Bioorganic & Medicinal Chemistry Letters 19 (2009) 2277–2281

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design of novel quinazoline derivatives and related analogues as potent and selective ALK5 inhibitors

F. Gellibert [a,*], M.-H. Fouchet [a], V.-L. Nguyen [a], R. Wang [b], G. Krysa [a], A.-C. de Gouville [a], S. Huet [a], N. Dodic [a]

[a] GlaxoSmithKline, 25-27 Avenue du Québec, 91951 Les Ulis, France
[b] GlaxoSmithKline, Research Triangle Park, NC 27703, USA

ARTICLE INFO

Article history:
Received 29 January 2009
Revised 20 February 2009
Accepted 22 February 2009
Available online 26 February 2009

Keywords:
Transforming growth factor-β
ALK5
P38MAP Kinase
Liver fibrosis

ABSTRACT

Starting from quinazoline **3a**, we designed potent and selective ALK5 inhibitors over p38MAP kinase from a rational drug design approach based on co-crystal structures in the human ALK5 kinase domain. The quinazoline **3d** exhibited also in vivo activity in an acute rat model of DMN-induced liver fibrosis when administered orally at 5 mg/kg (bid).

Transforming growth factor-β1 (TGF-β1) is a key mediator in progressive fibrosis in the kidney, liver, heart, lung, bone marrow and skin, which enhances extracellular matrix production by both increasing the transcription of matrix proteins, for example, fibronectin and collagen, and inhibiting enzymes responsible for matrix degradation.[1] More recently the role of TGF-β in cancer biology was described in the literature.[2] TGF-β1 signals through a family of transmembrane serine/threonine kinase receptors. These receptors can be divided into two classes, the type I or activin like kinase (ALK) receptors and type II receptors. Specifically, the binding of TGF-β1 to the type II receptor causes phosphorylation of the GS domain of the TGF-β type I receptor, ALK5. The ALK5 receptor, in turn, phosphorylates the cytoplasmic proteins smad2 and smad3 at two carboxyl terminal serines. The phosphorylated smad proteins form heteromeric complexes with smad4, after which the complex translocates into the nucleus to affect gene transcription.[3]

Therefore identification of small-molecule inhibitors of the kinase activity of the TGF-β type I receptor (also named activin-like kinase or ALK5) to block the pro-fibrotic effect of overexpression of TGF-β1 represents an attractive target for the treatment of fibrotic diseases[4] and cancer.[5]

To date several chemical series have been described in the literature with potent small-molecule ALK5 inhibitors (Fig. 1).[6] In order to identify new templates we decided to screen in-house kinase-focused libraries. Through this approach, we identified a quinazoline compound **3a** presenting both ALK5 and p38 moderate activities, with a binding affinity of 194 nM and 1270 nM, respectively. In this paper, we describe the rationale and exploration of structure–activity relationship (SAR) leading from **3a** to potent ALK5 inhibitors with improved selectivity over p38. Among the most potent analogues identified, the quinazoline **3d** was orally active in a rat model of liver-induced fibrosis when administered at 5 mg/kg bid.

The general synthetic route for the quinazoline compounds is shown in Scheme 1, and is illustrated for compounds **3a–d** and **8a–g**. Coupling of 4-aminopyridine with 4-chloroquinazoline intermediates **7a–d** using either standard Buchwald conditions ($Pd_2(dba)_3$, BINAP) in presence of sodium *tert*-butylate, or in DMF at 150 °C, afforded the quinazolines **3a–d**. The analogues **8a–g** were prepared from 4-chloro-2-(6-methyl-2-pyridinyl)quinazoline (**7d**) and the corresponding amines using the same reaction conditions as for **3a–d** (Scheme 1). Reaction of 6-methyl-2-pyridinecarboximidamide (**10**) with methyl acetoacetate and sodium ethylate in ethanol produced 6-methyl-2-(6-methyl-2-pyridinyl)-4(1*H*)-pyrimidinone (**11**) (Scheme 2). Subsequent activation of C-4 position with $POCl_3$, followed by the reaction of 4-aminopyridine with the 4-chloro intermediate **12** led to pyrimidine **13**. Coupling of the 2-amino-4-methyl-3-thiophenecarboxamide (**14**) and 3-amino-2-thiophenecarboxamide (**18**) with 6-methyl-2-pyridinecarboxylic acid using standard peptide coupling procedure (HOBT, EDCI, Et_3N), followed by cyclization with NaOH, gave, respectively, the thieno[2,3-*d*]pyrimidin-4(1*H*)-one (**15**) and thieno[3,2-*d*]pyrimidin-4(1*H*)-one (**19**). Chlorination followed by reaction with 4-aminopyridine led to the thienopyrimidine compounds **17** and **21**, respectively. The quinoline compound **25** was synthesized by

* Corresponding author.
E-mail address: francoise.gellibert@gsk.com (F. Gellibert).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.02.087

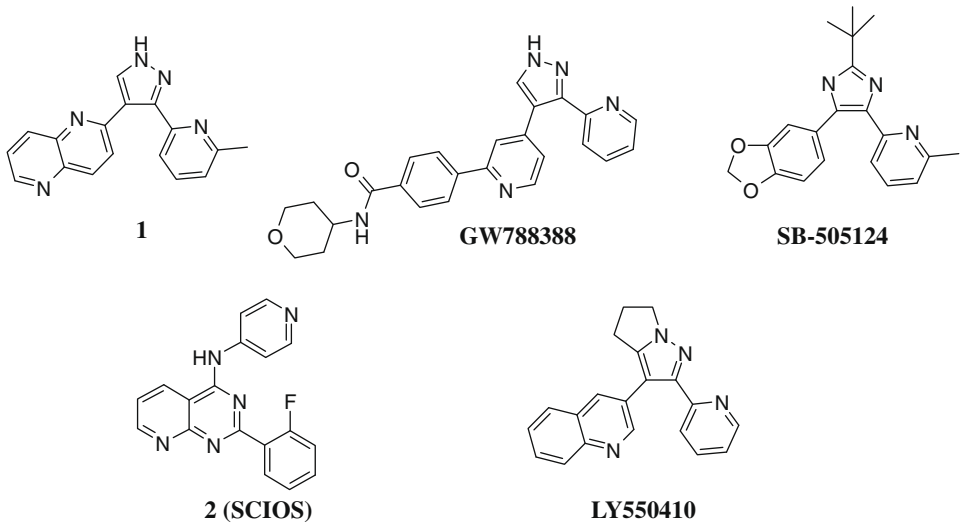
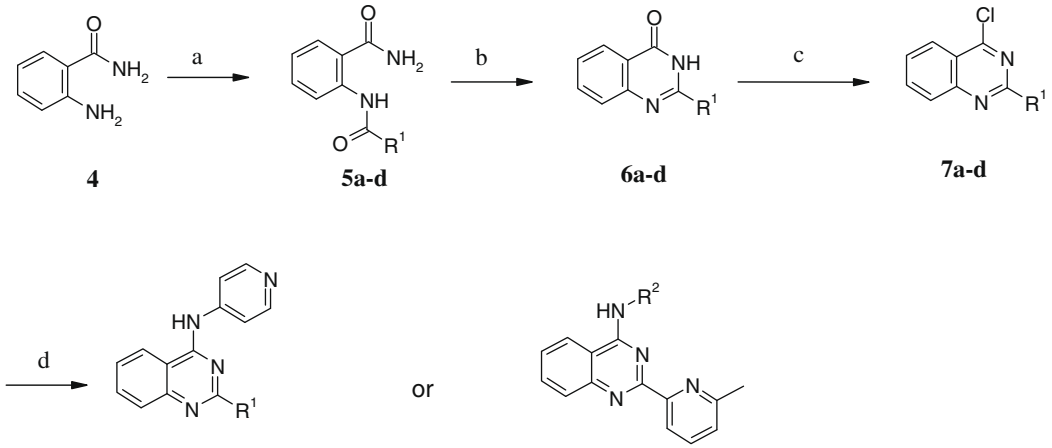


Figure 1. Small-molecules inhibitors of ALK-5 kinase domain.



3a-d
a, R^1=phenyl
b, R^1=3-chloro-phenyl
c, R^1=4-methyl-1,3-thiazol-2-yl
d, R^1=6-methyl-2-pyridinyl

8a-d
8a, R^2=3-methyl-4-pyridinyl
8b, R^2=3-phenylcarboxamide
8c, R^2=4-pyrimidinyl
8d, R^2=1H-indazol-5-yl
8e, R^2=1-methyl-1H-indazol-5-yl
8f, R^2=1,3-benzothiazol-6-yl
8g, R^2=1H-1,2,3-benzotriazol-5-yl

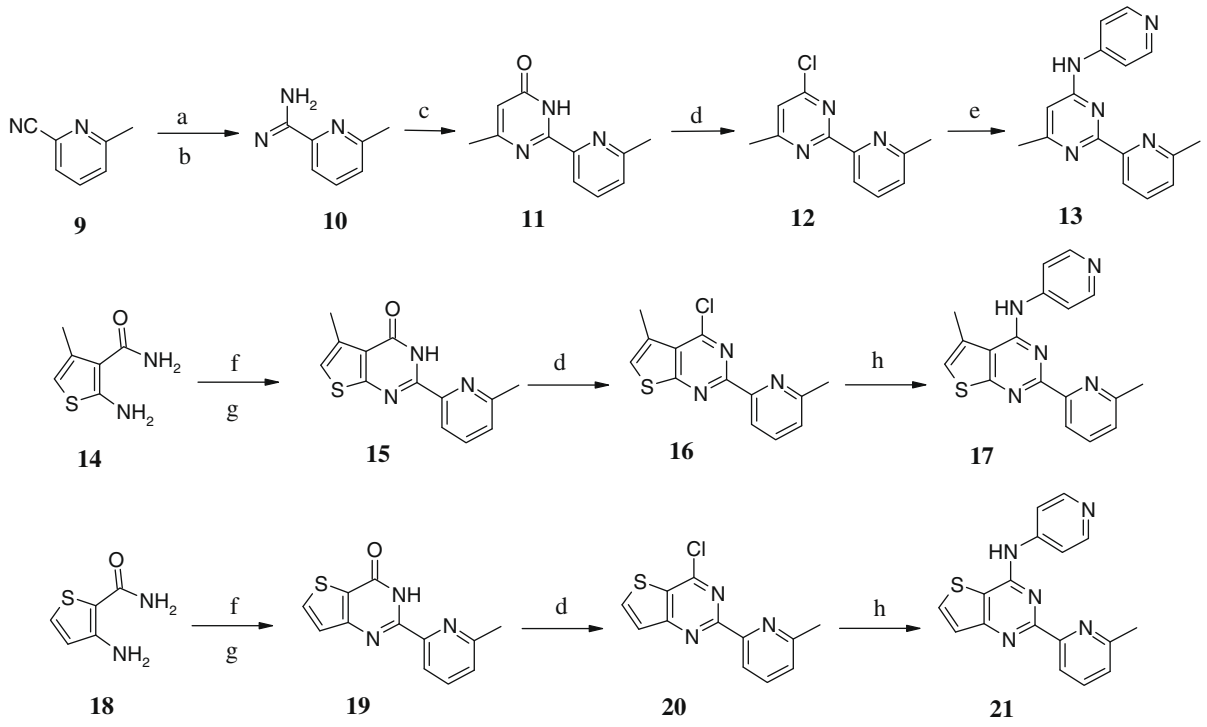
Scheme 1. Reagents and conditions: (a) R^1COOH, HOBT, EDCI, Et_3N, DMF; (b) NaOH, EtOH, reflux, 1 h; (c) $POCl_3$, toluene, reflux; (d) 4-aminopyridine or R^2NH2, DMF, 150 °C or 4-aminopyridine, $Pd_2(dba)_3$, BINAP, NaOtBu, toluene, reflux.

condensation of 2-aminobenzonitrile **22** with 1-(6-methyl-2-pyridinyl)ethanone, followed by cyclization of the ketimide **23** and coupling of the resulting 4-aminoquinoline **24** with 4-bromo-pyridine (Scheme 3).

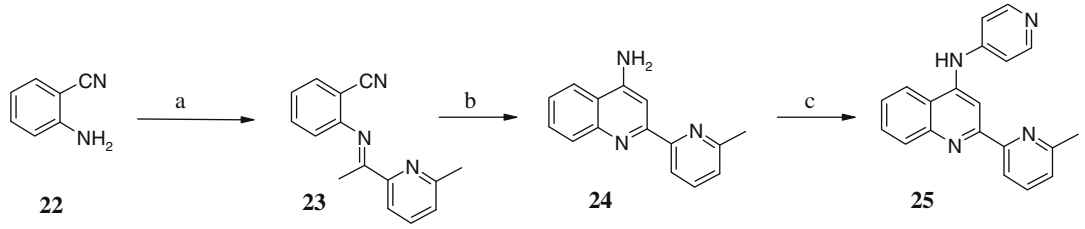
All the compounds (Tables 1–3) were tested in a kinase assay using purified human ALK5 kinase domain produced in Sf9 insect cells.[7] Binding to p38MAP kinase was tested on purified recombinant GST-p38 α.[8] These compounds were also evaluated in a TGF-β dependent transcriptional luciferase assay in HepG2 cells.[9]

We previously demonstrated in the naphthyridine series,[6a] that p38 activity could be reduced by replacing a 2-phenyl group with a pyridin-2-yl or a 6-methyl-pyridin-2-yl. Indeed, the 6-methylpyridine moiety is engaged in the 'selectivity pocket' of ALK5 containing Ser280 while for p38 the corresponding residue is Thr106. As expected, this structural modification when applied to the quinazoline series (Table 1), leads to compound **3d** displaying higher ALK5 binding affinity and approximately 40-fold increase in selectivity over p38 compared to **3a**. Interestingly **3d** is also 26-fold more potent in the cell-based assay than the phenyl **3a**. Although the 3-chlorophenyl **3b** and the 4-methyl-1,3-thiazol-2-yl **3c** derivatives are also potent ALK5 inhibitors with no significant p38 activity, these derivatives exhibit only low to moderate cellular activity compared to **3d**, respectively. Having established for this new chemical series that the 6-methyl-pyridin-2-yl group was important not only for the selectivity over p38 but also for ALK5 inhibition, we decided to explore the SAR for the two other heterocycles.

Interestingly, the pyrimidin-4-yl **8c** and *N*-1*H*-indazol-5-yl **8d** derivatives are potent ALK5 inhibitors in both the binding and cell-based assay with ALK5 inhibition comparable to **3d** (Table 2). It appears that substitution of the 4-aminopyridine with a



Scheme 2. Reagents and conditions: (a) Na, EtOH; (b) NH_4Cl, EtOH, reflux; (c) methyl acetoacetate, EtONa, EtOH, reflux; (d) $POCl_3$, toluene, reflux; (e) 4-aminopyridine, BINAP, $Pd_2(dba)_3$, tBuONa, toluene, reflux; (f) 6-methyl-2-pyridinecarboxylic acid, HOBT, EDCI, Et_3N, DMF; (g) NaOH, EtOH, reflux, 1 h; (h) 4-aminopyridine, DMF, 150 °C.



Scheme 3. Reagents and conditions: (a) 1-(6-methyl-2-pyridinyl)ethanone, *para*-toluene sulfonic acid, toluene, reflux; (b) LDA, ether, −78 °C to rt; (c) 4-bromopyridine, BINAP, $Pd_2(dba)_3$, tBuONa, toluene, reflux.

methyl in the ortho position **8a**, or N-methylation of the indazole ring **8e** decreases significantly the binding affinity. Replacement of the indazole core with benzothiazole **8f** or benzotriazole **8g** leads also to a drop of the binding affinity for ALK5 by 26- to 149-fold over **8c**, respectively. All these quinazoline derivatives **8a–g** are poor p38 inhibitors with activity higher than 10 μM.

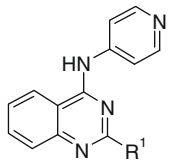
In an attempt to better understand the binding mode of these derivatives, the X-ray structures of compounds **3d** and **8d** bound to the ATP binding pocket of human ALK5 were solved[10] (Fig. 2). Both compounds form hydrogen bonds with the hinge region as reported previously for the other ALK5 inhibitors. The 4-pyridine nitrogen of **3d** is involved in a hydrogen bond with His 283 backbone NH, while the nitrogens N5 and N6 of **8d** form H-bond with His 283 backbone NH and CO, respectively. The second additional interaction observed for **3d** and **8d** involves the 6-methyl-pyridin-2-yl nitrogen that forms a water-mediated network of hydrogen bonds with the enzyme. The water molecule donates hydrogen bonds to the pyridyl nitrogen of **3d** and **8d** and the carboxyl oxygen of Glu245, and accepts hydrogen bonds from the phenol of Tyr249 and the backbone NH of Asp351. Another interaction with a water molecule is only observed for **3d**, involving the NH spacer at C-4 position of the quinazoline.

From this model and docking studies (data not shown), one explanation for the reduction in the binding to ALK5 observed for **8e**, **8f** and **8g** could be attributed to unfavourable interaction with the hinge region resulting from the substitution or replacement of the NH indazole.

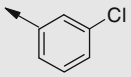
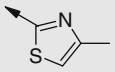
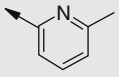
Replacement of the quinazoline core with quinoline or thienopyrimidines was very well tolerated (Table 3) which is consistent with the proposed binding mode of these derivatives where the main interactions with the binding site are maintained. These compounds can be differentiated by their cellular activity, with only the thienopyrimidine **21** and the quinoline **25** being equipotent to **3d**. The selectivity against p38 was also maintained for these new derivatives (**13**, **17**, **21** and **25**) with activity at concentrations higher than 10 μM (data not shown).

Among the most potent ALK5 inhibitors described above with IC_{50} < 100 nM, only quinazoline **3d** exhibited adequate pharmacokinetic parameters in rat for dosing orally (e.g., a bioavailability of 40% and oral exposure with an AUC of 524 ng h/ml at 5 mg/kg). The

Table 1
Replacement of the phenyl ring

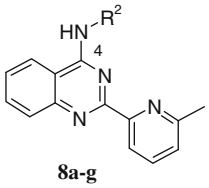


3a-d

Compd	R^1	ALK5 binding IC50[a] (μM)	p38 α binding IC50[a] (μM)	TGF-β cellular assay IC50[b] (μM)
3a		0.194	1.27	2.465
3b		0.049	>15	1.612
3c		0.020	>15	0.342
3d		0.025	6.72	0.095

[a] Values are the mean of two or more separate experiments, pIC_{50} were calculated and mean pIC_{50} was converted in IC_{50}.
[b] Averaged values of two or more separate experiments.

Table 2
Study of the diversity at position 4



8a-g

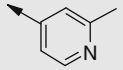
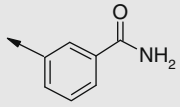
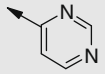
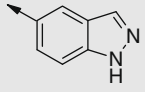
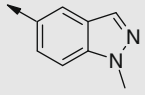
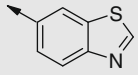
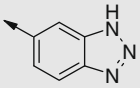
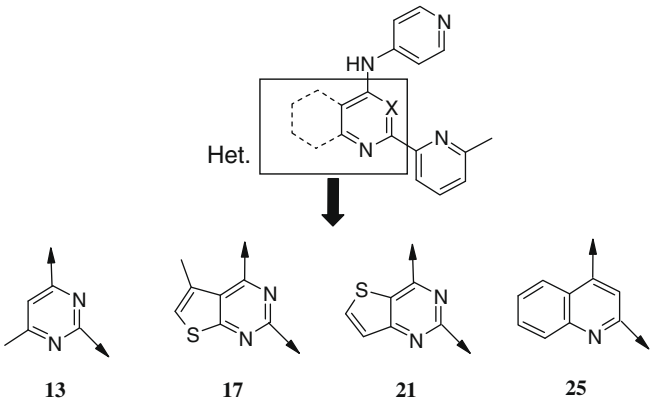
Compd	R^2	ALK5 binding IC50[a] (μM)	TGF-β cellular assay IC50[b] (μM)
8a		0.199	1.017
8b		0.263	0.545
8c		0.040	0.060
8d		0.025	0.090
8e		0.588	0.794
8f		0.646	>5

Table 2 (*continued*)

Compd	R^2	ALK5 binding IC50[a] (μM)	TGF-β cellular assay IC50[b] (μM)
8g		3.715	>5

[a] Values are the mean of two or more separate experiments, pIC_{50} were calculated and mean pIC_{50} was converted in IC_{50}.
[b] Averaged values of two or more separate experiments.

Table 3
Replacement of the quinazoline core with other heterocycles



13 **17** **21** **25**

Compd	ALK5 binding IC50[a] (μM)	TGF-β cellular assay IC50[b] (μM)
13	0.093	0.745
17	0.016	0.318
21	0.022	0.060
25	0.022	0.058

[a] Values are the mean of two or more separate experiments, pIC_{50} were calculated and mean pIC_{50} was converted in IC_{50}.
[b] Averaged values of two or more separate experiments.

in vivo efficacy of **3d** was tested in an acute model of dimethylnitrosamine (DMN)-induced liver disease.[11] DMN given for three consecutive days to rats increased collagen IA1 mRNA expression in the liver by about 10-fold at day 8. In this model, **3d** inhibits in a dose-dependent manner the DMN-induced collagen IA1 mRNA overexpression with 66% and 73% inhibition at the doses of 5 and 10 mg/kg po bid, respectively (Fig. 3).

The quinazoline **3d** was tested against a panel of more than 15 kinases[12] (Table 4). Although good selectivity was obtained against most of them, significant percentage of inhibition (93%) was observed for ROCK1 at 10 μM.

In summary, a series of quinazoline was optimized and led to novel and potent ALK5 inhibitors. The improvement of the selectivity over p38 was achieved by targeting the selectivity pocket with a 6-methyl-2-pyridine. Among these compounds, **3d**, exhibited antifibrotic activity when administered orally at 5 mg/kg bid in a model of DMN-induced liver fibrosis in the rat despite a modest oral exposure at this low dose. Due to the pleiotropic effects of TGF-β, one concern is now to demonstrate that chronic administration of a ALK5 inhibitor is devoid of toxicity, therefore as a representative compound of a novel chemical series **3d** has been progressed in toxicity studies that will be reported in due time.

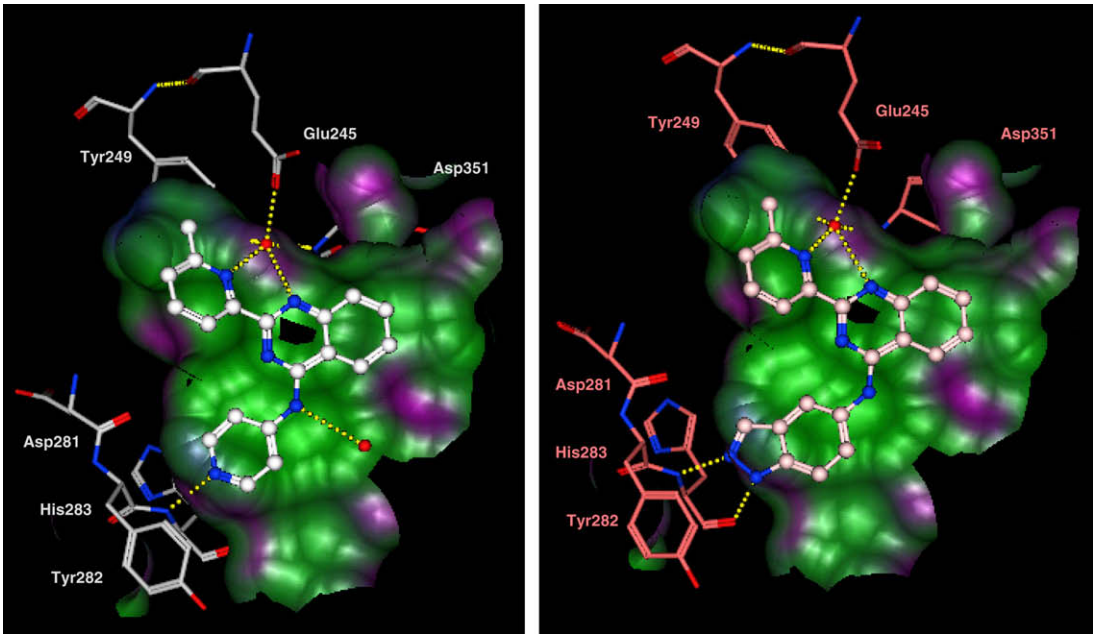


Figure 2. X-ray crystal structure of quinazoline compounds **3d** and **8d** bound respectively to the ATP binding site of the kinase domain of human ALK-5.

Table 4
Selectivity of compound **3d** against a panel of kinases[a]

Compd	AMPK	CDK2	CSK1	CSK2	GSK3	JNK1	LCK	MKK1	MSK1	MAPK2	P70S6K	P38 β	P38 γ	P38 δ	PKC	PDK1	Phos.K	ROCK	SGK
3d	26	2	25	−2	38	1	5	1	67	15	34	−5	1	−11	28	7	−6	93	5

[a] Values are %inhibition at 10 μM using 100 μM ATP. For kinases used and assay details see Ref. 12.

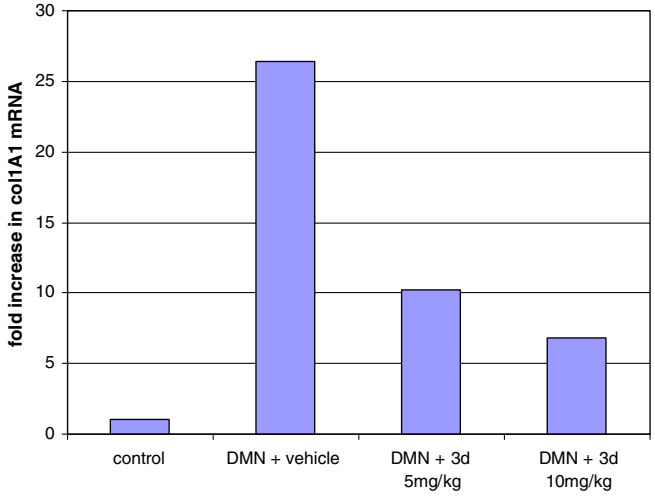


Figure 3. Effect of compound **3d** administered orally (bid) in the acute DMN rat model. Experiments were performed using 5–8 animals per group. Difference in Col1A1 mRNA between control and DMN + vehicle, DMN + **3d** was statistically significantly ($p < 0.0005$ and $p < 0.05$, respectively).

Acknowledgements

We acknowledge Géraldine Poulain and Christelle Sury-Martin for their contribution to the synthesis of these compounds, and the DMPK team for performing the pharmacokinetic study.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.02.087.

References and notes

1. (a) Massague, J.; Blain, S. A.; Lo, R. S. *Cell* **2000**, *103*, 295; (b) Border, W. A.; Noble, N. A. *N. Eng. J. Med.* **1994**, *331*, 1286; (c) Blobe, G. C.; Schiemann, W. P.; Lodish, H. F. *N. Eng. J. Med.* **2000**, *342*, 1350.
2. (a) Roberts, A.; Mishra, L. *Oncogene* **2005**, *24*, 5667; (b) Lahn, M.; Kloeker, S.; Berry, B. S. *Exp. Opin. Invest. Drugs* **2005**, *14*, 629; Dumont, N.; Arteaga, C. L. *Cancer Cell* **2003**, *3*, 531.
3. Derynck, R.; Zhang, Y.; Feng, X.-H. *Cell* **1998**, *95*, 737.
4. (a) Grygielko, E. T.; Martin, V. M.; Tweed, C. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 943; (b) de Gouville, A.-C.; Boullay, V.; Krysa, G., et al *Br. J. Pharmacol.* **2005**, *145*, 166; (c) Bonniaud, P.; Margetts, P. J.; Kolb, M., et al *Am. J. Resp. Crit. Care Med.* **2005**, *171*, 889; (d) Laping, N. J. *Curr. Opin. Pharmacol.* **2003**, *3*, 204.
5. Downing, J. R. *N. Eng. J. Med.* **2004**, *351*, 528.
6. (a) Gellibert, F.; Woolven, J.; Fouchet, M. H.; Mathews, N.; Goodland, H.; Lovegrove, V.; Laroze, A.; Nguyen, V. L.; Sautet, S.; Wang, R.; Janson, C.; Smith, W.; Krysa, G.; Boullay, V.; de Gouville, A.-C.; Huet, S.; Hartley, D. *J. Med. Chem.* **2004**, *47*, 4494; (b) Gellibert, F. et al *J. Med. Chem.* **2006**, *49*, 2210; (c) Byfield, S. D.; Major, C.; Laping, N. J.; Roberts, A. B. *Mol. Pharmacol.* **2004**, *65*, 744; (d) Sawyer, J. S. et al *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3581; (e) Munchhof, M. J. WO 2004147574.
7. Description of assay conditions for ALK5 binding assay can be found in Supplementary data.
8. Description of assay conditions for p38alpha Fluorescence Polarization assay can be found in Supplementary data.
9. Description of assay condition for TGF-β cellular assay can be found in Supplementary data.
10. The coordinates for compounds **3d** and **8d** have been deposited with the RCSB Protein Data Bank, PDB codes are 1SWZE and 1JYJE, respectively.
11. Description of assay conditions for acute DMN model can be found in Supplementary data.
12. Davies, S. P.; Reddy, H.; Caivano, M.; Cohen, P. *Biochem. J.* **2000**, *351*, 95.