Design and Synthesis of A Biotinylated Probe of COX-2 Inhibitor Nimesulide Analog JCC76

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Design and synthesis of a biotinylated probe of COX-2 inhibitor nimesulide analog JCC76

Bo Zhong, Rati Lama, Kerri M. Smith, Yan Xu, Bin Su

Numerous studies have demonstrated overexpression of cyclooxygenase 2 (COX 2) in solid malignancies including breast, prostate, colon, pancreas, non-small cell lung, bladder, and endometrium. Prostaglandin 2 (PGE2), the product of COX 2, promotes tumor invasiveness, angiogenesis, and progression in various cancers. It is therefore reasonable to conclude that inhibition of COX 2 would arrest carcinogenesis and thus, prevent cancer development and regress cancer once developed. Corresponding to these observations and theory, epidemiological, clinical, and preclinical studies provide compelling evidence that the use of COX 2 selective inhibitors may reduce the incidence of mammary cancer and colorectal cancer. Theoretically, COX 2 inhibitors achieve all the anti cancer or cancer preventive activity by targeting COX 2. However, these small molecules may also block other cellular machineries to affect the cancer cell function, which may lead to cell growth inhibition, apoptosis or necrosis. With the extensive studies of COX 2 inhibitors about their anti cancer or cancer prevention activity, molecular targets of the drugs beyond COX 2 seems play a very important role for their antineoplastic activity. Many researchers even suggested that COX independent effects might be fully responsible for the anti cancer properties of some COX 2 inhibitors. The phenomenon is more like an off target effect, which also underscores the needs to explore targets beyond COX 2, especially in view of emerging toxicity of the COX 2 inhibitors. Developing nonCOX 2 inhibitory analogs as new anti cancer drug based on COX 2 inhibitors as lead compound is feasible and practicable. In fact, this principle has been successfully applied in the discovery of some new anti cancer drugs. For instance, COX 2 inhibitor Celecoxib weakly inhibits PDK 1 kinase in prostate cancer cells. It has been used as a molecular scaffold to develop more potent PDK 1 inhibitors (no COX 2 inhibitory activity) which block PI3 K/Akt pathway. The generated new analog, such as OSU 03012 is 20 fold more active than Celecoxib to inhibit tumor cell proliferation and to induce cell apoptosis, which is currently in a phase I clinical trial. Nimesulide (4 nitro 2 phenoxymethanesulfoanilide) is a nonsteroidal anti inflammatory drug with a preferential COX 2 inhibitory activity and is available in some Asian and European countries since 1985. Studies suggest that nimesulide could induce apoptosis in liver and lung cancer cells; it also suppressed the development of 2 amino 1 methyl 6 phenylimidazo[4,5 b]pyridine (PhIP) induced mammary gland carcinogenesis in rats. NonCOX 2 active nimesulide derivatives have been synthesized. Some analogs were much more active than nimesulide in suppressing breast cancer cell growth. Interestingly, the most potent analog JCC76 was selectively potent in Her2 overexpressed breast cancer cells. Both in vitro and in vivo anticancer properties of JCC76 make it a potential new drug candidate or drug lead for Her2 overexpressed breast cancer.

To rational design more potent analogs of JCC76 based on its binding pocket and optimize the structure activity relationship (SAR), it is a prerequisite to identify the specific molecular target(s) of JCC76. Although JCC76 exhibited good selectivity to Her2 over expressed breast cancer cells, it did not show any significant block...
cer cells. In an effort to identify the bio molecule(s) that bind to JCC76, we planned to use biotin streptavidin affinity purification. For this purpose, biotinylated derivative of JCC76 with considerable bioactivity should be designed and synthesized. Generally, a biotinylated linker can be incorporated at the suitable position of JCC76, so that the biological activity of the small molecule can be conserved. Moreover, a linker between small molecule and biotin should have appropriate length in order to provide enough space for biotin to interact with streptavidin (Fig. 1).

The previous structure-activity analysis indicated that the inhibition of breast cancer cell growth by nimesulide derivatives requires a hydrophobic group substituted bulky phenyl ring, a methanesulfonyl and a hydrophobic carboxamide moiety. Those three moieties might not be suitable for incorporation of biotinylated linker. N methyl group on the sulfonamide group is the only position feasible for the introduction of biotin (Fig. 2).

Replacement of N methyl group with hydrogen or alkyl chains of different length generated a series of novel derivatives. The synthetic approach used is shown in Scheme 1. The key intermediate 5 was prepared according to the procedure previously used for synthesis of JCC76. The starting material 1 was treated with 2,5 dimethylbenzylbromide in the presence of potassium carbonate to afford compound 2, followed by mesylation and hydrolysis to generate compound 3. Instead of N methylation of sulfonamide group, compound 3 was directly reduced in the presence of Zn/FeCl₃ to generate substituted aniline 4. Coupling with cyclohexanecarbonyl chlo...
Table 1
IC₅₀ of inhibition of SKBR-3 breast cancer cells growth by JCC76 probes

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Inhibition of SKBR-3 cell growth (IC₅₀ μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JCC76</td>
<td>Me</td>
<td>3.43 ± 1.57</td>
</tr>
<tr>
<td>S</td>
<td>H</td>
<td>83.68 ± 42.77</td>
</tr>
<tr>
<td>6a</td>
<td></td>
<td>81.68 ± 31.18</td>
</tr>
<tr>
<td>6b</td>
<td></td>
<td>12.95 ± 4.92</td>
</tr>
<tr>
<td>8a</td>
<td></td>
<td>6.00 ± 2.52</td>
</tr>
<tr>
<td>8b</td>
<td></td>
<td>45.28 ± 12.47</td>
</tr>
<tr>
<td>8c</td>
<td></td>
<td>2.21 ± 0.78</td>
</tr>
</tbody>
</table>

SKBR-3 cells were treated with indicated compounds at various concentrations by triplicates for 48 h and cell viability was measured by MTT assay.¹²

chloride afforded 5. N alkylation of sulfonamide group with alkyl bromide/iodide in the presence of potassium carbonate yielded 6a c. Conversion of hydroxyl group in 6c to amino group followed by coupling with alkyl or aromatic carbonyl chloride generated 8a b.¹²

The anti proliferation effect of compounds 5, 6a, b, and 8a, b was evaluated against breast cancer cell line SKBR 3. The results are summarized in Table 1. It can be seen that alkyl group on the nitrogen of sulfonamide appreciably affects the biological activity of JCC76 derivatives. Replacement of methyl group with hydrogen or hydrophobic alkyl chain C₆H₁₃ almost abolishes the inhibitory activity. Replacement of methyl group with hydrophobic (CH₃)₂O (CH₂)₃OCH₃ to afford 6b and results in an about fourfold loss in inhibitory potency. It seems that hydrophobic linker is better than hydrophilic linker. However, it is difficult to further modify the end structure of the hydrophilic linker 6b. Extension of the linear C₆ chain with another alkyl chain or an aromatic ring through for mation of an amide bond restores the biological activity to some extent. Compared to 6a, compound 8b is about twofold more potent and compound 8a even exhibits 14 fold more potency. Notice ably, the inhibitory potency of compound 8a is comparable to that of JCC76, which indicates that the long chain in 8a is well tolerated. A survey of several JCC76 derivatives with different N alkyl chains shows that an appropriate alkyl group can be introduced in lead compound JCC76 without jeopardizing the biological activity.

Based on the information obtained from this preliminary SAR study, we designed and synthesized compound 8c, a novel biotinylated derivative of JCC76. PyBOP mediated coupling of intermediate 7c with biotin efficiently yielded compound 8c.²¹ This compound has a very similar long chain as in compound 8a. Hypothetically, with this long chain the biotin moiety should protrude for the interaction with streptavidin. The anti proliferation effect of 8c on SKBR 3 was evaluated and its IC₅₀ value was determined to be 2.21 ± 0.78 μM. This biotinylated derivative has comparable cell growth inhibition potency with lead compound JCC76. Biotin did not show any inhibitive activity even at 100 μM, which further prove that JCC76 was the moiety that cause the anti proliferation activity of the biotinylated probe.

In conclusion, we synthesized a biotin tagged probe of anti proliferative agent JCC76 in this work. The biotin moiety was successfully introduced in the molecule without loss of the biological activity of the parental compound. JCC76 is nonCOX 2 active agent developed based on COX 2 inhibitor nimesulide as a lead compound. It selectively inhibits Her2 over expressed breast cancer cell proliferation.¹⁰,²⁰ but its specific anti cancer molecular target(s) still remain unclear. There are numerous studies demonstrate that COX inhibitors exhibit anti cancer activity via COX independent mechanism(s).¹⁰,²⁰ However, it is difficult to elucidate the specific nonCOX targets of these small molecules. Our work can provide a feasible approach for the identification of the new molecular targets of the COX inhibitors. We currently focus on the purification and identification of JCC76 targeted protein(s) by protein pull down and proteomic approach based on the biotin tagged JCC76 probe.

Acknowledgment

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References and notes


20. Compound 5: White powder, $^1$H NMR (400 MHz, CDCl$_3$) δ 7.970 (1H, d, $J = 2.4$ Hz), 7.453 (1H, d, $J = 8.4$ Hz), 7.232 (1H, s), 7.126 (3H, m), 6.696 (1H, dd, $J = 2.4, 8.4$ Hz), 6.555 (1H, s), 5.047 (2H, s), 2.834 (3H, s), 2.313 (3H, s), 2.242 (1H, tt, $J = 3.6, 12$ Hz), 1.245–1.985 (10H, m); Compound 6a: White powder, $^1$H NMR (400 MHz, CDCl$_3$) δ 8.027 (1H, s), 7.883 (1H, s), 7.134 (4H, m), 6.686 (1H, d, $J = 8.4$ Hz), 5.677 (1H, b), 5.024 (2H, s), 3.487 (2H, b), 3.184 (2H, d, $J = 6.4$ Hz), 2.691 (3H, s), 2.312 (7H, m), 2.178–1.290 (26H, m), 0.878 (3H, t, $J = 6.8$ Hz); Compound 8a: White powder, $^1$H NMR (400 MHz, CDCl$_3$) δ 8.294 (1H, s), 8.006 (1H, s), 7.854 (5H, m), 7.528 (2H, m), 7.185 (1H, d, $J = 8.4$ Hz), 7.077 (3H, m), 6.696 (1H, dd, $J = 2, 8.4$ Hz), 7.057 (1H, s), 3.455 (4H, m), 2.680 (3H, s), 2.289 (7H, m), 1.931–1.192 (18H, m); Compound 8c: White powder, $^1$H NMR (400 MHz, CDCl$_3$) δ 8.201 (1H, s), 8.085 (1H, s), 7.203 (1H, d, $J = 8.4$ Hz), 7.061 (3H, m), 6.763 (1H, dd, $J = 2, 8.4$ Hz), 6.186 (1H, s), 6.115 (1H, t, $J = 6$ Hz), 5.223 (1H, s), 5.040 (2H, s), 4.498 (1H, m), 4.314 (1H, m), 3.488 (2H, m), 3.149 (3H, m), 2.900 (1H, dd, $J = 4.8, 128$ Hz), 2.717 (1H, d, $J = 12.4$ Hz), 2.684 (3H, s), 2.314 (7H, m), 2.195 (2H, t, $J = 7.2$ Hz), 1.951–1.283 (24H, m); HRMS calculated for C$_{39}$H$_{58}$N$_5$O$_6$S$_2$ (M+H$^+$) $^756.3828$, found 756.3847.

22. The effect of JCC76 probes on SKBR-3 breast cancer cell viability was assessed by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay (MTT) in triplicates. Cells were grown in custom medium in 96-well, flat-bottomed plates for 24 h, and were exposed to various concentrations of nimesulide derivatives dissolved in DMSO (final concentration $\leq 0.1\%$) in media for 72 h. Controls received DMSO vehicle at a concentration equal to that in drug-treated cells. The medium was removed, replaced by 200 l of 0.5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide in fresh media, and cells were incubated in the CO$_2$ incubator at 37 °C for 2 h. Supernatants were removed from the wells, and the reduced 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide dye was solubilized in 200 µl/well DMSO. Absorbance at 570 nm was determined on a plate reader.