 0.04 | **9c** | >10 | >10 |
| **6a** | >10 | >10 | ***iso*CA-4** | 0.001 ± 0.001 | 0.085 ± 0.02 |
| **6b** | 0.85 ± 0.11 | 4.45 ± 0.54 | **CA-4** | 0.007 ± 0.005 | 0.53 ± 0.14 |

[a] The $IC_{50}$ values represent the concentration that causes 50% inhibition of cell viability. Cells were treated with the compounds for 48 h. All data (mean ± SD) are the average of three determinations. Cancer cell lines: PC-3 (human prostate cancer), HeLa (human cervix cancer).

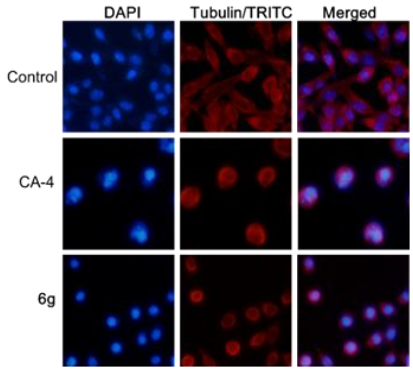



**Figure 4.** Effects of compound **6g** (1.0 μM) and CA-4 (0.02 μM) on inhibition of tubulin polymerization in PC-3 cells by immunofluorescence. The cell nucleus was visualized with DAPI (blue, left panel), and microtubules were visualized with an anti-α-tubulin antibody (TRITC, red, middle panel). The right panel represents a merge of the corresponding left and middle panels. Images were acquired with a fluorescence microscope using a 20× objective.

### 2.2.3. Immunohistochemistry studies

To visualize the tubulin polymerization inhibition of these novel compounds in cells, we investigated immunocytochemistry studies to examine effects of compound **6g** (1.0 μM) and CA-4 (0.02 μM) on microtubule network in PC-3 cells by immunofluorescence. As shown in **Figure 4**, control cells displayed a normal distribution of microtubular network with a slim and fibrous shape. However, cells treated with **6g** and CA-4 showed a disrupted microtubule network becoming short and wrapped around cell nucleus, demonstrating that 6g would inhibit the tubulin polymerization of cells.

### 2.2.4. Cell cycle analysis

To confirm the biological mechanism of these novel compounds, we further used flow cytometry to analyze the cell cycle distribution of PC-3 cells after treatment with **6g** (0.5 and 1.0 μM) and CA-4 (0.05 μM) for 24 h. Representative FACS measurements from a standard propidium iodide (PI) procedure were depicted in **Figure 5**. A distinct cell cycle arrest at G2/M phase (71.9%) was observed in PC-3 cells treated with CA-4, compared to control group (14.0%). Similarly, the cell accumulation percentage at the G2/M phase increased from 63.5% to 84.5% with a concentration rising from 0.5 to 1.0 μM of **6g**. The observed effects of **6g** on cell cycle progression correlated well with its cytotoxic and anti-tubulin activities, in agreement with the similar properties reported previously for the majority of antimitotic agents.

## *2.3. Molecular modeling*

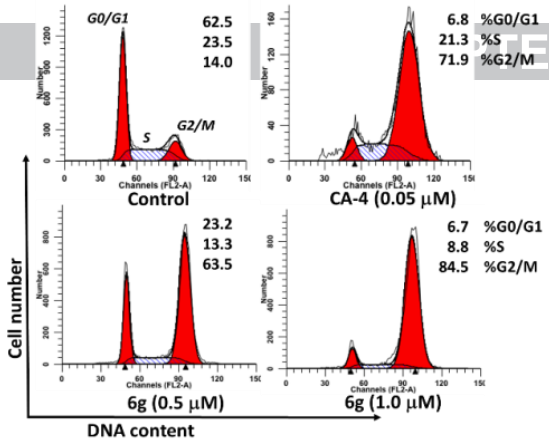



**Figure 6.** Flow cytometric analysis of cell cycle distribution in PC-3 cells. The cultured cells were treated with CA-4 and **6g** at the indicated concentrations for 24 h, then analyzed by a standard propidium iodide (PI) procedure using a FACS Calibur (BD Biosciences).

To elucidate the binding modeling of these novel analogues with the tubulin colchicine binding site, the X-ray crystal structure (PDB: 1SA0) was employed for performing the docking studies of the most potent compound **6g** using the Surflex-Dock program in the Sybyl-X 2.0 software. Taking into consideration the mixed enantiomers of **6g** in cytotoxic activity, we executed the docking studies of both enantiomers of **(*S*)-6g** and **(*R*)-6g**. **Figure 6a** exhibits **(*S*)-6g** and **(*R*)-6g** can bind well with the colchicines binding site of tubulin. The *meta*-methoxy groups on ring-A of both **(*S*)-6g** and **(*R*)-6g** formed two hydrogen bonds with the key amino acid Cys241 about 2.0 Å and 2.1 Å bond distance, respectively. Meanwhile, another two hydrogen bonds formed between the oxygen atoms of the hydroxyl groups and amino acid Val181 about 2.0 Å and 1.8 Å bond distance, respectively. It is noteworthy that **(*R*)-6g** and **(*S*)-6g** bear almost the same overlay form at the colchicine binding site with a sole difference on the direction of dihydroisoxazole which have little influence on binding with colchicine binding site. The similar results (Figure S1) also were carried out on *R*/*S* enantiomers of **5g**, a dihydrofuran analogue exhibiting a good cytotoxic activity against PC-3 cells. That will be a possible explanation for the few distinction in cytotoxic activity between *R*/*S* enantiomers of **5b** and **5n**. **Figure 6b** shows that **(*S*)-6g**, **(*R*)-6g** superimposed well with *iso*CA-4 and CA-4 at the colchicine binding site with similar distorted conformations. These docking studies suggested that the mechanism of **6g** inhibition tubulin may be the same as that of *iso*CA-4 and CA-4.

## 3. Conclusions

In the current work, based on $Mn(OAc)_3$ mediated and 1,3-dipolar [3+2] cycloaddition reactions, we synthesized thirty-eight *iso*CA-4 analogues belonging to two series of novel dihydrofuran and dihydroisoxazole and evaluated their activities as inhibitors of tubulin polymerization. Most compounds exhibited potential cytotoxic activity. Among them, **6g** was found to be the most potent cytotoxic agents against PC-3 cells with $IC_{50}$ value of 0.47 μM, and **5p** exhibted best activity on HeLa cells with $IC_{50}$ vaule of 2.32 μM. Tubulin polymerization assay revealed that **6g** was a dose-dependent and effective inhibitor of tubulin assembly. As probes in mechanism studies, immunohistochemistry studies and cell cycle analysis indicated that **6g** disrupted microtubule network severely and significantly arrested most cells in the G2/M phase of the cell cycle in PC-3 cells. In addition, molecular docking studies showed that two chiral isomers of **6g** binded similarly and efficiently at colchicine binding site on tubulin and superimposed well with *iso*CA-4 and CA-4 in similar distorted conformations. Although the anticancer activity evaluation of the two series of novel compounds did not perform as excellently as *iso*CA-4, our work expanded the research of olefin modification of tubulin polymerization agent *iso*CA-4 and provided fundamental data and additional insight for new developments of tubulin inhibitors.

## 4. Experimental section

### *4.1. Chemistry*

#### 4.1.1. General

All the compounds were inspected by a 500 MHz Bruker NMR spectrometer with $^1H$, $^{13}C$ and dept135 NMR in $CDCl_3$ solution ($\delta$: 7.26). Chemical shift values are given in $\delta$ (ppm) and coupling constants ($J$) are mentioned in Hz. MS (ESI) was measured on an ESI-Thermo Fisher LTQ Fleet instrument spectrometer. HR-ESI-MS spectra were recorded on a LTQ Orbitrap (Thermo Scientific). Analytical and preparative chical HPLC were performed on Waters 515-2996 or Shimadzu Essentia Prep LC-16 with columns of *S*-Chiral A/B or Daicel OD-H. The reaction progress was supervised by TLC (Qingdao Marine Chemical Group, Co.), spots were observed under UV light (254, 365 nm) and colorated with 5% phosphomolybdic acid. Silica gels (300-400 mesh, Qingdao Marine Chemical Ltd.) were used for column chromatography (CC). All reagents were purchased from commercial sources and used without further refinement. While the purifications and desiccations of commercially available solvents were accomplished by standard techniques before using. Positive control drug *iso*CA-4 was obtained by the deprotection of **4**, and it's $^1H$ NMR and MS (ESI) data were carried out for supporting the structure.

#### 4.1.2. *Synthesis of (3-((tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone **(3)***

*Tert*-butyldimethylsilyl chloride (TBSCl, 6.03 g, 40 mmol) was added to a solution of 5-bromo-2-methoxyphenol (4.06 g, 20 mmol) in *N*,*N*-dimethylformamide (DMF, 20 mL), followed by triethylamine (5.6 mL, 40 mmol). After 1.5 h, the mixture was extracted with diethyl ether (3×20 mL), and the combined organic phases were evaporated under reduced pressure to give crude product of **1**. Next, 1.6 mol/L *n*-butyl lithium in hexane (27.5 mL, 44 mmol) was added to a stirred suspension of crude products of **1** in dry tetrahydrofuran (THF, 20 mL) at -78 °C under argon atmosphere. After 1 h, 3,4,5-trimethoxybenzaldehyde (5.47 g, 26 mmol, 20 mL) in dry THF was slowly added to the resulting yellowish solution, allowing the reaction to slowly reach room temperature. After 24 h, the mixture was poured over ice and extracted with $CH_2Cl_2$. The organic phases were dried over anhydrous $Na_2SO_4$, filtered, and evaporated under reduced pressure to give crude product of **2**.

Pyridinium dichromate (PDC, 11.3 g, 30 mmol) was added to a solution of crude products of **2** and 4 Å molecular sieves in $CH_2Cl_2$ (50 mL) at 0 °C. After 24 h, the reaction mixture was filtered through pads of celite. The filtrates were evaporated under reduced pressure, and crude products were purified by column chromatography on silica gel using petroleum ether/ethyl acetate (8:1) as eluent.

That gave compound **3** as a white solid (4.30 g, 49%); $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$ = 7.42 (dd, $J$ = 8.3, 1.8 Hz, 1H), 7.35 (d, $J$ = 1.8 Hz, 1H), 7.01 (s, 2H), 6.88 (d, $J$ = 8.4 Hz, 1H), 3.91 (s, 3H), 3.87 (s, 3H), 3.86 (s, 6H), 0.98 (s, 9H), 0.16 ppm (s, 6H); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ = 194.53, 154.94, 152.80 (2C), 144.69, 141.55, 133.39, 130.44, 125.31, 122.34, 110.75, 107.44

(2C), 60.96, 56.28 (2C), 55.51, 25.68 (3C), 18.44, -4.54 ppm ($2C_{C\text{-}Si}$); MS (ESI): *m/z* calcd for $C_{23}H_{33}O_6Si^+$ $[M+H]^+$ 433.60, found 433.18.

4.1.3. *Synthesis of tert-butyl(2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)vinyl)phenoxy)dimethylsilane **(4)***

A solution of methyltriphenylphosphonium iodide ($Ph_3PCH_3I$, 11.32 g, 28 mmol) in dry THF (50 mL) was cooled to -78 °C under argon atmosphere. Then, 1.6 mol/L *n*-butyl lithium in hexane (12.5 mL, 20 mmol) was added dropwise, and the resulting yellow solution was stirred for 1 h. Then a solution of **3** (4.30 g, 10 mmol) in dry THF was added and warmed to room temperature. Once completed, the mixtures were cooled to 0 °C, stopped with saturated $NH_4Cl$ solution and extracted with $CH_2Cl_2$ (3×50 mL). The organic phases were washed with brine, dried over anhydrous $Na_2SO_4$, filtered, and evaporated under reduced pressure. Crude products were purified by column chromatography on silica gel using petroleum ether/ethyl acetate (10:1) as eluent. That gave compound **4** as a pale green oil (4.0 g, 93%); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 6.91 (dd, *J* = 8.3, 2.2 Hz, 1H), 6.85 (d, *J* = 2.2 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 1H), 6.55 (s, 2H), 5.34 (d, *J* = 1.1 Hz, 1H), 5.30 (d, *J* = 1.1 Hz, 1H), 3.87 (s, 3H), 3.81 (s, 3H), 3.80 (s, 6H), 0.98 (s, 9H), 0.15 ppm (s, 6H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 152.84 (2C), 150.88, 149.56, 144.55, 137.79, 137.52, 134.11, 121.78, 120.95, 112.45, 111.55, 105.69 (2C), 60.90, 56.09(2C), 55.49, 25.75 (3C), 18.47, -4.54 ppm ($2C_{C\text{-}Si}$); MS (ESI): *m/z* calcd for $C_{24}H_{35}O_5Si^+$ $[M+H]^+$ 431.62, found 431.31.

4.1.4. *Synthesis of **isocombretastatin-4***

Compound **4** (50 mg, 116 μM) were dissolved in tetrahydrofuran (5 mL), followed by 1.0 mol/L TBAF (175 μL, 0.175 mmol) in THF for 0.5 h. Water (10 mL) was added to this solution and extracted with ethyl acetate (3×10 mL). The combined organic phases were dried over anhydrous $Na_2SO_4$, filtered and evaporated. Crude products were purified by column chromatography on silica gel using petroleum ether/ethyl acetate (3:1).That gave the compound *iso*CA-4 as a white soild (34 mg, 92%); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 6.97 (d, *J* = 1.8 Hz, 1H), 6.84 (dd, *J* = 8.4, 1.9 Hz, 1H), 6.80 (d, *J* = 8.3 Hz, 1H), 6.55 (s, 2H), 5.37 (s, 1H), 5.30 (s, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.81 ppm (s, 6H); MS (ESI): *m/z* calcd for $C_{18}H_{19}O_5^-$ $[M-H]^-$ 315.35, found 315.09.

4.1.5. *Synthesis of 5-(hex-1-en-2-yl)-1,2,3-trimethoxybenzene **(7)***

The preparation of **7** shared a same method with intermediate **4** except the absent of purification during process in the second preparation of **4**. That gave compound **7** as a pale green liquild (1.0 g, 20%); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 6.60 (s, 2H), 5.19 (s, 1H), 5.01 (s, 1H), 3.85 (s, 6H), 3.84 (s, 3H), 2.45 (t, *J* = 7.5 Hz, 2H), 1.47 – 1.40 (m, 2H), 1.38 – 1.30 (m, 2H), 0.89 ppm (t, *J* = 7.3 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 152.95 (2C), 148.81, 137.52, 137.50, 111.75, 103.45 (2C), 60.86, 56.09 (2C), 35.22, 30.47, 22.43, 13.95 ppm; MS (ESI): *m/z* calcd for $C_{15}H_{23}O_3^+$ $[M+H]^+$ 251.35, found 251.18.

4.1.6. *General procedure for the synthesis of compounds **5a-p** and **8a-c***

A solution of manganese(III) acetate dehydrate (109 mg, 0.41 mmol or 156 mg, 0.58 mmol) in glacial acetic acid (2 mL) was heated under argon atmosphere at 80 °C until it dissolved completely. After the solution was cooled down to 70 °C, a solution of **4** (50 mg, 0.12 mmol) or **7** (30 mg, 0.12 mmol) and acylacetonitriles or □-diketones (0.18 mmol) in acetic acid were added to this mixture, and the temperature rised to 80 °C. The reaction completed when the dark brown colour of the solution disappeared (in 10-90 min). Acetic acid was evaporated under reduced pressure, and water (10 mL) was added to the residue. Then the mixture was neutralized with sated $NaHCO_3$ solution and extracted with $CHCl_3$ (3×10 mL). The combined organic phases were evaporated under reduced pressure.

The residues from reactions of **4** and acylacetonitriles or β-diketones were dissolved in THF (2 mL), followed by 1.0 mol/L TBAF (175 μL, 0.175 mmol) in THF in 0.5 h. Water (10 mL) was added to this solution and extracted with ethyl acetate (3×10 mL). The combined organic phases were dried over anhydrous $Na_2SO_4$, filtered and evaporated. Crude products were purified by column chromatography on silica gel using petroleum ether/ethyl acetate as eluent.

4.1.6.1. *2-(tert-butyl)-5-(3-hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydrofuran-3-carbonitrile **(5a)***

This compound was obtained from reaction of **4** with 4,4-dimethyl-3-oxopentanenitrile after 50 min, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 5:1. Reddish brown solid. Yield: 35 mg, 68%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 6.90 (d, *J* = 1.8 Hz, 1H), 6.80 (dd, *J* = 6.9, 5.2 Hz, 2H), 6.55 (s, 2H), 5.69 (s, 1H), 3.88 (s, 3H), 3.84 (s, 3H), 3.81 (s, 6H), 3.56 (d, *J* = 14.3 Hz, 1H), 3.49 (d, *J* = 14.3 Hz, 1H), 1.38 ppm (s, 9H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 177.12, 153.10 (2C), 146.31, 145.54, 140.14, 137.49, 137.41, 117.36, 117.02, 112.43, 110.20, 102.87 (2C), 91.95, 77.66, 60.87, 56.18 (2C), 56.00, 46.01, 34.84, 28.11 ppm (3C); MS (ESI): *m/z* calcd for $C_{25}H_{30}NO_6^+$ $[M+H]^+$ 440.52, found 440.28.

4.1.6.2. *5-(3-hydroxy-4-methoxyphenyl)-2-phenyl-5-(3,4,5-trimethoxyphenyl)-4,5-dihydrofuran-3-carbonitrile **(5b)***

This compound was obtained from reaction of **4** with 3-oxo-3-phenylpropanenitrile after 1 h, followed by TBAF 0.5 h. Eluent: petroleum ether/acetone = 10:1. Yellow solid. (***R*)-5b** and (***S*)-5b** were prepared by chical HPLC performed on Waters 515-2996 with columns of *S*-Chiral B. (***R*)-5b**: yield: 18 mg, 34%; mp: 148-150 °C; $[\alpha]_D^{30}$= -74.99° (*c* 0.036 in $CHCl_3$); (***S*)-5b**: yield: 17 mg, 31%; mp: 147-149 °C; $[\alpha]_D^{30}$= +98.22° (*c* 0.036 in $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 8.07 (dd, *J* = 7.7, 1.7 Hz, 2H), 7.48 (s, 2H), 7.47 (s, 1H), 7.00 (d, *J* = 2.1 Hz, 1H), 6.90 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 1H), 6.64 (s, 2H), 5.82 (s, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.81 (s, 6H), 3.75 (d, *J* = 14.9 Hz, 1H), 3.70 ppm (d, *J* = 14.8 Hz, 1H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 165.25, 153.19 (2C), 146.52, 145.65, 139.62, 137.71, 136.83, 131.57, 128.85 (2C), 127.98, 127.13 (2C), 117.44, 117.39, 112.60, 110.34, 103.17 (2C), 92.84, 79.22, 60.87, 56.27 (2C), 56.01, 45.61 ppm. MS (ESI): *m/z* calcd for $C_{27}H_{24}NO_6^-$ $[M-H]^-$ 458.49, found 458.15; HRMS (ESI): *m/z* calcd for $C_{27}H_{26}NO_6^+$ $[M+H]^+$ 460.17546, found 460.17460; Chiral HPLC: (***S*)-5b**: $t_R$ = 10.2 min, purity = 99.7%; (***R*)-5b**: $t_R$ = 10.9 min, purity = 98.6%@ 220 nm, 25% ethanol in hexane.

4.1.6.3. *5-(3-hydroxy-4-methoxyphenyl)-2-(p-tolyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydrofuran-3-carbonitrile **(5c)***

This compound was obtained from reaction of **4** with 3-oxo-3-(*p*-tolyl)propanenitrile after 1.5 h, followed by TBAF 0.5 h. Eluent: petroleum ether/ ethyl acetate = 3:1. Yellow solid. Yield: 52 mg, 94%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.96 (d, *J* = 8.3 Hz, 2H), 7.27 (d, *J* = 8.2 Hz, 2H), 6.99 (d, *J* = 2.3 Hz, 1H), 6.89 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 1H), 6.62 (s, 2H), 5.73 (s, 1H), 3.87 (d, *J* = 2.0 Hz, 3H), 3.83 (s, 3H), 3.81 (s, 6H), 3.72 (d, *J* = 14.7 Hz, 1H), 3.67 (d, *J* = 14.7 Hz, 1H), 2.41 ppm (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 165.42, 153.17 (2C), 146.44, 145.61, 142.14, 139.66, 137.73, 136.96, 129.51 (2C), 127.11 (2C), 125.25, 117.59, 117.45, 112.60, 110.29, 103.22

(2C), 92.66, 78.25, 60.85, 56.26 (2C), 56.02, 45.61, 21.65 ppm; MS (ESI): *m/z* calcd for $C_{28}H_{27}NNaO_6^+$ $[M+Na]^+$ 496.51, found 496.32.

4.1.6.4. *5-(3-hydroxy-4-methoxyphenyl)-2-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydrofuran-3-carbonitrile* ***(5d)***

This compound was obtained from reaction of **4** with 3-(4-methoxyphenyl)-3-oxopropanenitrile after 10 min, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 3:1. Yellow solid. Yield: 39 mg, 68%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 8.04 (d, *J* = 8.9 Hz, 2H), 6.99 (d, *J* = 2.3 Hz, 1H), 6.97 (d, *J* = 8.9 Hz, 2H), 6.89 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 1H), 6.62 (s, 2H), 5.71 (s, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H), 3.81 (s, 6H), 3.71 (d, *J* = 14.6 Hz, 1H), 3.66 ppm (d, *J* = 14.6 Hz, 1H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 165.16, 162.08, 153.16 (2C), 146.41, 145.59, 139.68, 137.72, 137.00, 128.98 (2C), 120.60, 117.91, 117.46, 114.19 (2C), 112.60, 110.27, 103.23 (2C), 92.58, 76.95, 60.86, 56.26 (2C), 56.02, 55.49, 45.55 ppm. MS (ESI): *m/z* calcd for $C_{28}H_{27}NNaO_7^+$ $[M+Na]^+$ 512.51, found 512.23.

4.1.6.5. *2-(4-fluorophenyl)-5-(3-hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydrofuran-3-carbonitrile* ***(5e)***

This compound was obtained from reaction of **4** with 3-(4-fluorophenyl)-3-oxopropanenitrile after 1 h, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 4:1. Yellow solid. Yield: 45 mg, 82%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 8.11 – 8.05 (m, 2H), 7.18 – 7.13 (m, 2H), 6.98 (d, *J* = 2.3 Hz, 1H), 6.87 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 1H), 6.61 (s, 2H), 5.76 (s, 1H), 3.87 (s, 3H), 3.83 (s, 3H), 3.81 (s, 6H), 3.73 (d, *J* = 14.8 Hz, 1H), 3.68 ppm (d, *J* = 14.8 Hz, 1H). $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 164.41, ($J_{C\text{-}F}$ = 254.1 Hz), 164.17, 153.22 (2C), 146.53, 145.67, 139.39, 137.89, 136.73, 129.50, 129.43, 124.30 ($J_{C\text{-}F}$ = 3.3 Hz), 117.44, 117.26, 116.16, 115.98, 112.56, 110.33, 103.30 (2C), 93.02, 78.98 ($J_{C\text{-}F}$ = 1.3 Hz), 60.84, 56.29, 56.01 (2C), 45.53 ppm. MS (ESI): *m/z* calcd for $C_{27}H_{23}FNO_6^-$ $[M-H]^-$ 476.48, found 476.30.

4.1.6.6. *2-(2-chlorophenyl)-5-(3-hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydrofuran-3-carbonitrile* ***(5f)***

This compound was obtained from reaction of **4** with 3-(2-chlorophenyl)-3-oxopropanenitrile after 10 min, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 4:1. Yellow solid. Yield: 26 mg, 45%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.55 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.49 (dd, *J* = 8.0, 0.7 Hz, 1H), 7.42 (td, *J* = 7.8, 1.5 Hz, 1H), 7.35 (td, *J* = 7.5, 1.1 Hz, 1H), 6.99 (d, *J* = 2.2 Hz, 1H), 6.90 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.83 (d, *J* = 8.5 Hz, 1H), 6.64 (s, 2H), 5.68 (s, 1H), 3.89 (s, 3H), 3.85 (s, 3H), 3.82 (s, 6H), 3.78 (d, *J* = 14.9 Hz, 1H), 3.70 ppm (d, *J* = 14.9 Hz, 1H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 165.63, 153.17 (2C), 146.52, 145.59, 139.60, 137.69, 136.83, 133.16, 132.10, 130.96, 130.61, 127.71, 127.00, 117.70, 115.83, 112.69, 110.25, 103.29 (2C), 94.41, 84.75 , 60.89, 56.27 (2C), 56.03, 45.26 ppm. MS (ESI): *m/z* calcd for $C_{27}H_{25}ClNO_6^+$ $[M+H]^+$ 494.95, found 494.07.

4.1.6.7. *2-(4-chlorophenyl)-5-(3-hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydrofuran-3-carbonitrile* ***(5g)***

This compound was obtained from reaction of **4** with 3-(4-chlorophenyl)-3-oxopropanenitrile after 10 min, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 3:1. Yellow solid. Yield: 36 mg, 62%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 8.01 (d, *J* = 8.6 Hz, 2H), 7.45 (d, *J* = 8.7 Hz, 2H), 6.97 (d, *J* = 2.2 Hz, 1H), 6.87 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 1H), 6.59 (s, 2H), 3.88 (s, 3H), 3.84 (s, 3H), 3.81 (s, 6H), 3.74 (d, *J* = 14.9 Hz, 1H), 3.68 ppm (d, *J* = 15.0 Hz, 1H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 164.03, 153.22 (2C), 146.51, 145.64, 139.30, 137.86, 137.65, 136.65, 129.18 (2C), 128.42 (2C), 126.40, 117.45, 117.08, 112.53, 110.29, 103.22 (2C), 93.07, 79.78, 60.88, 56.29 (2C), 56.03, 45.61 ppm. MS (ESI): *m/z* calcd for $C_{27}H_{24}ClNNaO_6^+$ $[M+Na]^+$ 516.93, found 516.20.

4.1.6.8. *2-(3,4-dichlorophenyl)-5-(3-hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydrofuran-3-carbonitrile* ***(5h)***

This compound was obtained from reaction of **4** with 3-(3,4-dichlorophenyl)-3-oxopropanenitrile after 10 min, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 3:1. Yellow solid. Yield: 33 mg, 53%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 8.07 (d, *J* = 2.0 Hz, 1H), 7.95 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.55 (d, *J* = 8.5 Hz, 1H), 6.95 (d, *J* = 2.0 Hz, 1H), 6.85 – 6.81(m, 2H), 6.58 (s, 2H), 5.72 (s, 1H), 3.89 (s, 3H), 3.84 (s, 3H), 3.81 (s, 6H), 3.74 (d, *J* = 11.7 Hz, 1H), 3.70 ppm (d, *J* = 12.3 Hz, 1H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 162.64, 153.25 (2C), 146.60, 145.68, 139.02, 137.95, 136.38, 135.82, 133.42, 130.97, 128.76, 127.74, 126.26, 117.50, 116.61, 112.52, 110.32, 103.23 (2C), 93.40, 81.02, 60.88, 56.31 (2C), 56.04, 45.62 ppm; MS (ESI): *m/z* calcd for $C_{27}H_{24}Cl_2NO_6^+$ $[M+H]^+$ 529.39, found 528.13.

4.1.6.9. *2-(4-bromophenyl)-5-(3-hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydrofuran-3-carbonitrile* ***(5i)***

This compound was obtained from reaction of **4** with 3-(4-bromophenyl)-3-oxopropanenitrile after 10 min, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 3:1. Yellow solid. Yield: 31 mg, 49%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.93 (d, *J* = 8.6 Hz, 2H), 7.61 (d, *J* = 8.6 Hz, 2H), 6.97 (d, *J* = 2.2 Hz, 1H), 6.86 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 1H), 6.59 (s, 2H), 5.69 (s, 1H), 3.88 (s, 3H), 3.84 (s, 3H), 3.81 (s, 6H), 3.73 (d, *J* = 15.0 Hz, 1H), 3.67 ppm (d, *J* = 14.9 Hz, 1H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 164.09, 153.22 (2C), 146.52, 145.65, 139.29, 137.88, 136.64, 132.15 (2C), 128.55 (2C), 126.83, 126.11, 117.45, 117.05, 112.53, 110.29, 103.23 (2C), 93.08, 79.92, 60.88, 56.29 (2C), 56.04, 45.63 ppm; MS (ESI): *m/z* calcd for $C_{27}H_{24}BrNNaO_6^+$ $[M+Na]^+$ 561.38, found 560.15.

4.1.6.10. *5-(3-hydroxy-4-methoxyphenyl)-2-(thiophen-2-yl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydrofuran-3-carbonitrile* ***(5j)***

This compound was obtained from reaction of 4 with 3-oxo-3-(thiophen-2-yl)propanenitrile after 1.5 h, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 2:1. Yellow solid. Yield: 41 mg, 76%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.93 (d, *J* = 3.7 Hz, 1H), 7.54 (d, *J* = 4.9 Hz, 1H), 7.17 (dd, *J* = 4.8, 4.0 Hz, 1H), 6.98 (d, *J* = 2.3 Hz, 1H), 6.88 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 1H), 6.63 (s, 2H), 5.65 (s, 1H), 3.89 (s, 3H), 3.83 (s, 3H), 3.82 (s, 6H), 3.73 (d, *J* = 14.7 Hz, 1H), 3.66 ppm (d, *J* = 14.7 Hz, 1H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 160.65, 153.18 (2C), 146.47, 145.59, 139.21, 137.77, 136.57, 130.20, 130.05, 129.75, 128.30, 117.56, 117.00, 112.60, 110.27, 103.12 (2C), 93.68, 77.66, 60.86, 56.25 (2C), 56.03, 45.38 ppm; MS (ESI): *m/z* calcd for $C_{25}H_{24}NO_6S^+$ $[M+H]^+$ 466.53, found 466.23.

4.1.6.11. *1-(5-(3-hydroxy-4-methoxyphenyl)-2-methyl-5-(3,4,5-trimethoxyphenyl)-4,5-dihydrofuran-3-yl)ethan-1-one* ***(5k)***

This compound was obtained from reaction of **4** with pentane-2,4-dione after 1 h, followed by TBAF 0.5 h. Eluent: petroleum ether/acetone = 2:1. Yellow solid. Yield: 30 mg, 63%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 6.90 (d, *J* = 1.9 Hz, 1H), 6.77 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.73 (d, *J* = 8.5 Hz, 1H), 6.56 (s, 2H), 6.27 (s, 1H), 3.77 (s, 6H), 3.77 (s, 6H), 3.58 (d, *J* = 14.5 Hz, 1H), 3.53 (d, *J* = 14.4 Hz, 1H), 2.32 (s, 3H), 2.10 ppm (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 194.35, 165.96, 152.99 (2C), 146.36, 145.62, 140.69, 138.01, 137.26, 117.18, 112.52, 112.45, 110.37, 103.08 (2C), 91.48, 60.77, 56.17 (2C), 55.91, 44.75, 29.32, 15.27 ppm; MS (ESI): *m/z* calcd for $C_{23}H_{25}O_7^-$ $[M-H]^-$ 413.45, found 413.50.

4.1.6.12. *2,2,2-trifluoro-1-(5-(3-hydroxy-4-methoxyphenyl)-2-methyl-5-(3,4,5-trimethoxyphenyl)-4,5-dihydrofuran-3-yl)ethan-1-one* ***(5l)***

This compound was obtained from reaction of **4** with 1,1,1-trifluoropentane-2,4-dione after 1 h, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 4:1. Yellow solid. Yield: 8 mg, 15%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 6.91 (s, 1H), 6.81 (s, 2H), 6.56 (s, 2H), 5.71 (s, 1H), 3.88 (s, 3H), 3.84 (s, 3H), 3.82 (s, 6H), 3.74 (d, *J* = 14.4 Hz, 1H), 3.68 (d, *J* = 14.2 Hz, 1H), 2.46 ppm (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 176.17, 175.89, 153.20 (2C), 146.46, 145.62, 139.40, 137.80, 136.93, 117.37, 116.71 ($J_{C\text{-}F}$ = 291.7), 112.42, 110.28 , 105.69, 103.20 (2C), 94.51, 60.85, 56.27 (2C), 56.00, 42.16 ($J_{C\text{-}F}$ = 2.8 Hz), 15.98 ppm; MS (ESI): *m/z* calcd for $C_{23}H_{22}F3O_7^-$ $[M\text{-}H]^-$ 467.42, found 467.10.

4.1.6.13. *1-(2-ethyl-5-(3-hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydrofuran-3-yl)propan-1-one* ***(5m)***

This compound was obtained from reaction of **4** with heptane-3,5-dione after 20 min, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 4:1. Yellow solid. Yield: 44 mg, 86%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 6.96 (d, *J* = 2.2 Hz, 1H), 6.84 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.79 (d, *J* = 8.5 Hz, 1H), 6.61 (s, 2H), 5.64 (s, 1H), 3.87 (s, 3H), 3.83 (s, 3H), 3.82 (s, 6H), 3.64 (d, *J* = 14.3 Hz, 1H), 3.56 (d, *J* = 14.3 Hz, 1H), 2.92-2.83 (m, 1H), 2.80 – 2.71 (m, 1H), 2.48 (q, *J* = 7.3 Hz, 2H), 1.26 (t, *J* = 7.5 Hz, 3H), 1.08 ppm (t, *J* = 7.3 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 197.14, 169.96, 153.03 (2C), 146.07, 145.42, 141.03, 138.38, 137.24, 117.44, 112.49, 110.14, 110.06, 102.98 (2C), 91.00, 60.86, 56.19 (2C), 56.00, 45.11, 34.64, 22.14, 11.32, 7.95 ppm;. MS (ESI): *m/z* calcd for $C_{25}H_{30}NaO_7^+$ $[M+Na]^+$ 465.50, found 465.27.

4.1.6.14. *2-(3-hydroxy-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-3,5,6,7-tetrahydrobenzofuran-4(2H)-one* ***(5n)***

This compound was obtained from reaction of **4** with cyclohexane-1,3-dione after 20 min, followed by TBAF 0.5 h. Eluent: petroleum ether/ ethyl acetate = 4:1. Yellow solid. (***R***)-**5n** and (***R***)-**5n** were prepared by chical HPLC performed on Waters 515-2996 with columns of S-Chiral A. (***R***)-**5n**: yield: 9 mg, 18%; mp: 62– 63 $^o$C, $[\alpha]_D^{30}$ =-56.36$^o$ (*c* 0.036 in $CHCl_3$); (***S***)-**5n**: yield: 10 mg, 20%; mp: 62– 63 $^o$C, $[\alpha]_D^{30}$=+65.66$^o$ (*c* 0.036 in $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 6.83 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.75 (dd, *J* = 6.6, 5.6 Hz, 2H), 6.54 (s, 2H), 3.79 (s, 3H), 3.77 (s, 6H), 3.74 (s, 3H), 3.47 (s, 2H), 2.54 (t, *J* = 5.9 Hz, 2H), 2.34 (t, *J* = 6.4 Hz, 2H), 2.08 – 2.02 (m, 2H), 0.91 (s, 9H), 0.07 ppm (s, 6H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 195.70, 175.88, 153.02 (2C), 146.47, 145.65, 140.11, 137.68, 137.43, 117.31, 112.90, 112.60, 110.37, 103.24 (2C), 95.48, 60.80, 56.19 (2C), 55.95, 40.56, 36.32, 24.04, 21.72 ppm. HRMS (ESI): *m/z* calcd for $C_{24}H_{27}O_7^+$ $[M+H]^+$ 427.17513, found 427.17480. Chiral HPLC: (***S***)-**5n**: $t_R$ = 9.65 min, purity = 98.2%; (***R***)-**5n**: $t_R$ = 12.8 min, purity = 95.1%@ 220 nm, 50% *iso*propanol in hexane.

4.1.6.15. *(5-(3-hydroxy-4-methoxyphenyl)-2-methyl-5-(3,4,5-trimethoxyphenyl)-4,5-dihydrofuran-3-yl)(phenyl)methanone* ***(5o)***

This compound was obtained from reaction of **4** with 1-phenylbutane-1,3-dione after 1.5 h, followed by TBAF 0.5 h. Eluent: petroleum ether/ ethyl acetate = 2:1. Yellow solid. Yield: 22 mg, 40%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.61 (dd, *J* = 8.0, 1.5 Hz, 2H), 7.52 – 7.45 (m, 3H), 7.04 (d, *J* = 2.3 Hz, 1H), 6.91 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 1H), 6.68 (s, 2H), 5.64 (s, 1H), 3.88 (s, 3H), 3.84-3.78 (m, 11H), 1.93 ppm (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 194.47, 164.42, 153.11 (2C), 146.12, 145.52, 140.69, 138.27, 137.40, 130.98, 130.69, 129.29 (2C), 128.50 (2C), 117.36, 115.06, 112.45, 110.25, 103.14 (2C), 91.60, 60.84, 56.24 (2C), 56.00, 45.29, 28.80 ppm; MS (ESI): *m/z* calcd for $C_{28}H_{28}NaO_7^+$ $[M+Na]^+$ 499.51, found 499.10.

4.1.6.16. *(5-(3-hydroxy-4-methoxyphenyl)-2-phenyl-5-(3,4,5-trimethoxyphenyl)-4,5-dihydrofuran-3-yl)(phenyl)methanone* ***(5p)***

This compound was obtained from reaction of **4** with 1,3-diphenylpropane-1,3-dione after 1.5 h, followed by TBAF 0.5 h. Eluent: petroleum ether/ ethyl acetate = 2:1. Yellow solid. Yield: 23 mg, 37%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.38 (d, *J* = 7.3 Hz, 2H), 7.30 (d, *J* = 7.3 Hz, 2H), 7.20 (t, *J* = 7.4 Hz, 2H), 7.12 – 6.99 (m, 6H), 6.85 (d, *J* = 8.5 Hz, 1H), 6.74 (s, 2H), 5.68 (s, 1H), 3.95 – 3.86 (m, 5H), 3.84 ppm (s, 9H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 193.42, 164.24, 153.11 (2C), 146.14, 145.52, 140.53, 138.93, 138.01, 137.37, 131.23, 130.19, 129.96, 129.53 (2C), 128.96 (2C), 127.74 (2C), 127.69 (2C), 117.45, 112.61, 112.18, 110.27, 103.22 (2C), 91.46, 60.88, 56.25 (2C), 56.01, 46.47 ppm; MS (ESI): *m/z* calcd for $C_{33}H_{30}NaO_7^+$ $[M+Na]^+$ 561.59, found 561.32.

4.1.6.17. *2-(tert-butyl)-5-butyl-5-(3,4,5-trimethoxyphenyl)-4,5-dihydrofuran-3-carbonitrile* ***(8a)***

This compound was obtained from reaction between **7** and 4,4-dimethyl-3-oxopentanenitrile after 30 min. Eluent: petroleum ether/ ethyl acetate = 8:1. Yellow liquid. Yield: 12 mg, 47%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 6.47 (s, 2H), 3.85 (s, 6H), 3.84 (s, 3H), 3.05 (s, 2H), 1.94 – 1.81 (m, 2H), 1.36 (s, 9H), 1.34 – 1.19 (m, 4H), 0.86 ppm (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 177.49, 153.24 (2C), 140.90 , 137.12 , 117.33, 101.58 (2C), 91.41, 77.31, 60.87, 56.16 (2C), 44.68, 42.19, 34.92, 28.13 (3C), 25.61, 22.68, 13.95 ppm; MS (ESI): *m/z* calcd for $C_{22}H_{31}NNaO_4^+$ $[M+Na]^+$ 396.48, found 396.20.

4.1.6.18. *5-butyl-2-(4-chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydrofuran-3-carbonitrile* ***(8b)***

This compound was obtained from reaction between **7** and 3-(4-chlorophenyl)-3-oxopropanenitrile after 30 min. Eluent: petroleum ether/ethyl acetate = 8:1. White viscous liquid. Yield: 11 mg, 39%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.98 (d, *J* = 8.6 Hz, 2H), 7.45 (d, *J* = 8.6 Hz, 2H), 6.53 (s, 2H), 3.85 (s, 6H), 3.84 (s, 3H), 3.26 (s, 2H), 2.08 – 1.94 (m, 2H), 1.44 – 1.13 (m, 4H), 0.86 ppm (t, *J* = 7.3 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 164.34, 153.35 (2C), 140.01, 137.50, 137.39, 129.17 (2C), 128.33 (2C), 126.62, 117.36, 101.70 (2C), 92.71, 79.60, 60.90, 56.27 (2C), 44.70, 42.23, 25.81, 22.72, 13.98 ppm; MS (ESI): *m/z* calcd for $C_{24}H_{26}ClNNaO_4^+$ $[M+Na]^+$ 450.91, found 450.10.

4.1.6.19. *(5-butyl-2-phenyl-5-(3,4,5-trimethoxyphenyl)-4,5-dihydrofuran-3-yl)(phenyl)methanone* ***(8c)***

This compound was obtained from reaction between **7** and 1,3-diphenylpropane-1,3-dione after 30 min. Eluent: petroleum ether/ethyl acetate = 8:1. Yellow solid. Yield: 10 mg, 31%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.39 (s, 1H), 7.37 (s, 1H), 7.27 (d, *J* = 7.1 Hz, 2H), 7.20 (t, *J* = 7.4 Hz, 2H), , 7.11 – 7.04 (m, 4H), 6.69 (s, 2H), 3.89 (s, 6H), 3.87 (s, 3H), 3.55 (d, *J* = 14.9 Hz, 1H), 3.51 (d, *J* = 14.9 Hz, 1H), 2.16 – 1.99 (m, 2H), 1.50 – 1.26 (m, 4H), 0.90 ppm (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 193.58, 164.91, 153.18 (2C), 141.15, 139.12, 137.03, 131.08, 130.34, 130.05, 129.37 (2C), 128.87 (2C), 127.74 (2C), 127.65 (2C), 112.13, 102.10 (2C), 91.24, 60.92, 56.27 (2C), 45.24, 42.69, 26.09, 22.87, 14.06 ppm; MS (ESI): *m/z* calcd for $C_{30}H_{32}NaO_5^+$ $[M+Na]^+$ 495.57, found 495.35.

4.1.7. *General procedure for the synthesis of the residue of 1,3-dipole congeners.*

A stirred solution of aldehyde (3.6 mmol) in diethyl ether (3 mL) added 50% hydroxylamine in $H_2O$ (440 µL, 7.2 mmol) in one portion. After 10 min, the reaction mixture dried over $MgSO_4$, filtered, and the ether was removed under reduced pressure to give the residue of oximes. Subsequently, the residue was dissolved in DMF (3 mL), and *N*-chlorosuccinimide (NCS, 529 mg, 3.96 mmol) was added in one portion. The resulting solution was stirred for 0.5 h at room temperature. Then, the solution was diluted with water (30 mL) and extracted with ethyl acetate (3×30 mL). The organic layers were combined, washed with water (2×30 mL) and brine (2×30 mL), dried over anhydrous $Na_2SO_4$, filtered and evaporated under reduced pressure to give the residue of 1, 3-dipole.

4.1.8. *General procedure for the synthesis of compounds **6a-n** and **9a-c***

A mixture of **4** (50 mg, 0.12 mmol) or **7** (30 mg, 0.12 mmol) and the residue of 1,3-dipole congeners (about 0.7 mmol) in $CH_2Cl_2$ (2 mL) was cooled to 0 °C, followed by triethylamine ($Et_3N$, 114 µL, 0.82 mmol). After the mixture was stirred at 25 °C for 1 h, water (10 mL) was added. The organic phase was washed with water and brine, then, evaporated under reduced pressure.

The residues from reactions of **4** and 1,3-dipole congeners were dissolved in THF (2 mL), followed by 1.0 mol/L TBAF in THF (175 µL, 0.175 mmol) for 0.5 h. Water (10 mL) was added to this solution and extracted with ethyl acetate (3×10 mL). The combined organic phases were dried over anhydrous $Na_2SO_4$, filtered and evaporated under reduced pressure. Crude products were purified by column chromatography on silica gel using petroleum ether/ethyl acetate as eluent.

4.1.8.1. *2-methoxy-5-(3-phenyl-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)phenol **(6a)***

This compound was obtained from reaction of **4** with the residue of *N*-hydroxybenzimidoyl chloride after 1 h, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 3:1. White solid. Yield: 25 mg, 50%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.69 (dd, *J* = 6.6, 3.0 Hz, 2H), 7.40 (d, *J* = 2.6 Hz, 2H), 7.39 (s, 1H), 7.01 (d, *J* = 2.3 Hz, 1H), 6.95 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 1H), 6.70 (s, 2H), 3.97 (d, *J* = 16.5 Hz, 1H), 3.89 (d, *J* = 16.5 Hz, 1H), 3.87 (s, 3H), 3.83 (s, 6H), 3.82 ppm (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 156.37, 153.09 (2C), 146.18, 145.47, 139.93, 137.34, 137.13, 130.22, 129.56, 128.76 (2C), 126.72 (2C), 117.92, 112.88, 110.30, 103.50 (2C), 91.78, 60.85, 56.26 (2C), 56.01, 48.41 ppm; MS (ESI): *m/z* calcd for $C_{25}H_{26}NO_6^+$ $[M+H]^+$ 436.48, found 436.26.

4.1.8.2. *2-methoxy-5-(3-(p-tolyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)phenol **(6b)***

This compound was obtained from reaction of **4** with the residue of *N*-hydroxy-4-methylbenzimidoyl chloride after 1 h, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 5:1-3:1. Reddish brown solid. Yield: 44 mg, 85%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.57 (d, *J* = 8.2 Hz, 2H), 7.19 (d, *J* = 8.1 Hz, 2H), 7.01 (d, *J* = 2.3 Hz, 1H), 6.95 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.80 (d, *J* = 8.5 Hz, 1H), 6.70 (s, 2H), 5.66 (s, 1H), 3.95 (d, *J* = 16.6 Hz, 1H), 3.88 – 3.86 (m, 4H), 3.83 (s, 6H), 3.82 (s, 3H), 2.36 ppm (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 156.32, 153.08 (2C), 146.15, 145.47, 140.46, 140.03, 137.40, 137.28, 129.43 (2C), 126.76, 126.66 (2C) 117.92, 112.91, 110.31, 103.61 (2C), 91.56, 60.82, 56.27 (2C), 56.00, 48.51, 21.45 ppm; MS (ESI): *m/z* calcd for $C_{26}H_{28}NO_6^+$ $[M+H]^+$ 450.51, found 450.23.

4.1.8.3. *2-methoxy-5-(3-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)phenol **(6c)***

This compound was obtained from reaction of **4** with the residue of *N*-hydroxy-4-methoxybenzimidoyl chloride after 1 h, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 5:1 to 2:1. Yellow solid. Yield: 34 mg, 62%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.62 (d, *J* = 8.9 Hz, 2H), 7.01 (d, *J* = 2.3 Hz, 1H), 6.95 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.90 (d, *J* = 8.9 Hz, 2H), 6.80 (d, *J* = 8.5 Hz, 1H), 6.70 (s, 2H), 3.94 (d, *J* = 16.4 Hz, 1H), 3.87 – 3.84 (m, 4H), 3.83 (s, 6H), 3.83 (s, 3H), 3.82 ppm (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 161.16, 155.93, 153.08 (2C), 146.13, 145.45, 140.05, 137.40, 137.33, 128.25 (2C), 122.14, 117.92, 114.17 (2C), 112.90, 110.30, 103.62 (2C), 91.42, 60.82, 56.28 (2C), 56.00, 55.39, 48.63 ppm; MS (ESI): *m/z* calcd for $C_{26}H_{26}NO_7^-$ $[M-H]^-$ 464.49, found 464.25.

4.1.8.4. *2-methoxy-5-(3-(2-nitrophenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)phenol **(6d)***

This compound was obtained from reaction of **4** with the residue of *N*-hydroxy-2-nitrobenzimidoyl chloride after 1 h, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 3:1. Yellow solid. Yield: 12 mg, 22%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 8.00 (d, *J* = 8.1 Hz, 1H), 7.64 (t, *J* = 7.5 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 2H), 6.98 (dd, *J* = 11.5, 3.0 Hz, 2H), 6.85 (d, *J* = 8.3 Hz, 1H), 6.66 (s, 2H), 5.67 (s, 1H), 3.89 (s, 3H), 3.86 – 3.81 ppm (m, 11H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 154.83, 153.10 (2C), 148.05, 146.26, 145.45, 139.47, 137.47, 136.57, 133.35, 131.25, 130.69, 125.41, 124.74, 118.06, 112.97, 110.34, 103.63 (2C), 92.91, 60.87, 56.26 (2C), 56.01, 50.02 ppm; MS (ESI): *m/z* calcd for $C_{25}H_{25}N_2O_8^+$ $[M+H]^+$ 481.48, found 481.15.

4.1.8.5. *2-methoxy-5-(3-(4-nitrophenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)phenol **(6e)***

This compound was obtained from reaction of **4** with the residue of *N*-hydroxy-4-nitrobenzimidoyl chloride after 1 h, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 5:1-2:1. Yellow solid. Yield: 33 mg, 60%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 8.25 (d, *J* = 8.9 Hz, 2H), 7.85 (d, *J* = 8.9 Hz, 2H), 7.00 (d, *J* = 2.3 Hz, 1H), 6.92 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 1H), 6.68 (s, 2H), 5.64 (s, 1H), 3.99 (d, *J* = 16.5 Hz, 1H), 3.91 (d, *J* = 16.6 Hz, 1H), 3.88 (s, 3H), 3.84 (s, 6H), 3.83 ppm (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 154.76, 153.24 (2C), 148.54, 146.38, 145.61, 139.23, 137.71, 136.55, 135.68, 127.40 (2C), 124.03 (2C), 117.85, 112.73, 110.35, 103.53 (2C), 93.13, 60.85, 56.33 (2C), 56.03, 47.83 ppm; MS (ESI): *m/z* calcd for $C_{25}H_{23}N_2O_8^-$ $[M-H]^-$ 479.47, found 479.25.

4.1.8.6. *5-(3-(2-chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)-2-methoxyphenol **(6f)***

This compound was obtained from reaction of **4** with the residue of 2-chloro-*N*-hydroxybenzimidoyl chloride after 1 h, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 4:1. Yellow solid. Yield: 14 mg, 26%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.64 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.40 (dd, *J* = 7.9, 0.9 Hz, 1H), 7.35 – 7.27 (m, 2H), 7.03 (d, *J* = 2.2 Hz, 1H), 6.97 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 1H), 6.69 (s, 2H), 5.68 (s, 1H), 4.13 (d, *J* = 16.9 Hz, 1H), 4.04 (d, *J* = 16.9 Hz, 1H), 3.88 (s, 3H), 3.84 (s, 6H), 3.83 ppm (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 156.51, 153.08 (2C), 146.20, 145.46, 139.69, 137.37, 136.77, 132.80, 130.98, 130.68, 130.57, 129.06, 127.04, 117.97, 112.92, 110.30, 103.49 (2C), 92.47, 60.85, 56.26 (2C), 56.00, 50.41 ppm; MS (ESI): *m/z* calcd for $C_{25}H_{25}ClNO_6^+$ $[M+H]^+$ 470.93, found 470.21.

4.1.8.7. *5-(3-(4-chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)-2-methoxyphenol **(6g)***

This compound was obtained from reaction of **4** with the residue of 4-chloro-*N*-hydroxybenzimidoyl chloride after 1 h,

followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 4:1. Yellow solid. Yield: 35 mg, 65%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.62 (d, $J$ = 8.6 Hz, 2H), 7.36 (d, $J$ = 8.6 Hz, 2H), 7.00 (d, $J$ = 2.3 Hz, 1H), 6.93 (dd, $J$ = 8.4, 2.3 Hz, 1H), 6.81 (d, $J$ = 8.5 Hz, 1H), 6.68 (s, 2H), 5.64 (s, 1H), 3.93 – 3.83 (m, 11H), 3.82 ppm (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 155.41, 153.14 (2C), 146.23, 145.51, 139.70, 137.50, 136.99, 136.16, 129.02 (2C), 128.10, 127.93 (2C), 117.87, 112.81, 110.31, 103.53 (2C), 92.11, 60.83, 56.29 (2C), 56.01, 48.24 ppm; MS (ESI): *m/z* calcd for $C_{25}H_{23}ClNO_6^-$ $[M-H]^-$ 468.91, found 468.24.

4.1.8.8. *5-(3-(4-chloro-2-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)-2-methoxyphenol* ***(6h)***

This compound was obtained from reaction of **4** with the residue of 4-chloro-*N*-hydroxy-2-methoxybenzimidoyl chloride after 1 h, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 3:1. Yellow solid. Yield: 30 mg, 52%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.74 (d, $J$ = 2.6 Hz, 1H), 7.30 (dd, $J$ = 8.9, 2.7 Hz, 1H), 7.02 (d, $J$ = 2.2 Hz, 1H), 6.95 (dd, $J$ = 8.4, 2.3 Hz, 1H), 6.85 (d, $J$ = 8.9 Hz, 1H), 6.81 (d, $J$ = 8.5 Hz, 1H), 6.68 (s, 2H), 5.61 (s, 1H), 4.05 (d, $J$ = 17.3 Hz, 1H), 3.93 (d, $J$ = 17.3 Hz, 1H), 3.88 (s, 3H), 3.84 (s, 9H), 3.83 ppm (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 156.07, 155.04, 153.05 (2C), 146.10, 145.38, 139.97, 137.37, 137.15, 130.89, 128.99, 126.03, 120.08, 118.08, 112.94, 112.70, 110.26, 103.62 (2C), 91.86, 60.84, 56.29 (2C), 56.00, 55.95, 50.44 ppm; MS (ESI): *m/z* calcd for $C_{26}H_{27}ClNO_7^+$ $[M+H]^+$ 500.95, found 500.21.

4.1.8.9. *5-(3-(2-chloro-3,4,5-trimethoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)-2-methoxyphenol* ***(6i)***

This compound was obtained from reaction of **4** with the residue of 2-chloro-*N*-hydroxy-3,4,5-trimethoxybenzimidoyl chloride after 1 h, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 3:1. Yellow solid. Yield: 12 mg, 18%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.03 (s, 1H), 7.03 (d, $J$ = 2.3 Hz, 1H), 6.96 (dd, $J$ = 8.4, 2.1 Hz, 1H), 6.83 (d, $J$ = 8.4 Hz, 1H), 6.69 (s, 2H), 5.62 (s, 1H), 4.14 (d, $J$ = 17.0 Hz, 1H), 4.07 (d, $J$ = 17.0 Hz, 1H), 3.91 (s, 3H), 3.89 (s, 3H), 3.89 (s, 3H), 3.86 (s, 3H), 3.84 ppm (s, 9H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 156.64, 153.09 (2C), 152.17, 150.39, 146.19, 145.46, 144.82, 139.66, 136.90, 129.51, 124.32, 119.66, 117.99, 112.92, 110.29, 108.62, 103.63 (2C), 92.58, 61.20 (2C), 60.84, 56.29 (3C), 56.01, 50.46 ppm; MS (ESI): *m/z* calcd for $C_{28}H_{31}ClNO_9^+$ $[M+H]^+$ 561.00, found 560.26.

4.1.8.10. *5-(3-(4-hydroxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)-2-methoxyphenol* ***(6j)***

This compound was obtained from reaction of **4** with the residue of *N*,4-dihydroxybenzimidoyl chloride after 1 h, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 3:1. Reddish brown solid. Yield: 19 mg, 35%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.55 (d, $J$ = 8.7 Hz, 2H), 7.00 (d, $J$ = 2.3 Hz, 1H), 6.94 (dd, $J$ = 8.4, 2.3 Hz, 1H), 6.84 (d, $J$ = 8.7 Hz, 2H), 6.80 (d, $J$ = 8.5 Hz, 1H), 6.69 (s, 2H), 6.05 (s, 1H), 5.66 (s, 1H), 3.93 (d, $J$ = 16.4 Hz, 1H), 3.87 – 3.83 (m, 4H), 3.82 ppm (s, 9H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 157.79, 156.15, 153.05 (2C), 146.16, 145.43, 140.03, 137.29, 137.28, 128.49 (2C), 121.87, 117.95, 115.77 (2C), 112.89, 110.32, 103.57 (2C), 91.42, 60.85, 56.26 (2C), 56.01, 48.64 ppm; MS (ESI): *m/z* calcd for $C_{25}H_{26}NO_7^+$ $[M+H]^+$ 452.48, found 452.25.

4.1.8.11. *5,5'-(5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole-3,5-diyl)bis(2-methoxyphenol)* ***(6k)***

This compound was obtained from reaction of **4** with the residue of *N*,3-dihydroxy-4-methoxybenzimidoyl chloride after 1 h, followed by TBAF 0.5 h. Yellow solid. Eluent: petroleum ether/ethyl acetate = 3:1. Yield: 29 mg, 52%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.28 (d, $J$ = 1.9 Hz, 1H), 7.18 (dd, $J$ = 8.4, 1.9 Hz, 1H), 7.00 (d, $J$ = 2.1 Hz, 1H), 6.94 (dd, $J$ = 8.4, 2.2 Hz, 1H), 6.84 (d, $J$ = 8.4 Hz, 1H), 6.79 (d, $J$ = 8.5 Hz, 1H), 6.69 (s, 2H), 5.73 (s, 1H), 5.67 (s, 1H), 3.93 – 3.90 (m, 4H), 3.86 – 3.83 (m, 10H), 3.81 ppm (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 156.02, 153.05 (2C), 148.26, 146.13, 145.72, 145.42, 140.04, 137.28, 137.24, 122.92, 119.28, 117.91, 112.89, 112.84, 110.51, 110.29, 103.49 (2C), 91.51, 60.83, 56.25 (2C), 56.03, 56.00, 48.57 ppm; MS (ESI): *m/z* calcd for $C_{26}H_{28}NO_8^+$ $[M+H]^+$ 482.51, found 482.26.

4.1.8.12. *4-(5-(3-hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-3-yl)-2-methoxyphenol* ***(6l)***

This compound was obtained from reaction of **4** with the residue of *N*,4-dihydroxy-3-methoxybenzimidoyl chloride after 1 h, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 3:1. Yellow solid. Yield: 22 mg, 40%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.42 (d, $J$ = 1.7 Hz, 1H), 7.01 (d, $J$ = 2.3 Hz, 1H), 6.99 (dd, $J$ = 8.2, 1.9 Hz, 1H), 6.94 (dd, $J$ = 8.4, 2.3 Hz, 1H), 6.89 (d, $J$ = 8.2 Hz, 1H), 6.80 (d, $J$ = 8.5 Hz, 1H), 6.69 (s, 2H), 5.71 (s, 2H), 3.95 – 3.91 (m, 4H), 3.88 – 3.83 (m, 10H), 3.82 ppm (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 156.26, 153.07 (2C), 147.81, 146.84, 146.14, 145.44, 139.97, 137.34, 137.25, 121.82, 121.03, 117.92, 114.10, 112.90, 110.27, 108.25, 103.55 (2C), 91.53, 60.84, 56.26 (2C), 56.07, 56.00, 48.59 ppm; MS (ESI): *m/z* calcd for $C_{26}H_{28}NO_8^+$ $[M+H]^+$ 482.51, found 482.23.

4.1.8.13. *4-(5-(3-hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-3-yl)benzene-1,2-diol* ***(6m)***

This compound was obtained from reaction of **4** with the residue of *N*,3,4-trihydroxybenzimidoyl chloride after 1 h, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 3:1. Yellow solid. Yield: 20 mg, 36%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.39 (d, $J$ = 1.9 Hz, 1H), 7.03 – 6.99 (m, 2H), 6.92 (dd, $J$ = 8.4, 2.3 Hz, 1H), 6.86 (d, $J$ = 8.3 Hz, 1H), 6.79 (d, $J$ = 8.5 Hz, 1H), 6.66 (s, 2H), 5.75 (s, 1H), 3.92 (d, $J$ = 16.5 Hz, 1H), 3.86 – 3.84 (m, 4H), 3.82 (s, 3H), 3.80 ppm (s, 6H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 156.68, 153.05 (2C), 146.64, 146.23, 145.41, 143.91, 139.78, 137.30, 136.87, 121.79, 120.56, 117.98 , 115.16, 113.30, 112.91, 110.35, 103.55 (2C), 91.61, 60.87, 56.26 (2C), 56.00, 48.59 ppm; MS (ESI): *m/z* calcd for $C_{25}H_{26}NO_8^+$ $[M+H]^+$ 468.48, found 468.24.

4.1.8.14. *2-methoxy-5-(3-(pyridin-2-yl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)phenol* ***(6n)***

This compound was obtained from reaction of **4** with the residue of *N*-hydroxypicolinimidoyl chloride after 1 h, followed by TBAF 0.5 h. Eluent: petroleum ether/ethyl acetate = 3:1. White solid. Yield: 29 mg, 58%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 8.61 – 8.56 (m, 1H), 8.06 (d, $J$ = 8.0 Hz, 1H), 7.72 (td, $J$ = 7.8, 1.7 Hz, 1H), 7.28 (ddd, $J$ = 7.5, 4.9, 1.0 Hz, 1H), 7.02 (d, $J$ = 2.3 Hz, 1H), 6.94 (dd, $J$ = 8.4, 2.3 Hz, 1H), 6.80 (d, $J$ = 8.5 Hz, 1H), 6.71 (s, 2H), 4.15 (d, $J$ = 17.3 Hz, 1H), 4.09 (d, $J$ = 17.3 Hz, 1H), 3.85 (s, 3H), 3.83 (s, 6H), 3.82 ppm (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 158.24, 153.13, 153.09 (2C), 149.36, 149.28, 146.17, 145.50, 139.87, 137.29, 136.50, 124.36, 121.79, 117.75, 112.85, 110.29, 103.38 (2C), 92.61, 60.83, 56.22 (2C), 55.97, 47.79 ppm; MS (ESI): *m/z* calcd for $C_{24}H_{25}N_2O_6^+$ $[M+H]^+$ 437.47, found 437.16.

4.1.8.15. *5-butyl-3-(4-chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole* ***(9a)***

This compound was obtained from reaction between **7** and the residue of 4-chloro-*N*-hydroxybenzimidoyl chloride after 1 h. Eluent: petroleum ether/ethyl acetate = 8:1. Yellow solid. Yield: 9 mg, 31%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.58 (d, *J* = 8.5 Hz, 2H), 7.35 (d, *J* = 8.5 Hz, 2H), 6.65 (s, 2H), 3.88 (s, 6H), 3.83 (s, 3H), 3.46 (s, 2H), 2.05 – 1.97 (m, 2H), 1.42 – 1.17 (m, 4H), 0.87 ppm (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 155.25, 153.24 (2C), 140.66, 137.00, 135.96, 128.97 (2C), 128.31, 127.80 (2C), 102.13 (2C), 91.06, 60.86, 56.28 (2C), 47.16, 41.60, 26.19, 22.83, 13.99 ppm; MS (ESI): *m/z* calcd for $C_{22}H_{27}ClNO_4^+$ $[M+H]^+$ 404.91, found 404.17.

4.1.8.16. *5-butyl-3-(4-chloro-2-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole* ***(9b)***

This compound was obtained from reaction between **7** and the residue of 4-chloro-*N*-hydroxy-2-methoxybenzimidoyl chloride after 1 h. Eluent: petroleum ether/ethyl acetate = 10:1. Yellow solid. Yield: 13 mg, 42%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.71 (d, *J* = 2.6 Hz, 1H), 7.28 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.83 (d, *J* = 8.9 Hz, 1H), 6.64 (s, 2H), 3.88 (s, 6H), 3.83 (s, 3H), 3.82 (s, 3H), 3.58 (d, *J* = 17.3 Hz, 1H), 3.51 (d, *J* = 17.3 Hz, 1H), 2.02 – 1.95 (m, 2H), 1.40 – 1.20 (m, 4H), 0.87 ppm (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 156.04, 154.80, 153.14 (2C), 140.93, 136.87, 130.71, 128.87, 125.91, 120.49, 112.70, 102.24 (2C), 90.78, 60.86, 56.28 (2C), 55.90, 49.53, 41.37, 26.21, 22.88, 14.01 ppm; MS (ESI): *m/z* calcd for $C_{23}H_{29}ClNO_5^+$ $[M+H]^+$ 434.94, found 434.22.

4.1.8.17. *4-(5-butyl-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-3-yl)-2-methoxyphenol* ***(9c)***

This compound was obtained from reaction between **7** and the residue of *N*,3-dihydroxy-4-methoxybenzimidoyl chloride after 1 h, followed by TBAF 0.5 h. Yellow solid. Eluent: petroleum ether/ethyl acetate = 4:1. Yield: 12 mg, 41%; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.24 (d, *J* = 1.9 Hz, 1H), 7.14 (dd, *J* = 8.4, 1.8 Hz, 1H), 6.82 (d, *J* = 8.4 Hz, 1H), 6.65 (s, 2H), 5.77 (s, 1H), 3.88 (s, 3H), 3.87 (s, 6H), 3.82 (s, 3H), 3.43 (s, 2H), 2.02 – 1.95 (m, 2H), 1.42 – 1.18 (m, 4H), 0.85 ppm (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 155.83, 153.15 (2C), 148.15, 145.70, 141.01, 136.86, 123.16, 119.10, 112.73, 110.48, 102.22 (2C), 90.42, 60.85, 56.25 (2C), 56.01, 47.52, 41.54, 26.22, 22.86, 13.99 ppm; MS (ESI): *m/z* calcd for $C_{23}H_{28}NO_6^-$ $[M-H]^-$ 414.48, found 414.21.

4.1.9. *X-ray experimental*

Single crystals of **(*S*)-5b** obtained from solvent volatilization in $CH_3OH$. An appropriate crystal was selected and analysed on a SuperNova, Dual, Cu at zero, Eos diffractometer. The crystal was held at 293(2) K in the period of data collection. Using Olex2[62], the structure was solved with the Superflip[63] structure solution program using Charge Flipping and refined with the ShelXL [64] refinement package using Least Squares minimisation.

*4.2. Biological evaluation*

4.2.1. *Cell Culture*

PC-3 cell line was originally acquired from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. HeLa cell line was donated by Prof. Lei group of college of life sciences, Northwest A&F University. The two cell lines were grown in RPMI-1640 (Gibco) including 10% (v/v) thermally inactivated fetal bovine serum (FBS), penicillin (100 KU/L) and streptomycin (100 KU/L) at 37 $^o$C in a 5% $CO_2$ humidified incubator.

4.2.2. *Cytotoxic activity (SRB)*

*In vitro* antiproliferative activity was evaluated by using the SRB colorimetric assay as previous methods[61, 65].

4.2.3. *Tubulin polymerization assay*

Effects of compounds on tubulin polymerization *in vitro* were performed in the light of the fluorescence based tubulin polymerization assay kit's protocol (Cat. #BK011P, Cytoskeleton Inc.). Tubulin (10 mg/mL) containing buffer 1, tubulin glycerol buffer and GTP stock (100 mM) was incubated with **6g** (10, 20 and 40 μM), paclitaxel (3 μM), CA-4 (3 μM), *iso*CA-4 (3 μM) and DMSO for 80 minutes at 37 $^o$C. Paclitaxel, CA-4 and *iso*CA-4 were used as reference compounds in this assay. The fluorescence increase was monitored every minute for characterization of tubulin polymerization at 360 nm excitation wavelength and 460 nm emission wavelength by microplate spectrophotometer (Synergy HTX, BioTek Instruments, Inc). The $IC_{50}$ value of compound **6g** was determined by five concentrations (3, 5, 10, 20, 40 μM) against tubulin polymerization.

4.2.4. *Immunohistochemistry studies*

PC-3 cells were incubated in 12-well plates in the presence or absence of tested compounds (**6g**: 1.0 μM and CA-4: 0.02 μM) for 48 h. After 4% paraformaldehyde was used to fix cells for 30 min and rinsed thrice with PBS containing 5% bovine serum albumin (BSA), 0.1% Triton X-100 in PBS was employed for permeabilization at 4 $^o$C in the dark for 30 min and rinsed again with PBS containing 5% BSA. Then the cells were blocked with PBS containing 5% BSA for 30 min. Anti-α-tubulin antibodies (1: 500, Abcam) and the secondary antibody (goat anti-rabbit IgG, 1: 500, ZSGB-BIO) were added sequentially to each well and rinsed with PBS before incubating for overnight at 4 $^o$C and 1 h at room temperature, respectively. At last, the cells were stained with DAPI for 5 min at room temperature. Images of results were acquire using a fluorescence microscope with a 20× objective

4.2.5. *Cell cycle analysis*

2.5 mL aliquots of PC-3 cells containing $1.0\times10^5$ cells/mL were plated in 6-well plates and incubated at 37 $^o$C for 24 h. Propidium iodide staining assay (Sigma) was used for the cell cycle arrest of PC-3 cells as described previously. The cultured cells were treated with **6g** and CA-4 at the indicated concentrations (**6g**: 0.5, 1.0 μM; CA-4: 0.05 μM) for 48 h. After that, centrifugation, fixation using 70% ethanol at 4 $^o$C overnight and resuspension process in PBS containing 100 μL RNase A and 400 μL PI were carried out in sequence. For cell cycle distribution analysis, cellular DNA content was collected twenty thousand events per sample by a FACS Calibur flow cytometer (BD Biosciences). The results were analyzed using Modfit LT 3.0 software.

4.2.6. *Statistics analysis*

All the data reported were shown as the arithmetic mean of date from three separate determinations where each group owns three parallels. The form of results presented as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad software with the 2-tailed Student *t*-test.

*4.3. Molecular modeling*

All docking studies were performed by using the Surflex-Dock program in the Sybyl-X 2.0 software. The X-ray crystal structure of tubulin protein (PDB ID: 1SA0) was acquired from RSCB Protein Data Bank (http:// www.rcsb.org/pdb). The analysis of results was carried out by USCF Chimera software, which was downloaded from http://www.cgl.ucsf.edu/chimera.

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# Constructing novel dihydrofuran and dihydroisoxazole analogues of *iso*combretastatin-4 as tubulin polymerization inhibitors through [3+2] reactions

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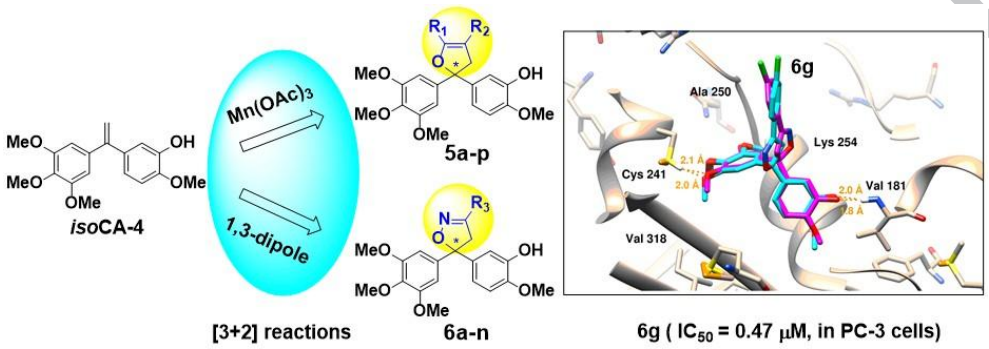
Graphical abstract:

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