Bioorganic & Medicinal Chemistry Letters 20 (2010) 2311–2315

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design and synthesis of conformationally constrained N,N-disubstituted 1,4-diazepanes as potent orexin receptor antagonists

Paul J. Coleman [a,*], John D. Schreier [a], Georgia B. McGaughey [b], Michael J. Bogusky [a], Christopher D. Cox [a], George D. Hartman [a], Richard G. Ball [c], C. Meacham Harrell [d], Duane R. Reiss [d], Thomayant Prueksaritanont [e], Christopher J. Winrow [d], John J. Renger [d]

[a] Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 4, Sumneytown Pike, West Point, PA 19486, USA
[b] Department of Chemical Modeling and Informatics, Merck Research Laboratories, PO Box 4, Sumneytown Pike, West Point, PA 19486, USA
[c] Department of Pharmaceutical Research and Development, Merck Research Laboratories, PO Box 4, Sumneytown Pike, West Point, PA 19486, USA
[d] Department of Depression and Circadian Disorders, Merck Research Laboratories, PO Box 4, Sumneytown Pike, West Point, PA 19486, USA
[e] Department of Drug Metabolism, Merck Research Laboratories, PO Box 4, Sumneytown Pike, West Point, PA 19486, USA

ARTICLE INFO

Article history:
Received 7 January 2010
Revised 27 January 2010
Accepted 28 January 2010
Available online 8 February 2010

Keywords:
Orexin receptor
Sleep disorders
Diazepanes

ABSTRACT

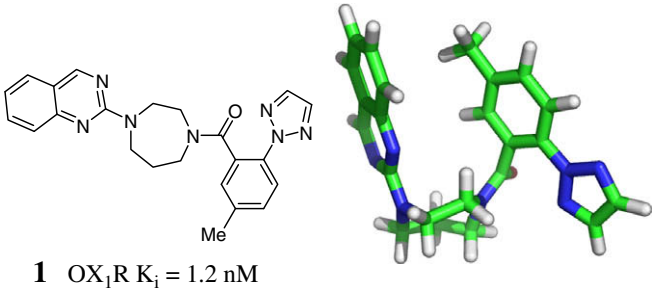
Orexins are neuropeptides that regulate wakefulness and arousal. Small molecule antagonists of orexin receptors may provide a novel therapy for the treatment of insomnia and other sleep disorders. In this Letter we describe the design and synthesis of conformationally constrained N,N-disubstituted 1,4-diazepanes as orexin receptor antagonists. The design of these constrained analogs was guided by an understanding of the preferred solution and solid state conformation of the diazepane central ring.

© 2010 Elsevier Ltd. All rights reserved.

Orexins (or hypocretins) are two neuropeptides that are secreted by a restricted group of neurons in the lateral hypothalamus. These neurons project to diverse regions of the central nervous system including areas of the brain that modulate the sleep-wake cycle.[1] The orexin neuropeptides, Orexin A and Orexin B, bind to two G protein-coupled receptors, OX_1R and OX_2R. There is strong genetic and pharmacological evidence that orexins promote and maintain wakefulness.[2] Indeed, blockade of orexin signaling by small molecule antagonists has been shown to promote sleep in preclinical species and in human clinical trials.[3]

In a previous publication from this laboratory, we described the discovery of a novel series of diazepane dual orexin receptor antagonists including 2-{4-[5-methyl-2-(2*H*-1,2,3-triazolyl-2-yl)benzoyl]-1,4-diazepan-1-yl}quinazoline, **1**.[4] Compound **1** is a brain-penetrant, potent dual orexin receptor antagonist that has excellent OX_2R/OX_1R receptor affinity (Fig. 1). Indeed, when dosed orally to rats with EEG-telemetry transmitters, compound **1** decreased wakefulness and increased slow-wave sleep at 100 mg/kg. Despite the excellent intrinsic potency associated with **1**, this compound had poor in vitro metabolic stability and limited (<20%) oral bioavailability in dogs and rats.

Spectroscopic, X-ray, and molecular modelling studies indicate that N,N-disubstituted 1,4-diazepane antagonists such as **1** adopt a low energy conformation that is characterized by an intramolecular π-stacking interaction and a twist-boat ring conformation.[5] This structure suggested to us that constraining the conformationally mobile seven-membered diazepane ring could favorably impact receptor potency and potentially address pharmacokinetic limitations.[6] Herein, we describe the synthesis and characterization of a series of bridged diazepanes. These efforts identified a po-

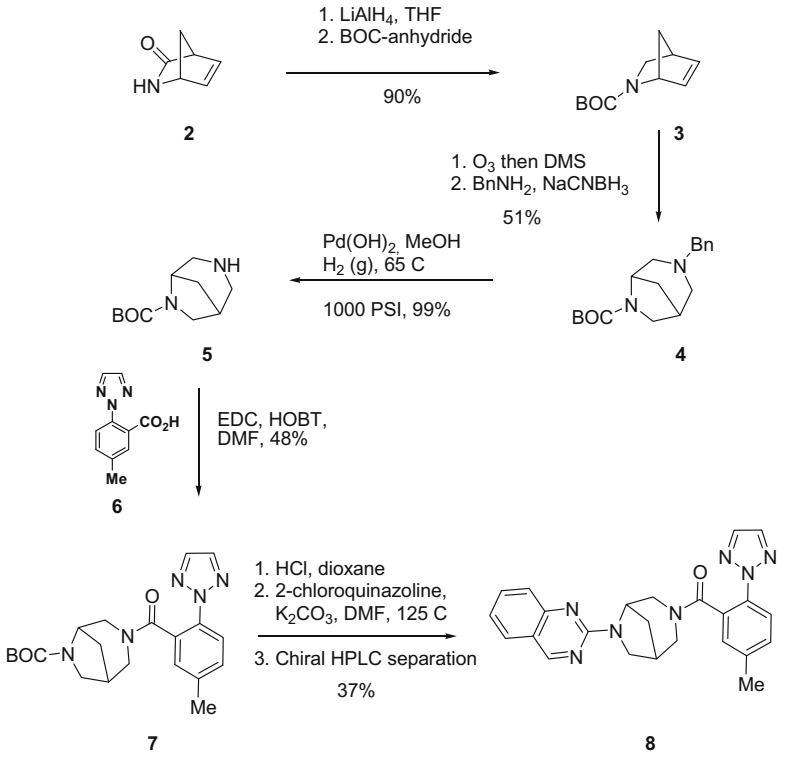


1 OX_1R K_i = 1.2 nM
OX_2R K_i = 0.6 nM

Figure 1. Structure and X-ray of orexin receptor antagonist **1**.

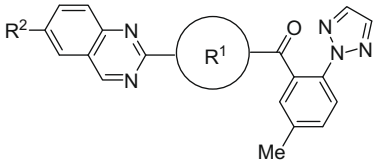
* Corresponding author. Tel.: +1 215 652 4618.
E-mail address: paul_coleman@merck.com (P.J. Coleman).

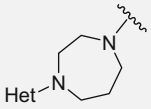
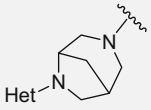
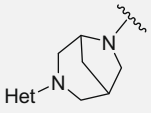
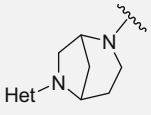
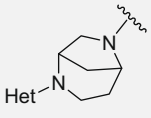
0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.01.138



Scheme 1. Synthesis of 3,6-diazabicyclo[3.2.1]octanes as orexin receptor antagonists.

Table 1
Diazabicyclo[3.2.1]octane orexin receptor antagonists



Compd	R^1	R^2	OX_1R (K_i, nM)	OX_2R (K_i, nM)	FLIPR	
					OX_1R (IC_{50}, nM)	OX_2R (IC_{50}, nM)
1		H	1.2	0.6	29	27
1a		F	1.0	0.2	36	27
8a (*rac*)		H	2.3	3.6	42	65
8b (*end A*)		H	110	155	2500	6200
8c (*end B*)		H	0.7	0.9	24	44
9		H	12	11	77	110
14 (*rac*)		F	870	260	>5700	>10,000
15 (*rac*)		F	1700	3500	3900	3700

tent, constrained analog of **1** with improved physicochemical properties and enhanced brain penetration.

In order to constrain the flexible diazepane central ring in these dual orexin receptor antagonists, we designed and synthesized bridged diazepanes where we systematically varied the linkage of a one carbon bridge on the seven-membered diazepane ring. Preliminary modeling studies suggested that several of these constrained diazepane rings would enforce the low energy conformation previously observed with compound **1**.[7]

A series of 3,6-diazabicyclo[3.2.1]octanes were prepared according to Scheme 1. In order to enable comparison between various constrained cores, the 2-(2*H*-1,2,3-triazol-2-yl)-5-methylbenzamide was maintained as a conserved feature. Further, the bridged diazepane was coupled with either an unsubstituted quinazoline or with a 6-fluoroquinazoline.[8] A solution of 2-azabicyclo[2.2.1]hept-5-en-3-one **2** was reduced with lithium aluminum hydride and the amine product protected with BOC-anhydride. Ozonolysis of alkene **3** followed by double, in situ reductive alkylation between the unpurified dialdehyde and benzylamine afforded the bridged diazepane **4** in moderate yield.[9] Hydrogenolysis of the benzyl group provided **5**. Amide coupling with 2-(2*H*-1,2,3-triazol-2-yl)-5-methylbenzoic acid **6**, followed by acid deprotection, nucleophilic aromatic substitution using 2-chloroquinazoline (or 6-fluoro-2-chloroquinazoline) afforded the receptor antagonist **8** in Table 1. *Rac*-**8a** could be resolved by HPLC on a chiral stationary support to afford enantiomers **8b** and **8c**.[10] The sequence of transformations could be reversed and intermediate **5** treated with 2-chloroquinazoline followed by BOC-deprotection and amide coupling to give compound **9** (Table 1).

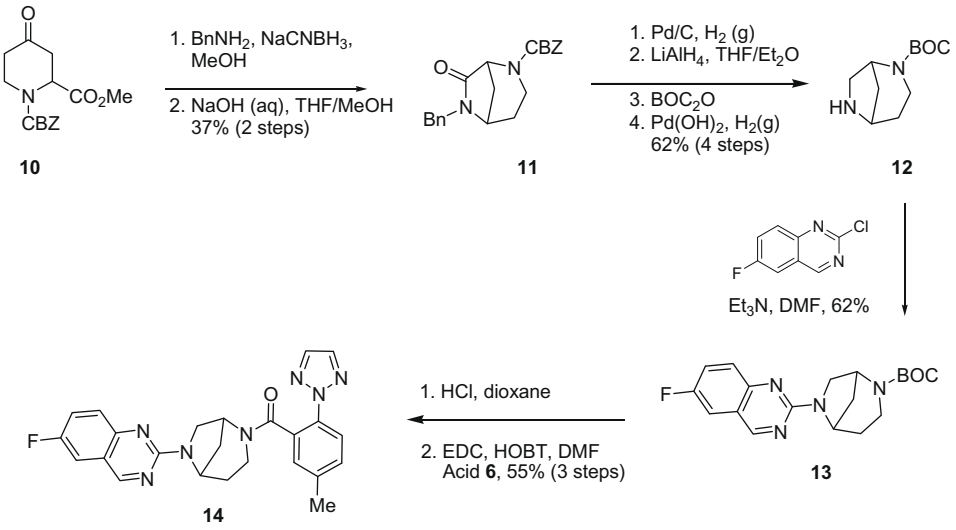
Synthesis of the 2,3-diazabicyclo[3.2.1]octanes is shown in Scheme 2. Piperidone **10** underwent reductive alkylation with benzylamine and intramolecular cyclization of **11** under basic conditions gave bicyclic lactam **11**. Removal of the CBZ-group, lactam reduction, BOC-protection, and debenzylation afforded **12**. Nucleophilic aromatic substitution with 6-fluoro-2-chloroquinazoline provided **13**. BOC-deprotection and standard amide coupling provided **14**. The reaction sequence could be easily reordered from intermediate **12** (amide formation and heteroarylation) to provide **15** (Table 1).

Results: Rigidifying the conformationally mobile diazepane in **1** by installing a methylene bridge provides the compounds shown in Table 1. To measure potency, we utilized a binding assay that measures displacement of a high-affinity radioligand bound to human orexin receptors (expressed as K_i), and a FLIPR (Fluorometric Imaging Plate Reader) assay in which calcium flux is measured as a functional determinant of orexin antagonism (expressed as IC_{50}).[11] These compounds all incorporated a 2-triazolyl-5-methylbenzamide discovered from previous optimization studies. The most potent compound in this group was compound **8a**. This racemic mixture could be resolved by chiral HPLC and provided two enantiomers **8b** and **8c**. The two stereoisomers differed in receptor affinity by greater than 50-fold favoring single enantiomer **8c** which was equipotent to **1** with respect to OX_2R binding and slightly more potent on OX_1R. In contrast, bridged compounds **9**, **14**, and **15** lost significant receptor potency (Fig. 1).

Detailed ^{1}H NMR studies were performed on compound **8c** to determine its solution conformation. In solution, **8c** exists as a mixture of four components in equilibrium at −35 °C related to restricted rotation of the amide and 2-aminoquinazoline rotamers. The two major rotamers possess several strong NOE correlations between the phenyl triazole ring and the quinazoline protons (Fig. 2). For the two major rotamers, the ^{1}H NMR methyl resonances from the phenyltriazole group (1.37 ppm major; 1.17 ppm minor) and the aromatic singlets (6.11 ppm, major; 5.97 ppm, minor) are shifted upfield which is characteristic of an aromatic ring current shift. Reference compound **C** was prepared to determine the inherent chemical shifts of the C6 phenyl and aromatic methyl protons in a molecule in which a π-stacking interaction is not available.

Attempts to generate diffraction quality crystals of **8c** were unsuccessful; however we were able to crystallize a Cl-analog of **8c**, which replaces the methyl group in **8c** with a chlorine atom to give **16** (Fig. 3). This molecule retains the binding affinity of **8c** and the structure of this molecule was solved by X-ray crystallography.[12] The solid state structure of **16** was consistent with the ^{1}H NMR structure of **8c** and superimposes well on the X-ray generated for compound **1** (Fig. 3). Based on the solved X-ray, the stereochemistry of **29** was assigned as *R, R*.

We were unsuccessful in obtaining X-ray structures of the less active, constrained diazepanes **9**, **14**, and **15**. Interestingly, detailed ^{1}H NMR studies were done on **15**, and no significant NOE's were observed between the phenyltriazole and quinazoline resonances indicating the ring systems were not proximal. Further, the key chemical shifts of the methyl resonances from the phenyl triazole (major rotamer 2.45 ppm; minor rotamer 2.41 ppm) and the aromatic singlets (major rotamer 7.27 ppm; minor rotamer



Scheme 2. Synthesis of 2,3-diazabicyclo[3.2.1]octanes as orexin receptor antagonists.

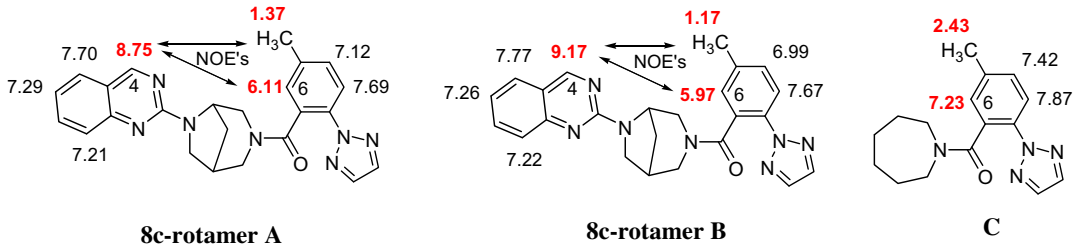


Figure 2. ^{1}H NMR chemical shifts for compound **8c** and reference compound C.

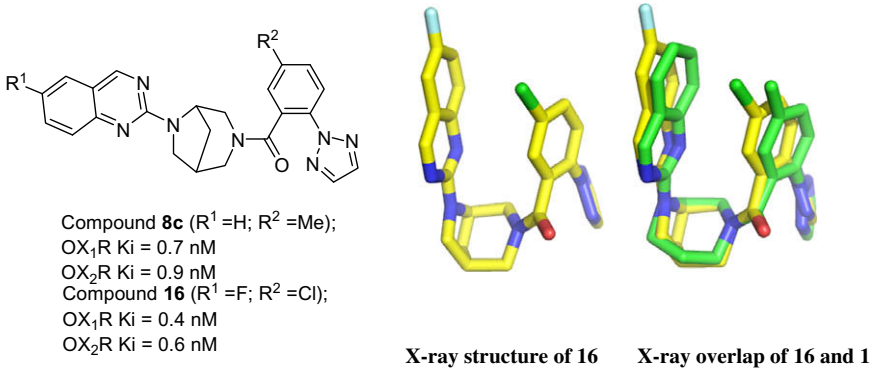


Figure 3. X-ray of orexin receptor antagonist **16**.

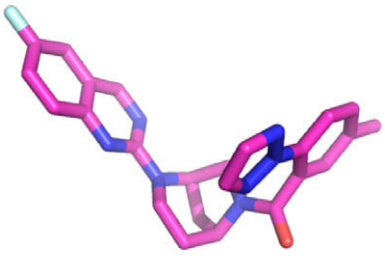


Figure 4. Minimized Structure of **15**.

7.25 ppm) do not indicate ring current shifts. Modeling studies on **15** (Fig. 4) provided a minimized structure where aryl-aryl overlap was minimal (Fig. 4).

Compound **8c** emerged as the most promising compound identified from these studies and was further characterized in terms of the physicochemical properties, pharmacokinetic parameters, and brain penetration. Despite introduction of the conformational constraint, none of the bridged compounds were significantly more potent than the lead molecule **1**.

Introduction of a ring constraint altered the physicochemical properties of **8c** relative to **1**. The experimentally measured Log *P* of **8c** was lower than **1** (Table 2). Brain, plasma, and CSF concentrations of compounds were assessed for compounds **1** and **8c** in anesthetized Sprague–Dawley rats.[13] Although total brain concentration is an important indicator of CNS penetration, we postulated that CSF concentrations of an orexin antagonist would be critical component for predicting receptor-available free compound in the brain.[14] Compounds were administered by intravenous infusion at 2 mg/kg and then the animals were sacrificed and brain, plasma, and CSF levels were measured. Both **1** and **8c** were brain-penetrant with brain-plasma ratios of 0.7–0.8 (Table 2). Neither were substrates of the P-glycoprotein transporter.[15] CSF concentrations of **8c** were threefold higher than **1** and consistent with the higher unbound concentration measured in plasma.

The pharmacokinetics of bridged compound **8c** were evaluated after oral administration to rats and dogs. As previously noted, **1** had poor pharmacokinetics in the rat with low oral bioavailability (F = 2%). Orexin receptor antagonist **1** also had poor pharmacokinetics in the dog with moderate clearance and short $T_{1/2}$ (Table 3). Unfortunately, bridged diazepane antagonist **8c** did not exhibit improved pharmacokinetics in the rat or dog. Bioavailability and plasma exposure remained low in both species with moderate to high plasma clearance.

In summary, we have designed and synthesized constrained derivatives of compound **1** that were inspired by an understanding of the solution and solid state conformations of **1**. The 3,6-diazabicyclo[2.2.1]octane core in **8c** enforces the energetically favorable π–π interaction seen in **1** and maintains binding affinity for OX_1R and OX_2R. Solution conformational analysis of **8c** and X-ray crystallography of analog **16** demonstrate that these conformationally-restricted analogs have good overlap with the lowest energy

Table 2
Rat brain penetration and physical properties of key compounds

Compd	Rat PK at 2 mpk, 30 min; iv infusion			PB%	Log *P*
	Plasma (nM)	Brain (nM)	CSF (nM)	Human (rat)	
1	664	462	19	97.6 (97.1)	2.91
8c	835	690	60	93.6 (92.7)	2.60

Table 3
Dog and rat pharmacokinetics for bridged compound **8c** versus **1**

Compd	Species	%*F*	Cl (mL/min/kg)	$T_{1/2}$ (h)	AUC_{0-24} (μM h)	C_{max} (μM)
1	Dog	16	12	1.3	0.44	0.23
	Rat	2	53	0.3	0.19	0.30
8c	Dog	8	24	0.9	0.49	0.51
	Rat	2	45	0.7	0.23	0.15

Rats dosed orally at 10 mpk, po in VitE-TPGS and dosed iv at 2 mpk in DMSO.

conformation of diazepane **1**. These findings further substantiate our hypothesis that the π–π interaction in the diazepane series leads to a highly favorable binding conformation with the orexin receptors. While compound **8c** does not have improved pharmacokinetics in rats and dogs, the bridged core provides reduced plasma protein binding and enhanced brain penetration. Further investigation of the in vitro and in vivo properties of diazepane-based scaffolds as orexin receptor antagonists will be reported in due course.

References and notes

1. For reviews: (a) Boss, C.; Brisbare-Roch, C.; Jenck, F. *J. Med. Chem.* **2009**, *52*, 891; (b) Boss, C.; Brisbare-Roch, C.; Jenck, F.; Aissaoui, H.; Koberstein, R.; Sifferlen, T.; Weller, T. *Chimia* **2008**, *62*, 974; (c) Roecker, A. J.; Coleman, P. J. *Curr. Top. Med. Chem.* **2008**, *8*, 977.
2. (a) De Lecea, L.; Kilduff, T. S.; Peyron, C.; Gao, X.-B.; Foye, P. E.; Danielson, P. E.; Fukuhara, C.; Battenberg, E. L. F.; Gautvik, V. T.; Bartlett, F. S., II; Frankel, W. N.; Van Den Pol, A. N.; Bloom, F. E.; Gautvik, K. M.; Sutcliffe, J. G. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 322; (b) Sakurai, T.; Amemiya, A.; Ishii, M.; Matsuzaki, I.; Chemelli, R.; Tanaka, H.; Williams, S. C.; Richardson, J. A.; Kozlowski, G. P.; Wilson, S.; Arch, J. R. S.; Buckingham, R. E.; Haynes, A. C.; Carr, S. A.; Annan, R. S.; McNulty, D. E.; Liu, W.; Terrett, J. A.; Elshourbagy, N. A.; Bergsma, D. J.; Yanagisawa, M. *Cell* **1998**, *92*, 573.
3. Brisbare-Roch, C.; Dingemanse, J.; Koberstein, R.; Hoever, P.; Aissaoui, H.; Flores, S.; Mueller, C.; Nayler, O.; van Gerven, J.; de Haas, S. L.; Hess, P.; Qiu, C.; Buchmann, S.; Scherz, M.; Weller, T.; Fischli, W.; Clozel, M.; Jenck, F. *Nat. Med.* **2007**, *13*, 150.
4. Whitman, D. B.; Cox, C. D.; Breslin, M. J.; Brashear, K. M.; Schreier, J. D.; Bogusky, M. J.; Bednar, R. A.; Lemaire, W.; Bruno, J. G.; Hartman, G. D.; Reiss, D. R.; Harrell, C. M.; Kraus, R. L.; Li, Y.; Garson, S. L.; Doran, S. M.; Prueksaritanont, T.; Li, C.; Winrow, C. J.; Koblan, K. S.; Renger, J. J.; Coleman, P. J. *ChemMedChem* **2009**, *4*, 1069.
5. Cox, C. D.; McGaughey, G. B.; Bogusky, M. J.; Whitman, D. B.; Ball, R. G.; Winrow, C. J.; Renger, J. J.; Coleman, P. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2997.
6. Reduction in numbers of rotatable bonds has been associated with improved oral bioavailability. See: Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, 2615.
7. Conformational searching using the mixed torsion/low-mode sampling algorithm as implemented in Schrödinger, v9.0 (Schrödinger, LLC, v9.0) was performed using the OPLS force field in a constant dielectric of **1**. All conformers within 5 kcal/mol of the global minimum were saved and visually inspected for evidence of pi-stacking. See: Kolossváry, I.; Guida, W. C. *J. Am. Chem. Soc.* **1996**, *118*, 5011.
8. The 6-fluoroquinazoline imparted additional binding affinity for OX_2R (e.g. Table 1; compound **1** vs **1a**). For preparation of 6-fluoro-2-chloroquinazoline see: Eur. Pat. Appl. 248554 A2, 1987.
9. (a) Bunnelle, W. H.; Daanen, J. F.; Ryther, K. B.; Schrimpf, M. R.; Dart, M. J.; Gelain, A.; Meyer, M. D.; Frost, J. M.; Anderson, D. J.; Buckley, M.; Curzon, P.; Cao, Y.-J.; Puttfarcken, P.; Searle, X.; Ji, J.; Putman, C. B.; Surowy, C.; Toma, L.; Barlocco, D. *J. Med. Chem.* **2007**, *50*, 3627; (b) Fray, A. H.; Augeri, D. J.; Kleinman, E. F. *J. Org. Chem.* **1988**, *53*, 896.
10. Racemic **8a** was resolved by chiral HPLC on a ChiralPak AD column (2 × 25 cm) eluting with 40% hexanes/60% EtOAc. The first eluting isomer (**8b**) has a retention time = 14.3 min and the second eluting enantiomer (**8c**) has a retention time = 17.9 min.
11. For detailed descriptions of these assays see: Supplementary data in Ref. 4.
12. *Compound* **16**, $C_{23}H_{19}Cl\ F\ N_7O$, *Mr* = 463.900, orthorhombic, *P*212121, *a* = 10.1978(7), *b* = 10.4373(6), *c* = 19.6892(11) Å, *V* = 2095.7(2) Å^3, *Z* = 4, *Dx* = 1.470 gcm^{-3}, monochromatized radiation λ(Cu) = 1.5418 Å, μ = 1.97 mm^{-1}, *F*(0 0 0) = 960, *T* = 100 K. Data were collected on a Oxford Diffraction CCD diffractometer to a θ limit of 66.4838° which yielded 6974 reflections. There are 2548 unique reflections with 2287 observed at the 2σ level. The structure was solved by direct methods (SHELXS-97, Sheldrick, G.M. *Acta Crystallogr.*, 1990, A46, 467–473) and refined using full-matrix least-squares on *F2* (SHELXL-97, Sheldrick, G.M. SHELXL-97. *Program for the Refinement of Crystal Structures*. Univ. of Göttingen, Germany). The final model was refined using 298 parameters and all 2548 data. All non-hydrogen atoms were refined with anisotropic thermal displacements. The final agreement statistics are: *R* = 0.068 (based on 2287 reflections with $I > 2\sigma(I)$), *wR* = 0.177, *S* = 1.10 with (Δ/σ)max = 0.01. The maximum peak height in a final difference Fourier map is 0.774 eÅ^{-3} and this peak is without chemical significance. CCDC 758078 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
13. Seven to ten week old Sprague–Dawley rats were anesthetized with isoflurane (5% with oxygen at 2 L/min). Once anesthetized, the rat was shaved from the ears to the shoulders and then placed on a circulating water warming blanket set at 37 °C. Anesthesia was maintained using 2% isoflurane by means of a nose cone. The tail vein was cannulated with a 25 G winged infusion needle connected via tubing to a syringe containing the test compound. The syringe was placed on a Harvard Apparatus infusion pump set to deliver 2 mg/kg over 30 min. At the end of the infusion, CSF was collected from the cisterna magna by needle puncture with slow aspiration using a 25 G butterfly connected by tubing to a 1 cc syringe. The CSF sample was dispensed into a vial and immediately frozen on dry ice. A 1 ml blood sample was then obtained via cardiac puncture, centrifuged, plasma collected and frozen on dry ice. The brain was rapidly harvested and frozen on dry ice. CSF, plasma, and brain samples were then analyzed to determine compound levels.
14. Martin, I. *Drug Discovery Today* **2004**, *9*, 161.
15. The MDR1 B–A/A–B ratio for **8c** = 0.9 with an apparent permeability of 37×10^{-6} cm/s in LLC-PK1 cells. For a description of this method see (a) Hochman, J. H.; Yamazaki, M.; Ohe, T.; Lin, J. H. *Curr. Drug Metab.* **2002**, *3*, 257; (b) Lin, J. H.; Yamazaki, M. *Drug Metab. Rev.* **2003**, *35*, 417.