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Discovery of Selective Nonpeptidergic Neuropeptide FF2 Receptor Agonists

Gilles Gaubert,[†] Fabio Bertozzi,[†] Nicholas M. Kelly,[†] Jan Pawlas,[†] Audra L. Scully,[‡] Norman R. Nash,[‡] Luis R. Gardell,[‡] Jelveh Lameh,[‡] and Roger Olsson*,[†]

[†]*ACADIA Pharmaceuticals AB, Medeon Science Park, S-205 12 Malmo, Sweden, and* [‡]*ACADIA Pharmaceuticals Inc., 3911 Sorrento Valley Boulevard, San Diego, California 92121*

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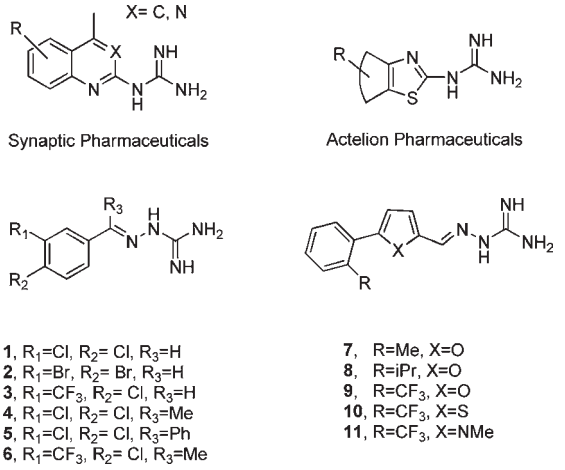
Abstract: We report the discovery and initial characterization of a novel class of selective NPFF2 agonists. HTS screening using R-SAT, a whole cell based functional assay, identified a class of aryliminoguanidines as NPFF1 and NPFF2 ligands. Subsequent optimization led to molecules exhibiting selective NPFF2 agonistic activity. Systemic administration showed that selective NPFF2 agonists (**1** and **3**) are active in various pain models in vivo, whereas administration of a nonselective NPFF1 and NPFF2 agonist (**9**) increases sensitivity to noxious and non-noxious stimuli.

The octapeptide neuropeptide FF (NPFF,[a] FLFQPQRF-NH2) is generally considered to be a pain modulating peptide,[1] but it also displays potent effects on the cardiovascular system.[2] Its effects on nociception are believed to be mediated in the spinal cord and brain, while its cardiovascular effects may be centrally or peripherally mediated.[2] These distinct actions are believed to be mediated via interaction with two specific G-protein-coupled receptors (GPCRs), NPFF1 and NPFF2, which are present in the central nervous system (CNS) and peripherally.[3] In the CNS, NPFF2s have been found in the most external layers of the dorsal horn of the spinal cord, the parafascicular thalamic nucleus, laterodorsal thalamic nucleus, and presubiculum of the hippocampus, whereas NPFF1s have been detected in septal, thalamic, and hypothalamic areas but not in the spinal cord.[4] Hence, identifying selective agonists and antagonists of NPFF1 and NPFF2 would not only be useful in elucidating these receptors specific physiological roles but also provide leads for therapeutically interesting molecules. In addition, nonpeptidergic, metabolically stable ligands would be preferable as tool compounds, compared to peptides based on the natural ligand NPFF, because they would not be subject to peptidolytic degradation, which could have been the cause of the transient effects previously observed of NPFF.[2]

To date, small nonpeptidergic ligands for NPFF1 and NPFF2 have been primarily described in patents. Notably, substituted quinolino- or quinazolinoguanidines that interact agonistically or antagonistically with both receptors or antagonistically with NPFF1 and agonistically with NPFF2 were reported in a 2003 patent (Chart 1).[5] In another patent, published a year later, thiazoleguanidine derivatives were described as NPFF1 antagonists, but no data regarding their NPFF2 activity were provided (Chart 1).[6]

Chart 1



Here, we report the discovery of selective NPFF2 agonists and initial characterization of their structure−activity relationships (SARs) and in vivo effects in pain models.

A chemical library containing 250 000 small druglike molecules was screened for agonist activity using receptor selection and amplification technology (R-SAT) assays. R-SAT is a functional cell-based assay that allows one to monitor receptor-dependent proliferative responses of various receptor classes, including GPCRs and nuclear receptors.[7]

Aryliminoguanidines (compounds **1**−**11**, Chart 1) were prepared in moderate to excellent yields (48−95%) by reacting the corresponding aldehydes or ketones with aminoguanidine hydrochloride in ethanol under microwave irradiation in a sealed vial (130 °C, 12 min, pressure increased up to 4 bar) or conventional heating (70−90 °C, 18 h) (Scheme 1). With conventional heating, the reactions involving ketones required slightly higher temperatures (90 °C, 18 h). Commercially available aldehydes and ketones were used in the reactions except for aldehydes **12**−**15**, required for the preparation of **7**−**11** (Scheme 2). Aryliodide **16** was synthesized following an aromatic Finkelstein methodology as reported by Klapars et al.[8] from the bromide analogue. A combination of copper iodide, sodium iodide, and ligand **L1** was used in the reaction, which afforded an excellent yield of 95%. Aldehydes **12**−**15** were prepared using a modified Heck cross-coupling procedure based largely on the method published by McClure et al.[9] with different substrates. In our hands, for our specific substrates, the air stable ligand **L2** (i.e., cyclohexyl JohnPhos)[10] proved to be a superior catalyst than the oxidation-prone tricyclohexylphosphine used in the original report. The yields of the coupling reactions were moderate (45−55%). Notably, aldehyde **15** could not be purified completely (it was contaminated with approximately 5% of the starting aromatic iodide) and was used in the last step without further purification.

Compounds **1**−**11** are a representative set of the most active compounds selected from approximately 500 compounds synthesized throughout the project. These compounds were

*To whom correspondence should be addressed. Phone: +46406013400. Fax: +46406013401. E-mail: roger@acadia-pharm.com.

[a] Abbreviations: CNS, central nervous system; GPCR, G-protein-coupled receptor; ip, intraperitoneal; MPE, maximal possible effect; NPFF, neuropeptide FF; NPFF1, neuropeptide FF1 receptor; NPFF2, neuropeptide FF2 receptor; R-SAT, receptor selection and amplification technology; SAR, structure−activity relationship; SNL, spinal nerve ligation.

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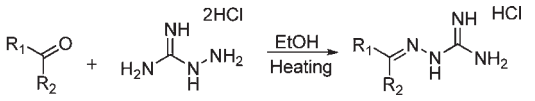
Scheme 1[a]

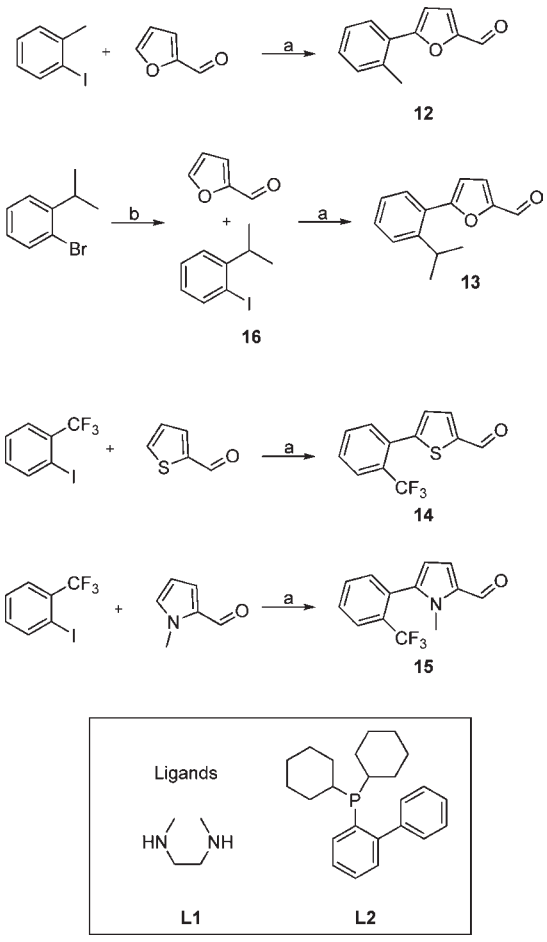


[a] Reaction conditions: microwave irradiation in a sealed vial, 130 °C, 12 min; or conventional heating, 70−90 °C, 18 h.

first tested in functional R-SAT assays, for in vitro agonist and antagonist activities. Dose−response curves were generated by testing the compounds in seven concentrations, each in triplicate, at human NPFF1 and NPFF2 receptors (Table 1). To simplify the SAR characterization, the aryliminoguanidines were divided into two subclasses; substituted phenyliminoguanidines (**1**−**6**) and 5-aryl substituted-five-membered heteroaromatic iminoguanidines (**7**−**11**). In the first subclass (**1**−**6**) the active and NPFF2 selective compounds bore inductive electron withdrawing substituents in the 3 and 4 positions such as chloro, bromo, or trifluoromethyl groups. If there was no substitutents or if alkyl, aryl, amino, or ether substituents were present at these positions, the compounds displayed low partial agonistic activity, a 10-fold lower potency, and lower selectivity toward the NPFF2 receptor. Moreover, the presence of additional substituents at the 2-position, the 6-position, or both was generally detrimental for NPFF2 agonistic activity. For example, guanabenz (Chart 2), a drug used for treating hypertension, showed no activity at NPFF2 or NPFF1. Guanabenz is described as an α2-selective adrenergic agonist; therefore, **1** was tested at all the α-adrenergic receptors, but no crossover activity was observed. Dichloro compound **1** was also N-methylated at the iminoguanidine in all permutations, but all the resulting analogues showed little or no activity. In addition, the 5-HT_4 partial agonist tegaserod (Chart 2), used for treating irritable bowel syndrome, was tested at NPFF2 and was found to be inactive (Table 1). Compounds **4** and **6** (acetophenone derivatives) showed similar potency to the compounds originating from aldehydes **1** and **3** but with slightly less efficacy, especially **3** compared to **6**. However, compounds originating from the aldehyde **1**, acetophenone **4**, and benzophenone **5** showed similar activities at NPFF2, but the phenyl in **5** increased the activity at NPFF1, resulting in **5** being equipotent at NPFF2 and NPFF1. Members of the second subclass of the aryliminoguanidines (**7**−**11**) are 5-aryl substituted-five-membered heteroaromatic iminoguanidines. In general, these compounds exhibited improved NPFF2 agonist properties, but at the cost of selectivity toward NPFF1 (in accordance with the effects of the phenyl group introduced in the benzophenone **5** of the first subclass). Compounds **9**−**11** showed that similar molecules such as furan, thiophene, or *N*-methylpyrrole derivatives had similar activities in R-SAT. Moreover, only compounds with ortho-substitution on the phenyl moiety were the most active compounds (e.g., 5-phenylfuranyliminoguanididine the nonsubstituted analogue of **7** showed >10-fold lower potency), and the most active compounds **8** and **9** have an isopropyl or trifluoromethyl substituent, respectively. These observations imply that the restriction in the conformation could be beneficial for NPFF1 and NPFF2 agonistic activity.

On the basis of the initial R-SAT data, three compounds (**1**, **3**, and **9**) were selected for further evaluation. Compound **3** showed full agonistic activity at human NPFF2 and minimal activity at NPFF1. Compound **1** displayed partial efficacy at NPFF2 and was inactive at NPFF1. Compound **1** was further tested as an antagonist at NPFF1 and was found to be inactive. Biaryl **9** demonstrated full efficacy at NPFF2 and partial efficacy at NPFF1 (Table 1). These results were confirmed in a secondary assay, cAMP inhibition. The selective NPFF2 agonist **3** showed full efficacy (89%) at NPFF2

Scheme 2[a]



[a] Reaction conditions: (a) $PdCl_2$, **L2**, Bu_4NBr, AcOK, DMF, 110 °C, 10 h, 45−55% yield of **12**−**15**; (b) CuI, **L1**, NaI, dioxane, 110 °C, 20 h.

Table 1. In Vitro Activities of NPFF2 Agonists Using R-SAT[a]

	NPFF2		NPFF1	
compd	pEC_{50}	% efficacy	pEC_{50}	% efficacy
1	6.0 ± 0.2	68 ± 10	nd	18 ± 7
2	5.9 ± 0.1	90 ± 15		na
3	6.3 ± 0.2	114 ± 28	< 5.5	46 ± 0
4	6.3 ± 0.1	64 ± 4		na
5	6.1 ± 0.1	63 ± 7	6.1 ± 0.1	46 ± 7
6	6.4 ± 0.2	66 ± 6		na
7	7.0 ± 0.2	90 ± 6	7.1 ± 0.2	55 ± 6
8	6.7 ± 0.1	70 ± 7	6.8 ± 0.1	62 ± 7
9	7.0 ± 0.4	73 ± 13	7.4 ± 0.2	55 ± 6
10	6.8 ± 0.1	55 ± 6	6.9 ± 0.1	44 ± 6
11	6.9 ± 0.1	72 ± 7	6.5 ± 0.1	36 ± 7
guanabenz		na		na
tegaserod		na		na

[a] nd, not determined. na, not active at 10 mM. R-SAT was performed as described in ref 7. Values represent the mean ± SEM of at least three independent experiments.

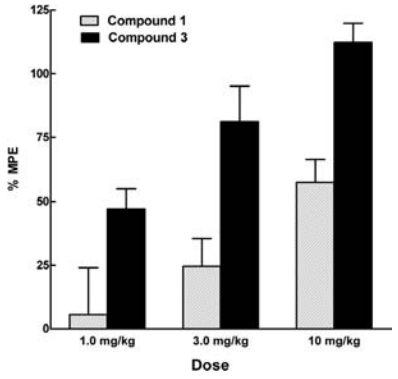


Figure 1. Selective NPFF2 agonists **1** and **3** attenuate carrageenan-induced thermal hypersensitivity, % MPE (% maximum possible effect), ip administration.

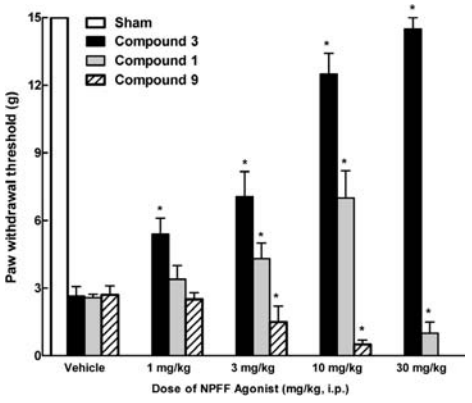
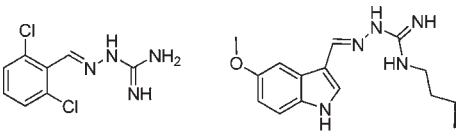


Figure 2. Selective NPFF2 agonists **1** and **3** attenuate and non-selective NPFF2 agonist **9** produces SNL-induced mechanical hypersensitivity.

Chart 2



Guanabenz Tegaserod

and only weak agonist activity (42% at the highest dose) at NPFF1. The nonselective **9** was fully efficacious at both NPFF receptors in cAMP assays, showing 114% and 93% efficacy at NPFF2 and NPFF1, respectively.

The selective NPFF2 agonists **1** and **3** were tested in a rat model of acute inflammatory pain, and both compounds displayed dose dependent inhibition of carrageenan induced thermal hypersensitivity measured using a metal plate maintained at 52 °C in "hot plate test" (Figure 1). The result from the acute inflammatory pain model corroborated the in vitro data; hence, the partial agonist **1** displayed a partial inhibition whereas the full agonist **3** showed maximum response (Figure 1).

Three compounds (**1**, **3**, and **9**) were tested in a rat model of neuropathic pain developed by Kim and Chung[11] (Figure 2). This model requires the ligation of the L_5 and L_6 spinal nerves distal to the dorsal root ganglion. At 10−14 days following spinal nerve ligation (SNL) surgery, the rats were reassessed for their response thresholds to mechanical stimuli with a series of calibrated von Frey filaments. Paw withdrawal thresholds to probing were determined according to a previously described method,[12] and a significant reduction in the paw withdrawal threshold was interpreted as the presence of tactile allodynia. The NPFF2 full agonist **3** completely attenuated SNL-induced hypersensitivity at 30 mg/kg, whereas treatment with the partial agonist **1** yielded an inverted U-shaped dose−response curve, displaying a maximum effect at 10 mg/kg. However, the nonselective NPFF1 and NPFF2 agonist **9** dose dependently potentiated the SNL-induced hypersensitivity in rats (Figure 2). Furthermore, **9** exerted tactile allodynia in sham-operated rats, an effect that was completely blocked by the selective NPFF1 antagonist dansylPQRamide (30 mg/kg, ip administration).

The stability in human and rat liver microsomes was analyzed for **1**. The compound showed moderate to low clearance (less than 30% liver blood flow) in human and rat microsomes, 26 and 18 μL/(min·mg), respectively, indicating that **1** is a stable compound. The in vitro data were corroborated by the result from a pharmacokinetic study, in which $F = 43\%$ and $T_{1/2} = 2.5$ h in rat were obtained for **1**. Moreover, experimentally physicochemical properties of **1** ($pK_a = 8.4$, $\log D = 1.8$, and $\log P = 2.8$) all appeared to be suitable for a potential drug.

In conclusion, we have described the discovery and characterization of the first small molecule nonpeptidic selective NPFF2 agonists. Compounds **1** and **3** showed dose dependent attenuation of hypersensitivity in acute inflammatory and neuropathic pain rat models. In contrast, the NPFF1 and NPFF2 agonist **9** dose-dependently potentiated SNL-induced hypersensitivity and induced tactile allodynia in sham-operated rats. The identification of potent, selective, and metabolically stable small-molecule NPFF2 agonists will provide useful pharmacological tools for elucidating the complex physiological functions of the NPFF receptors.

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Supporting Information Available: Experimental details and characterization data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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