Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 6691–6696
Bioorganic & Medicinal Chemistry Letters

Design and synthesis of a functionally selective D3 agonist and its in vivo delivery via the intranasal route

Julian Blagg,[a,*] Charlotte M. N. Allerton,[a] David V. J. Batchelor,[a] Andrew D. Baxter,[a,†] Denise J. Burring,[a] Christopher L. Carr,[a] Andrew S. Cook,[a] Carly L. Nichols,[a] Joanne Phipps,[a] Vivienne G. Sanderson,[a] Hugh Verrier[a] and Stephen Wong[b]

[a]*Pfizer Global Research & Development, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK*
[b]*Department of Neurosciences Biology, Pfizer Global Research & Development, Groton, CT 06340, USA*

Received 17 September 2007; revised 16 October 2007; accepted 16 October 2007
Available online 22 October 2007

Abstract—This paper reports the synthesis and biological activity of a novel series of aryl-morpholine dopamine receptor agonists. Several compounds show high levels of functional selectivity for the D3 over the D2 dopamine receptor. Compound **26** has >1000-fold functional selectivity and has been successfully progressed in vivo using an intranasal delivery route.

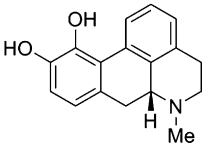
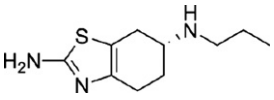
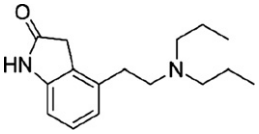
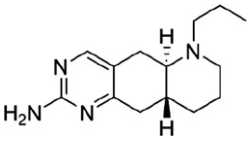
The dopaminergic system is characterised by 5 receptor subtypes, which cluster into 2 families. The D1-like receptors (D1 and D5) and the D2-like receptors (D2, D3 and D4). Non-selective dopamine agonists, including Apomorphine and Pramipexole, have been investigated for the treatment of male erectile dysfunction (MED) (Table 1).[1] Apomorphine has been shown to facilitate penile erection in clinical studies in men.[2] Uprima™ (apomorphine in the form of a sublingual tablet) was marketed for the treatment of MED; however, poor efficacy[3] and competition from Viagra™ were cited amongst the reasons for its withdrawal. The efficacy of apomorphine is compromised by dose-limiting side effects (e.g., nausea, dizziness and hypotension)[4] and by a relatively short duration of exposure in man ($T_{1/2}$ = 35 min). We set out to examine the hypothesis that whilst both the D2 and D3 receptors may be involved in the erectile process, D2 receptor activation is responsible for the dose-limiting side effects of non-selective dopamine agonists.

We hypothesised that a selective D3 agonist would prove effective in the treatment of MED whilst reducing the levels of nausea, emesis and cardiovascular effects observed with non-selective agents. Dopamine agonists exemplified in the literature demonstrated marginal selectivity for D3 over D2 receptor activation in our functional assays, whilst Apomorphine and Quinelorane demonstrated essentially balanced activity (Table 1). The marginal in vitro functional selectivity of Pramipexole for the D3 receptor translated to an equivalent pro-erectile efficacy in in vivo animal models of MED to Apomorphine. In addition, there was some indication of a better therapeutic ratio over nausea, emesis and blood pressure effects.[5] These preliminary findings prompted our search for a tool compound with significantly greater functional selectivity for activation of the D3 over the D2 receptor. Such a tool compound would enable definitive in vivo demonstration of efficacy with a widened therapeutic index. Literature reports of dopamine subtype selective agonists have been extensively reviewed.[6] However, many disclosures have been supported by in vitro binding as opposed to in vitro functional data. Herein we report the discovery of a series of aryl-morpholines, several of which show impressive levels of in vitro functional selectivity for D3 over D2 receptor activation in the same assay format and conditions where literature agents (Table 1) show marginal or no functional selectivity. In addition, the compounds described here show good selectivity over a range of other aminergic GPCR targets.

Keywords: Dopamine D3 receptor agonist; Arylmorpholine; Synthesis; Intranasal delivery.
* Corresponding author at present address: Cancer Research UK Centre for Cancer Therapeutics, The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey SM2 5NG, UK. Tel.: +44 (0)20 8722 4051; fax: +44 0208 722 4126; e-mail: julian.blagg@icr.ac.uk
† Present address: Serentis Ltd, 23 Cambridge Science Park, Milton Road, Cambridge CB4 0EY, UK.

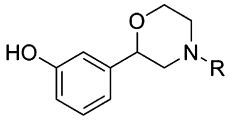
0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.10.059

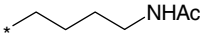
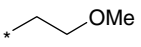
Table 1. D3/D2 Selectivity data for some literature dopamine agonists

Compound	Structure	Average functional EC_{50} (nM)		D3/D2
		D2	D3	
Apomorphine		20	7	3
Pramipexole		27	1	27
Ropinerole		83	4	21
Quinelorane		2	1	2

Our initial starting point was to carry out a mini-high throughput functional screen versus the D3 receptor. In vitro functional potency was determined by inhibition of forskolin stimulated cAMP accumulation in recombinant cell lines expressing either the human D3 receptor (expressed in CHO cells) or the human D2 receptor (expressed in rat pituitary cells).[5] The compounds screened comprised a subset of the Pfizer compound file chosen for their propensity to interact with aminergic GPCR targets. Selectivity versus the D2 receptor was subsequently used to triage the hits. The most promising hit matter discovered from this screening campaign contained the aryl morpholine template and included the racemic compounds **1**, **2** and **3** (Table 2). The optimal *N*-alkyl chain was quickly established as *n*-propyl **6** by in-house screening of analogues bearing varied substituents on the morpholine nitrogen and by reference to literature SAR from *N*-propyl-3-hydroxyphenyl piperidine (3-PPP)[7] and tricyclic dopamine agonists[8] where an *N*-propyl chain favoured dopamine activity. Compounds with longer or shorter alkyl chains had significantly reduced functional activity. Compounds **1**, **2**, **3** and **6** all triggered a full agonist response at the human recombinant D3 receptor. Compound **1** displayed weak agonism at the D2 receptor; no evidence for switching to functional antagonism at either the D3 or D2 receptors was observed.

Table 2. Mini-HTS results and subsequent SAR

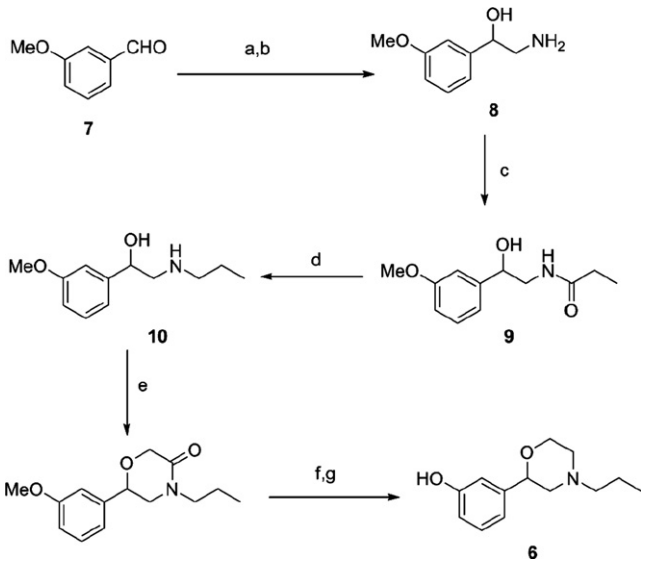


Compound	R group	Functional D3 EC_{50}	Functional D2 EC_{50} (μM)
1	H	864 nM	7.2
2	Et	547 nM	>30
3	Allyl	1312 nM	>100
4		>10 μM	>10
5		>3000 nM	>10
6	*n*-propyl	27 nM	>10

Synthesis of the racemic aryl morpholines was achieved according to the route exemplified for compound **6** (Scheme 1). Aryl aldehyde **7** was converted to the amino-alcohol **8** via intermediate cyanohydrin formation. Preparation of the amide **9** using propionyl chloride was followed by borane reduction to give the propyl amino-alcohol intermediate **10**. Cyclisation was carried out with chloro-acetylchloride, followed by reduction to the target morpholine ring system. Deprotection of the phenol in refluxing HBr gave the racemate **6** (Scheme 1). The route depicted in Scheme 1 was also used as the basis for synthesis of potential replacements for the phenol (Table 3). These compounds were prepared by incorporation of the appropriate aromatic functionality into the starting aldehyde **7** or by functional group manipulation of the phenol **6** (routes not shown).[9]

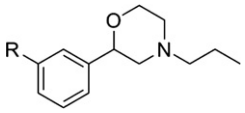
The phenol proved essential for potent and full agonism at the D3 receptor. None of the replacements described here that bear polar groups, H-bond donor or H-bond acceptor functionality designed to mimic the phenol retained significant functional activity.

Separation of the racemate **6** by chiral HPLC (Chiralpak AD column)[10] gave the enantiomers **26** and **27**



Scheme 1. Reagents and conditions: (a) 2 M HCl, Na_2SO_3, KCN, THF, rt, 30 min; (b) BH_3-THF (1 M in THF), reflux, 1.5 h under N_2 69% combined yield for a and b; (c) Propionyl chloride, Et_3N, DCM, rt, 5 h 67%; (d) $BH_3.SMe_2$, THF, 93%; (e) chloro-acetyl chloride, NaOH, H_2O, DCM, rt, 30 min; then aqKOH, IPA, rt, 2 h, 90%; (f) BH_3-THF, (1 M in THF) reflux, 3 h 93%; (g) aqHBr, reflux, 1 h, 85%.

Table 3. D3 and D2 functional assay results for compounds **6**–**25**



Compound	R group	Functional D3 EC_{50} (nM)	Functional D2 EC_{50}
6	OH	27	>10 μM
11	H	>1000	>10 μM
12	Cl	>2800	>10 μM
13	OMe	>1000	>10 μM
14	OCF_3	>1000	>1000 nM
15	SMe	>1000	>10 μM
16	SO_2Me	>1000	>10 μM
17	CO_2Me	>1000	NT[a]
18	CO_2H	>1000	>10 μM
19	CH_2OH	>1000	NT[a]
20	CN	>1000	NT[a]
21	$CONH_2$	>1000	NT[a]
22	NH_2	>1000	>1000 nM
23	$NHSO_2Me$	>1000	>1000 nM
24		>1000	NT[a]
25		>1000	>10 μM

[a] Not tested.

which demonstrated that all activity resides in the ***R***-enantiomer (**26**), (Fig. 1). The absolute configuration of the active enantiomer (crystallized as its HCl salt) was confirmed by small molecule X-ray analysis (Fig. 2).[9,11]

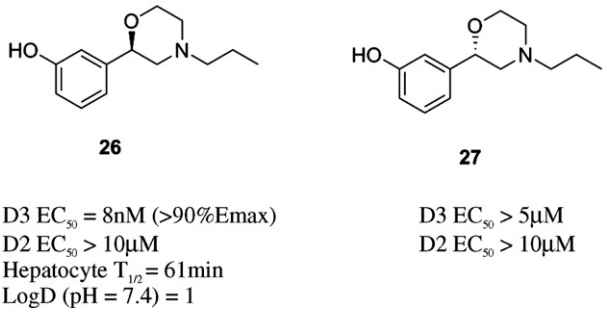


Figure 1. Profile of individual enantiomers **26** and **27**.

Compound **26** met our potency and selectivity criteria. It is a moderately lipophilic base ($\mathrm{Log}\,D_{7.4\ \mathrm{octanol}} = 1$; $pK_a = 7.4$), with high aqueous solubility (>1 g/ml), good Caco-2 flux ($\mathrm{Papp} = 38 \times 10^{-6}$ cm s^{-1} at 1 μM) and low plasma protein binding (41%, 76% and 65% in rat, dog and human plasma, respectively). Compound **26** displayed minimal binding activity ($IC_{50} > 10$ μM) against a wide panel of targets (Cerep wide ligand profile) including dopamine D1 and D5 receptors. Weak binding activity was observed at the human $5HT_{1A}$ receptor (16% at 100 nM and 95% at 10 μM). The lack of significant $5HT_{1A}$ activity was further confirmed using human recombinant assays to detect antagonism ($IC_{50} > 20$ μM) and agonist activity (inactive at 1 μM). In addition, compound **26** displayed weak binding activity at a range of other selectivity targets; for example: Rat $5HT_{2A}$ receptor: $K_i = 970$ nM; rat $alpha_{1A}$ receptor: $K_i > 1$ μM, human $alpha_{2A}$ receptor: $K_i > 1$μM; human histamine H_1 receptor: $K_i > 2$ μM; human muscarinic M_1 receptor: $K_i > 1$μM; dofetilide binding: $K_i > 4$ μM. The promising pharmacology and physiochemical properties prompted in vivo evaluation to explore the efficacy-toleration profile. Multiple dose evaluation in animal models of MED via subcutaneous administration gave an ED_{50} of approximately 0.2 to 0.5 nM free drug concentration. No nausea, emesis or hypotensive effects were

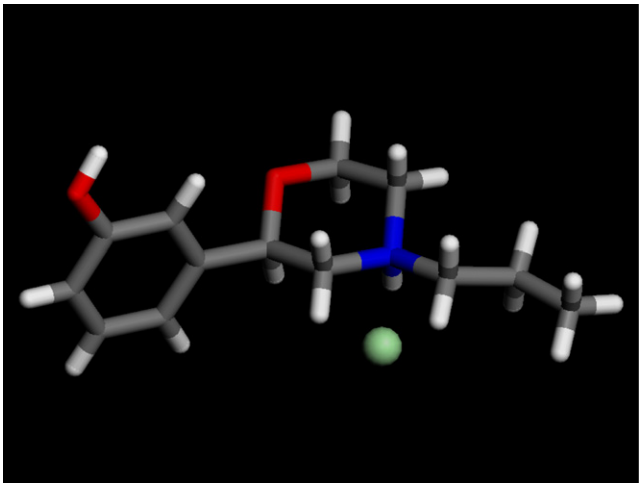
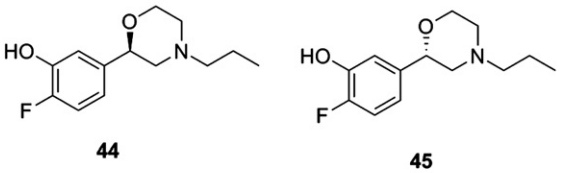


Figure 2. X-ray structure of active phenol enantiomer **26**.[11]

seen at these efficacious exposure levels or at significantly higher exposures (up to 2.7 μM free drug concentrations following subcutaneous dosing in the dog) indicative of a much wider therapeutic index than non-selective dopamine agonists.[5] However, compound **26** suffered from poor oral bioavailability due to clearance at, or greater than, liver blood flow in rat and dog. Unsurprisingly, glucuronidation of the phenolic group was the major contributor to the rapid first pass clearance. A programme was initiated to reduce the rate of phenol conjugation by modulating the phenolic pK_a and by introduction of proximal steric bulk to disrupt interaction of the phenol with the glucuronidase active site.[12,13] Maintaining functional D3 potency whilst altering the pharmacokinetic profile proved difficult. Di-substituted compounds for example **30**, **31** and **38** resulted in significant loss of potency (Table 4). Compound **41**, a potent full agonist; contains the well-known *ortho*-hydroxyaniline toxicophore which limited its potential for further investigation. Substitution *ortho*- to the morpholine ring (positions R^1 and R^5) significantly reduced activity possibly due to restriction of the torsional rotation between the aryl moiety and the morpholine ring. However, compound **29** demonstrated that an R^3 fluoro group is well tolerated and we further examined the effect of an *ortho*-fluoro substituent on the pharmacokinetic profile.

Compound **29** was separated into enantiomers **44** and **45** (Chiralpak AD column),[14] again all activity resided in one isomer **44**. However, the poor stability of **44** in human hepatocytes, coupled with its stability in microsomal preparations, indicated that stability to glucuronidation or other Phase II conjugative pathways was unlikely to have been improved. This compound was not progressed further (Fig. 3).



D3 EC_{50} = 36nM (>90%Emax)
D2 EC_{50} > 10μM
Hepatocyte $T_{1/2}$ = 21min

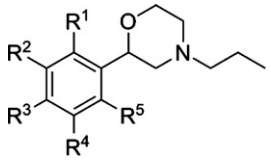
D3 EC_{50} > 10μM
D2 EC_{50} > 10μM

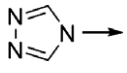
Figure 3. Data for separated enantiomers of compound **29**.

There is precedent in the literature from Terbutaline[15] and Phenylephrine[16] (Fig. 4) that conjugative clearance of a mono-phenol can be improved by addition of a second phenolic group in the *meta* position. Application of this strategy to compound **26** resulted in compound **32** with maintained activity (Table 4). Separation of **32** into enantiomers **46** and **47** (Chiralpak AD column)[17] again showed that all activity resides in one isomer. In this case improved rat and human hepatocyte stability was achieved; however, in vivo clearance in rat significantly exceeded rat liver blood flow indicating that the improved in vitro stability was unlikely to translate in vivo in human (Fig. 4).

The programme of phenol replacements and aryl ring modifications described here did not solve the issue of the high first pass clearance for compound **26**. One solution was to opt for a non-oral delivery route following the precedented bioavailability improvements observed with, for example, intranasal Apomorphine.[18] Advantages of the intranasal dosing route include the avoidance of first pass metabolism, consequent improved bioavailability and a rapid onset of action.[19,20] Intranasal

Table 4. D3 functional assay results for compounds **28–43**



Compound	R^1	R^2	R^3	R^4	R^5	Functional D3 EC_{50}
28	Me	OH	H	H	H	>1000 nM
29	H	OH	F	H	H	35 nM
30	H	OH	Cl	H	H	>688 nM
31	H	OH	CH_2OH	H	H	>5900 nM
32	H	OH	H	OH	H	37 nM
33	H	OH	H	CN	H	>10 μM
34	H	OH	H	$CONH_2$	H	>10 μM
35	H	OH	H	H	Br	>10 μM
36	H	H	OH	H	H	>1000 nM
37	H	CN	OH	H	H	>1000 nM
38	H	$CONH_2$	OH	H	H	427 nM
39	H	COMe	OH	H	H	>1000 nM
40	H	CH_2OH	OH	H	H	>1000 nM
41	H	NH_2	OH	H	H	40 nM
42	H	$NHSO_2Me$	OH	H	H	>1000 nM
43	H		OH	H	H	>1000 nM

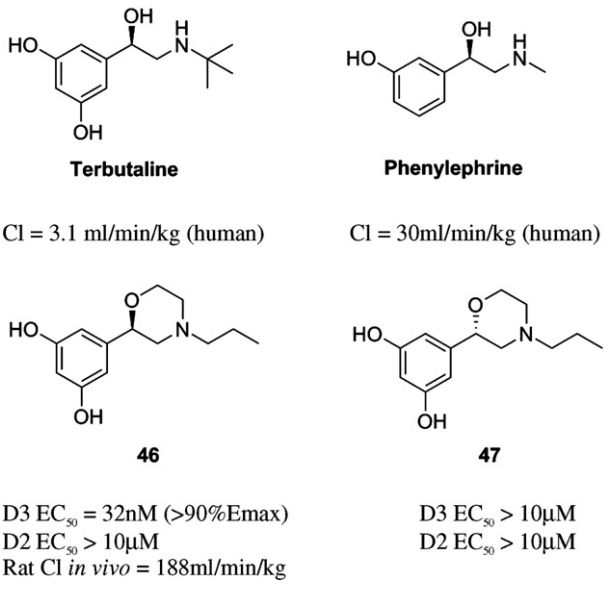


Figure 4. The effect of an additional *meta*-phenol on clearance.

delivery is greatly facilitated for highly soluble compounds and the aqueous solubility of compound **26** (>1 g/ml) was very favourable in this regard. Rat and dog intranasal pharmacokinetics (Table 5) gave encouragement that the intranasal route would indeed be a viable development option.[21]

Based upon these in vivo animal data, the predicted human intranasal bioavailability was an encouraging 25% compared to a predicted 0% oral bioavailability. The disappearance half-life values in rat, dog and human hepatocytes for compound **26** were 28, 18 and 61 min, respectively, indicative of high hepatic extraction in all species. In vivo pharmacokinetic studies in rat and dog demonstrated plasma clearance close to hepatic blood flow as expected. Thus, compound **26** was predicted to exhibit plasma clearance values approximating to liver blood flow in humans. The volume of distribution in humans was predicted to be in the range 1.4–6.6 L/kg. Together these figures predict a half-life in humans in the range of 0.6–3 h.

In conclusion, we have described the synthesis and properties of the first potent, full D3 agonist with high functional selectivity over D2 receptor agonism. High first pass clearance of the lead compound, **26**, could not be overcome by the structural modifications presented here. However, intranasal administration enabled significant systemic exposure to be achieved in rat and dog. The absence of nausea, emesis and hypotensive effects in preclinical animal models with compound **26** at free drug concentrations, significantly exceeding those required for erectile efficacy in the same species, indicates that these adverse events may be linked to D2 receptor activation. The efficacy of compound **26** in animal models of male erectile dysfunction indicates that activation of the D3 receptor may play a significant role in male erectile dysfunction. However, the hypothesis that selective activation of the D3 receptor in man will demonstrate efficacy in the absence of dose limiting side effects remains to be proven.

Table 5. Intranasal pharmacokinetics of compound **26**

	Rat 2 mg/kg	Dog 0.3 mg/kg
T_{max}	5 min	5 min
$T_{1/2}$	0.3 h	1.6 h
Intranasal bioavailability	20%	33%

References and notes

1. (a) Giuliano, F.; Allard, J.; Rampin, O.; Droupy, S.; Benoit, G.; Alexandre, L.; Bernabe, J. *Int. J. Impot. Res.* **2001**, *13*, 110; (b) Melis, M. A.; Argiolas, A. *Neurosci. Biobehav. Rev.* **1995**, *19*, 19; (c) Rosen, R. C.; Ashton, A. K. *Arch. Sex. Behav.* **1993**, *22*, 521.
2. Dula, E.; Bukofzer, S.; Perdok, R.; George, M. *Eur. Urol.* **2001**, *39*, 558.
3. MacLennan, K. M.; Boshier, A.; Wilton, L. V.; Shakir, S. A. W. *Brit. J. Urol. Int.* **2006**, *98*, 125.
4. Montorsi, F. *Int. J. Impot. Res.* **2003**, *15*, S7.
5. Van der Graaf, P. H.; Wayman, C. P.; Baxter, A. D.; Cook, A. S.; Wong, S. K. *Chem. Abstr.* **2003**, *139*, 63348, WO 03/051370, 2003.
6. Boeckler, F.; Gmeiner, P. *Pharmacol. Therapeut.* **2006**, *112*, 281.
7. Cannon, J. G. Progress in Drug Research. In *Chapter 9: Dopamine Agonists: Structure–activity Relationships*; Jucker, E., Ed.; Birkhauser Verlag: Basel, 1985; vol. 29, p 381.
8. Van Vliet, L. A.; Rodenhuis, N.; Dijkstra, D.; Wikstrom, H.; Pugsley, T. A.; Serpa, K. A.; Meltzer, L. T.; Heffner, T. G.; Wise, L. D.; Lijiness, M. E.; Huff, R. M.; Svensson, K. *J. Med. Chem.* **2000**, *43*, 2871.
9. Allerton, C. M. N.; Baxter, A. D.; Cook, A. S.; Hepworth, D.; Wong, S. K. *Chem. Abstr.* **2004**, *141*, 71567, WO 04/052372, 2004.
10. The enantiomers were separated by chiral column chromatography at room temperature (Chiralpak AD250, 20 mm column) eluting with hexane: isopropyl alcohol: diethylamine (70:30:0.05) to give ***R***(-)-3-(4-propylmorpholin-2-yl)phenol (**26**) (e.e. >99.5%) followed by ***S***(**+**)- 3-(4-propylmorpholin-2-yl)phenol (**27**) (e.e. >99%).
11. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited at the Cambridge Crystallographic Data Centre for small molecules and allocated the deposition number CCDC 661368. Copies of data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK.
12. Yin, H.; Bennet, G.; Jones, J. P. *Chem-Biol. Interact.* **1994**, *90*, 47.
13. Soffers, A. E. M. F.; Veeger, C.; Rietjens, I. M. C. M. *Xenobiotica* **1994**, *24*, 759.
14. The enantiomers were separated by chiral column chromatography at room temperature (Chiralpak AD250, 20 mm column) eluting with hexane: isopropyl alcohol (90:10) to give ***R***-(**+**)-2-Fluoro-5-(4-propylmorpholin-2-yl)phenol (**44**) (e.e. >99.5%) followed by ***S***-(−)-2-Fluoro-5-(4-propylmorpholin-2-yl)phenol (**45**) (e.e. >99%).
15. *The Pharmacological Basis of Therapeutics* (IX edition), Gilman, A. Ed.; McGraw-Hill, **1996**, 1785.
16. *The Pharmacological Basis of Therapeutics*, (X edition), Gilman, A. Ed.; McGraw-Hill, **2001**, 1994.
17. The enantiomers were separated by chiral column chromatography at room temperature (Chiralpak AD250, 20 mm column) eluting with hexane: isopropyl alcohol

(80:20) to give ***R***-5-(4-propylmorpholin-2-yl)benzene-1,3-diol (**46**) (e.e. = 99%) followed by ***S***-5-(4-propylmorpholin-2-yl)benzene-1,3-diol (**47**) (e.e. = 96%).
18. Sam, E.; Jeanjean, A. P.; Maloteaux, J. M.; Verbeke, N. *Eur. J. Drug Metab. Ph.* **1995**, *20*, 27.
19. Arora, P.; Garg, S.; Sharma, S. *Drug Discov. Today* **2002**, *7*, 967.
20. Behl, C. R.; Pimplaskar, H. J.; Sileno, A. P.; DeMeireles, J.; Romeo, V. D. *Adv. Drug Delivery Rev.* **1998**, *29*, 89.
21. The pharmacokinetics of compound **26** were determined following single intravenous and intranasal doses to male rats and to male ($n = 2$) and female ($n = 2$) dogs. Compound **26** was freshly prepared in saline solution. In rat, the intravenous dose was administered as a bolus dose of 1 mg/kg. The intranasal dose was administered via a Gilson pipette (20 μl) into one nostril to give a final dose of 0.5 and 2 mg/kg. In dog, the intravenous dose was administered as an intravenous infusion over a 10-min period at a rate of 3 ml/kg/h of a 0.2 mg/ml solution, to give a final dose of 0.1 mg/kg. The intranasal dose was administered via a Pfeiffer device (100 μl) into one nostril to give a final dose of 0.1 and 0.3 mg/kg.