Design, synthesis, and structure-activity relationship studies of novel diaryl ether amides as potential antitumor agents

Man-Yi Zheng, Zhi-Ning Huang, Shao-Mei Yang, Han-Liang, Lu-Xu, Bao-Rui Wang, Li-Juan Wang, Hai-Li Wang, Shan-Hua Li & Fu-Nan Li

Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Xiamen University, Xiamen, P. R. China
Department of Basic Medical Sciences, College of Medicine, Xiamen University, Xiamen, P. R. China
Fujian Provincial Key Laboratory of Innovative Drug Target Research, School of Pharmaceutical Sciences, Xiamen University, Xiamen, P. R. China
E-mail: fnlee5@xmu.edu.cn; lish@xmu.edu.cn

Sorafenib is a drug that has been verified to be effective on hepatoma cells. Three series of diaryl ethers have been designed and synthesized based on the structure of sorafenib. The compound shows better inhibitory potency against HepG2 cells (IC\textsubscript{50} = 1.96 \mu M) than sorafenib (IC\textsubscript{50} = 9.61 \mu M). These results have been verified with MTT assay and colony-forming assay. Moreover, compound 5m exhibits good antitumor activities against PLC/PRF5, HeLa, A549, and HT-29 cell lines. The excellent bioactivity of compound 5m confirms that a single optical conformation is superior to the racemate. A western blotting analysis indicates that compound 5m induces the apoptosis of HepG2 cells by enhancing the protein levels of p21 and Cl-caspase3.

**Keywords**: Diaryl ethers, structural modification, antitumor activity

Hepatocellular carcinoma (HCC), which is the main primary liver cancer, has strong invasiveness, metastasis, and poor prognosis. The treatment of HCC remains a significant challenge because of the lack of balance of effectiveness, selectivity, and toxicity. A successful and complete treatment method needs to be determined through clinical practice and examinations of the pathophysiological mechanisms.

Sorafenib, which is a drug that is frequently used clinically to treat HCC and renal cancer, is a potential multitarget antitumor agent that interests many researchers. Sorafenib can double block tumors by restraining a number of kinases, including Raf, vascular endothelial growth factor receptor, platelet-derived growth factor receptor, and Kit, which are involved in the proliferation and angiogenesis of tumors and inhibiting the Ras/Raf/mitogen-activated protein kinase and extracellular signal-regulated kinase signaling pathway. Sorafenib is considered a good template for modification due to its relatively weak activity and controversial adverse effects, including myelosuppression, neutropenia, and liver dysfunction. Many sorafenib analogs that perform better in both bioactivity and toxicity aspects have been described.

Diaryl ether is an important elementary structure that is found in many natural products and biological molecules. Moreover, diaryl ether derivatives possess a wide spectrum of pharmacological activities, including antibacterial, anti-inflammatory, and anticancer. We considered the diaryl ether skeleton in our structural modification due to its specific and effective ability to inhibit the proliferation of human tumor cell lines.

In recent years, more and more novel antitumor agents have been reported. We have reported that diaryl ether analogs and N-(piperidine-4-yl)benzamides exhibit highly potent and competitive antitumor activity effects and structure-activity relationships. Recently, we attempted to structurally optimize our amide series through the incorporation of new scaffolds that were expected to improve the metabolic and pharmacokinetic profiles of the amide series.

Sorafenib was modified by the strategy shown in Figure 1. First, we divided sorafenib into three parts. Due to the preponderant basic structure of diaryl ether, we changed the skeleton of 4-phenoxypridine in the A region. Then, acylamino was substituted in the B region as an isosteric replacement of carbamido.
Finally, in the C region, we chose a benzyl group to create a basic and simple structural core in the design template. However, we thought that a 2-substituted-1,2,3,4-tetrahydronaphthalene might be a new and innovative substitute that would aid in the exploration of the chiral field. We designed and synthesized two dozen compounds in this study by inserting electron-donating, halogen, and electron-withdrawing groups in the benzene ring in the C region. We compared these compounds with sorafenib and secondarily considered the structural modifications at the phenoxyl in the A region. In summary, we examined the chemical synthesis, cytotoxic activities, structure-activity relationships, and potential biological targets of the new diaryl ethers.

Experimental Section

Chemistry

All reagents were purchased and used without further purification unless otherwise noted. Reactions were monitored by thin-layer chromatography (TLC) on Yan Tai silica gel GF-254 thin-layer plates. $^1$H and $^{13}$C NMR spectra were determined with a Bruker Avance III 600 MHz spectrometer (400 MHz or 600 MHz for $^1$H and 150 MHz for $^{13}$C). Chemical shifts are expressed in $\delta$ (ppm) values, using tetramethylsilane (TMS) as the internal standard; coupling constants ($J$) are given in Hz. Signal multiplicities are characterized as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br s (broad signal). High-resolution mass spectral (HRMS) were acquired on a Q-Exactive series MS instrument with UV detection at 254 nm in low-resonance electrospray mode (ESI).

4-Phenoxybenzoic acid, 4a

To a solution of iodobenzene (1 mL, 8.9 mol), $p$-hydroxybenzoic acid (1.85 g, 13.4 mol), CuI (170 mg, 0.9 mol), Cs$_2$CO$_3$ (5.81 g, 17.8 mol) and N,N-dimethyl glycine (276 mg, 2.7 mmol) in 1,4-dioxane were added under nitrogen atmosphere at 90°C and the mixture was stirred for 24 h. 1,4-Dioxane was evaporated and the residue was extracted with EtOAc and water. The organic phase was treated with brine, dried over anhydrous MgSO$_4$ and concentrated. Then the residue was purified by column chromatography to get compound 3a as yellow oil. It was dissolved in water (8 mL) and ethanol (8 mL), NaOH (1.82 g, 4.5 mol) added and the reaction mass refluxed for 3 h. The mixture was cooled and 50 mL water added, and the mass acidified with 2M HCl. The aqueous mixture was extracted with EtOAc. The organic phase was combined and washed with brine, dried over anhydrous MgSO$_4$ and concentrated. Then the residue was purified by column chromatography to get compound 4a (87%) as a white solid. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 12.82 (br s, 1H, OH), 7.95 (d, $J = 8.8$ Hz, 2H, Ar-H), 7.45 (t, $J = 7.5$ Hz, 2H, Ar-H), 7.23 (t, $J = 7.3$ Hz, 1H, Ar-H), 7.11 (d, $J = 7.7$ Hz, 2H, Ar-H), 7.02 (d, $J = 8.8$ Hz, 2H, Ar-H).

N-Benzyl-4-phenoxybenzamide, 5a

To a solution of phenylmethanamine (51 mg, 0.47 mmol) and compound 4a (100 mg, 0.47 mmol) in CH$_2$Cl$_2$ (5 mL) at 0°C were added 1-hydroxybenzotriazole HOBt (76 mg, 0.56 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydro-chloride (EDCI) (107 mg, 0.56 mmol) and 4-methylmorpholine (155 µL, 1.41 mmol). The reaction mixture was stirred at RT overnight, then washed with 10% aqueous HCl, 5% aqueous NaHCO$_3$, H$_2$O and brine, dried over anhydrous MgSO$_4$, and concentrated in vacuo. The residue was purified by column chromatography to provide the title compound (70%) as a white solid. $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 7.79 (d, $J = 9.3$, 2H, Ar-H), 7.40 (t, $J = 5.5$ Hz, 2H, Ar-H), 7.38-7.35 (m, 4H, Ar-H), 7.33 (m, 1H, Ar-H), 7.19 (t, $J = 8.5$ Hz, 1H, Ar-H), 7.06 (d, $J = 6.8$ Hz, 2H, Ar-H), 7.02 (d, $J = 8.8$ Hz, 2H, Ar-H), 6.40 (t, $J = 5.2$ Hz, 2H, CH$_2$); $^{13}$C NMR (150 MHz, CDCl$_3$): $\delta$ 166.7, 160.5, 156.0, 138.3, 130.0, 128.9, 128.8, 127.9, 127.6, 124.3, 119.8, 117.8,
44.1; HRMS (ESI): m/z Calcd for C_{20}H_{18}NO_2 [M+H^+] 304.1338. Found 304.1331.

4-Phenoxy-N-phenylbenzamide, 5e: Titled compound 5e (90%) was prepared by the synthetic procedure of 5a as white solid. 1H NMR (600 MHz, CDCl_3): δ 7.86 (d, J = 9.0 Hz, 2H, Ar-H), 7.77 (br s, 1H, NH), 7.63 (d, J = 7.5 Hz, 2H, Ar-H), 7.40 (q, J = 7.3 Hz, 4H, Ar-H), 7.19 (t, J = 7.3 Hz, 1H, Ar-H), 7.15 (t, J = 7.3 Hz, 1H, Ar-H), 7.08 (t, J = 8.8 Hz, 4H, Ar-H); 13C NMR (150 MHz, CDCl_3): δ 166.1, 160.4, 156.1, 137.7, 136.8, 130.0, 129.2, 129.0, 128.9, 128.8, 127.3, 126.3, 124.2, 119.7, 117.9, 48.0, 30.3, 29.3, 20.2; HRMS (ESI): m/z Calcd for C_{20}H_{18}NO_2 [M+H^+] 344.1645. Found 344.1637.

N-(3-Methoxyphenyl)-4-phenoxybenzamide, 7a
To a solution of compound 4a (100 mg, 0.47 mmol) in CH_2Cl_2 (5 mL) was added slowly thionyl dichloride (35 µL, 0.47 mmol) at 100°C. The reaction mixture was stirred at 100°C for 2 h, and the solvent removed to get compound 6, which was used for the next step immediately after its high instability.

To a solution of 3-methoxyaniline (58 mg, 0.47 mmol) was added Et_3N (200 µL, 1.41 mmol), followed by drop-wise addition of acid chloride in anhydrous CH_2Cl_2. The solution was stirred for 2 h at RT. It was then washed successively with 10% HCl, 5% NaOH, water and brine, dried over anhydrous MgSO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel to afford compounds 7a (36%) as a yellow solid. 1H NMR (600 MHz, CDCl_3): δ 8.14 (br s, 1H, NH), 7.83 (d, J = 8.0 Hz, 2H, Ar-H), 7.43 (t, J = 2.1 Hz, 1H, Ar-H), 7.39 (t, J = 7.5 Hz, 2H, Ar-H), 7.22 (t, J = 5.9 Hz, 1H, Ar-H), 7.19 (t, J = 7.5 Hz, 1H, Ar-H), 7.13 (d, J = 7.0 Hz, 1H, Ar-H), 7.06 (d, J = 8.5 Hz, 2H, Ar-H), 6.99 (d, J = 8.8 Hz, 2H, Ar-H), 6.69 (dd, J = 8.2, 2.5 Hz, 1H, Ar-H), 3.79 (s, 3H, CH_3); 13C NMR (150 MHz, CDCl_3): δ 165.2, 160.7, 160.1, 155.7, 139.2, 130.0, 129.6, 129.1, 129.0, 124.3, 119.8, 117.7, 112.4, 110.4, 105.9, 55.2; HRMS (ESI): m/z Calcd for C_{23}H_{20}NO_2 [M+H^+] 320.1279. Found 320.1279.

Cell growth inhibition rate (MTT assay)
The compounds were evaluated for their anti-proliferation activity on cancer cell lines like HepG2 (liver hepatoma cells), A549 (lung cancer), HT-29 (colon adenocarcinoma cancer). All the cell lines were incubated at 37°C in 5% CO_2. The culture medium used was the DMEM supplemented with 10% of Fetal Bovine Serum (FBS) (except HT-29 cell line for RPMI-1640).

The cells were seeded in 96-well plate at 5000 cells/well and incubated 24 h. The cells were treated with...
compounds at 20 µM concentration, while control cells were treated with equal volume of DMSO. After 48 h treatment, 75 µL MTT solution (5 mg/mL) was added to each well, and incubated for 4 h, then the culture media were removed and dissolved in 100 µL DMSO. After 10 min, the OD (optical density) was detected at 490 nm on microplate reader. If the inhibitory rate of tested compounds was >50%, we evaluated their IC<sub>50</sub> values.

**Colony forming assay**

The cells were seeded in 6-well plates at 1000 cells/well and incubated in different concentrations of compound 5m for one week. Then the cells were washed with PBS, fixed by 70% ethanol and stained with 0.25% crystal violet. The number of colonies was analyzed. These experiments were performed in triplicate.

**Western blotting**

The cells were lysed with sodium dodecyl sulfate buffer containing 200 mM Tris (pH 6.8), 40% glycerol, and 10% mercaptoethanol. The proteins (30 µg) from the cell extracts were separated on a 10% or 15% sodium dodecyl sulfate/polyacrylamide gel and then transferred to Immobilon-P membranes. The membranes were blocked with 10% nonfat milk in Tris-buffered saline (TBS) containing 0.1% Tween-20 (TTBS) for 1 h at RT and then incubated overnight at 4°C with primary antibodies diluted 1:1,000-2,000 in TTBS containing 5% nonfat milk. After three washes in TTBS, the membranes were incubated with a horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibody (diluted 1:10,000 in TTBS containing 5% nonfat milk) for 1 h at RT, and the antigen-antibody complexes were visualized with an ECL kit. β-Actin was analyzed as a loading control.

**Flow cytometric analysis of apoptosis**

As described above, the cells in the exponential growth phase were treated with compound 5m for 24 h, and cell death or apoptosis was evaluated. Cell suspensions (0.3 mL, 1 × 10<sup>6</sup> cells/mL) were placed in the flow cytometry apparatus. Before detection, the horizontal coordinate was set for propidium iodide (PI) and the vertical coordinate was set for SSC (side scatter, side light scatter). The PI-positive cells were gated to include the apoptotic and necrotic cells. A map was set to the linear model, and a horizontal histogram for PI in the third fluorescence channel (FL3, 610 nm) was obtained. Finally, a log ploidy determination of the diploid and tetraploid cells was performed. A tube of unstained cells was used as a blank control to adjust the test conditions.

**Results and Discussion**

**Chemistry**

All of the target compounds were prepared from the general intermediate 4-phenoxybenzoic acid (4a), which was obtained through an Ullmann coupling reaction<sup>20</sup>. Iodobenzene was subjected to a condensation reaction with compound 2 to obtain compound 3a. Compound 3a was further hydrolyzed to generate compound 4a with a yield of 57% (Scheme I).

The target compounds were obtained through the three routes outlined in Scheme II and Scheme III. Compounds 5a–o were obtained with a condensation reaction of commercially available amines and intermediate 4 with 1.1–1.2 equivalents (equiv) of hydroxybenzotriazole, 1.0 equiv of 1-ethyl-3-(3-dimethylaminopropyl)carbo diimide, and 3.0 equiv of triethylamine or N-methylmorpholine with yields of 30% to 91% (Scheme II).

Compounds 7a–j were obtained as shown in Scheme III. Compound 4a was treated with thionyl...
chloride in ethyl acetate to obtain the corresponding acyl chloride 6, and then 6 was reacted with various amines in the presence of triethylamine in anhydrous dichloromethane to obtain the target compounds 7a–j with yields of 21% to 47%.

**In vitro antitumor activity**

Table I shows the *in vitro* antitumor activities of the three series of 4-phenoxy-N-phenylbenzamide derivatives. In the beginning, we explored the bioactivities of compounds 5a and 5e. After a preliminary experiment, compound 5a and its derivatives exhibited poor inhibition of the proliferation of HepG2 cells (5b–5d). Hence, we stopped further biological experiments on compounds 5a–5d. However, 5e showed good inhibition of the activity of HepG2 and A549 cells with IC₅₀ values of 0.61, 7.54, 10.34, and 7.01 µM, respectively. These studies have made it clear that compounds with electron-donating substitutions at meta-positions had better inhibitory activities on HT-29 cells than those at ortho- and para-positions (7a vs. 7b, 7d vs. 7e vs. 7f, 7g vs. 7h). Simultaneously, we found that inserting another group into C-ring resulted in a decrease in the bioactivities on HT-29 cells while there was a methoxyl at the meta-substituent on the C-ring (7a vs. 7c, 7d, and 7e), probably because of methoxysteric hindrance. Most
Table I — Inhibition of growth by diaryl ether derivatives in HepG2, A549, and HT-29 cells

<table>
<thead>
<tr>
<th>Compd</th>
<th>R</th>
<th>R'</th>
<th>HepG2&lt;sup&gt;a&lt;/sup&gt;</th>
<th>A549&lt;sup&gt;a&lt;/sup&gt;</th>
<th>HT-29&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inhibition (%)</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (µM)</td>
<td>Inhibition (%)</td>
</tr>
<tr>
<td>5a</td>
<td>H</td>
<td></td>
<td>23.56</td>
<td>NT</td>
<td>37.60</td>
</tr>
<tr>
<td>5b</td>
<td>H</td>
<td>F</td>
<td>18.74</td>
<td>NT</td>
<td>27.60</td>
</tr>
<tr>
<td>5c</td>
<td>H</td>
<td>Cl</td>
<td>14.45</td>
<td>NT</td>
<td>55.50</td>
</tr>
<tr>
<td>5d</td>
<td>H</td>
<td>OH</td>
<td>9.13</td>
<td>NT</td>
<td>66.09</td>
</tr>
<tr>
<td>5e</td>
<td>H</td>
<td></td>
<td>54.39</td>
<td>19.80</td>
<td>77.27</td>
</tr>
<tr>
<td>5f</td>
<td>H</td>
<td>OH</td>
<td>37.36</td>
<td>NT</td>
<td>84.98</td>
</tr>
<tr>
<td>5g</td>
<td>H</td>
<td>Br</td>
<td>25.56</td>
<td>NT</td>
<td>90.92</td>
</tr>
<tr>
<td>5h</td>
<td>H</td>
<td>CF&lt;sub&gt;3&lt;/sub&gt;</td>
<td>42.92</td>
<td>NT</td>
<td>81.95</td>
</tr>
<tr>
<td>5i</td>
<td>H</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>16.16</td>
<td>NT</td>
<td>60.54</td>
</tr>
<tr>
<td>5j</td>
<td>H</td>
<td></td>
<td>31.77</td>
<td>NT</td>
<td>87.89</td>
</tr>
<tr>
<td>5k</td>
<td>H</td>
<td></td>
<td>74.01</td>
<td>9.07</td>
<td>102.69</td>
</tr>
<tr>
<td>5l</td>
<td>F</td>
<td></td>
<td>71.30</td>
<td>13.22</td>
<td>15.64</td>
</tr>
<tr>
<td>5m</td>
<td>H</td>
<td></td>
<td>64.12</td>
<td>1.96</td>
<td>99.03</td>
</tr>
<tr>
<td>5n</td>
<td>H</td>
<td></td>
<td>57.68</td>
<td>15.16</td>
<td>62.45</td>
</tr>
<tr>
<td>5o</td>
<td>H</td>
<td>OH</td>
<td>33.67</td>
<td>NT</td>
<td>100.33</td>
</tr>
</tbody>
</table>

(Contd.)
importantly, this revealed that a halogen or electron-withdrawing group at the meta-position on ring C (5g and 5h) resulted in good inhibitory potency of HT-29 and A549 cells. These results suggested that 4-phenoxy-N-(1,2,3,4-tetrahydronaphthalen-1-yl) benzamide derivatives (5j–n) needed to be used in the rest of the investigation. Compared with compounds 5a and 5j, compound 5k exhibited higher anti-proliferative activities against HepG2, A549, and HT-29 cells with IC_{50} values of 9.07, 14.54, and 5.21 µM, respectively. We separated the chiral carbon of compound 5k into two monomers (5m and 5n). Surprisingly, compound 5m with an IC_{50} value of 1.96 µM exhibited more significant antitumor activities on HepG2 cells than both 5k and 5n. Thus, optical rotation was effective on HepG2 cells, perhaps because of its spatial structure. Because the structural optimization of the para-position on the A-ring (5i) was not so potent, we did not examine it further. In addition, we investigated whether 4-phenoxy-N-(5,6,7,8-tetrahydronaphthalen-1-yl) benzamide derivatives were more effective. The results revealed that a

---

Table I — Inhibition of growth by diaryl ether derivatives in HepG2, A549, and HT-29 cells (Contd.)

<table>
<thead>
<tr>
<th>Compd</th>
<th>R</th>
<th>R'</th>
<th>HepG2&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>IC_{50} (µM)</th>
<th>A549&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>IC_{50} (µM)</th>
<th>HT-29&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>IC_{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>H</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>51.37</td>
<td>18.03</td>
<td>19.57</td>
<td>NT</td>
<td>71.07</td>
<td>7.54</td>
</tr>
<tr>
<td>7b</td>
<td>H</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>5.94</td>
<td>NT</td>
<td>29.07</td>
<td>NT</td>
<td>52.24</td>
<td>39.00</td>
</tr>
<tr>
<td>7c</td>
<td>H</td>
<td>Cl</td>
<td>17.71</td>
<td>NT</td>
<td>11.50</td>
<td>NT</td>
<td>83.87</td>
<td>19.26</td>
</tr>
<tr>
<td>7d</td>
<td>H</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>29.32</td>
<td>NT</td>
<td>17.05</td>
<td>NT</td>
<td>54.48</td>
<td>37.93</td>
</tr>
<tr>
<td>7e</td>
<td>H</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>22.78</td>
<td>NT</td>
<td>15.04</td>
<td>NT</td>
<td>72.94</td>
<td>10.34</td>
</tr>
<tr>
<td>7f</td>
<td>H</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.97</td>
<td>NT</td>
<td>−10.50</td>
<td>NT</td>
<td>47.74</td>
<td>NT</td>
</tr>
<tr>
<td>7g</td>
<td>H</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>21.14</td>
<td>NT</td>
<td>26.80</td>
<td>NT</td>
<td>82.26</td>
<td>7.01</td>
</tr>
<tr>
<td>7h</td>
<td>H</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>21.14</td>
<td>NT</td>
<td>19.57</td>
<td>NT</td>
<td>40.81</td>
<td>NT</td>
</tr>
<tr>
<td>7i</td>
<td>H</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>44.97</td>
<td>NT</td>
<td>19.48</td>
<td>NT</td>
<td>40.81</td>
<td>NT</td>
</tr>
<tr>
<td>7j</td>
<td>H</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>26.99</td>
<td>NT</td>
<td>−1.30</td>
<td>NT</td>
<td>40.16</td>
<td>NT</td>
</tr>
</tbody>
</table>

Sorafenib — — 65.18 9.61 NT NT NT NT

<sup>a</sup> Each compound was tested in triplicate
hydroxy-substituent on ring-C or -D might increase the bioactivity on HT-29 cells, but more phenyl rings did not produce better results (5f vs. 5o). In summary, we chose compound 5m for further studies, based on the results of Table I.

The chiral structure of compound 5m is presented in Figure 2A. The significant inhibitory abilities of compound 5m against several different tumor cell lines, including hepatoma (HepG2, Hep3B, PLC/PRF/5, and SMMC-7721), lung cancer (A549), colon adenocarcinoma (HT-29), cervical cancer (HeLa), and human skin melanoma (A375), were tested, and the results are shown in Figure 2B. As far as can be understood, compound 5m was universally effective for inhibiting different hepatoma cell lines, especially HepG2 cells, with an IC50 value of 1.96 µM, and compound 5m was greatly effective against Hela and A549 cells with IC50 values of 2.86 and 1.50 µM, respectively. These results indicated that compound 5m had better inhibition than sorafenib, but it did not have obvious specificity. The proliferation and colony-forming assay results showed great potency of compound 5m against HepG2 cells and suggested that further biological study was warranted (Figure 2C,D).

Western blotting showed that the expression of p21 and Cl-caspase3, which promote the apoptosis of hepatoma cells, increased when HepG2 cells were exposed to compound 5m in both time-dependent and dose-dependent manners (Figure 3A-D). A flow cytometry analysis of apoptosis confirmed that compound 5m promotes the apoptosis of HepG2 cells (Figure 3E,F). The expression of the cyclin-dependent kinase p21, which is tightly controlled by p53, accompanies the growth arrest at the S phase of the cell cycle21,22. These results suggested that p21 is associated with Ras-MAPK signal pathways23, and this needs to be investigated further.

In conclusion, three series of diaryl ether derivatives, the structure of which were based on sorafenib, were designed, synthesized, and evaluated through several bioactivity tests, such as MTT assays and western blotting. Most of these compounds were preliminarily identified to be potent against the proliferation of some tumors. Compound 5m, which was the most potent inhibitor against HepG2 cells in

![Figure 2](https://example.com/figure2.png)

**Figure 2** — Compound 5m inhibits proliferation of various cancer cells. (A) The structure of compound 5m. (B) IC50 of compound 5m against various cancer cells. (C) The growth curves of HepG2 cells treatment with different dose compound 5m. (D) Colony forming assay performed by different dose compound 5m treatment and control HepG2 cells (n = 3).
In our study, exhibited better inhibition of the proliferation of HepG2 cells than sorafenib ($IC_{50} = 9.61 \mu M$) with $IC_{50}$ values of 1.96 $\mu M$. The advantages of compound 5m included the fact that it was easy to synthesize and purify and that it had high bioactivity. In addition, compound 5m played a role in the apoptosis of cancer cells by inducing the expression of p21 and Cl-caspase3 proteins, and these results suggested the need for further study of the targets and pathological mechanisms of compound 5m. In addition, we established the structure-activity relationship of the synthesized amide analogs through structural optimization. Currently, studies on the therapeutic application of the potent amide analogs are in progress.

**Conclusions**

Based on sorafenib, we designed and synthesized three series of diaryl ether derivatives of tumor inhibitors. After several bioactivity tests, it was found
that compound $5m$ exhibited better tumor inhibitor activity against HepG2 cells ($IC_{50} = 1.96 \, \mu M$) than other compounds. Through western blot analysis, it was found that compound $5m$ could induce the expression of p21 and Cl-caspase3, which may result in the apoptosis of HepG2 cells.

Supplementary Information
Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

Acknowledgments
This work was supported by the National Natural Science Foundation of China (NSFC-81472230), the National Training Program of Innovation and Entrepreneurship for Undergraduates (2016x0644), and the Natural Science Foundation of Fujian Province of China (2015Y0081, 2015J01350).

The authors have declared no conflicts of interest.

References