Bioorganic & Medicinal Chemistry Letters 20 (2010) 5502–5505

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

The discovery and synthesis of highly potent subtype selective phosphodiesterase 4D inhibitors

Renee Aspiotis *, Denis Deschênes, Daniel Dubé, Yves Girard, Zheng Huang, France Laliberté, Susana Liu, Robert Papp, Donald W. Nicholson, Robert N. Young

Merck Frosst Centre for Therapeutic Research, 16711 Trans-Canada Hwy, Kirkland, Québec., Canada H9H 3L1

ARTICLE INFO

Article history:
Received 1 June 2010
Revised 14 July 2010
Accepted 16 July 2010
Available online 21 July 2010

Keywords:
PDE4D
Phosphodiesterase 4D inhibitors
Asthma
COPD
Quinoline

ABSTRACT

The SAR study of a series of 6-aryloxymethyl-8-aryl substituted quinolines is described. Optimization of the series led to the discovery of compound **26b**, a highly potent (IC_{50} = 0.6 nM) and selective PDE4D inhibitor with a 75-fold selectivity over the A, B, and C subtypes and over 18,000-fold selectivity against other PDE family members. Rat pharmacokinetics and tissue distribution are also summarized.

The phosphodiesterases (PDEs) are metallo hydrolases responsible for the degradation of the secondary messengers adenosine and guanosine 3′,5′-cyclic monophosphates (cAMP and cGMP, respectively).[1] Comprised of multiple isoforms, the PDEs are classed into 11 families (PDE1-11) according to their substrate specificities, tissue and subcellular distribution, amino acid sequences, and response to exogenous and endogenous substrates.[1,2]

Among the most targeted PDEs are the type 4 cAMP-specific phosphodiesterases (PDE4s), the predominant isoenzyme in airway smooth muscle cells as well as immune and inflammatory cells.[3] There are four genes comprising the PDE4 family, PDE4A, PDE4B, PDE4C, and PDE4D and all are expressed as multiple splice variants. Inhibition of this family of enzymes significantly increases intracellular cAMP levels, leading to the down regulation of various cytokines and proinflammatory mediators, and the suppression of infiltration of neutrophils and eosinophils in inflamed airways.[4] Indeed, several PDE4 drug candidates have been identified as promising anti-inflammatory agents for the treatment of asthma, chronic obstructive pulmonary disease (COPD), and rheumatoid arthritis, including the archetype rolipram and its second generation inhibitors cilomilast and roflumilast.[5]

A key obstacle limiting the practical application of PDE4 inhibitors is their reported dose-limiting side effects, namely nausea and emesis.[6] All of the earlier chemotypes do not discriminate between the PDE4 subtypes (A, B, C, or D), yet recent studies from knockout mice together with siRNA targeting and distribution studies demonstrate that the PDE4 subtypes have distinct functional roles in inflammatory, immunocompetent, and smooth muscle cells.[7] Targeting one of the four subtypes thus may be advantageous in maximizing the therapeutic window of efficacy over its reported adverse effects.

Within the last decade, considerable effort has been directed toward understanding the biological role of PDE4D. PDE4D accounts for at least 80% of PDE activity in inflammatory cells.[8] In knockout mice, the PDE4D gene was directly associated with potent anti-inflammatory effects and the loss of airway hyperactivity.[9] In humans, a genome-wide association analysis identified PDE4D as an asthma-susceptibility gene.[10] PDE4D has also been implicated in depression[11], long-term memory formation[12], prostate cancer[13], Type II diabetes[14], cystic fibrosis[15], and controversially in stroke.[16] One potential caveat of PDE4D inhibition is its proposed association with the central nervous system-related emesis and cardiac side effects.[17] While it is not entirely clear whether PDE4D inhibition alone is the sole basis of the said adverse events, its emerging therapeutic potential prompted us to begin a medicinal chemistry effort to identify a subtype selective PDE4D inhibitor.

Examples of PDE4D selective inhibitors have been reported in the literature (Fig. 1). The prototypical example is Cilomilast **1** with a ~7-fold reported selectivity for the D subtype (IC_{50} = 12 nM) relative to PDE4A, B and C.[19a] Nicotinamide **2** has also been shown to be a potent subtype selective PDE4D inhibitor with a minimum 140-fold selectivity over its respective subtypes.[15] In addition,

* Corresponding author.
E-mail address: renee_aspiotis@merck.com (R. Aspiotis).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.07.076

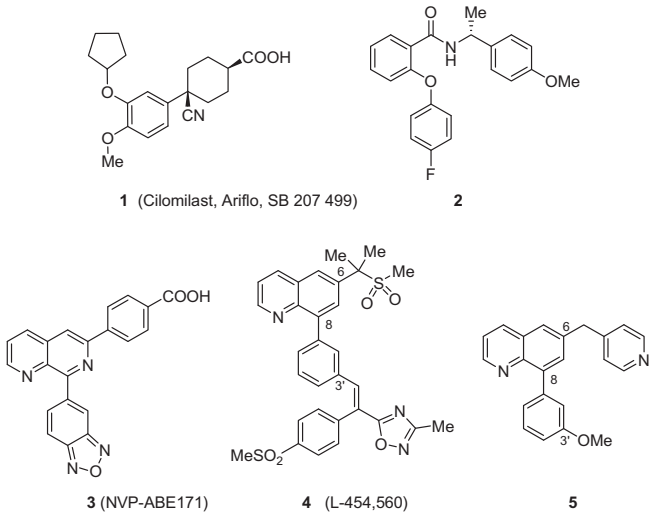


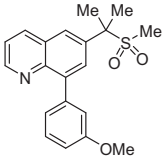
Figure 1.

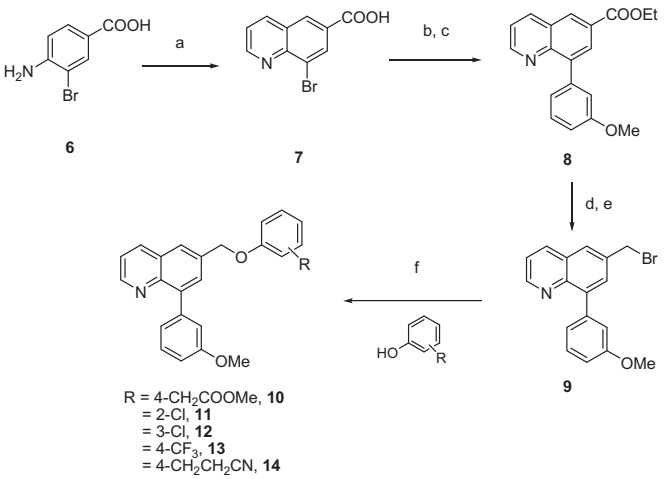
Hersperger et al. have developed potent, subtype selective D inhibitors such as **3** (NVP-ABE171) which has activity in both human inflammatory cells and in in vivo models of lung inflammation.[18b,18c] Our quest for a PDE4D selective inhibitor stemmed from the simple pyridylmethylquinoline **5**[19] which was shown to be a potent pan-selective PDE4 inhibitor. We were intrigued by the fact that this truncated version of our initial clinical candidate **4** (L-454,560)[19b] retained much of its activity on the D subtype relative to B (twofold loss in D vs fivefold loss in B, Fig. 1 and Table 1). A library was thus set out to further explore the 6- and 8-position of the quinoline core in hope that we could gain additional selectivity and binding with respect to the D subtype. Herein, we wish to report the discovery of 6-aryloxymethyl-8-aryl substituted quinolines as a new structural class of potent, subtype selective PDE4D inhibitors.

Table 1
Potency and selectivity profiles of PDE4 inhibitors against the four human subtypes A, B, C, and D

Compound	IC_{50}[a,b] (nM)				Ratio B/D
	PDE4A	PDE4B	PDE4C	PDE4D	
5	1.5	2.3	3.3	2.5	0.9
4	1.4	0.5	9.1	1.2	0.4
27[c]	9.9	10.7	69.5	9.3	1.2
10	4.6	7.4	31.2	0.4	18
11	126	87	469	5.0	17
12	319	389	102.1	12.1	32
13	338	418	218	48.7	9
14	122	86.5	414	2.0	44
18	307	120	1977	22.2	5
19	350	96.3	28,300	17.0	6
20	224	92.7	1235	10.5	9
21	166	43.3	1115	4.5	10
22	819	369	1341	18.3	20
23	60.4	57.6	103	0.9	61
24	12.8	15.3	36.2	0.8	20
25b	5.7	8.7	32.2	0.2	36
26b	63.7	45.0	151	0.6	75

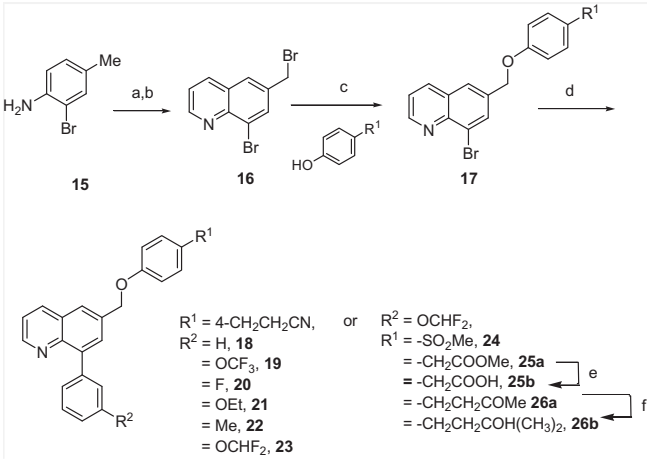
[a] Values are means of at least two experiments.
[b] See Ref. 22 for assay conditions against the PDE4 subtypes.
[c] Structure of compound **27**.





Scheme 1. Reagents and conditions: (a) glycerol, 3-NO_2PhSO_3Na, H_2SO_4/H_2O, heat; (b) EtOH, H_2SO_4, reflux; (c) 3-$MeOC_6H_4B(OH)_2$ $Pd(dppf)Cl_2$ (cat.), Na_2CO_3, EtOH/tol (1:1), reflux; (d) $LiAlH_4$, THF, −78 °C; (e) HBr, HOAc, 100 °C; (f) Cs_2CO_3, DMF, rt, o/n.

The syntheses of **4**[19b] and **5**[19a] have been previously described. Access to the quinoline compounds **10–14** can be similarly obtained as illustrated in Scheme 1. Thus, treatment of commercially available aniline **6** with excess glycerol under Skraup conditions afforded the corresponding 6-carboxy-8-bromoquinoline intermediate **7**. A direct Suzuki coupling on this intermediate proved problematic upon scaling so the crude acid **7** was converted to its ethyl ester using standard Fischer conditions. A subsequent Suzuki coupling with commercially available 3-methoxyphenyl boronic acid using $Pd(dppf)Cl_2$ catalyst easily afforded the 3′-methoxy-8-arylquinoline intermediate **8**. Careful reduction of the ethyl ester with $LiAlH_4$ at −78 °C followed by conversion to the methylbromide intermediate **9** set the stage for the final alkylation step, in which treatment with an appropriately substituted phenol using Cs_2CO_3



Scheme 2. Reagents and conditions: (a) glycerol, $FeSO_4{\cdot}7H_2O$, $MeSO_3H$, 3-NO_2PhSO_3Na, 130–135 °C; (b) Vazo-52®, NBS, chlorobenzene, 50 °C; (c) Cs_2CO_3, DMF, 50 °C; (d) 3-substituted phenyl boronic acid or pinacol boronate ester, $Pd(dppf)Cl_2$ (cat.), Na_2CO_3, DMF, 90 °C; (e) LiOH, THF, MeOH, 50 °C; (f) MeLi, $CeCl_3$, THF, −78 °C–rt, 4 h.

as the base in DMF afforded the desired phenolic ether analogs **10–14**.

A more convergent method of accessing 6-aryloxymethyl-8-aryl substituted quinolines **18–26** is depicted in Scheme 2. The bromomethylquinoline starting material **16** was synthesized in two steps using an optimized process route by condensing 2-bromo-4-methylaniline **15** with glycerol followed by a radical bromination of the corresponding 6-methyl-8-bromoquinoline.[20] Subsequent displacement of the intermediate bromomethylquinoline **16** with an appropriately functionalized 4-substituted phenol followed by standard Suzuki coupling using either a commercially available boronic acid or a pre-synthesized pinacol boronate ester, as in the case for $R^2 = OCHF_2$, afforded compounds **18–26**. Hydrolysis of the methyl ester **25a** with LiOH afforded the final acid **25b**, whereas the tertiary alcohol **26b** was easily obtained by reacting methyl ketone **26a** with a methyl cerium reagent derived from MeLi and $CeCl_3$.

All of the compounds were evaluated for their inhibitory activity against the four subtypes PDE4A, B, C, and D (Table 1).[22] For comparison, the data for **4** and **5** are also included.[19b] The B/D ratio was used as a selectivity gauge as the catalytic active sites of these two subtypes are said to be most comparable.[21]

Previous work toward the design of **4** (L-454,560) revealed that the bulky 3′ substituent could be replaced with a smaller methoxy group with an average ~10-fold loss in intrinsic potency across the four PDE4 subtypes (compound **4** vs **27**). Interestingly, potency across the four subtypes could be restored with a 6-pyridylmethyl substituent as depicted in **5**. A closer observation of the overall loss in potency on going from **4** to **5** suggested that the D subtype was less affected by the change in the 6-position on the quinoline ring compared to the B subtype (twofold loss in D vs fivefold loss in B). This subtle finding prompted us to further explore the 6-position of the quinoline ring in hope that the compound's affinity for the B subtype could be tuned out; the bromomethylquinoline intermediate **9** served as a useful tool for this purpose.

Many thio, sulfinyl, sulfonyl, and alkoxy ethers were synthesized, of which the phenolic ether template prevailed (Table 1, compounds **10–14**). In particular, a *para*-phenolic ether bearing a 2-cyanoethyl moiety **14** was deemed most interesting in terms of its potency (2.0 nM) and selectivity for the D subtype (44-fold over B). We then decided to further optimize the 3′ position on the 8-aryl ring of the quinoline core (compounds **18**–**23**), keeping the substituents small, and quickly found that a difluoromethoxy substituent **23** was a suitable replacement for the methoxy analog in **14**. A further reoptimization of the 2-cyanoethyl moiety in the quinoline compound **23** afforded subnanomolar PDE4D inhibitors **24**, **25b**, and **26b** with moderate to good 4D selectivity. In particular, the highly potent tertiary alcohol **26b** (0.6 nM) proved most interesting with an observed 75-fold selectivity for PDE4D.

Compound **26b** was further profiled against a panel of PDE family members and was shown be a highly specific PDE4D inhibitor, with a minimum 18,000-fold selectivity for the 4D isoform (Table 2).

Table 2
Potency and selectivity profile of compound **26b** against a panel of human PDE family members

PDEs	IC_{50}[a] (nM)	PDEs	IC_{50}[a] (nM)
1A	⩾58,000	7A2	⩾75,000
rh2A3[b]	⩾57,000	8A	⩾29,000
3A	⩾29,000	9A	⩾55,000
4D	0.6	10A2	⩾43,000
5A	11,000	11A	⩾95,000

[a] Values are means of at least two experiments.
[b] rh2A3, recombinant human 2A3.

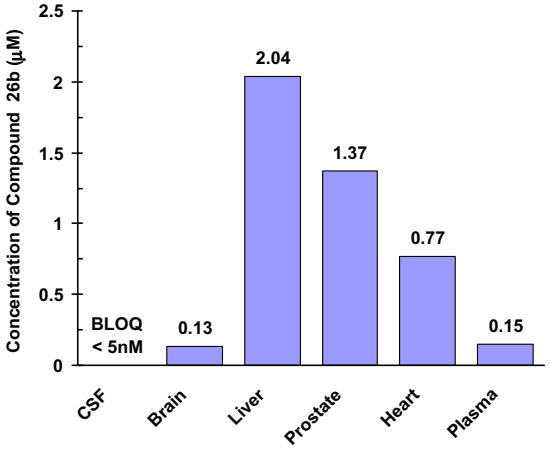


Figure 2. Tissue, CSF and plasma levels of compound **26b** 1 h post-interperitoneal dosing in fasted male Wistar–Han rats ($n = 2$). Dose = 3 mg/kg, dose volume = 3 mL/kg in 60% PEG200 (Ref. 23).

Preliminary pharmacokinetic testing demonstrated oral bioavailability in rats with $F = 25\%$, $V_{dss} = 4.87$ L/kg, CL = 44 mL/min/kg, and $t_{1/2} = 3.5$ h.[23] Drug levels were also assessed in a panel of tissues where PDE4D activity may play a physiologically important role (Fig. 2). In summary, compound **26b** exhibited favorable distribution to tissues relative to plasma 1 h post-interperitoneal dosing, with high levels noted in the liver, prostate, and heart (2.04, 1.37, and 0.77 μM, respectively). Interestingly, PDE4D has been shown to be over-expressed in human prostate cancer tissues and cell lines. Moreover, PDE4D can be selectively knocked down with shRNAs resulting in the reduction of growth and proliferation of prostate cancer cells in vivo and in vitro.[13] Thus, a subtype selective PDE4D inhibitor such as **26b** may serve as a useful tool in determining the potential of PDE4D inhibitors as anti-prostate cancer agents.

In summary, we have identified highly potent and selective inhibitors of PDE4 targeting the D subtype. The 6-bromomethyl-8-bromoquinoline intermediate **16** served as a useful tool in rapidly addressing substituents at both the 6- and 8-position of the quinoline core. The tertiary alcohol **26b** was found to be most interesting, with an $IC_{50} = 0.6$ nM on PDE4D, a 75-fold selectivity over the subtypes A, B, C and over 18,000-fold selectivity against other PDE family members. Future studies using this compound as a biological tool may further delineate the biological relevance of inhibiting PDE4D in vitro and in vivo.

Acknowledgments

The author thanks Dr. Michel Gallant and Dr. David Powell for helpful discussions and the proof reading of the manuscript. In addition, Dr. Izzat Raheem is thanked for helping with the profiling of compound **26b** against other PDE family members.

References and notes

1. (a) Bender, A. T.; Beavo, J. A. *Pharmacol. Rev.* **2006**, *58*, 488; (b) Conti, M.; Beavo, J. *Annu. Rev. Biochem.* **2007**, *76*, 481.
2. (a) Ke, H.; Wang, H. *Curr. Top. Med. Chem.* **2007**, *7*, 391; (b) Lugnier, C. *Pharmacol. Ther.* **2006**, *109*, 366.
3. (a) Houslay, M. D.; Schafer, P.; Zhand, K. Y. J. *Drug Discovery Today* **2005**, *10*, 1503; (b) Srivani, P.; Usharani, D.; Jemmis, E. D.; Sastry, G. N. *Curr. Pharm. Des.* **2008**, *14*, 3854.
4. (a) Torphy, T. J. *Am. J. Respir. Crit. Care Med.* **1998**, *157*, 351; (b) Claveau, D.; Chen, S. L.; O'Keefe, S.; Zaller, D. M.; Styhler, A.; Liu, S.; Huang, Z.; Nicholson, D. W.; Mancini, J. A. *J. Pharmacol. Exp. Ther.* **2004**, *310*, 752.
5. (a) Huang, Z.; Mancini, J. A. *Curr. Med. Chem.* **2006**, *13*, 3253; (b) Dastidar, S. G.; Rajagopal, D.; Ray, A. *Curr. Opin. Invest. Drugs* **2007**, *8*, 364.

6. (a) Spina, D. *Br. J. Pharmacol.* **2008**, *155*, 308; (b) Giembycz, M. A. *Br. J. Pharmacol.* **2008**, *155*, 288.
7. (a) Peter, D.; Jin, S. L.; Conti, M.; Hatzelmann, A.; Zitt, C. *J. Immunol.* **2007**, *178*, 4820; (b) Manning, C. D.; Burman, M.; Christensen, S. B.; Cieslinski, L. B.; Essayan, D. M.; Grous, M.; Torphy, T. J.; Barnette, M. S. *Br. J. Pharmacol.* **1999**, *128*, 1393; (c) Houslay, M. D.; Baillie, G. S.; Maurice, D. H. *Circ. Res.* **2007**, *100*, 950.
8. Liu, H.; Maurice, D. H. *J. Biol. Chem.* **1999**, *274*, 10557.
9. (a) Méhats, C.; Jin, S. L.; Wahlstrom, J.; Law, E.; Umetsu, D. T.; Conti, M. *FASEB J.* **2003**, *17*, 1831; (b) Hansen, G.; Jin, S.-L. C.; Umetsu, D. T.; Conti, M. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6751.
10. Himes, B. E.; Hunninghake, G. M.; Baurley, J. W.; Rafaels, N. M.; Sleiman, P.; Strachan, D. P.; Wilk, J. B.; Willis-Owen, S. A. G.; Klanderman, B.; Lasky-Su, J.; Lazarus, R.; Murphy, A. J.; Soto-Quiros, M. E.; Avila, L.; Beaty, T.; Mathias, R. A.; Ruczinski, I.; Barnes, K. C.; Celedón, J. C.; Cookson, W. O. C.; Gauderman, W. J.; Gilliland, F. D.; Hakonarson, H.; Lange, C.; Moffatt, M. F.; O'Connor, G. T.; Raby, B. A.; Silverman, E. K.; Weiss, S. T. *Am. J. Hum. Genet.* **2009**, *84*, 581.
11. (a) Zhang, H.-T. *Curr. Pharm. Des.* **2009**, *15*, 1688; (b) Zhang, H. T.; Huang, Y.; Jin, S. L.; Frith, S. A.; Suvarna, N.; Conti, M.; O'Donnell, J. M. *Neuropsychopharmacology* **2002**, *27*, 587.
12. Rutten, K.; Misner, D. L.; Works, M.; Blokland, A.; Novak, T. J.; Santarelli, L.; Wallace, T. L. *Eur. J. Neurosci.* **2008**, *28*, 625.
13. Rahrmann, E. P.; Collier, L. S.; Knutson, T. P.; Doyal, M. E.; Kuslak, S. L.; Green, L. E.; Malinowski, R. L.; Roethe, L.; Akagi, K.; Waknitz, M.; Huang, W.; Largaespada, D. A.; Marker, P. C. *Cancer Res.* **2009**, *69*, 4388.
14. Ong, W. K.; Gribble, F. M.; Reimann, F.; Lynch, M. J.; Houslay, M. D.; Baillie, G. S.; Furman, B. L.; Pyne, N. J. *Br. J. Pharmacol.* **2009**, *157*, 633.
15. Liu, S.; Veilleux, A.; Zhang, L.; Young, A.; Kwok, E.; Laliberté, F.; Chung, C.; Tota, M. R.; Dubé, D.; Friesen, R. W.; Huang, Z. *J. Pharmacol. Exp. Ther.* **2005**, *314*, 846.
16. (a) Gretarsdottir, S.; Thorleifsson, G.; Reynisdottir, S. T.; Manolescu, A.; Jonsdottir, S.; Jonsdottir, T.; Gudmundsdottir, T.; Bjarnadottir, S. M.; Einarsson, O. B.; Gudjonsdottir, H. M.; Hawkins, M.; Gudmundsson, G.; Gudmundsdottir, H.; Andrason, H.; Gudmundsdottir, A. S.; Sigurdardottir, M.; Chou, T. T.; Nahmias, J.; Goss, S.; Sveinbjörnsdottir, S.; Valdimarsson, E. M.; Jakobsson, F.; Agnarsson, U.; Gudnason, V.; Thorgeirsson, G.; Fingerle, J.; Gurney, M.; Gudbjartsson, D.; Frigge, M. L.; Kong, A.; Stefansson, K.; Gulcher, J. R. *Nat. Genet.* **2003**, *35*, 131; (b) Rosand, J.; Bayley, N.; Rost, N.; de Bakker, P. I. *Nat. Genet.* **2006**, *38*, 1091; (c) Munshi, A.; Kaul, S. *Int. J. Stroke* **2008**, *3*, 188.
17. (a) Robichaud, A.; Stamatiou, P. B.; Jin, S. L.; Lachance, N.; MacDonald, D.; Laliberté, F.; Liu, S.; Huang, Z.; Conti, M.; Chan, C. C. *J. Clin. Invest.* **2002**, *110*, 1045; (b) Lehnart, S. E.; Marks, A. R. *Expert Opin. Ther. Targets* **2006**, *10*, 677.
18. (a) Kroegel, C.; Foerster, M. *Expert Opin. Investig. Drugs* **2007**, *16*, 109; (b) Hersperger, R.; Dawson, J.; Mueller, T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 233; (c) Trifilieff, A.; Wyss, D.; Walker, C.; Mazzoni, L.; Hersperger, R. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 241.
19. (a) Wilhelm, R. S.; Fatheree, P. R.; Chin, R. L.; U.S. Patent 5455,252, 1995.; (b) Macdonald, D.; Mastracchio, A.; Perrier, H.; Dubé, D.; Gallant, M.; Lacombe, P.; Deschênes, D.; Roy, B.; Scheigetz, J.; Bateman, K.; Li, C.; Trimble, L. A.; Day, S.; Chauret, N.; Nicoll-Griffith, D. A.; Silva, J. M.; Huang, Z.; Laliberté, F.; Liu, S.; Ethier, D.; Pon, D.; Muise, E.; Boulet, L.; Chan, C. C.; Styhler, A.; Charleson, S.; Mancini, J.; Masson, P.; Claveau, D.; Nicholson, D.; Turner, M.; Young, R. N.; Girard, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5241.
20. Conlon, D. A.; Drahus-Paone, A.; Ho, G.-J.; Pipik, B.; Helmy, R.; McNamara, J. M.; Shi, Y.-J.; Williams, J. M.; Macdonald, D.; Deschênes, D.; Gallant, M.; Anthony Mastracchio, A.; Roy, B.; Scheigetz, J. *Org. Process Res. Dev.* **2006**, *10*, 36.
21. Wang, H.; Peng, M.-S.; Chen, Y.; Geng, J.; Robinson, H.; Houslay, M. D.; Cai, J.; Ke, H. *Biochem. J.* **2007**, *408*, 193.
22. Claveau, D.; Chen, S. L.; O'Keefe, S.; Zaller, D. M.; Styhler, A.; Liu, S.; Huang, Z.; Nicholson, D. W.; Mancini, J. A. *J. Pharmacol. Exp. Ther.* **2004**, *310*, 752.
23. Compound **26b** was administered separately to male Wistar–Han rats at 3 mg/kg by oral gavage, 3 mg/kg interperitoneally and 1 mg/kg intravenously. Plasma samples were collected in EDTA tubes at specified time-points up to 24 h postdose. The plasma samples were extracted using protein precipitation. Tissue samples were homogenized in water using a bead beating device and aliquots extracted by protein precipitation with acetonitrile. All bioanalytical measurements were made using a ThermoFisher Transcend LX2 Parallel UHPLC system (Franklin, MA, USA) coupled to an Applied Biosystems API4000 triple quadrupole mass spectrometer (Foster City, CA, USA). Standards and quality controls were prepared in the corresponding blank tissue matrix.