The design, synthesis and biological evaluation of 7-alkoxy-4-heteroarylamino-3-cyanoquinolines as dual inhibitors of c-Src and iNOS

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ABSTRACT

Because both c-Src and iNOS are key regulatory enzymes in tumorigenesis, a new series of 4-heterocycle amine-3-quinolinecarbonitriles as potent dual inhibitors of both enzymes were designed, synthesized and evaluated as multiple targets agents in cancer therapy. All compounds were evaluated by two related enzyme inhibition assays and an anti-proliferation assay in vitro. The results showed that most compounds inhibited c-Src and iNOS well. The best compound 8 inhibited both enzymes with the IC50 values of 34.8 nM and 26.7 μM. Several compounds also showed moderate anti-proliferation at 10 μM against colon and liver cancer cell lines.

SFKs (Src family kinases) belong to the non-receptor protein tyrosine kinases (PTKs), and they transfer the γ-phosphoryl group from ATP to the hydroxyl group of specific tyrosine (Tyr) residues on substrate proteins.1 SFKs express predominantly in differentiated cell types and regulate many fundamental cellular processes, including cell growth, differentiation, cell shape, migration, survival, and specialized cell signals.2 They were also found to be over-activated in various human cancers, including colon,3 pancreatic,4 lung,5 and ovarian cancers.6 These observations have prompted many investigations of c-Src inhibitors aimed toward curing cancer, for example bosutinib (SKI-606, 1) of Wyeth has been evaluating in a Phase III clinical trial in the treatment of CML.7,8 CPU-Y020 (2) is another efficient Src inhibitor reported by our group which is under preclinical evaluations (Fig. 1).9

iNOS is the inducible hypotype of nitric oxide synthase (NOS), and is expressed in a wide array of cells and tissues, for example, macrophages,10 Kupffer cells, hepatocytes,11 neutrophils, pulmonary artery epithelium, colonic epithelium, and vasculature.12 Unfortunately, iNOS also expresses highly in various neoplastic diseases.13 The role of iNOS during tumor development is highly complex and remains obscure. However, there is no doubt that the tumor metastatic activity, host defense mechanisms, and the level of differentiation are all correlated with over-expressed iNOS.14 The attempt to suppress two distinct cancer related-enzymes with a single agent was an attractive idea. It is reasonable that the dual inhibitors of both c-Src and iNOS were more effective for blocking multiple signaling pathways in cancer intervention.

Figure 1. The structures of bosutinib (SKI-606, 1) and CPU-Y020 (2).
The selective inhibitors against iNOS previously.\textsuperscript{15–17} In order to retain derivatives, etc (multifunctional heterocycle amines, like 2-aminothiazole and its piperine from the conventional 4-aniline group to some other carbonitrile as the leading scaffold, and made functional hops from the 4-position of 3-quinolinecarbonitrile and the 2-position of 2-aminobenzothiazole and 2,5-diaminobenzimidazole were chosen as the hopped groups that were introduced into the leading scaffold (Fig. 3). The relatively more active 6'-amino group was connected to the 4-position of 3-quinolinecarbonitrile and the 2'-amino group was directed out of the molecule.

![Figure 3](image-url) The design of the dual inhibitors of Src and iNOS.

The preparation of the designed compounds was carried out smoothly. 2,6-Diaminobenzothiazole 7\textit{a} and 2,5-diaminobenzimidazole 7\textit{b} were synthesized by selective nitration at 6-position of 2-aminobenzothiazole and 5-position of 2-aminobenzimidazole,\textsuperscript{18} and the nitro group intermediates were reduced by iron powder (Scheme 1) to achieve the heterocycle diamine 7. The 3-quinolinecarbonitrile core was prepared using the modified cyclic method as described by us previously.\textsuperscript{9} Then the two main fragments were connected in refluxing isopropanol. After that, the protective group was removed and an alkaline side chain at C-7 position were introduced (Scheme 2).

The compounds were tested in two related enzyme inhibition assays to determine their inhibition activities on Src and iNOS: a Protein Tyrosine Kinase Inhibition Assay (c-Src, and Tyrosine Kinase Assay Kit, Invitrogen) and an iNOS Inhibition Assay (mice macrophage ANA-1, and Nitric Oxide Synthase Assay Kit, Beyotime China) were carried out following the reported methods.\textsuperscript{19–21}

The IC\textsubscript{50} values of compounds 8–18 (Table 1) clearly showed that most compounds inhibited c-Src and iNOS with the IC\textsubscript{50} values range in the domains of 20–80 nM and 10–50 μM, respectively. However, the Src inhibition activities turned to be less potent than SKI-606 (1, Table 1)\textsuperscript{7} for all their IC\textsubscript{50} values were more than 20 nM. The iNOS inhibition activities were similar to those typical iNOS inhibitors, such as 2-aminothiazole (3)\textsuperscript{16} and L-canavanine (4, Table 1).\textsuperscript{17} Most of the IC\textsubscript{50} values towards iNOS ranged 10–50 μM.

When a 2,6-diaminobenzothiazole group was at C-4 position, and a methoxyl group was at C-6 position, compound 14 showed the best Src inhibition activity with an IC\textsubscript{50} of 20.7 nM while its iNOS inhibition activity was only 105 μM. But when the C-6 methoxyl group was changed into a methyl group, the compound 11's Src inhibition activity decreased to 129 nM while its iNOS inhibition activity increased to 17.3 μM. When n equaled 3 and the terminal alkaline group piperazine was changed to 3',5'-dimethylpiperezine, compound 13 showed the IC\textsubscript{50} values of 36.6 nM and 89.1 μM towards Src and iNOS.

Other C-7 alkaline substituted compounds like compounds 9, 10 and 12's Src inhibition activities were 3- to 5-fold weaker than compound 13 while their iNOS inhibition activities were more potent than compound 13. When n equaled 2 and the terminal alkaline group was piperidine, compound 8 showed IC\textsubscript{50} values of 34.8 nM and 26.7 μM towards the two enzymes and that was better compound 13.

The changed 4-heteroaromatic amines of these compounds affected the inhibition activity towards Src kinase. When the 4-aminolino group was changed into 2,5'-diaminobenzimidazole, compound 16 and 17 only showed weaker inhibition against Src kinase with IC\textsubscript{50} values of 143 and 159 nM. But it was interesting that compounds 16 and 17 showed better inhibition activities against iNOS with the IC\textsubscript{50} values of 13.2 and 24.8 μM compared with the 2,6-diaminobenzothiazole substituted compounds.

In general, most compounds could inhibit both enzymes in vitro screening, while compound 8 showed the best activities against both enzymes with the IC\textsubscript{50} values of 34.8 nM and 26.7 μM, respectively. The new compounds were more potent against Src than their activities against iNOS, as indicated by the IC\textsubscript{50} values in

![Scheme 1](image-url) Reagents: (a) HNO\textsubscript{3}, H\textsubscript{2}SO\textsubscript{4}; (b) Fe, HCl, ethanol.
the nanomolar range. This result may be explained as that the scaffold of the series was mainly designed to fit in the hinge region near the ATP binding site in Src kinase domain, and what is more, the agents only possess some inhibition groups against iNOS. Thus, there were no breakthrough findings with respect to iNOS inhibitors.

The anticancer activities of these compounds were also evaluated by a SRB (Sulforhodamine B) anti-proliferation assay. However, there were no predominant compounds discovered according to the assay results. All compounds were less active than SKI-606 in the anti-proliferation screening assay according to the percentages of survival cancer cells at 10 μM treated by the compounds. Only a few compounds, such as compounds 8, 9 and 15, exhibited moderate inhibition activities towards the colon cancer HT-29 and liver cancer HepG2 cell lines at 10 μM in vitro assays (Table 2).

Table 2
The percentages of survival cells at 10 μM treated by compounds 8, 9, 15 and bosutinib (1) against 5 different human cancer cell lines

<table>
<thead>
<tr>
<th>Compound</th>
<th>Lung A549 (%)</th>
<th>Stomach AGS (%)</th>
<th>Liver HepG2 (%)</th>
<th>Colon HT-29 (%)</th>
<th>Prostate PC-3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>55.39</td>
<td>61.01</td>
<td>37.10</td>
<td>42.70</td>
<td>77.68</td>
</tr>
<tr>
<td>9</td>
<td>64.14</td>
<td>68.13</td>
<td>40.45</td>
<td>31.60</td>
<td>59.45</td>
</tr>
<tr>
<td>15</td>
<td>53.64</td>
<td>65.46</td>
<td>42.16</td>
<td>60.59</td>
<td>62.99</td>
</tr>
<tr>
<td>1 (Bosutinib)</td>
<td>9.32</td>
<td>3.80</td>
<td>9.20</td>
<td>2.84</td>
<td>7.81</td>
</tr>
</tbody>
</table>
The discrepancy between enzymatic and cell activity is likely attributed to the poor cell permeabilities of the compounds. The 2-aminothiazole and 2-aminothiazole at the heads of the molecules act as the 4-heteroaromatic amine groups in the designed scaffold exhibit both acidic and alkaline properties in solution, and could be easily protonated in the artificial environment of the cell culture medium, which imitates the natural intercellular substance of the cells. The molecular polarizations of these compounds would be enhanced, and with the extra positive charges on the molecules, it would be difficult for the agents to permeate across the cell membranes. As a result, the anti-proliferation activities of the compounds decreased though they showed moderate enzyme inhibition activities towards both Src and iNOS.

In conclusion, a series of dual inhibitors of Src kinase and iNOS as anticancer agents were proposed in this study. The designed compounds were synthesized and evaluated by related biological assays. Most of these compounds were effective to permeate across the cell membranes. As a result, the anti-proliferation activities of the compounds decreased though they showed moderate enzyme inhibition activities towards both Src and iNOS.

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References and notes