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# Synthesis and biological evaluation of novel benzamide derivatives as potent smoothened antagonists

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ABSTRACT

A series of novel benzamide derivatives were prepared and evaluated using cell-based measurements. Among these compounds, **10f** significantly inhibited Hedgehog signaling and showed equivalent or more potency than GDC-0449 in different tests. Furthermore, compound **10f** potently inhibited the proliferation of Daoy, a medulloblastoma cell line that is reported to be resistant to GDC-0449, which indicated a promising prospect in the treatment of Hedgehog signaling pathway related cancer in clinical trial.



In addition to the important role in the regulation of stem cell and progenitor cell differentiation during multiple developmental processes, Hedgehog (Hh) signaling pathway has also been implicated in various type of cancers, such as skin cancer,[1] breast cancer,[2] prostate cancer[3] and brain cancer.[4] Typically, Hedgehog pathway is activated when the secreted Hedgehog ligands directly bind to 12-pass transmembrane protein Patched1 (PTCH1),[5] relieving its repression of the G protein-coupled receptor-like protein Smoothened (SMO),[6] which subsequently signals to the glioma-associated (Gli) family of transcription factors and induces a change in the transcriptional profile of the cell.

Antagonists of SMO showed effectively anticancer activity, and a number of these antagonists were under investigation in clinical trials, such as CUR61414[7] and IPI-926.[8] More recently, one known SMO antagonist Vismodegib (GDC-0449) has achieved promising results for the treatment of patients with advanced BCC, highlighted by a 55% overall response rate in early-stage clinical studies,[9] and has been approved by FDA for the treatment of metastatic basal cell carcinoma and locally advanced basal cell carcinoma.[10] These data suggests that SMO is a promising therapeutic target for Hh signaling pathway related diseases.

Aiming to design, synthesize and obtain novel SMO inhibitor for the treatment of Hh dependent malignancies, we initially replaced the C-3 substitution on the middle phenyl group of GDC-0449 with urea or amide spacer, and expected that the terminal NH group of the urea or amide moiety may form additional hydrogen bond(s) at the receptor site to improve SMO antagonist activity. The SMO antagonist activity was identified by dual luciferase assay on Shh-light 2 cells (NIH3T3 cell line stably transfected with 8*Gli-binding site luciferase and Renilla reporters).[11] The following initial hit offered considerable potency in Light 2 assay with $IC_{50}$ = 300 nM (Fig. 1).

Encouraged by this result, a series of compounds based on the lead structure was synthesized. To begin with, the target amide derivatives **6a–d** were prepared according to the route described

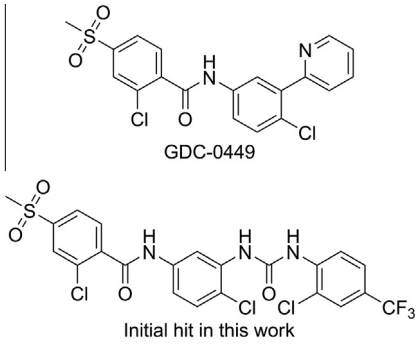


**Figure 1.** Structures of GDC-0449 and the initial hit identified from the cell-based Gli-Luciferase assay.

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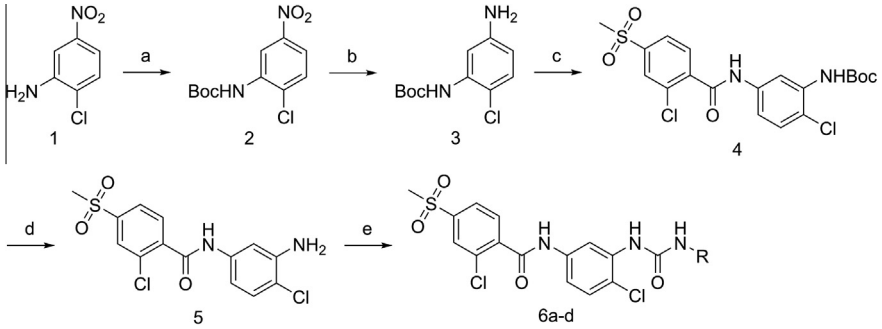
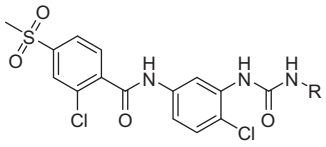
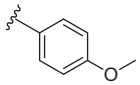
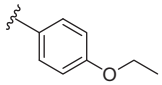
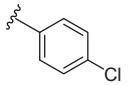
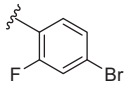
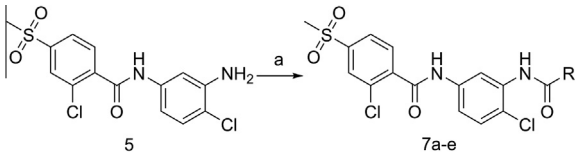


**Scheme 1.** Reagents and conditions: (a) $Boc_2O$, $Et_3N$, DMAP, THF, 25 °C, 98.2%; (b) Fe, $NH_4Cl$ in $H_2O$, ethanol, 90 °C, 52%; (c) 2-chloro-4-(methylsulfonyl)benzoyl chloride, $Et_3N$, DMAC, 78.6%, (d) TFA/$CH_2Cl_2$ = 10%, 25 °C, 87.1%; (e) substituted phenyl isocyanates, pyridine, 25 °C.

**Table 1**
Activities of compounds against the Hh pathway at a concentration of 2 μM



| Entry | R | Gli-LUC Shh-light 2 inhibition (%) |
|---|---|---|
| **6a** |  | 73.8 ± 3 |
| **6b** |  | 66.1 ± 2 |
| **6c** |  | 0 |
| **6d** |  | 55.4 ± 5 |



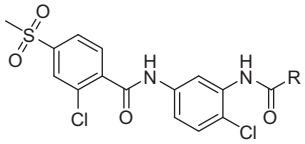
**Scheme 2.** Reagents and conditions: (a) aromatic acyl chlorides, THF, $Et_3N$, 25 °C.

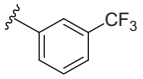
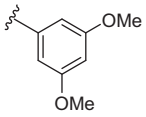
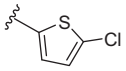
in Scheme 1. The amino group of 2-chloro-5-nitroaniline (**1**) was protected by Boc, and then the nitro group was reduced to amino group to give compound **3**. Compound **3** was reacted with 2-chloro-4-(methylsulfonyl) benzoyl chloride in the presence of triethylamine to obtain compound **4**. After deprotection of the Boc group of compound **4** with TFA in dichloromethane, the resulting amine (**5**) is finally condensed with appropriate aryl isocyanate to gain the target urea derivatives **6a**–**d**.

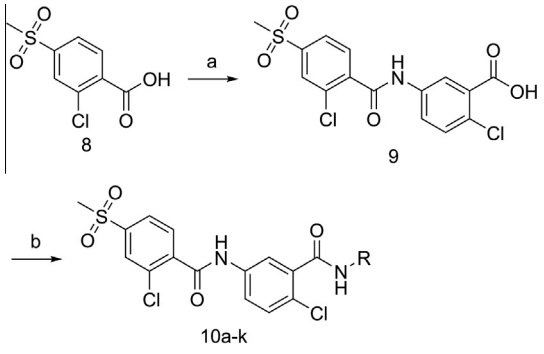
Dual luciferase assay was performed to measure the inhibition activities of each compound at concentration of 2 μM. Except compound **6c**, compounds **6a**, **6b** and **6d** exhibited good Hh signaling inhibition activities (Table 1). However, when the concentrations of **6a**–**d** decreased to 0.5 μM, none of the above compounds showed satisfying potency (data not shown).

Subsequently, we preceded our investigation by modifying the urea moiety to two different amide-based derivatives with

**Table 2**
Activities of compounds against the Hh pathway at a concentration of 2 μM



| Entry | R | Gli-LUC Shh-light2 inhibition (%) |
|---|---|---|
| **7a** |  | 28.4 ± 10 |
| **7b** |  | 3.6 ± 10 |
| **7c** |  | 53.2 ± 5 |
| **7d** |  | 30.5 ± 6 |
| **7e** |  | 51.4 ± 7 |



**Scheme 3.** Reagents and conditions: (a) (i) $SOCl_2$, reflux; (ii) 5-amino-2-chlorobenzoic acid, $Et_3N$, DMAC, 25 °C, 79.8%, (b) (iii) HOBT, DCC, DMF, 0 °C; (iv) aromatic amines, 0 °C; (v) 25 °C.

opposite directions. One group of amide-based derivative was prepared according to the route described in Scheme 2. Compound **5** was reacted with aromatic acyl chlorides in the presence of triethylamine to yield **7a**–**e**. Nevertheless, the inhibition activities of compounds **7a**–**e** in the Light 2 assay (Table 2) were all far behind the reported potency of GDC-0449 ($IC_{50}$ = 20 nM).[12]