Bioorganic & Medicinal Chemistry Letters 18 (2008) 4610–4614

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Knowledge-based design of 7-azaindoles as selective B-Raf inhibitors

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ARTICLE INFO

Article history:
Received 6 June 2008
Revised 3 July 2008
Accepted 8 July 2008
Available online 10 July 2008

Keywords:
B-Raf kinase
7-Azaindole
Knowledge-based design

ABSTRACT

The synthesis of a 7-azaindole series of novel, potent B-Raf kinase inhibitors using knowledge-based design was carried out. Compound **6h** exhibits not only excellent potency in both the enzyme assay (IC_{50} = 2.5 nM) and the cellular assay (IC_{50} = 63 nM), but also has an outstanding selectivity profile against other kinases.

As a target family, kinases have been widely accepted as one of the largest druggable gene families for drug discovery.[1] In order to find a molecule for each druggable kinase target, High-Throughput Screening can be used to obtain a primary starting point, which is further subjected to multidimensional optimization. As more and more crystal structures of protein kinases complexed with ATP or an ATP-competitive inhibitor have been published in the Protein Data Bank and accumulated in individual research groups, knowledge-based design is emerging as the predominant design tool.[2] In particular, the refined pharmacophore map and molecular recognition of protein kinase binding pockets have recently been published,[3] which provide deep insight for medicinal chemists to carry out relatively reliable rational design of the desired inhibitors. Recent reviews describe the methodology very well.[4] Herein, we outline our strategy and illustrate a successful example of how we developed a potent and selective B-Raf kinase inhibitor using knowledge-based design.

Focusing B-Raf kinase as a drug intervention for cancer emanates from the biological elucidation of the Raf-MEK-ERK signaling pathway and the fact that Raf kinases play an important role in activating MEK and promoting cell proliferation and survival.[5] Furthermore, the small molecule, Sorafenib (Nexavar, BAY-43-9006), was approved as a Raf/VEGFR2 inhibitor for the treatment of renal cell carcinoma in 2005.[6] However, given the limited number of selective B-Raf kinase inhibitors so far reported (Fig. 1), we wanted to discover new selective B-Baf inhibitors, which could be used to enhance our understanding of the problems due to lack of selectivity.[7]

A 2-D pharmacophore map used in this study is described in Figure 2. It depicts the seven critical binding regions of the ATP-binding domain that can be accessed to generate ATP-competitive kinase inhibitors. The 7-azaindole series was elaborated by incorporating functional groups that were designed to interact with key features of these regions. The scaffold of an ATP-competitive kinase inhibitor typically included a hinge binder as the starting point for the design. Many heteroaromatic groups, such as pyridine, pyrimidine, aminopyrazole, quinoline, and quinazoline, can be incorporated into the molecule to access the hinge-binding region. We chose 7-azaindole as the hinge binder because this donor-acceptor combination generally provided greater potency than the others indicated above.[8]

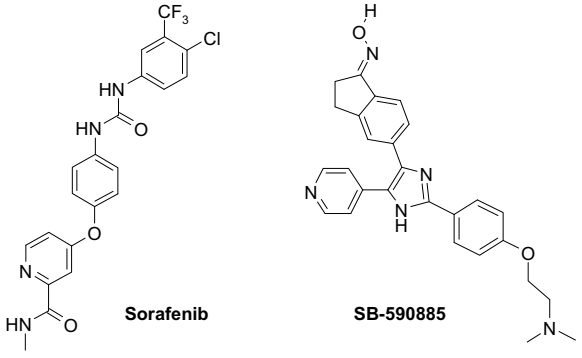

Figure 1. Non-selective B-Raf inhibitor Sorafenib and selective B-Raf inhibitor SB-590885.

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doi:10.1016/j.bmcl.2008.07.019

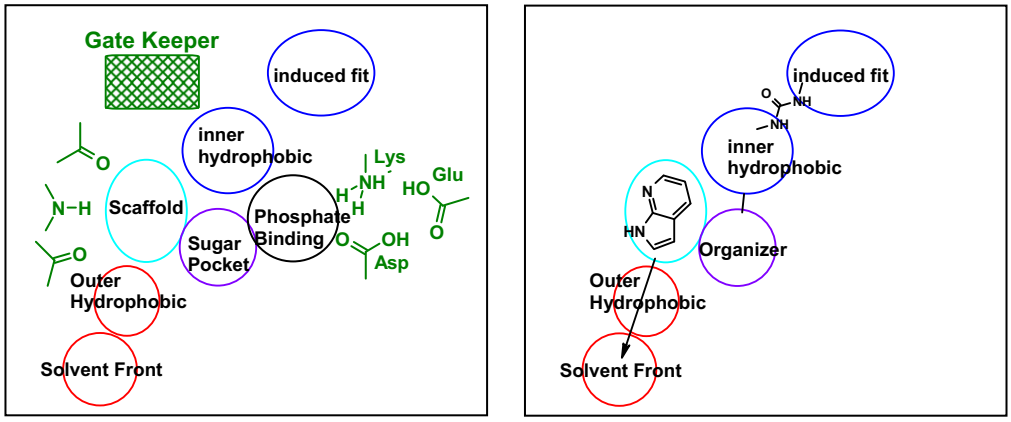
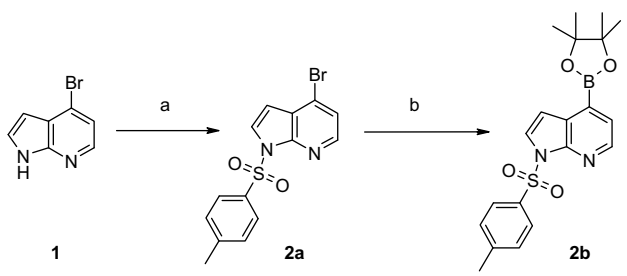


Figure 2. 2-D pharmacophore map of the ATP kinase domain and the proposed scaffold interactions created by the inhibitor.

As designated by its name, the *inner hydrophobic pocket* prefers an aromatic ring or one of its bioisosteres. On the other hand, the *induced fit pocket* is a relatively big pocket featuring a flexible phenylalanine (Phe594 in B-Raf kinase). With regard to potency for B-Raf kinase inhibitors, either substituted or unsubstituted hydrophobic aromatic rings are good options for binding in this region. A reasonable linker to connect the two parts is a urea moiety, which is found in the known B-Raf kinase inhibitor Sorafenib. Additionally, the urea moiety should pick up some additional donor-acceptor interactions with Glu500. If the molecule constructed from the above fragments is not potent enough, pursuing further interactions with a phenyl ring or other lipophilic ring systems in the *outer hydrophobic pocket* might be beneficial. However, this interaction may also deteriorate the selectivity over other kinases. Functional groups in the *solvent front area* offer further extension to the outside of ATP domain with water soluble residues.

Arranging these fragments together generated a molecule that was unsuitable for fitting into the ATP domain. We required an organizer motif which when substituted appropriately allowed each fragment to fit precisely into the desired pockets. Considering the tractability of the chemistry, we chose a 1,3,4-trisubstituted-1*H*-pyrazole as the organizer motif.

Synthetic methods for building the designed compounds were established and are summarized in Schemes 1–5.[9] The two different hinge binder pieces **2b** and **3c** used in this study were prepared as boronic esters, exemplified in Schemes 1 and 2, and incorporated into the final molecule by a Suzuki coupling reaction. 4-Bromo-1*H*-pyrrolo[2,3-*b*]pyridine **1** was prepared according to the literature from commercially available reagents.[10] The tosyl protecting group was incorporated using biphasic conditions to give **2a**, which was transferred to the corresponding boronic ester **2b** in good yield.[9] Ethyl 1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxylate



Scheme 1. Reagents and conditions: (a) TsCl, Bu_4NHSO_4 (cat), aqueous NaOH, CH_2Cl_2, rt, 30 min, 70%; (b) $Pd(dppf)Cl_2$, bis(pinacolato)diboron, KOAc, DMF, 90 °C, 48 h, 77%.

7-oxide **3a** was prepared as described in the literature,[11] and was readily brominated at the 4-position in good yield. Subsequent reaction with bis(pinacolato)diboron under catalytic conditions afforded the corresponding boronic ester **3c** for further coupling reaction.

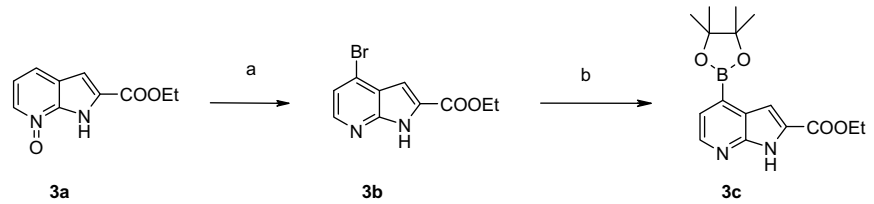
The core consisting of the pyrazole organizer and a phenyl ring at the 3-position was prepared as follows. Starting material **4a** was prepared according to the reported approach.[12] The ethyl group was easily installed by alkylation with ethyl iodide in the presence of a base and phase transfer catalyst. The undesired isomer was obtained as a minor byproduct and was readily removed from the product **4b** by silica gel chromatography. Reduction with Sn powder gave the amine analogue **4c**.

The final compounds were assembled using the chemistry indicated below in Scheme 4. Suzuki coupling between compounds **4b** and **2b** under microwave conditions afforded the adduct **5a**. Subsequent Sn powder reduction gave **5b** in moderate yield. The deprotection of 7-azaindole with aqueous NaOH at slightly elevated temperature provided compound **5c**, which reacted with isocyanates and isothiocyanates to give the final compounds **6a–l** (Table 1).

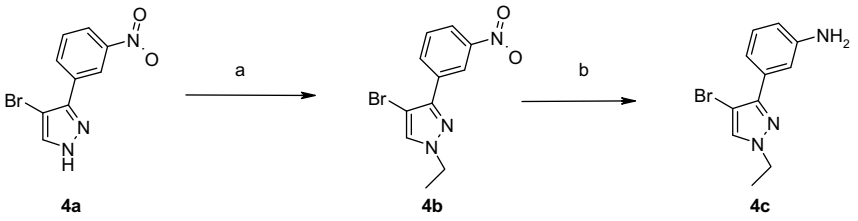
Synthesis of the 3-bromo-7-azaindole derivative **5e** required deprotection of the tosyl group, followed by treatment with NBS at room temperature as described in Scheme 5. Similarly, the final compound **6m** was obtained in the same fashion as described above.

The final compounds with a substituent at the 2-position of 7-azaindole were achieved via a Suzuki reaction between compounds **4c** and **3c**, followed by treatment with 4-trifluoromethylphenylisocyanate (Scheme 6). The corresponding carboxylic acid analogue **6o** was obtained by saponification of **6n** with aqueous NaOH at reflux.

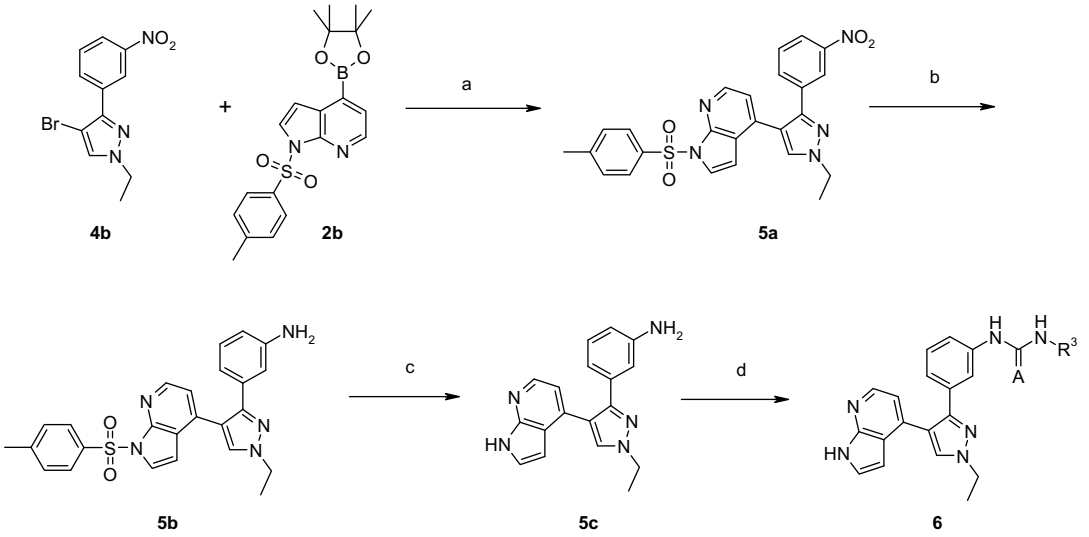
Thus, a novel series of 7-azaindole analogues **6a**–**6o** were prepared (Table 1). Compounds were tested against B-Raf in a fluorescence anisotropy binding assay.[9] The parent phenyl urea compound **6b** (IC_{50} = 17.4 nM) showed ~20-fold improved inhibitory activity compared to its thiourea analogue **6a**. Direct connection of an aromatic moiety (R^3) to the urea proved to be optimal based on the reduced inhibitory activities observed when the R^3 substituent was cyclohexyl **6c** or benzyl **6d**. A survey of substitutions was performed to find the optimal group for the *induced fit pocket* (**6d–6l**). Mono-substituted phenyl rings (**6f, 6h, 6i, 6l**) and di-substituted phenyl rings (**6g, 6j, 6k**) were generally very potent with IC_{50} values ranging from 2.1 to 25.7 nM, regardless of the positions of the substituents. However, by the incorporation of an oversized substituent, such as when R^3 was benzyloxyphenyl (**6e**), a dramatic drop in activity was observed. Among all of the



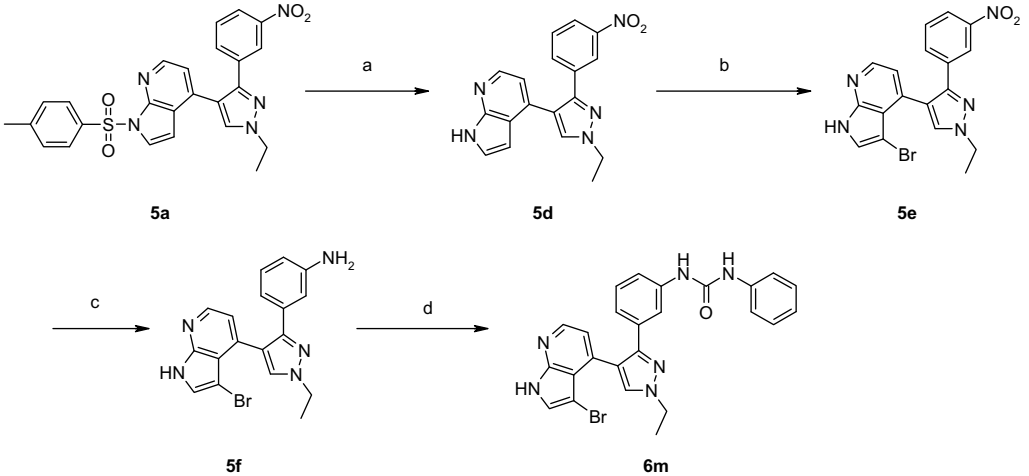
Scheme 2. Reagents and conditions: (a) TBAB, $(MeSO_2)_2O$, DMF, rt, 6 h, 77%; (b) $Pd(dppf)Cl_2$, bis(pinacolato)diboron, KOAc, DMF, 90 °C, 48 h, 68%.



Scheme 3. Reagents and conditions: (a) EtI, TBAB, aq NaOH, CH_2Cl_2, rt, 1 h, 81%; (b) Sn powder, aq HCl, EtOH, reflux, 4 h, 95%.

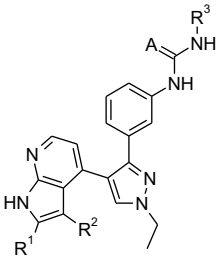


Scheme 4. Reagents and conditions: (a) $Pd(PPh_3)_4$, aq Na_2CO_3, DME, MW heating, 120 °C, 1 h, 88%; (b) Sn powder, aq HCl, EtOH, reflux, 4 h, 78%; (c) aq NaOH, MeOH, 40 °C, 1 h, 99%; (d) Isocyanates or isothiocyanate, pyridine, rt, 1 h.



Scheme 5. Reagents and conditions: (a) aq NaOH, MeOH, rt, 12 h, 89%; (b) NBS, THF, rt, 12 h; (c) Sn powder, aq HCl, EtOH, reflux, 12 h; (d) Phenylisocyanate, pyridine, rt, 1 h.

Table 1
B-Raf inhibitory activities of compounds **6a–6o**

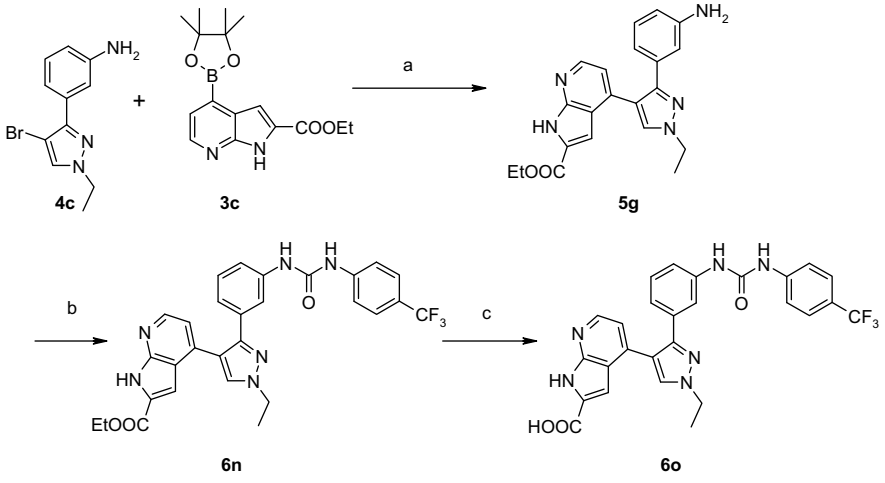


Compound	A	R^1	R^2	R^3	B-Raf (FP, IC_{50}) (nM)
6a	S	H	H	Phenyl	331
6b	O	H	H	Phenyl	17.4
6c	O	H	H	Cyclohexyl	41.7
6d	O	H	H	Phenyl	170
6e	O	H	H	4-Benzyloxyphenyl	110
6f	O	H	H	2-Chlorophenyl	5.3
6g	O	H	H	2,6-Difluorophenyl	25.7
6h	O	H	H	4-Trifluoromethylphenyl	2.5
6i	O	H	H	3-Chlorophenyl	10.5
6j	O	H	H	2-Fluoro-5-trifluoromethylphenyl	3.9
6k	O	H	H	4-Chloro-3-trifluoromethylphenyl	5.4
6l	O	H	H	3-Methoxylphenyl	2.1
6m	O	H	Br	Phenyl	79.4
6n	O	Ethyl formate	H	Phenyl	33.9
6o	O	Formic acid	H	Phenyl	45.7

lipophilic terminal substituted phenyl rings examined, compound **6h**[13] with a trifluoromethyl group at the 4′-position provided one of the best inhibitory activities (IC_{50} = 2.5 nM) against B-Raf. We chose **6h** over **6l** as the exemplar for this series due to a superior p450 inhibition profile.[14] Evaluation of this compound in a cellular assay through the measurement of phosphorylation of MEK1 demonstrated its excellent potency (IC_{50} = 63 nM).[9] Substituent R^2 may be affected by steric interactions as evidenced by the drop in inhibitory activity for 6 m containing a bromide at the 3-position of 7-azaindole. A substituent at the 2-position of 7-azaindole such as ethyl formate (**6n**) or formic acid (**6o**) was tolerated. Although these substituents did not add potency value, they could potentially be used to improve the physical and chemical properties.

Illustrated in Fig. 3 is a docking model of **6h** complexed with an inactive conformation of B-Raf.[15] From this docking analysis, it is noted that 7-azaindole binds to the kinase at the hinge region with its nitrogen and hydrogen atoms forming H-bond interactions with the backbone NH and CO of Cys531.[16] The pyrazole ring resides at the *sugar pocket*, and the connected phenyl at the 3-position is positioned into the *inner hydrophobic pocket*. The urea tethered to the *meta* position of the central phenyl ring is positioned deep into the *induced fit pocket*, surrounded by mostly lipophilic residues including Leu504, Val503, Leu566, and His573. The urea moiety appears to form interactions with Glu500. Based on this docking analysis, substituents at the 2-position of the 7-azaindole scaffold, such as ethyl formate, would reach the *outer hydrophobic pocket* and would be expected to be well tolerated despite the dissimilarity of the ethyl formate moiety to the preferred lipophilic ring systems.

The compounds described here were generally about 10-fold more potent as inhibitors of B-Raf than other kinases evaluated.



Scheme 6. Reagents and conditions: (a) $Pd(PPh_3)_4$, aq Na_2CO_3, DME, MW heating, 120 °C, 1 h, 17%; (b) 4-trifluoromethylphenylisocyanate, pyridine, rt, 1 h; (c) 6 N NaOH, MeOH, reflux, 1 h.

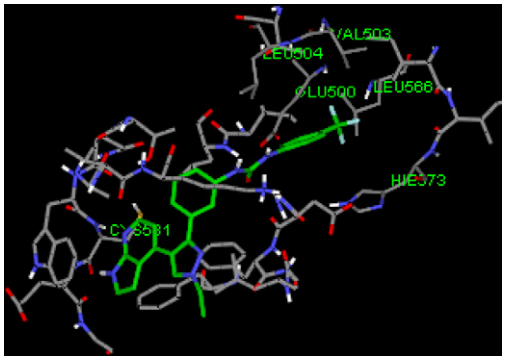


Figure 3. Homology model of compound **6h** bound to inactive form of B-Raf. Inhibitor atoms colored as follows: C, gray; N, blue; O, red.

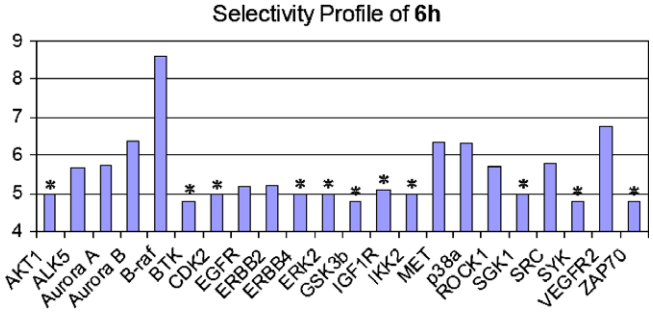


Figure 4. Kinase selectivity profile of compound **6h**. Potency is represented by pIC_{50} on the *Y* axis. * denotes no activity at maximum concentration (30 μM) tested.

For example, compound **6h** bearing a trifluoromethyl group at the 4′-position, which was proposed to fit the *induced fit pocket* very well, showed more than 1000-fold selectivity against a variety of other kinases screened, and almost 100-fold selectivity against VEGFR2 and c-Met (Fig. 4).

In conclusion, we discovered a series of novel, potent B-Raf inhibitors using knowledge-based design. As exemplified by compound **6h**, we achieved not only excellent potency in both enzyme and cellular assays, but also an outstanding selectivity profile against other kinases.

Acknowledgments

We are grateful to the enzymologists for contributions to the screening work and Hiroshi Sootome for the evaluation of cellular potency.

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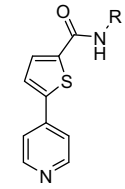
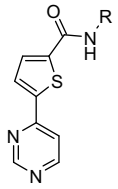
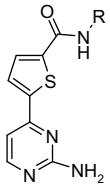
8. Four similarly substituted compounds with different hinge binders are shown below. The 7-azaindole hinge binder provides the most potent inhibitory activities against 4 kinases. Potency is represented by pIC_{50} value.



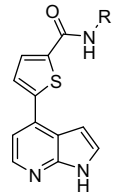
AKT1 FL	5.8
Aurora A	6.8
PKA	6.6
ROCK1	8.4



AKT1 FL	< 5.5
Aurora A	6.1
PKA	5.1
ROCK1	6.5



AKT1 FL	5.6
Aurora A	6.7
PKA	7.3
ROCK1	8.6



AKT1 FL	7.3
Aurora A	8.0
PKA	8.0
ROCK1	8.8

9. Tang, J.; Nakano, M.; Hamajima, T. PCT Int. Application, WO2007090141. Enzyme and cellular assay description can be found in this patent. Cellular assays: B-Raf mediated phosphorylation of MEK1 was measured in the cellular assay. Expression constructs for B-Raf and FLAG-tagged MEK1 were co-transfected in 3T3 cells and gene expression was induced using the GeneSwitch (TM) system for inducible mammalian expression (Invitrogen). Four hours following the induction of expression of B-Raf and MEK1, cells were exposed to the test compounds for 2 h. The cells were lysed, and then an immunoassay was performed using anti-phospho-MEK1/2 (Cell Signaling Technologies) to detect the percent inhibition of MEK1 phosphorylation.
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13. Analytic data of potent inhibitors **6h:** ^{1}H NMR (400 MHz, DMSO-d_6) ppm 1.49 (t, 3H, *J* = 7.3 Hz), 4.26 (q, 2H, *J* = 7.3 Hz), 6.26 (dd, 1H, *J* = 2.0, 3.3 Hz), 6.81 (d, 1H, *J* = 5.1 Hz), 6.90–6.95 (m, 1H), 7.18 (dd, 1H, *J* = 7.8, 8.0 Hz), 7.39 (dd, 1H, *J* = 2.5, 3.3 Hz), 7.47 (ddd, 1H, *J* = 1.0, 2.0, 8.1 Hz), 7.51–7.54 (m, 1H), 7.59–7.65 (m, 4H), 8.09 (d, 1H, *J* = 5.1 Hz), 8.18 (s, 1H), 8.80 (s, 1H), 9.00 (s, 1H), 11.62 (brs, 1H). LC/MS: *m*/*z* 491 $(M+1)^+$.
14. Compound **6h**: 2C9 (pIC_{50} = 5.9); 2D6 (pIC_{50} = 5.3). Compound **6l**: 2C9 (pIC_{50} = 6.5); 2D6 (pIC_{50} = 6.7).
15. Model built from X-ray crystal structure PDB ID 1UWH with MacroModel, Schrödinger LLC.
16. This was numbered as Cys532 in some recent papers, for example: Tsai, J.; Lee, J. T.; Wang, W.; Zhang, J.; Cho, H.; Mamo, S.; Bremer, R.; Gillette, S.; Kong, J.; Haass, N. K.; Sproesser, K.; Li, L.; Smalley, K. S. M.; Fong, D.; Zhu, Y.-L.; Marimuthu, A.; Nguyen, H.; Lam, B.; Liu, J.; Cheung, I.; Rice, J.; Suzuki, Y.; Luu, C.; Settachatgul, C.; Shellooe, R.; Cantwell, J.; Kim, S.-H.; Schlessinger, J.; Zhang, K. Y. J.; West, B. L.; Powell, B.; Habets, G.; Zhang, C.; Ibrahim, P. N.; Hirth, P.; Artis, D. R.; Herlyn, M.; Bollag, G. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 3041.