Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 916–920
Bioorganic & Medicinal Chemistry Letters

1,5-Biaryl pyrrole derivatives as EP_1 receptor antagonists. Structure–activity relationships of 6-substituted and 5,6-disubstituted benzoic acid derivatives

Adrian Hall,* Susan H. Brown, Iain P. Chessell, Anita Chowdhury, Nicholas M. Clayton, Tanya Coleman,† Gerard M. P. Giblin, Beverley Hammond, Mark P. Healy, Matthew R. Johnson, Ann Metcalf, Anton D. Michel, Alan Naylor, Riccardo Novelli, David J. Spalding, Jennifer Sweeting and Lisa Winyard

Neurology and Gastrointestinal Centre of Excellence for Drug Discovery, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK

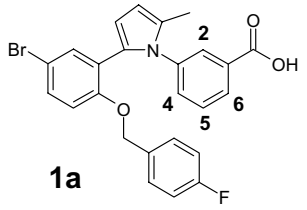
Received 26 October 2006; revised 17 November 2006; accepted 18 November 2006
Available online 22 November 2006

Abstract—Herein we describe the SAR of 1,5-biaryl pyrrole derivatives, with substituents in the 6-position of the benzoic acid moiety, as EP_1 receptor antagonists. Substitution at this position was well tolerated and led to the identification of several analogues with high affinity for the EP_1 receptor that displayed good efficacy in the established FCA model of inflammatory pain. Furthermore, several analogues were prepared which combined substitution at the 5- and 6-positions as well as derivatives with an aromatic ring fused to the 5- and 6-positions.

The EP_1 receptor is one of four 7-transmembrane receptor subtypes, known as $EP_{1\text{-}4}$,[1] that is activated by prostaglandin E_2 (PGE_2). The importance of PGE_2 as an inflammatory mediator has been demonstrated by the correlation between PGE_2 blood concentration and analgesia for cyclooxygenase (COX) inhibitors.[2] Evidence that the pro-inflammatory actions of PGE_2 are mediated by the EP_1 receptor has been provided by studies with knock-out (KO) mice and tool compounds. KO mice have implicated the EP_1 receptor subtype in the sensation of PGE_2-mediated inflammatory pain.[3] In conjunction with this, EP_1 receptor antagonists have shown efficacy in preclinical models of postoperative pain,[4] neuropathic pain[5] and allodynia.[6] We have previously shown that EP_1 antagonists are efficacious in preclinical models of inflammatory pain.[7]

There have been several reports of EP_1 antagonists in the recent literature.[8] We disclosed the structure–activity relationships (SAR), in vivo rat pharmacokinetic data and preclinical efficacy data of a series of 1,5-biaryl pyrrole EP_1 receptor antagonists, such as **1a** (Fig. 1).[7]

Compound **1a**[7] showed good in vitro affinity at the recombinant human EP_1 receptor with a pIC_{50} of 8.2 in a [^{3}H-]PGE_2 binding assay in CHO cell membranes.[7,8] Rat in vivo pharmacokinetic data showed **1a** to have moderate blood clearance (CL_b = 42 mL/min/kg) whilst data from the established complete Freund's adjuvant (CFA or FCA) model of inflammatory pain[7] showed **1a** to have an ED_{50} of 9.2 mg/kg when dosed orally to rats.



EP_1 binding pIC_{50} = 8.2
EP_1 pA_2 9.1

rat pharmacokinetics
CLb = 42 mL/min/kg
Vss = 0.7 L/kg

established FCA ED_{50} 9.2 mg/kg

Figure 1. Profile of lead compound **1a**.

Keywords: EP_1 antagonist; Pyrrole; Pain; Established FCA.
* Corresponding author. Tel.: +44 1279 643464; e-mail: adrian.2.hall@gsk.com
† Present address: AstraZeneca, Mereside, Alderley Park, Macclesfield, Cheshire SK11 4TG, UK.

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.11.059

Recently, we described the SAR of 4- and 5-substituted benzoic acid derivatives.[9]

We now describe further SAR from this series where substitution of the benzoic acid moiety at the 6-position was investigated. Furthermore, we describe the SAR for some 5,6-disubstituted and fused analogues. This programme of work was directed at finding an analogue of **1a** which had improved pharmacokinetics and improved in vivo efficacy. We were also interested in assessing whether these changes would impact in vitro affinity for the EP_1 receptor.

Compound affinities were determined using recombinant human EP_1 receptor stably expressed in CHO cell membranes.[8,10b]

Initial results demonstrated that installation of a fluorine atom at the 6-position, **1b**, led to a moderate improvement in affinity, Table 1. Fixing the 6-fluorobenzoic acid on the right hand side and exploring alternative X-groups on the left hand side phenyl ring revealed that Br and CF_3 were generally better than Me or F, **1b**–**f**. Returning to the 6-position of the benzoic acid, replacement of the fluorine atom by chlorine had little effect on activity, **1g** versus **1b**, Table 1.

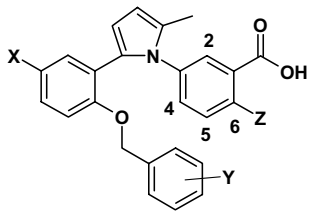
It was found that the electron-withdrawing halogen group in the 6-position could be replaced by various groups such as Me, OH, OMe, $OCHF_2$ and NHAc, **1h**–**p**. Addition of these groups led to the identification of several analogues with exceptionally high affinity, such as **1l**, **1o** and **1p**. Comparison of **1i** with **1j** revealed that Cl and Br (X-group on the left hand side) could be interchanged with minimal effect on affinity.

The benzyl substitution (Y-group) effect is well illustrated by the stepwise increase in affinity moving from **1m** to **1n** to **1o**.

Having identified several groups in the 6-position that increased in vitro affinity, we were eager to combine one of these substituents with the NH_2 group in the 5-position which we had previously found to increase in vitro affinity.[9] Previously we had found that when an NH_2 group was introduced to the 5-position of the benzoic acid (X = Br, Y = H), the pIC_{50} value increased from 8.1[7,8] to 8.5.[9] Hence, derivatives **2a**–**f** were prepared, where the 5-amino group was incorporated with the 6-methyl group, Table 2. The selection of the methyl group in the 6-position was based mainly on the availability of starting material, see later (Scheme 2). Although the in vitro affinity was good, it was not as high as might be anticipated and the addition of a second substituent did not give an additive rise in affinity, although no direct comparisons of **1h**–**j** were made. Again, affinity was lowest when X was Me, compound **2f**, Table 2.

As an alternative approach to combine substitution at the 5- and 6-position we decided to investigate the activity of fused systems such as the naphthyl derivatives, **3a**–**f**, Table 3. As the results show, the additional phenyl ring was well tolerated. As previously observed the 2,4-difluorobenzyl group yielded the most active compounds, whilst bromine was the best X-group and methyl the least active.

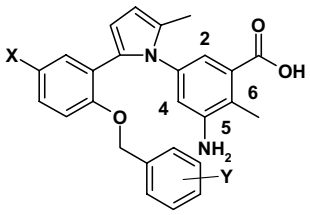
Table 1. In vitro EP_1 binding data for 6-substituted derivatives **1a**–**p**



Compound	X	Y	Z	Binding pIC_{50}[a]
1a	Br	4-F	H	8.2 ± 0.1
1b	Br	4-F	F	8.9 ± 0.2
1c	CF_3	H	F	8.7 ± 0.1
1d	CF_3	4-F	F	8.1 ± 0.2
1e	Me	4-F	F	7.8 ± 0.0
1f	F	4-F	F	7.9 ± 0.1
1g	Br	4-F	Cl	8.6 ± 0.1
1h	Cl	4-F	Me	8.3 ± 0.1
1i	Cl	2,4-DiF	Me	8.6 ± 0.1
1j	Br	2,4-DiF	Me	8.9 ± 0.1
1k	Br	2,4-DiF	OH	8.7 ± 0.1
1l	Br	2,4-DiF	OMe	9.4 ± 0.0
1m	Br	H	$OCHF_2$	8.3 ± 0.2
1n	Br	4-F	$OCHF_2$	8.7 ± 0.2
1o	Br	2,4-DiF	$OCHF_2$	9.2 ± 0.1
1p	Br	2,4-DiF	NHAc	9.4 ± 0.1

[a] Mean of at least three experiments ± standard deviation.

Table 2. In vitro EP_1 binding data for 5,6-disubstituted derivatives **2a–f**



Compound	X	Y	Binding pIC_{50}[a]
2a	Cl	H	8.7 ± 0.2
2b	Br	H	8.5 ± 0.1
2c	CF_3	H	8.4 ± 0.1
2d	Br	4-F	9.1 ± 0.1
2e	CF_3	4-F	8.4 ± 0.1
2f	Me	4-F	7.8 ± 0.1

[a] Mean of at least three experiments ± standard deviation.

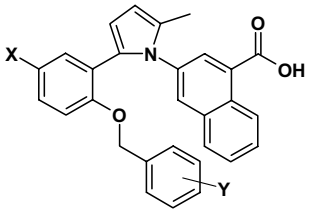
Due to the activity of the naphthyl derivatives, we investigated other fused ring systems, especially those that might mimic the hydrogen bond donating (HBD) ability of the acetamide group in the 5-position which we had previously discovered to impart high affinity.[9] Hence, the indole-4-carboxylic acid **4** was prepared in an attempt to increase in vitro affinity. Pleasingly, this compound showed excellent activity with a pIC_{50} value of 8.9, Figure 2. It is also worth noting that this level of activity was attained without the preferred substitution pattern on the left hand side of the molecule.

Based on a balance of in vitro affinity at the EP_1 receptor, minimal CYP450 interactions and in vitro metabolic stability, several compounds were profiled in a rat pharmacokinetic assay to investigate their oral exposure, Table 4.

Substitution of the 6-position generally resulted in good exposure, **1c** and **1h**, defined as a dose-normalized area under the curve (AUC) $\geqslant$10 min kg/L, with the exception of the methoxy derivative **1l**. The 5,6-disubstituted analogues **2a**, **2c** and **2d** displayed the highest exposure, whereas the naphthoic acids had considerably lower exposure.

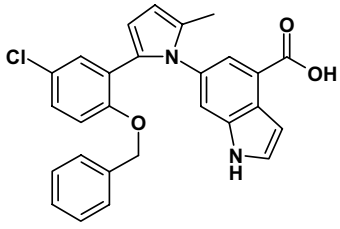
Compounds with acceptable oral exposure were assessed in the established FCA model of inflammatory pain.[7] Compounds were either assayed at a single dose of 5 mg/kg or in dose–response format, Table 5. The 6-methyl derivative **1h** showed considerably better efficacy than the 6-F derivative **1c**. Disappointingly, the disubstituted derivatives **2a**, **2c** and **2d** showed considerably less efficacy despite the excellent exposure shown. On the basis of these results, **1h** (GW855454X) was profiled in a dose–response assay, and showed improved efficacy relative to **1a**, with an ED_{50} of 2.5–4.9 mg/kg,[11] Table 5.

Table 3. In vitro EP_1 binding data for naphthoic acid derivatives **3a–f**



Compound	X	Y	Binding pIC_{50}[a]
3a	Cl	H	8.2 ± 0.1
3b	Br	H	7.9 ± 0.3
3c	Br	4-F	8.3 ± 0.2
3d	Br	2,4-DiF	8.6 ± 0.2
3e	Me	4-F	7.1 ± 0.1
3f	F	4-F	7.6 ± 0.2

[a] Mean of at least three experiments ± standard deviation.



4: binding pIC_{50} 8.9 (0.1)[a]

Figure 2. In vitro EP_1 binding data for compound **4**. [a]Mean of at least three experiments, standard deviation in parentheses.

Table 4. In vivo rat pharmacokinetic data for selected compounds[a]

Compound	AUC (0–*t*)/dose (min kg/L)	T_{max} (h)	C_{max} (μM)
1c	27 ± 8	0.6	0.87 ± 0.03
1h	18 ± 9	0.5	0.59 ± 0.18
1l	4 ± 1	0.5	0.34 ± 0.17
2a	39 ± 9	0.5	2.94 ± 1.16
2c	31 ± 3	0.5	0.91 ± 0.10
2d	52 ± 14	1.1	1.35 ± 0.32
3a	9 ± 3	4.0	0.22 ± 0.11
3c	9 ± 3	0.5	0.52 ± 0.25

[a] Mean of three experiments. Compounds administered orally in 1% aqueous methylcellulose at 3 mg/kg.

Table 5. In vivo rat pain data from FCA model of inflammatory pain[a]

Compound	% reversal of hypersensitivity at 5 mg/kg[a] (%)	ED_{50}[a,b] (mg/kg)
1c	56	n/t
1h	75	2.5–4.9[c]
2a	35	n/t
2c	40	n/t
2d	50	n/t

[a] Compounds administered orally in 1% aqueous methyl cellulose at doses of 1, 3 and 10 mg/kg or 3, 10 and 30 mg/kg or at a single dose of 5 mg/kg. Pain readout taken 1 h post-dose of test compound.
[b] n/t = not tested.
[c] See Ref. 11.

Due to the excellent efficacy of compound **1h** its pharmacokinetic profile was investigated in a rat iv-po crossover study, detailed in Table 6. In vitro, **1h** showed good metabolic stability with intrinsic clearance (CL_i) values $\leqslant$3.6 mL/min/g liver in rat, dog, monkey and human liver microsomes. Compound **1h** showed improved in vivo metabolic stability relative to **1a** with a large volume of distribution which is unusual for a carboxylic acid. The combination of the large volume of distribution and low clearance results in a half-life of 4.6 h.

Based on these data, the effects of **1h** were studied in a time-course (PK-PD) experiment over a 24 h time period using a dose of 30 mg/kg in the established FCA model of inflammatory pain. Results from this study are shown in Figure 3 and Table 7. As the results show, **1h** showed good reversal of the mechanical hyperalgesia induced by FCA at the 1 and 2 h time points. This effect correlates with peak blood and brain concentrations at the 1 h time point. Furthermore, the analgesia is maintained for approximately 8 h, at which point there are still considerable drug concentrations in both tissues, Figure 3 and Table 7. From these data the estimated blood EC_{50} is 3.5 μM at the 4 h time point.

Table 6. In vivo rat pharmacokinetic data for compound **1h**[a]

Compound	CL_b (mL/min/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	F_{po}
1h	22 ± 4	2.6 ± 0.9	4.6 ± 3.2	45%

[a] Compound administered intravenously in 5% (w/v) glucose containing 2% DMSO (v/v) at 1 mg/kg and orally in 1% aqueous methylcellulose at 3 mg/kg. Data are mean values of three experiments.

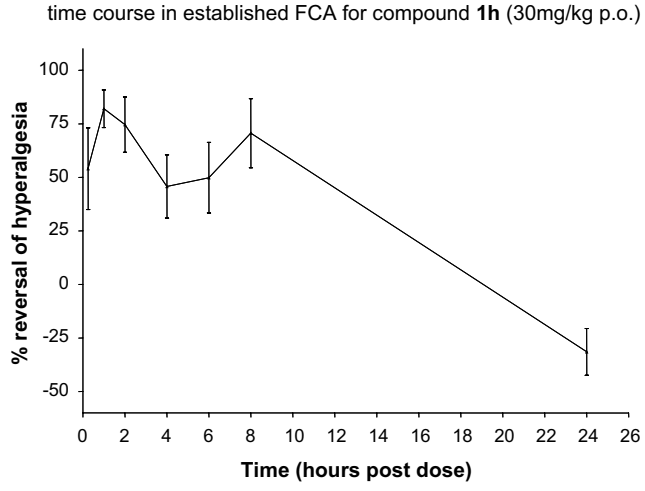


Figure 3. PK-PD data for **1h** in established FCA at 30 mg/kg (po).

Table 7. Rat blood and brain levels of compound **1h** (GW855454) from FCA study[a]

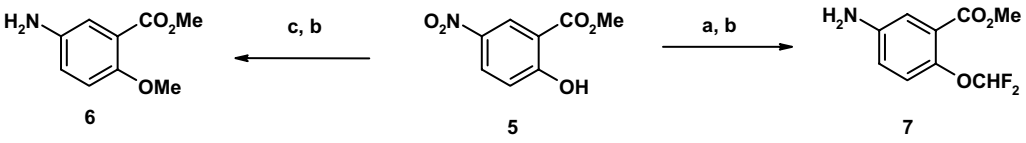
Time (h)	Blood concn[b] (μM)	Brain concn[c] (μM)	Br:Bl ratio[d]
0.25	7.358 ± 1.842	2.598 ± 0.441	0.4
1	10.089 ± 2.598	3.564 ± 0.669	0.4
2	5.930 ± 2.304	2.484 ± 0.516	0.4
4	3.561 ± 1.285	1.221 ± 0.618	0.4
6	2.309 ± 0.682	0.913 ± 0.282	0.4
8	1.600 ± 0.322	0.753 ± 0.080	0.4
24	0.037 ± 0.020	0.031[e]	0.5[e]

[a] Compound dosed orally in 1% aqueous methylcellulose at 30 mg/kg.
[b] Mean of seven data points.
[c] Mean of three data points.
[d] Br:Bl ratios calculated by dividing individual brain concentrations by the corresponding blood concentration for the same animal. Mean of three data points.
[e] Single data point.

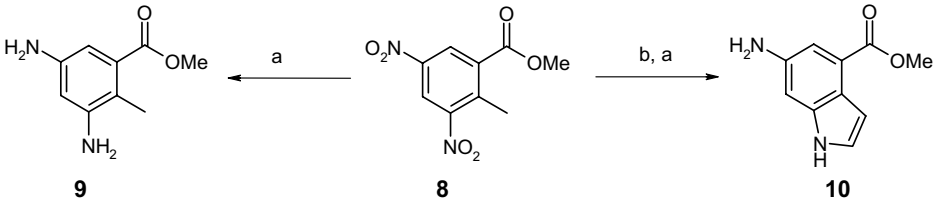
The in vitro selectivity profile of **1h** was ascertained at related prostanoid receptors (EP_2, EP_3, EP_4, IP, FP and TP). Functional antagonism at the EP_1, EP_3 and TP receptors was ascertained using a calcium mobilisation assay (FLIPR, recombinant human receptor expressed in CHO cells). In these assays **1h** demonstrated a pK_i of 9.0 ± 1.1 ($n = 2$) at the EP_1 receptor and a pK_i of 6.3 ± 0.0 ($n = 4$) at the EP_3 receptor. At the TP receptor **1h** showed a pIC_{50} of 7.4 and a pK_i of 7.8. No agonist activity was observed at the TP receptor. Compound **1h** showed no activity up to a concentration of 1 μM at the EP_2 or IP receptors and no activity at the FP receptor ($pIC_{50} < 5.5$). At the EP_4 receptor, **1h** showed weak activity with a pIC_{50} of 4.8 and pK_i of 5.1 in a binding assay and a pK_i of 5.2 in a cAMP mobilisation assay. No significant activity (<25%) was seen at a battery of 50 targets (Cerep) at a concentration of 1 μM.[12] Thus, **1h** shows excellent selectivity over a range of related and unrelated targets with the exception of the TP receptor.

The data for **1h** (GW855454X) thus support its use as a tool compound to investigate the in vivo effects of EP_1 receptor antagonism. In addition, despite being a carboxylic acid, **1h** shows considerable penetration into the central nervous system (CNS) as evidenced by the brain concentrations from the time-course study. Thus, **1h** may prove useful in elucidating the CNS effects of EP_1 receptor antagonism.

Compounds were prepared by Paal-Knorr condensation of the requisite 1,4-diketone with an appropriately functionalized aniline as described previously.[7] Full experimental procedures and characterizing data have been disclosed.[10] The anilines were generally commercially available or were prepared by literature procedures. Conveniently, if the aniline was not available it could



Scheme 1. Reagents and conditions: (a) $ClCF_2CO_2Na$, Na_2CO_3, DMF, 100 °C; (b) H_2, Pd/C, EtOH; (c) NaH, MeI, DMF.



Scheme 2. Reagents and conditions: (a) H_2, Pd/C, MeOH; (b) DMF–DMA, DMF, 60 °C, μwave, 15 min.

be prepared by reduction of the analogous nitro derivative.

Methyl 5-amino-2-(methyloxy)benzoate **6** and methyl 5-amino-2-[(difluoromethyl)oxy]benzoate **7** were prepared from **5** as detailed in Scheme 1. Paal-Knorr condensation[7] with the appropriate 1,4-diketone followed by ester hydrolysis yielded derivatives **1m**–**o**. The condensation reaction was conducted under thermal (PhMe, pTSA, reflux) or microwave (NMP, pTSA, 150 °C, 10 min) conditions.

Methyl 3,5-diamino-2-methylbenzoate **9** was prepared from **8** as detailed in Scheme 2. Paal-Knorr condensation took place selectively with the less hindered 5-amino moiety, to give derivatives **2a**–**f**, upon basic hydrolysis of the ester group. Methyl 6-amino-1*H*-indole-4-carboxylate **10** was prepared as described in Scheme 2 and used without purification to prepare compound **4**.

In conclusion, substitution of the benzoic acid moiety of the 1,5-biaryl pyrrole template led to the identification of compounds with sub-nanomolar affinity for the EP_1 receptor. Several analogues showed good oral exposure and efficacy in a preclinical model of inflammatory pain. Compound **1h** (GW855454X) demonstrated good metabolic stability, good bioavailability and excellent efficacy in established FCA model of inflammatory pain with an ED_{50} of 2.5–4.9 mg/kg. These data support the use of **1h** as a tool compound to elucidate the in vivo effects of EP_1 receptor antagonism.

Acknowledgments

The authors thank Emma Ward for generating the EP_1 and EP_3 FLIPR data and Davina Mitchell for generating the FP data.

References and notes

1. (a) Coleman, R. A.; Smith, W. L.; Narumiya, S. *Pharmacol. Rev.* **1994**, *46*, 205; (b) Breyer, R. M.; Bagdassarian, C. K.; Myers, S. A.; Breyer, M. D. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 661; (c) Narumiya, S.; Sugimoto, Y.; Ushikubi, F. *Physiol. Rev.* **1999**, *79*, 1193; (d) Coleman, R. A., Kennedy, I., Humphrey, P. P. A., Bunce, K., Lumley, P., Eds.; *Comprehensive Medicinal Chemistry*; 1990, Vol. 3, Pergamon: Oxford, UK, p 643.
2. Huntjens, D. R. H.; Danhof, M.; Della Pasqua, O. E. *Rheumatology* **2005**, *44*, 846.
3. Stock, L. S.; Shinjo, K.; Burkhardt, J.; Roach, M.; Taniguchi, K.; Ishikawa, T.; Kim, H.-S.; Flannery, P. J.; Coffman, T. M.; McNeish, J. D.; Audoly, L. P. *J. Clin. Invest.* **2001**, *107*, 325.
4. (a) Omote, K.; Kawamata, T.; Nakayama, Y.; Kawamata, M.; Hazama, K.; Namiki, A. *Anesth. Analg.* **2001**, *92*, 233; (b) Omote, K.; Yamamoto, H.; Kawamata, T.; Nakayama, Y.; Namiki, A. *Anesth. Analg.* **2002**, *95*, 1708.
5. Kawahara, H.; Sakamoto, T.; Takeda, S.; Onodera, H.; Imaki, J.; Ogawa, R. *Anesth. Analg.* **2001**, *93*, 1012.
6. Maruyama, T.; Koketsu, M.; Yaamamoto, H.; Yamamoto, K.; Yamamoto, L. T.; Hayashida, K.-i.; Ohchida, S.; Kondo, K. *Prostaglandins and Other Lipid Mediators* **1999**, *59*, 217.
7. Hall, A.; Atkinson, S.; Brown, S. H.; Chessell, I. P.; Chowdhury, A.; Clayton, N. M.; Coleman, T.; Giblin, G. M. P.; Gleave, R. J.; Hammond, B.; Healy, M. P.; Johnson, M. J.; Michel, A. D.; Naylor, A.; Novelli, R.; Spalding, D. J.; Tang, S. P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3657.
8. Hall, A.; Bit, R. A.; Brown, S. H.; Chaignot, H. M.; Chessell, I. P.; Coleman, T.; Giblin, G. M. P.; Hurst, D. N.; Kilford, I. R.; Lewell, X. Q.; Michel, A. D.; Mohamed, S.; Naylor, A.; Novelli, R.; Skinner, L.; Spalding, D. J.; Tang, S. P.; Wilson, R. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2666, and references therein.
9. Hall, A.; Brown, S. H.; Chessell, I. P.; Chowdhury, A.; Clayton, N. M.; Coleman, T.; Giblin, G. M. P.; Hammond, D.; Healy, M. P.; Johnson, M. R.; Metcalf, A.; Michel, A. D.; Naylor, A.; Novelli, R.; Spalding, D. J.; Sweeting, J. *Bioorg. Med. Chem. Lett.* **2006**, *16*. doi:10.1016/j.bmcl.2006.10.078.
10. (a) Giblin, G. M. P.; Hall, A.; Healy, M. P.; Lewell, X. Q.; Miller, N. D.; Novelli, R. WO2003/101959A1.; (b) Giblin, G. M. P.; Hall, A.; Healy, M. P.; Lewell, X. Q.; Miller, N. D.; Novelli, R.; King, F. D.; Naylor, A. WO2005/054191A1.
11. ED_{50} = 2.5 mg/kg when dose–response assay carried out at doses of 1, 3 and 10 mg/kg, ED_{50} = 4.9 mg/kg when dose–response assay carried out at 3, 10 and 30 mg/kg.
12. www.cerep.fr.