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ARTICLE TYPE

# Design, synthesis and evaluation of novel tetrahydrothieno[3,2-c]pyridine derivatives as potent smoothened antagonists†

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Hedgehog (Hh) signalling plays an important role in embryonic development and adult tissue homeostasis. Since activation of the Hh signalling pathway is implicated in several types of human cancers, inhibitors of this pathway could be promising anticancer agents. The smoothened (Smo) receptor mediates Hh signalling. In the present study, we synthesised a series of novel tetrahydrothieno[3,2-c]pyridine derivatives using a scaffold hopping strategy. Compounds with this novel scaffold demonstrated promising Hh and Smo inhibition, indicating that this novel scaffold can serve as a starting point for further optimisation.

## 1. Introduction

The hedgehog (Hh) pathway is a key signalling transduction pathway that regulates many critical cellular processes, including cell proliferation, embryological development, tissue growth and stem-cell maintenance [1-3]. Hh proteins were originally identified as a highly conserved protein family in fruit flies. This family includes sonic Hh, desert Hh and Indian Hh; all of these act as ligands of the 12-pass transmembrane protein Patched (Ptch) . In the absence of the Hh ligand, Ptch inhibits the activity of the membrane receptor , smoothened (Smo), which resembles G-protein coupled receptors in its general topology. Upon binding to an Hh ligand, Ptch relieves the suppression of Smo, which moves into the primary cilia where it promotes the dissociation of the complex of suppressor of fused and glioma-associated oncogene homologue (GLi). This allows GLi to translocate to the nucleus, where it regulates the expression of genes involved-in uncontrolled cellular proliferation and tumour growth most notably in basal cell carcinoma (BCC) [4,5] and medulloblastoma (MB) [6]. Thus, inhibition of aberrant Hh signalling provides a promising approach to the development of a novel anticancer therapy.

Recent research has focused on novel types of Smo antagonist [7-9]. The plant steroidal alkaloid, cyclopamine, was first identified as an inhibitor of Hh signalling because it produced teratogenic effects in sheep [10]. Cyclopamine was later shown to bind directly to Smo [11]. Subsequent research has identified a number of small-molecule Smo antagonists, which are progressing through clinical trials (Fig. 1) [12] ; these include the semi-synthetic cyclopamine analogue IPI-926 (Infinity), LY-2940680 (Lilly) and PF-04449913 (Pfizer). The cyclopamine analogue, IPI-926 , has demonstrated improved potency and drug-like properties [13]. Nevertheless, Infinity Pharmaceuticals reported disappointing results from a phase II study of IPI-926 in patients with metastatic chondrosarcoma and subsequently suspended the trials for myelofibrosis in 2012. To date , two Hh signalling pathway inhibitors, vismodegib (GDC-0449) and sonidegib (NVP-LDE225), have been approved by the United State Food and Drug Administration for the treatment of locally advanced BCC [14].

Unfortunately, the development of resistance to these compounds has become a major hurdle for their continued development. Treatment of an MB patient with GDC-0449 produced initially positive results, but relapse occurred owing to a point mutation in Smo (D473H), the molecular target of GDC-0449; this rendered the patient insensitive to further GDC-0449 treatment [15]. The molecular mechanisms explaining resistance to vismodegib were reported and published for the first time in 2014 [16]. The Smo mutations found in sonidegib resistant tumours, at position 477 [17], differed from those found during vismodegib treatment. This is the only acquired resistance reported so far in humans. Novel potent Hh signalling pathway inhibitors are still urgently needed as probes to identify clinical profiles for Hh signaling targeting strategy.

The aim of the present study was to establish a novel skeleton for Smo antagonists and to explore the diversity of the Hh signalling pathway inhibitor structure, laying a foundation for further exploration of the structure-activity relationship (SAR) in order to develop more effective Smo antagonists. Previous studies have identified Hh pathway inhibitor molecules using high-throughput screening campaigns [18,19]. The publication of the crystal structure of Smo protein [20] has provided further opportunities to identify novel Hh signalling pathway antagonists. Fragment-based design

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and screening is a highly attractive design tool in medicinal chemistry. Our investigation of a number of templates for novel Smo receptor antagonists found that the benzimidazole building block, was present in numerous Smo inhibitors [21,22], including the Pfizer compound, PF-04449913 . We replaced the pyridine group with the benzimidazole fragment of PF-04449913 via the isostere principle and expected that the benzimidazole group would form extra hydrogen bond(s) at the receptor binding site, thus increasing Smo antagonist activity. Additionally, nitrogen-or sulphur-containing heterocycles are regularly used as isosteres for amide groups in medicinal chemistry. Interestingly, the use of heterocycles as amide replacements was recently reported to improve Smo antagonist activity [23-25], and we speculated that the conceptual core of piperidine fused to thiophene would produce a number of new potential Hh signalling pathway inhibitors. Hence, the novel compounds were designed using the tetrahydrothieno[3,2-c]pyridine skeleton as a privileged scaffold and employing a combination of conceptual cyclisation and bioisosterism strategies, as shown in Fig. 2 . Subsequently, these molecules, with diverse side chains, were synthesised and their biological activities were evaluated .

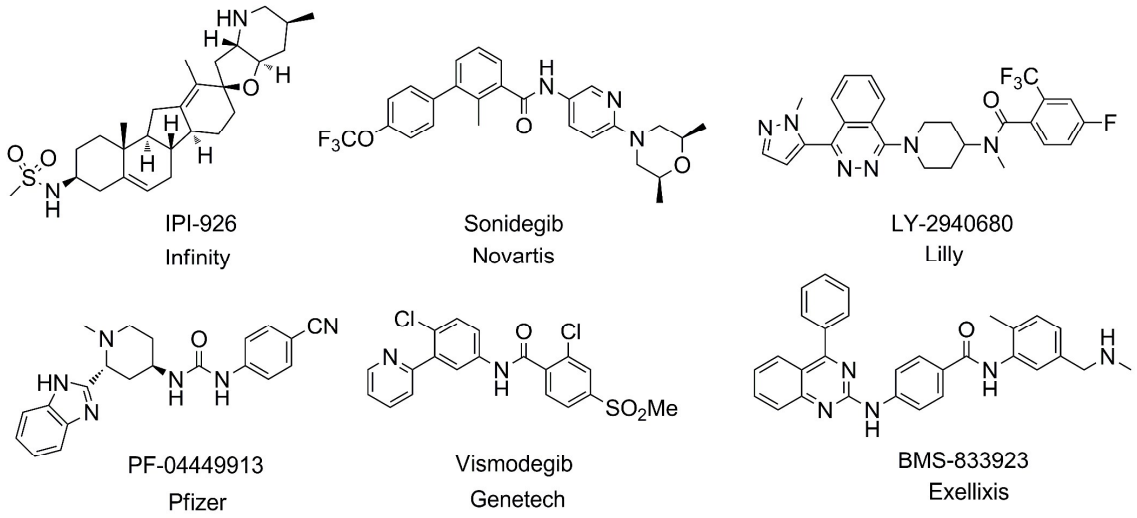


**Fig. 1** Structures of smoothened antagonists in advanced development

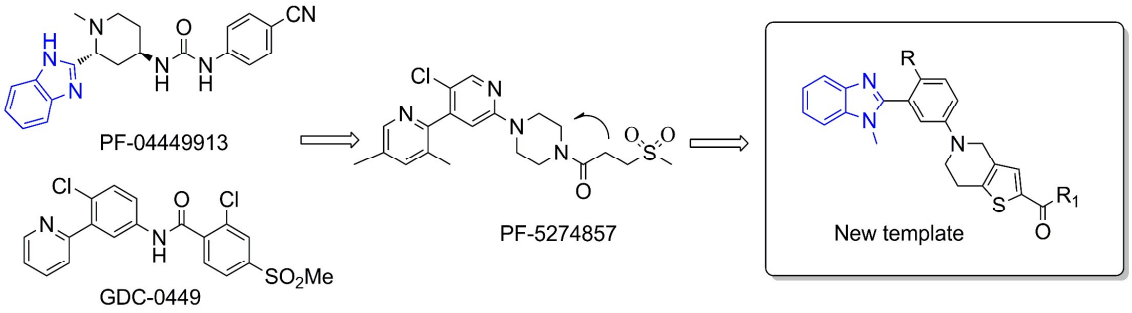


**Fig. 2.** The isostere and scaffold hopping approach used to design a novel series of smoothened antagonists

## 2. Results and Discussion

### 2.1. Chemistry

The general synthetic methods used to build the designed compounds are illustrated in Scheme 1. Production of the key intermediate (4) involved three transformations. Firstly, commercially available butyl-1-piperidinecar-boxylate (1) was chloroformylated using Vilsmeier-Haack reaction to yield intermediate (2); this was then reacted with ethyl mercaptoacetate in the presence of excess triethylamine to yield intermediate (3) and removal of the protecting group of intermediate (3) in the presence of trifluoroacetic acid /dichloromethane afforded the key intermediate (4). The intermediates (10a) and (10b) were prepared as described previously [22], by treating the 1,2-phenylenediamine (5) with 3-bromo-benzaldehyde or 5-bromo-2-chlorobenzaldehyde to afford (7a) and (7b) separately, followed by methylation with methyl iodide in the presence of KOH to produce compounds (8a) and (8b). These were then coupled with intermediate (4) using Buchwald coupling under palladium catalysis to afford the condensation products (9a) and (9b), which were further hydrolysed to

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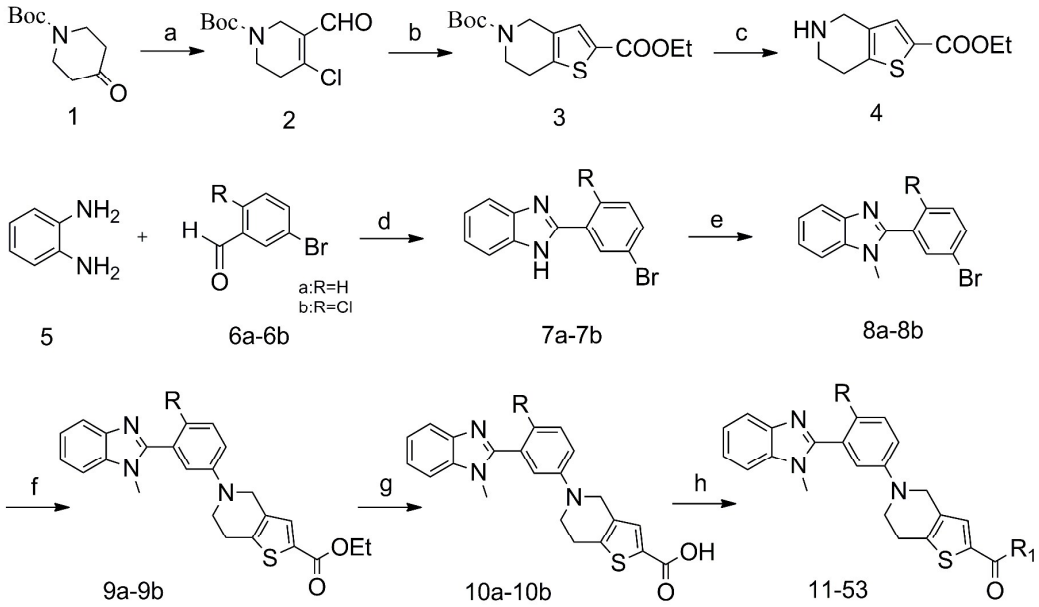
produce the free acids (10a) and (10b). The condensation of (10a-10b) with various amines afforded the target compounds (11-53). The structures of (11-53) were confirmed by $^{1}H$ nuclear magnetic resonance (NMR), $^{13}C$ NMR, and ESI-MS.




**Scheme 1** Reagents and conditions: (a) $POCl_3$, DMF, $CH_2Cl_2$, 0 °C to rt; (b) ethyl mercaptoacetate, $Et_3N$, EtOH, rt; (c) TFA, DCM, rt, 1 h; (d) sodium bisulfite, 3-br-benzaldehyde or 5-bromo-2-chlorobenzaldehyde, 70 °C, 3 h; (e) potassium hydroxide, DMSO, MeI, 2h ; (f) $Pd(OAc)_2$, BINAP, toluene, compound 4, 100 °C, 12 h ; (g) LiOH, THF, rt, overnight ; (h) HATU, $CHCl_3$, ethyl acetate, rt, overnight.

## 2.2. Biological evaluation

The Hh signalling pathway inhibitory activities of tetrahydr-othieno[3,2-c]pyridine derivatives were initially assessed using a luciferase reporter in NIH3T3 cells stably transfected with a Gli-reporter construct (Gli-luciferase reporter cell lines) [26-28]. This assay can effectively identify Smo antagonists and Hh inhibitors. These in vitro 50% inhibitory concentration ($IC_{50}$) values are illustrated in Table 1.

Our initial focus was on compounds bearing various basic substitutions at the carbonyl group. The clearest trend emerging from the resulting SAR was that some cyclic aliphatic amines pyrrole (15), methylpiperidine (16), morpholine (17), 4-hydroxy piperidine (18) and piperazine (19) showed higher activity than that shown by acyclic aliphatic amines (11-14). This finding indicated that the cyclic amine, piperazine (19), showed the most potent activity.

We then explored the 4-position displacement of piperazine, in order to identify some more potent inhibitors. N-ethyl piperazine (20), showed moderate activity ($IC_{50}$ = 0.35 μM) compared to that shown by N-methyl piperazine (19). However, 4-(methylsulfonyl)piperazine (21) showed promising potency ($IC_{50}$ = 0.22 μM). Surprisingly, conversion to the corresponding amide group (22) by the insertion of a carbonyl group between the methyl and 4-position of the piperazine group resulted in a loss of activity ($IC_{50}$ = 0.37 μM). Compound 24 also displayed diminished activity ($IC_{50}$ = 0.49 μM). In a similar manner, the larger Boc derivative (23) showed 12-fold lower activity ($IC_{50}$ = 2.08 μM) than that shown by compound (19) , perhaps owing to steric hindrance by Boc. These data demonstrated that a carbonyl group at that position reduced compound activity. N-phenyl piperazine (25), with $IC_{50}$ of 1.32 μM, displayed similar activity as that of compound (11). The subsequent introduction of a halogen atom to the phenyl ring produced 3-chlorine-phenyl piperazine (26) and 4-fluorine-phenyl piperazine (27), which displayed moderate activities ($IC_{50}$ = 0.38 μM and 0.32 μM , respectively). Interestingly, changing the position of the fluorine atom in compound (28) resulted in a detrimental effect on its biological activity with respect to that of compound (27), whereas; 3,4-dichlorophenyl piperazine (29) showed improved activity ($IC_{50}$ = 0.27 μM). Other substituents reduced potency, such as the methoxy group in the para or ortho position in compounds (30) and (31) , with $IC_{50}$ values of 1.26 μM and 2.35 μM, respectively. These data appeared to indicate that the introduction of a more electron-withdrawing group on phenyl improved the biological activity of these compounds. However, a decrease of Hh inhibition ($IC_{50}$ = 1.76 μM) was observed in the presence of a trifluoromethyl-phenyl group (32). Replacement of the phenyl group by a pyridinyl group (34) resulted in moderate activity ($IC_{50}$ = 0.34 μM). Addition of a second nitrogen to the heteroaryl group (35) improved the activity further, with $IC_{50}$ of 0.15 μM in this assay.

Simple anilines were also systematically explored (compounds 36-45). As illustrated in Table 1, the majority of these aniline analogues either retained or improved the Hh pathway inhibitory potency in these cell-based assays. The introduction of methyl on aniline reduced activity and lengthened the hydrocarbon chain , as demonstrated by compound (37); this was 2-fold less active ($IC_{50}$ = 1.63 μM) and the propyl derivative (38) displayed an even more marked drop in potency

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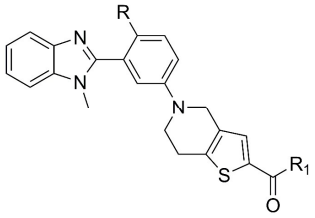
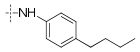
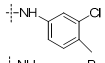
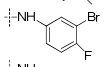
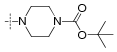
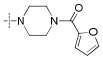
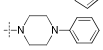
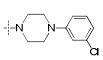
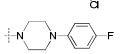
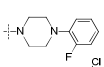
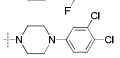
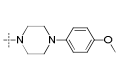
($IC_{50}$ = 3.95 μM). Addition of an ortho-fluorine (39), a para-bromine (41) and ortho-methoxy (42) had no significant impact on activity ($IC_{50}$ = 0.36 μM, 0.45 μM and 0.66 μM, respectively). Di-substituted analogues (40), (43) and (44) demonstrated dece-

**Table 1** SAR of designed compounds




| Compound | R | R1 | Gli-luc Reporter $IC_{50}$ [a] (μM) | Compound | R | R1 | Gli-luc Reporter $IC_{50}$ [a] (μM) |
|---|---|---|---|---|---|---|---|
| 11 | H |  | 1.41 ± 0.02 | 33 | H |  | >15 |
| 12 | H |  | 1.56 ± 0.13 | 34 | H |  | 0.34 ± 0.06 |
| 13 | H |  | 1.72 ± 0.01 | 35 | H |  | 0.15 ± 0.05 |
| 14 | H |  | 1.34 ± 0.03 | 36 | H |  | 0.85 ± 0.08 |
| 15 | H |  | 0.26 ± 0.05 | 37 | H |  | 1.63 ± 0.11 |
| 16 | H |  | 0.33 ± 0.11 | 38 | H |  | 3.95 ± 0.18 |
| 17 | H |  | 0.21 ± 0.01 | 39 | H |  | 0.36 ± 0.02 |
| 18 | H |  | 0.28 ± 0.07 | 40 | H |  | 0.27 ± 0.06 |
| 19 | H |  | 0.17 ± 0.06 | 41 | H |  | 0.45 ± 0.03 |
| 20 | H |  | 0.35 ± 0.12 | 42 | H |  | 0.66 ± 0.11 |
| 21 | H |  | 0.22 ± 0.07 | 43 | H |  | 0.31 ± 0.15 |
| 22 | H |  | 0.37 ± 0.09 | 44 | H |  | 0.35 ± 0.02 |
| 23 | H |  | 2.08 ± 0.02 | 45 | H |  | 0.20 ± 0.03 |
| 24 | H |  | 0.49 ± 0.01 | 46 | Cl |  | 0.22 ± 0.01 |
| 25 | H |  | 1.32 ± 0.02 | 47 | Cl |  | 0.06 ± 0.02 |
| 26 | H |  | 0.38 ± 0.05 | 48 | Cl |  | 0.19 ± 0.03 |
| 27 | H |  | 0.32 ± 0.04 | 49 | Cl |  | 0.13 ± 0.01 |
| 28 | H |  | 0.76 ± 0.16 | 50 | Cl |  | 0.24 ± 0.12 |
| 29 | H |  | 0.27 ± 0. 01 | 51 | Cl |  | 0.09 ± 0.01 |
| 30 | H |  | 1.26 ± 0.05 | 52 | Cl |  | 0.16 ± 0.02 |
| 31 | H |  | 2.35 ± 0.15 | 53 | Cl |  | 0.14 ± 0.03 |
| 32 | H |  | 1.76 ± 0.17 | 54 | Vismodegib[b] | | 0.025 |

[a] The values are an average of triplicate separate determinations.

[b]Used as a positive control.

**Table 2** In vitro inhibition of Smo for tetrahydrothieno[3,2-c]pyridine derivatives

| compound | SMO-BCB $IC_{50}$ [a] (μM) | compound | SMO-BCB $IC_{50}$ [a] (μM) |
|---|---|---|---|
| 15 | 0.352 | 47 | 0.018 |

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| | | | |
|---|---|---|---|
| 17 | 0.294 | 48 | 0.192 |
| 18 | 0.431 | 49 | 0.135 |
| 19 | 0.326 | 50 | 0.217 |
| 21 | 0.373 | 51 | 0.092 |
| 29 | 0.456 | 52 | 0.213 |
| 35 | 0.237 | 53 | 0.056 |
| 45 | 0.261 | Vismodegib[b] | 0.0051 |
| 46 | 0.315 | | |

[a]Inhibition of BODIPY-cyclopamine fluorescence signalling in the competitive displacement experiment using HEK293 cells over-expressing human Smo. Data are expressed from a single $IC_{50}$ determination.
[b]Used as a positive control.

nt potency ($IC_{50}$ = 0.27μM , 0.31 μM and 0.35 μM , respectively). An attempt to further improve activity using heteroaryl incorporated into aniline (45) produced good activity ($IC_{50}$ = 0.20 μM).

Next, the influence of a central aromatic ring substitution in the core region was explored (compounds 46-53). We prepared a set of compounds with a chlorine atom on the phenyl. This introduction afford good potency in the Gli-Luc reporter assay, with $IC_{50}$ values of 0.06 μM, 0.09 μM and 0.14 μM for compounds (47), (51) and (53). The activity thus approached that of vismodegib. These data showed that the presence of a chlorine atom in the central core region was important for inhibition of Hh signalling .

To further verify whether the Hh inhibitory activities of the target compounds in Table 1 derived from their inhibition to Smo receptor, the more potent sixteen compounds were evaluated in a fluorescent competitive displacement assay (Table 2). In this assay, displacement of boron-dipyrromethene (BODIPY)-cyclopamine by the selected compounds was evaluated in HEK293 cells over-expressing human Smo [29,30]. As a result, Compounds 47, 51 and 53 exhibited potent displacement of BODIPY-cyclopamine in these cells (0.018 μM, 0.092 μM and 0.056 μM, respectively), but vismodegib was more active in this assay (0.0051 μM). These data demonstrated that the Smo inhibition potency of these compounds correlated well with their Hh inhibition and suggested that the observed Hh activity in the reporter-gene cell assay was driven by Smo antagonism. Taken together, the results of the present study showed that compounds based on the tetrahydrothieno[3,2-c]pyridine template produced good Hh inhibition by acting as Smo antagonists. SAR studies of other parts of this novel scaffold are on-going in our laboratory and these results will be reported in due course .

## 3. Conclusions

In summary, the exploration of distinctive tetrahydrothieno[3,2-c] pyridine analogues led to the discovery of a novel class of heterocyclic amide compounds that act as potent Smo antagonists. Using this new scaffold, we produced a number of compounds with Hh inhibitory activities that approached that of the marketed drug, vismodegib (0.025 μM); these included (47), (51) and (53) , with $IC_{50}$ values of 0.06 μM , 0.09 μM and 0.14 μM, respectively. In the light of these encouraging results, exploration of other regions of this novel template is on-going in our laboratory and the results will be reported in due course.

## Experimental Section

Synthetic protocols for all compounds, analytical data, and procedures and methods for in vitro assays are available in the Supporting Information.

## Acknowledgments

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## References

1 X. Zen, J. A. Goetz, L. M. Suber, W. J. Scott , C. M. Schreiner and D. J. Robbins, *Nature*., 2001, **411**, 716-720.
2 M. Neeraj, P. Chandanamali and F. Naoaki, *J. Med. Chem*., 2009, **52**, 3829-3845.
3 O. Hideya and K. Mitsuo, *Cancer Sci*., 2011, **102**, 1756-1760.
4 H. Hahn, C. Wickin, P. G. Zaphiropoulos, M. R. Gailani, S. Shanley, A. Chidambaram, I. Vorechovsky, E. Holmberg, A. B. Unden, S. Gillies, K. Negus, I. Smyth, C. Pressman, D. J. Leffell, B. Gerrard, A. M. Goldstein, M. Dean, R. Toftgard, G. Chenevix-Trench, B. Wainwright and A. E. Bale, *Cell*., 1996, **85**, 841-851.
5 R. L. Johnson, A. L. Rothman, J. Xie, L. V.Goodrich, J. W. Bare, J. M. Bonifas, A. G. Quinn, R. M. Myers, D. R.Cox, Epstein, E. H and M. P. Scott, *Science*., 1996, **272**, 1668-1671.
6 C. Raffel, R. B. Jenkins, L. Frederick, D. Hebrink, B. Alderete, D. W.Fults and C. D. James, *Cancer Res*., 1997, **57**, 842-845.
7 G. Guerlet, T. Spangenberg, A. Mann, H. Faure and M. Ruat, *Bioorg. Med. Chem. Lett.,* 2011, **21**, *3608-3612.*
8 S. Malancona, S. Altamura, G. Filocamo, O. Kinzel, J. I. Hernando, M. Rowley, R. Scarpelli, C. Steinkühler, and P. Jones, *Bioorg. Med.Chem. Lett.,*2011, **21**, 4422-4428.
9 O. Kinzel, A. Alfieri, S. Altamura, M. Brunetti, S. Bufali, F. Colaceci, F. Ferrigno, G. Filocamo, M. Fonsi, P. Gallinari, S. Malancona, J. I. Hernando, E. Monteagudo, M. V. Orsale, M. C. Palumbi, V. Pucci, M. Rowley, R. Sasso, R. Scarpelli, C. Steinkühler and P. Jones, *Bioorg. Med. Chem. Lett*., 2011, **21**, 4429-4435.
10 D. M. Berman, S. S. Karhadkar, A. R. Hallahan, J. I. Pritchard, C. G. Eberhart, D. N. Watkins, J. K.Chen, M. K.Cooper, J. Taipale, J. M. Olson and P. A Beachy, *Science* .2002., **297**, 1559-1561.
11 J. K. Chen, J. Taipale, M. K. Cooper and P. A Beachy, *Genes Dev*. 2002., **16**, 2743-2748.
12 S. E. Gould, J. A. Low, J. C. Marsters Jr, K. Robarge, L. L. Rubin, F. J. D. Sauvage, D. P. Sutherlin, H. Wong and R. L. Yauch, *Expert Opin. Drug Discovery.,* 2014, **9**, 969–984.
13 M. R. Tremblay, M. Nevalainen, S. J. Nair, J. R. Porter, A. C. Castro, M. L. Behnke, L. C. Yu, M. Hagel, K. White, K. Faia, L. Grenier, M. J. Campbell, J. Cushing, C. N. Woodward, J. Hoyt, M. A. Foley, M. A. Read, J. R. Sydor, J. K. Tong, V. J. Palombella, K. McGovern and J. Adams, *J. Med. Chem*., 2008, **51**, 6646-6649.
14 U. Banerjee and M. K. Hadden, *Expert Opin. Drug Discov*., 2014, **9**, 751-771.

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15 R. L. Yauch, G. J. Dijkgraaf, B. Alicke, T. Januario, C.P. Ahn, T. Holcomb, K. Pujara, J. Stinson, C. A. Callahan, T. Tang, J. F. Bazan, Z. Kan, S. Seshagiri, C. L. Hann, S. E. Gould, J. A. Low, C.M. Rudin and F.J. de Sauvage, *Science*., 2009, **326** ,572-574.
16 S. Pricl, B. Cortelazzi, V. D. Col, D.Marson, E. Laurini, M. Fermeglia, L. Licitra, S . Pilotti, P. Bossi and F. perrone, *Mol. Oncol*., 2014, **9**, 389-397.
17 D. Amakye, Z. Jagani and D. Dorsch, *Nat. Med*. 2013, **19**, 1410–1422.
18 K. D. Robarge, S. A. Brunton, G. M. Castanedo, Y. Cui, M. S. Dina, R. Goldsmith, S. E. Gould, O. Guichert, J. L. Gunzner, J. Halladay, W. Jia, C. Khojasteh, M. F. Koehler, K. Kotkow, H. La, R.L. Lalonde, K. Lau, L. Lee, D. Marshall, J. C. Marsters Jr., L. J. Murray, C. Qian, L. L. Rub, L. Salphati,M. S. Stanley, J. H. Stibbard, D. P. Sutherlin, S. Ubhayaker, S. Wang, S. Wong and M. Xie, *Bioorg. Med. Chem. Lett.,*2009, **19**, 5576-5581.
19 S. Pan, X. Wu, J. Jiang, W. Gao, Y. Wan, D. Cheng, D. Han, J. Liu, N.P. Englund and Y. Wang, *ACS. Med. Chem. Lett.,*2010, **1**, 130-134.
20 C. Wang, H. Wu, V. Katritch, G.W. Han, X.P. Huang, W. Liu, F. Siu, B.L. Roth, V. Cherezov, R.C. Stevens, *Nature*., 2013, **497**, 338-343.
21 K. G. Petrov, Y. M. Zhang, M. Carter, G. S. Cockerill, S.Dickerson, C. A. Gauthier, Y. Guo, R. A. D. Mook, W.Rusnak, A. L. Walker, E. R. Wood and K. E. Lackey, *Bioorg. Med. Chem. Lett*., 2006, **16**, 4686-4691.
22 M. Betti, G. Castagnoli, A. Panico, S. Sanna Coccone and P.Wiedenau, *Org. Process Res. Dev*., 2014, **18**, 699-708.
23 M. L. Brown, W. Aaron, R. J. Austin, A. Chong, T. Huang, B. Jiang, J. A. Kaizerman, G. Lee, B. S. Lucas, D. L. McMinn, J. Orf, M. Rong, M. M.Toteva, G. Xu, Q. Ye, W. Zhong, M. R. DeGraffenreid, D. Wickramasinghe, J. P. Powers, R. Hungate and M. G.Johnson, *Bioorg. Med. Chem. Lett.,* 2011, **21**, 5206-5209.
24 A. Rohner, M.E. Spilker, J.L. Lam, B. Pascual, D. Bartkowski, Q.J. Li, A.H. Yang, G. Stevens, M. Xu, P.A. Wells, S. Planken, S. Nair, and S. Sun, Mol. *Cancer Ther*., 2012, **11**, 57-65.
25 M. Betti, G. Castagnoli, A. Panico, S. Sanna Coccone and P.Wiedenau, *Org. Process Res*., Dev. 2012, **16** , 1739-1745.
26 T. Ohashi, Y. Oguro, T. Tanaka, Z. Shiokawa, S. Shibata, Y. Sato, H. Yamakawa, H. Hattori, Y. Yamamoto, S. Kondo, M. Miyamoto, H. Tojo, A. Baba and S. Sasaki, *Bioorg.Med.Chem*., 2012, **20**, 5496.
27 K. Miller-Moslin, S. Peukert, R. K. Jain, M. A. McEwan, R. Karki, L. Llamas,N.Yusuff, F. He, Y. Li, Y. Sun, M. Dai, L. Perez, W. Michael, T. Sheng, H. Lei, R. Zhang, J. Williams, A. Bourret, A. Ramamurthy, J. Yuan, R. Guo, M. Matsumoto, A. Vattay, W. Maniara, A. Amaral and M. Dorsch, *J. Med. Chem*., 2009, **52**, 3954-3968.
28 J. Taipale, J. K. Chen, M. K. Cooper, B. Wang, R. K. Mann, L. Milenkovic, M. P. Scott and P.A. Beachy, *Nature*., 2000, **406** , 1005-1009.
29
30 H. Ma, W. Lu, Z. Sun, L. Luo, D. Geng, Z. Yang, E. Li, J. Zheng, M. Wang, H. Zhang, S. Yang and X. Zhang, *Eur. J. Med. Chem*., 2015, **89**, 721–732.
31 M. Fabrizio, F. Helene, R. Hermine, G. Tatiana, T. Elisabeth, S. Angele, M. Andre, S. Antonio, T. Maurizio and R. Martial, *Mol. Pharmacol*., 2010, **78**, 658–665.

## Footnotes

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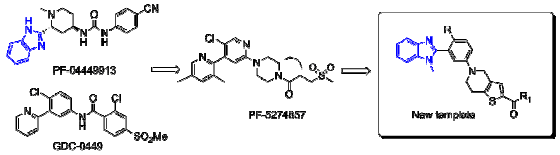
Graphical abstract




Compounds with this novel scaffold demonstrated promising Hh and Smo inhibition, indicating that this novel scaffold can serve as a starting point for further optimization.