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ROYAL SOCIETY OF CHEMISTRY

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View Article Online
DOI: 10.1039/C7MD00078B

Pyrazolo[3,4-*d*]pyrimidines as Sigma-1 Receptor Ligands for the Treatment of Pain. Part 2: Introduction of Cyclic Substituents in Position 4†

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Electronic Supplementary Information (ESI) available: Analytical and characterization data for all the compounds. See DOI: 10.1039/x0xx00000x

†The authors declare no competing interests.

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DOI: 10.1039/C7MD00078B

Abstract

The replacement of acylamino by cyclic substituents in the 4 position of the pyrazolo[3,4-*d*]pyrimidine scaffold, led to highly active sigma-1 receptor (σ_1R) ligands. Phenyl or pyrazolyl groups were the best in terms of affinity for the σ_1R and the 4-(1-methylpyrazol-5-yl) derivative, **12f**, was the most selective. Compound **12f** is also one of the best σ_1R ligands ever described in terms of lipophilic ligand efficiency, which translates into a good physicochemical and ADMET profile. In addition, **12f** was identified as an antagonist of the σ_1R in view of its potent antinociceptive profile in several pain models in mice.

Key words: sigma-1 receptor, antagonist, pyrazolo[3,4-d]pyrimidine, antinociceptive, pain.

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Introduction

After the discovery of the σ receptor (σR) four decades ago and the subsequent identification of two differentiated subtypes (σ_1 and σ_2),[1] several σ_1R ligands were identified and studied for treating different central nervous system (CNS)-related pathologies, such as schizophrenia and depression.[2] More recently, the σ_1R has been revealed to act as an intracellular chaperone[3] implicated in the modulation of the activity of different molecular targets involved in transmission and amplification (e.g., central sensitization) of nociceptive messages, supporting a role for σ_1R antagonists in the treatment of pain.[4]

However, the finding of σ_1R antagonists with adequate drug-like properties and good absorption, distribution, metabolism, excretion and toxicity (ADMET) profiles is not evident.[5] First, as described in the preceding article,[6] the establishment of the functional behavior of σ_1R ligands is not obvious, due to the unique characteristics of the protein, and often relies on in vivo testing. Second, it is well known that compounds with optimal lipophilicity have increased chances of success in development,[7] but the σ_1R binding site is highly lipophilic,[8] as can be inferred from the crystalline structure recently published[9] and the most accepted pharmacophores, such as Glennon's[10] and Laggner's models.[11] This lies together with the observation that the majority of the described σ_1R ligands are highly lipophilic and challenges the discovery of ligands with optimal drug-like properties. Third, the overlap of the σ_1R requirements with those of other receptor systems (e.g., monoaminergic receptors and transporters) and proteins responsible for off-target effects (e.g., hERG channel), complicates the finding of selective agents. Thus, although designing compounds active on the σ_1R may be relatively simple, it is much more challenging to find selective drug-like compounds.

In the preceding article, the design and synthesis of a series of 4-acylaminopyrazolo[3,4-*d*]pyrimidine derivatives as σ_1R ligands is described.[6] This series was designed based on Glennon pharmacophore and taking into account the structure-activity relationship (SAR) study that led to the selection of E-52862 (**1**), currently in phase II clinical trials for the treatment of different pain conditions..[12] Several 4-acylaminopyrazolo[3,4-*d*]pyrimidine derivatives, represented by **2** (Figure 1), showed interesting in

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vitro and ADMET profiles with the potential drawback of a certain unstability at acidic pH values. Although this would not preclude a potential development of this type of compounds, a search for more stable alternatives was undertaken. In this work, the replacement of the 4-acylamino group by cyclic substituents is described. This modification led to stable and highly active σ_1R ligands, showing as well good physicochemical and ADMET profiles.

Results and Discussion

Chemistry

The synthesis of the final compounds was effected from the commercially available 4-chloro (**3**) and 4-amino (**6**) pyrazolo[3,4-*d*]pyrimidine derivatives (Scheme 1).[13] Reaction of **3** with 1-(2-hydroxyethyl)piperidine (**4**) under Mitsunobu conditions afforded the 1-alkylated chloropyrimidine **5** in good yield, while reaction of **6** with 1-(2-chloroethyl)piperidine (**7**) provided **8**. As reported in the preceding article for the 4-acylaminopyrazolo[3,4-*d*]pyrimidine derivatives, the N^1-sustituted pyrazole was the major regioisomer.[6]

Derivatives **9**, in which the heterocycle is linked to the central scaffold by a nitrogen atom (Table 1), were prepared from **5** or **8** using different conditions depending on the type of reagent used. Thus, the saturated derivatives **9a-e** were prepared by nucleophilic substitution of **5** with the corresponding amines under mild basic conditions (Method A), the pyrrolidone **9f** was prepared from **5** using Buchwald-Hartwig conditions (Method B), the imidazole (**9g**) and benzimidazole (**9h**) derivatives were obtained via nucleophilic substitution using a strong base (Bu^tOK, Method C) and finally, the dimethylpyrrole **9i** was prepared by reaction of **8** with hexane-2,5-dione (Method D).

Derivatives in which the cyclic group is linked to the central scaffold by a carbon atom (Tables 2-4) were prepared from **5** using arylboronic derivatives (acids or esters, Method E, **10**, **11a-g**, **12a-k**) or aryltributylstannanes (Method F, **11h-j**, **12l-m**) under palladium catalysis and microwave irradiation. Suzuki or Stille coupling were selected depending on availability and price of the required reagents and

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afforded the desired compounds with moderate to good yields, which decreased on steric hindrance increase of the metalated reagents. General conditions used are depicted in Scheme 1, but in the case of **12f** the more reactive catalyst $PdCl_2(dppf)\cdot CH_2Cl_2$ was used for the coupling, as described in the experimental part.

SAR

In the previous article,[6] where the structure-activity relationship (SAR) study of 4-acylamino pyrazolo[3,4-*d*]pyrimidine derivatives is described, the 2-piperidinylethyl substituent was shown to be preferred in position 1. Thus, this group was selected for exploring the replacement of the amide moiety in position 4 by cyclic derivatives, a change expected to improve the chemical stability of **2** at acidic pH values.

All the compounds synthesized were evaluated in primary σ_1R binding assay using [^{3}H]-(+)-pentazocine[14] as radioligand (K_i values are given in Tables 1-3). Compounds having a K_i for the σ_1R lower than 500 nM were evaluated against the σ_2R at 1 μM using [^{3}H]-di-*o*-tolylguanidine[15] as radioligand. As discussed previously,[6] selectivity for the σ_1R vs the σ_2R is desired in view of the differentiated pharmacological properties of the two receptors.

The SAR study was initiated by the introduction of heterocycles linked to the pyrazolo[3,4-*d*]pyrimidine scaffold by a nitrogen atom (Table 1). The saturated derivatives piperidine (**9a**), difluoropiperidine (**9b**), 3,3-dimethylpyrrolidine (**9c**) and 3-phenylpyrrolidine (**9d**) were shown to be less active and selective than the parent pyvalamide **2**. This result cannot be attributed to steric factors, since as indicated in Figure 2A, compounds **2** and **9a** showed a good overlap and fitted the positive ionizable feature and two (HYD2 and HYD4) out of the four hydrophobic features of the Laggner[11] σ_1R pharmacophore. The introduction of polar substituents (**9e**,**f**) was more detrimental, matching the results obtained in the SAR study of the 4-acylamino derivatives. The low activity of the imidazol-1-yl (**9g**) and dimethylpyrrol-1-yl (**9i**) derivatives was recovered by **9h**. As shown in Figure 2B, the benzimidazole

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group of **9h** was able to cover the HYD4 hydrophobic feature, while the imidazole ring matched quite well the carbonyl region of the acylamino derivatives.

Next, the introduction of C-linked aryl and heteroaryl derivatives was explored. As shown in Table 2, different types of phenyl substituents were tolerated by the σ_1R, the less active derivatives being the two compounds containing a hydrogen acceptor moiety (OMe, **10f**; CN, **10g**) in position 4. However, none of the phenyl derivatives showed the desired selectivity for the σ_2R. The introduction of nitrogen atoms in the phenyl ring was subsequently explored (Table 3), but it was detrimental. Compound **11f**, with a substituent in position 2, was the most active among the pyridyl derivatives, but yet less potent than the phenyl analogues.

Finally, five membered heterocycles were introduced in position 4 (Table 4). The isothiazole (**12a**), oxazole (**12l**) and thiazole (**12m**) derivatives did not improve the pyridine analogues, while the intermediate affinity of pyrazole **12b** was impaired on alkylation (**12c-d**). The 2,5-dimethyloxazol-3-yl derivative **12e** provided an improvement in σ_1R affinity, while the 1-methylpyrazol-5-yl derivative **12f** was even more active, affording as well an improvement in selectivity *vs* the σ_2R. Introducing a trifluoromethyl substituent on the pyrazole ring of **12f** provided a decrease in activity (**12g**), as did changing to the imidazole isomers (**12i,j**). The 1-methylpyrrol-2-yl (**12h)** and 3-chlorothiophene (**12k)** recovered affinity, but less selectivity was observed again.

Chemical stability was assayed for all the compounds having a K_i value for the σ_1R below 100 nM and none of them showed any sign of degradation in solution at pH 2 or 7.4, indicating that, as expected, the replacement of amide groups by cyclic derivatives provided an improvement in stability.

The previous results show that the introduction of cyclic substituents in position 4 provides a decrease in selectivity for the σ_2R in relation to the amide analogues discussed in the previous paper. The higher selectivity of compound **12f** could be explained by the concurrence of two factors: its increased polarity (cLog*P* 1.3) and the presence of the pyrazole nitrogen atom, which matches quite well the carbonyl

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group of **2** (Figure 2C) and indicates that the σ_2R is less tolerant to polar groups in this region than the σ_1R.

Being the most selective compound, **12f** was further profiled in vitro and failed to show any significant affinity (inhibition at 10 μM < 50%) in a panel of 65 receptors[17] and no significant inhibition (below 50 % at 1 μM) in recombinant human cytochrome P450 (rhCYP) isoforms (1A2, 2C9, 2C19, 2D6, and 3A4),[18] suggesting low potential for drug-drug interactions. Compound **12f** showed a high metabolic stability in human liver microsomes (Cl_{int} = 2.4 μl/min/mg prot.), a good indicator for obtaining an appropriate pharmacokinetic profile in humans. It had also a good permeability in Caco-2 cells (Papp = 407 nm/s) and an efflux ratio of 0.7, indicating that it was not a P-gp substrate. Additionally, **12f** did not show *in vitro* cytotoxic potential in the MTT and Neutral Red uptake assays when tested up to 100 μM in HepG2 cells,[19] and lacked genotoxic potential in the SOS/*umu* and Ames bacterial mutation assays.[20] As in the previous series, this good in vitro ADMET profile was complemented by a low (IC_{50} > 10 μM) blockage of the human Ether-a-go-go-Related Gene (hERG) potassium channel.[21]

Compound **12f** (Figure 3) complied Lipinski's rules, showing a low lipophilicity which together with its good affinity provided one of the best lipophilic ligand efficiencies (LLE)[22] ever described for a σ_1R ligand (LLE: 6.1) and at the same time, its total polar surface area (tPSA) was maintained in the desired range for a good blood−brain barrier (BBB) penetration (59.2). In accordance with this reduced polarity, its thermodynamic solubility was high in phosphate buffer both at pH 7.4 (1.5 mg/mL) and pH 2 (2.4 mg/mL).

In view of its good in vitro profile, compound **12f** was advanced to in vivo testing in three pain models in mice in comparison to **1** (Table 5). It showed a similar activity to **1**, both i.p. and p.o., in the formalin model, where the intraplantar (i.pl.) injection of formalin elicits an early phase (phase I, results not shown) and a delayed phase (phase II) of pain characterized by paw licking, biting and other behaviours.[23] Compound **12f** was also active, after oral administration, in the mouse model of

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mechanical hypersensitivity induced by i.pl. capsaicin[24] and in the partial sciatic nerve ligation (PSNL) model in mice,[25] where it exhibited a substantial antiallodynic activity. The range of activity in the three models was similar to that shown by **1**, a result that reinforces the link of antagonistic behavior for the σ_1R and analgesia.

Conclusions

A change of the acylamino groups in the 4 position of the pyrazolo[3,4-*d*]pyrimidine scaffold, by cyclic substituents, led to highly active σ_1R ligands with improved chemical stability. The SAR study showed that phenyl or pyrazolyl groups were the best in terms of affinity for the σ_1R. Introduction of nitrogen atoms in the six membered rings was detrimental and only a few five membered heterocycles provided a good affinity. The 4-(1-methylpyrazol-5-yl) derivative, **12f**, was the most selective derivative both over the σ_2R and a wider panel of receptors. The compound showed as well a low lipophilicity, which together with its good affinity, provided one of the best lipophilic ligand efficiencies (LLE) ever described for a σ_1R ligand. These properties resulted in a good physicochemical profile, including a good thermodynamic solubility, a good ADMET profile and potent antinociceptive properties in several pain models in mice, indicative of its antagonistic nature.

Experimental

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Microwave assisted reactions were conducted with an Explorer Synthesizer from CEM. Flash chromatography was performed with a forced flow of the indicated solvent system on SDS silica gel Chromagel 60 ACC-(230-400 mesh) or on a CombiFlash Companion system with Redisep Rf disposable columns. ^{1}H spectra were recorded on an Agilent UNITY 300 MHz (spectrometer fitted with a 5 mm H/F/X ATB probe) or an Agilent Mercury 400 MHz (spectrometer fitted with a 5 mm ID/PFG probe) with 2 H lock in deuterated solvents. Chemical shifts (δ) are in parts per million. Commercially

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available reagents and solvents (HPLC grade) were used without further purification for all the analytical tests. Analytical UHPLC−MS were performed on a Waters Acquity H-Class-MS ZQ system using reverse phase C18 columns. Several analytical methods (1 to 6) were used depending on the particular compound, as detailed in the ESI. Mass spectra were obtained over the range m/z 100−800 at a sampling rate of 0.3 scans per second using Waters ZQ. Data were integrated and reported using Waters MassLynx software. All compounds display purity higher than 95 % as determined by these methods. Accurate mass measurements were carried out using an Agilent 6540 UHD Accurate-Mass QTOF system and obtained by electrospray ionization (ESI) in positive mode.

Calculation of molecular descriptors. clog*P* was calculated using ChemDraw Ultra 10.0.3. LLE was calculated with the equation: LLE = pIC_{50}– cLog*P*.

Determination of solubility. Solubility was measured as thermodynamic solubility from solid compound in phosphate buffer after 24 h of stirring at pH = 7.4 using HPLC.

Determination of chemical stability. Chemical stability was determined from 10 mM DMSO solution of test compounds in phosphate buffer at pH = 7.4 or 0.01 M HCl at pH = 2 by LC/MS.

4-Chloro-1-[2-(piperidin-1-yl)ethyl]-1*H*-pyrazolo[3,4-*d*]pyrimidine (5). To a stirred solution of 4-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidine (0.2 g, 1.29 mmol) in anhydrous THF (10 mL), 2-(piperidin-1-yl)ethanol (0.258 mL, 1.94 mmol) and triphenylphosphine (0.51 g, 1.94 mmol) were sequentially added. The reaction mixture was cooled to 0 ºC and diisopropylazodicarboxylate (0.38 mL, 1.94 mmol) was added dropwise. The mixture was stirred for 30 min. at 0 ºC and kept overnight at 4 ºC. The solvent was removed at reduced pressure and the residue was dissolved in dichloromethane and washed with 1 M HCl. The aqueous phase was separated, basified and extracted with dichloromethane. The organic phase was separated, dried and the solvent was removed under reduced pressure to give a residue, that was purified by flash chromatography eluting with (EtOAc/Petroleum ether, 8:2) to yield **5** (146 mg, 42 %) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (1H, s, 6-H), 8.15 (1H, s, 3-H), 4.67 (2H, t, *J*=6.7 Hz, 1-

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Et), 2.93 (2H, t, *J*=6.6 Hz, 2-Et), 2.65 – 2.42 (4H, m, 2'-H, 6'-H), 1.59 – 1.50 (4H, m, 3'-H, 5'-H), 1.47 – 1.34 (2H, m, 4'-H). LC/MS (Method 7) Rt 2.70 min, purity 100%. MS (ESI): *m/z* $[M+H]^+$ 266.2

1-[2-(Piperidin-1-yl)ethyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**8**). NaH (60 % in mineral oil, 651 mg, 16.3 mol) was added under argon atmosphere to a solution of 4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidine (**6**, 2.0 g, 14.8 mmol) in anhydrous DMF (25 mL) at 0 ºC. The mixture was left to reach room temperature for 1 h, and then, after cooling to 0 ºC again, a solution of 1-(2-chloroethyl)piperidine (**7**, 2.19 g, 14.8 mmol) in anhydrous DMF (10 mL) was added dropwise. The mixture was stirred at room temperature for 18 h and then quenched with H_2O. The mixture was concentrated in vacuum and partitioned between EtOAc and H_2O. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated to dryness to yield **8** as a solid (1.63 g, 45% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (1H, s, 3-H), 7.94 (1H, s, 6-H), 5.55 (2H, br s, NH2), 4.62 (2H, t, *J*=7.0 Hz, 1Et), 3.05 – 2.98 (2H, m, 2Et), 2.66 – 2.51 (4H, m, 2'-H, 6'-H), 1.66 – 1.61 (4H, m, 3'-H, 5'-H), 1.47 – 1.42 (2H, m, 4'-H) LC/MS (Method 7) Rt 1.79 min, purity 100%. MS (ESI): *m/z* $[M+H]^+$ 247.2.

Method A: 4-(4,4-Difluoropiperidin-1-yl)-1-[2-(piperidin-1-yl)ethyl]-1*H*-pyrazolo[3,4-*d*]pyrimidine (9b). A suspension of 4,4-difluoropiperidine hydrochloride (44 mg, 0.28 mmol) and K_2CO_3 (78 mg, 0.56 mmol) in acetonitrile (3 mL) was stirred for 15 min. Then, compound **5** (50 mg, 0.19 mmol) in acetonitrile (2 mL) was added and the mixture stirred at room temperature overnight. The suspension thus obtained was filtered and the solvent was removed under reduced pressure to give **9b** as an oil (65 mg, quantitative). ^{1}H NMR (400 MHz, CDCl3) δ 8.38 (1H, s, 6-H), 7.94 (1H, s, 3-H), 4.54 (2H, t, *J*=7.2 Hz, 1Et), 4.14 – 4.06 (4H, m, 2''-H, 6''-H), 2.85 (2H, t, *J*=7.2 Hz, 2Et), 2.48 (4H, t, *J*=5.4 Hz, 2'-H, 6'-H), 2.20 – 2.05 (4H, m, 3'-H, 5'-H), 1.59 – 1.48 (4H, m, 3''-H, 5''-H), 1.45 – 1.36 (2H, m, 4''-H). ^{13}C NMR($CDCl_3$, 101 MHz): δ (ppm) 156.73 (C-4), 155.06 (C-6), 154.45 (C-7a), 131.96 (C-3), 121.65 (t, *J*=242.2 Hz, C-4'), 100.62 (C-3a), 57.77 (2Et), 54.63 (C-2'', C-6'')', 44.87 (1Et), 42.61 (t, *J*=4.7 Hz, C-2', C-6'), 34.11 (t, *J*=23.5 Hz, C-3', C-5'), 26.09 (C-3'', C-5''), 24.38 (C-4''). LC/MS MS (Method

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1) Rt 1.69 min, purity 98 %. HRMS (ESI): *m/z* calculated for $C_{17}H_{25}N_6F_2$ $[M+H]^+$ 351.2103, found 351.2108.

Method B: 1-{1-[2-(Piperidin-1-yl)ethyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl}pyrrolidin-2-one (9f). A mixture of compound **5** (50 mg,0.19 mmol), pyrrolidin-2-one (0.07 mL), palladium diacetate (4.22 mg, 1.7 mmol), Xantphos (16 mg, 2.7 mmol) and Cs_2CO_3 (67 mg, 0.20 mmol) in dry toluene (3 mL) was stirred at 120 ºC for 20 min under microwave irradiation (150 W) and argon atmosphere. The mixture was filtered on decalite and the solvent was removed under reduced pressure to give a crude product that was purified by flash chromatography to give **9f** (12 mg, 20 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (1H, s, 6'-H), 8.63 (1H, s, 3'-H), 4.63 (2H, t, *J*=7.1 Hz, 1Et), 4.20 (2H, t, *J*=7.1 Hz, 5-H), 2.93 (2H, t, *J*=7.1 Hz, 2Et), 2.75 (2H, t, *J*=8.1 Hz, 3-H), 2.60 – 2.46 (4H, m, 6"-H, 2"-H), 2.35 – 2.15 (2H, m, 4-H), 1.66 – 1.48 (4H, m, 5"-H, 3"-H), 1.48 – 1.35 (2H, m, 4"-H).^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 175.17 (C-2), 154.53 (C-4'), 154.30 (C-6'), 151.52 (C-7a'), 136.71 (C-3'), 104.80 (C-3a'), 57.55 (2Et), 54.51 (C-6", C-2"), 48.12 (C-5), 44.59 (1Et), 33.57 (C-3), 25.81 (C-5", C-3"), 24.19 (C-4"), 18.46 (C-4). LC/MS (Method 1) Rt 1.45 min, purity 96 %. HRMS (ESI): *m/z* calculated for $C_{16}H_{23}N_6O$ $[M+H]^+$ 315.1955, found 315.1933.

Method C: 4-(1*H*-Imidazol-1-yl)-1-[2-(piperidin-1-yl)ethyl]-1*H*-pyrazolo[3,4-*d*]pyrimidine (9g). Potassium tert-butoxide (51 mg, 0.45 mmol) was added to a solution of 1*H*-imidazole (31 mg, 0.45 mmol) in acetonitrile (5 mL) at room temperature and the mixture was stirred for 15 min. Then, compound **5** (100 mg, 0.37 mmol) was added and the mixture was stirred overnight at room temperature. Water (0.5 mL) was added and the solvent was removed at reduced pressure. The resulting solid was extracted with dichloromethane and the organic phase was washed with brine and removed under reduced pressure to give **9g** as a pale yellow solid (110 mg, quantitative). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 (1H, s, 6-H), 8.65 (1H, dd, *J*=1.3, 0.9 Hz, 2'-H), 8.25 (1H, s, 3-H), 7.93 (1H, t, *J*=1.5 Hz, 4'-H), 7.31 (1H, dd, *J*=1.6, 0.9 Hz, 5'-H), 4.67 (2H, t, *J*=6.9 Hz, 1Et), 2.89 (2H, t, *J*=6.9 Hz, 2Et), 2.47 (4H, t, *J*=5.3 Hz, 6"-H, 2"-H), 1.54 – 1.44 (4H, m, 5"-H, 3"-H), 1.45 – 1.33 (2H, m, 4"-H). ^{13}C NMR

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(101 MHz, $CDCl_3$) δ (ppm) 155.32 (C-4), 154.91 (C-6), 149.16 (C-7a), 136.53 (C-3), 131.90 (C-2'), 130.93 (C-4'), 117.08 (C-5'), 103.26 (C-3a), 57.70 (2Et), 54.62 (C-6", C-2"), 45.38 (1Et), 26.07 (C-5", C-3"), 24.34 C-(4"). LC/MS (Method 1) Rt 1.10 min, purity 97 %. HRMS (ESI): *m/*z calculated for $C_{15}H_{20}N_7$ $[M+H]^+$ 298.1775, found 298.1778.

Method D: 4-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-1-[2-(piperidin-1-yl)ethyl]-1*H*-pyrazolo[3,4-d]pyrimidine (9i). A mixture of compound **8** (50 mg, 0.20 mmol) in hexane-2,5-dione (1.5 mL) was stirred at 180 ºC for 20 min under microwave irradiation (150 W). The mixture was acidified with 1M HCl and extracted with dichloromethane. The aqueous phase was basified with 20% NaOH and extracted with dichloromethane. The organic phase was removed under reduced pressure and the residue was purified by flash chromatography to give **9i** as a brown solid (21 mg, 32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 (1H, s, 6-H), 8.29 (1H, br s, 4'-H), 8.18 (1H, s, 3-H), 6.46 – 6.33 (1H, m, 3'-H), 4.62 (2H, t, *J*=6.8 Hz, 1Et), 2.96 (2H, t, *J*=6.9 Hz, 2Et), 2.57 (3H, s, Me), 2.54 (4H, t, *J*=5.3 Hz, 2"-H, 6"-H), 2.29 (3H, s, Me), 1.62 – 1.49 (4H, m, 3"-H, 5"-H), 1.49 – 1.34 (2H, m, 4"-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 158.78 (C-4), 154.96 (C-6), 153.25 (C-7a), 133.56 (C-3), 132.05 (C-5'), 127.27 (C-2'), 117.34 (C-4'), 110.87 (C-3'), 107.36 (C-3a), 57.86 (2Et), 54.63 (C-2", C-6"), 44.30 (1Et), 25.86 (C-3", C-5"), 24.30 (C-4"), 14.19 (Me), 13.07 (Me). LC/MS (Method A) Rt 1.33 min, purity 97 %. HRMS (ESI): *m/*z calculatedd for $C_{18}H_{25}N_6$ $[M+H]^+$ 325.2135, found 325.2141.

Method E: 4-Phenyl-1-[2-(piperidin-1-yl)ethyl]-1*H*-pyrazolo[3,4-*d*]pyrimidine (10a). A mixture of compound **5** (50 mg,0.19 mmol), phenylboronic acid (73 mg, 0.60 mmol) and anhydrous K_2CO_3 (82 mg, 0.59 mmol) in toluene (3 mL) was introduced in a microwave vial. It was degassed by argon for 30 min followed by the addition of $Pd(PPh_3)_4$ (4.0 mg, 0.003 mmol), after which the mixture was degassed for 10 additional min. The mixture was stirred at 150 ºC for 30 min under microwave irradiation (150 W). After cooling to rt, the mixture was filtered on decalite and the solvent was removed at reduced pressure. The crude was purified by flash chromatography to provide **10a** as a white solid (19.1 mg, 33%). ^{1}H NMR (300 MHz, CD_3OD) δ 9.00 (1H, s, 6-H), 8.53 (1H, s, 3-H), 8.32 – 8.16 (2H, m, Ph), 7.63

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(3H, dq, *J*=5.3, 2.0 Hz, Ph), 4.70 (2H, t, *J*=6.8 Hz, 1Et), 2.94 (2H, t, *J*=6.8 Hz, 2Et), 2.54 (4H, t, *J*=5.2 Hz, 6'-H, 2'-H), 1.64 – 1.49 (4H, m, 5'-H, 3'-H), 1.49 – 1.37 (2H, m, 4'-H). LC/MS (Method 1) Rt 1.60 min, purity 99 %. HRMS (ESI): *m/z* calculated for $C_{18}H_{22}N_5$ $[M+H]^+$ 308.1870, found 308.1876.

Method E: 4-(1-Methyl-1*H*-pyrazol-5-yl)-1-[2-(piperidin-1-yl)ethyl]-1*H*-pyrazolo[3,4-*d*]pyrimidine (12f). A mixture of compound **5** (0.28 g, 1.05 mmol), 1-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (0.33 g, 1.58 mmol), Na_2CO_3 (0.28 g, 2.63 mmol) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.09 g, 0.10 mmol) in CH_3CN/H_2O (1:1, 3mL), was placed in a vial, capped and heated at 110 ºC for 10 min under microwave irradiation (5-250 W). After cooling to room temperature, the mixture was poured into water (5 mL) and extracted with CH_2Cl_2 (4x5 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The crude residue was purified by medium pressure flash chromatography (Biotage SP1 25+M SiO_2 column, 0-10% MeOH/CH_2Cl_2) to furnish compound **12f** as a pale yellow solid (215 mg, 66% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (1H, s, 6-H), 8.24 (1H, s, 3-H), 7.64 (1H, d, *J*=2.1 Hz, 3'-H), 7.01 (1H, d, *J*=2.1 Hz, 4'-H), 4.66 (2H, t, *J*=7.0 Hz, 1Et), 4.38 (3H, s, NMe), 2.90 (2H, t, *J*=7.0 Hz, 2Et), 2.49 (4H, t, *J*=5.2 Hz, 2''-H, 6''-H), 1.57 – 1.46 (4H, m, 3''-H, 5''-H), 1.45 – 1.36 (2H, m, 4''-H).

^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 154.62 (C-6), 153.56 (C-7a), 151.39 (C-4), 138.82 (C-3'), 137.50 (C-5'), 132.51 (C-3), 111.34 (C-3a), 110.29 (C-4'), 57.78 (2Et), 54.64 (C-2'', C-6''), 45.00 (1Et), 40.37 (NMe), 26.06 (C-3'', C-5''), 24.34 (C-4''). The assignment of signals is according to the numbering of Figure 3. LC/MS (Method 6) Rt 18.15 min, purity 99 %. HRMS (ESI): *m/z* calculated for $C_{16}H_{22}N_7$ $[M+H]^+$ 312.1931, found 312.1938.

Method F: 2-{1-[2-(Piperidin-1-yl)ethyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl}oxazole (12l). A mixture of compound **5** (50 mg, 0.188 mmol), 2-(tri-*n*-butylstannyl)oxazole (0.059 mL, 0.282 mmol) in toluene (3 mL) was introduced in a microwave vial. It was degassed by argon for 30 min followed by the addition of $Pd(PPh_3)_4$ (22 mg, 19 mmol), after which the mixture was degassed for 10 additional min. The mixture was stirred at 100 ºC for 30 min under microwave irradiation (150 W). The solvent

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was removed and the crude thus obtained was purified by flash chromatography to provide **12l** as a cream-colored solid (37 mg, 66 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.12 (1H, s, 6'-H), 8.71 (1H, s, 3'-H), 8.09 – 7.88 (1H, m, 5-H), 7.60 – 7.39 (1H, m, 4-H), 5.06 – 4.68 (2H, m, 1Et), 3.10 (2H, s, 2Et), 2.76 – 2.35 (4H, m, 2"-H, 6"-H), 1.86 – 1.52 (4H, m, 3"-H, 5"-H), 1.52 – 1.32 (2H, m, 4"-H). LC/MS (Method 1) Rt 1.46 min, purity 98 %. HRMS (ESI): *m/z* calculated for $C_{15}H_{19}N_6O$ $[M+H]^+$ 299.1615, found 299.1620.

In vitro and in vivo tests: In vitro and in vivo (formalin, capsaicin and PSNL) tests were performed using the same methods described in the previous article.[6]

Acknowledgment.

We thank Adriana Port, Raquel Enrech, Xavier Monroy, Pilar Pérez, Enrique Hernández, Eva Ayet, Ariadna Balada, Raquel Fernández-Reinoso, Sandra Yeste, Enrique Portillo, Beatriz de la Puente and Daniel Zamanillo, for their expert contribution to analytical, in vitro and in vivo studies, Joan Andreu Morató, Monica Carro, and Edmundo Ortega for their excellent technical assistance and Carlos Pérez and Eduardo Villarroel for their contribution to compound management. We thank also the support from Centro de Desarrollo Tecnológico e Industrial (CDTI, PROJECT IDI-20110577).

Abbreviations.

ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
BBB	Blood-Brain Barrier
Cl_{int}	Intrinsic clearance
cLog*P*	Calculated logarithm of the octanol/water partition coefficient
CNS	Central Nervous System
rhCYP	Recombinant human cytochrome P450

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3D	Three-dimensional
hERG	human Ether-a-go-go-Related Gene
HRMS	High Resolution Mass Spectrometry
HLM	Human Liver Microsomes
LLE	Lipophilic Ligand Efficiency
Papp	Apparent permeability coefficient
PSNL	Partial Sciatic Nerve Ligation
SAR	Structure-Activity Relationships
Rt	Retention time
σR, σ_1R, σ_2R	Sigma, Sigma-1 and Sigma-2 Receptor, respectively
tPSA	Total Polar Surface Area

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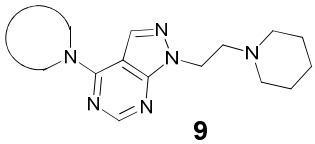
Table 1. Cyclic nitrogenated derivatives in position 4.



9

comp		K_i σ_1[a] (*h*, nM)	% 1 μM σ_2[b] (gp)
1		17	---
2		51	---
9a	Piperidin-1-yl	200	67
9b	4,4-Difluoropiperidin-1-yl	108	67
9c	3,3-Dimethylpyrrolidin-1-yl	165	58
9d	3-Phenylpyrrolidin-1-yl	294	71
9e	3-Methylaminopyrrolidin-1-yl	25770	---
9f	2-Oxopyrrolidin-1-yl	6194	
9g	Imidazol-1-yl	13803	---
9h	Benzimidazol-1-yl	112	66
9i	2,5-Dimethylpyrrol-1-yl	740	---

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[a]Binding affinity (K_i) to human σ_1R in transfected HEK-293 membranes using [^{3}H](+)-pentazocine as radioligand. Each value is the mean of two determinations. [b]Binding affinity (% inhibition at 1 μM) to σ_2R in guinea pig brain membranes using [^{3}H]-di-*o*-tolylguanidine as radioligand. Each value is the mean of two determinations.

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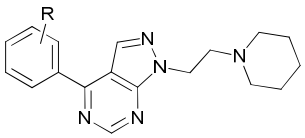
Table 2. Substituted phenyl moieties in 4-position.



10

comp	R	$K_i\ \sigma_1$[a] (*h*, nM)	% 1 μM σ_2[b] (gp)	cLog*P*
10a	H	100	69	2.8
10b	2-Methyl	74	83	3.1
10c	3-Methyl	82	82	3.4
10d	2-Ethoxy	300	81	2.9
10e	4-Fluoro	95	75	3.0
10f	4-Methoxy	531	58	2.9
10g	4-Cyano	558	47	2.3
10h	2-Chloro	76	92	3.4
10i	4-Chloro	43	74	3.6
10j	2-Chloro-5-methyl	31	95	3.9
10k	2,5-Dimethyl	63	89	3.6
10l	2,4-Difluoro	93	83	3.2

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10m	4-Fluoro-2-methoxy	39	87	2.6
10n	4-Fluoro-2-methyl	48	86	3.2
10o	4-Chloro-2-fluoro	112	73	3.8
10p	4-Fluoro-3-trifluoromethyl	57	87	3.9
10q	2-Trifluoromethyl	125	86	3.8

[a,b] See footnotes of Table 1.

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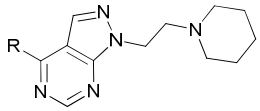
Table 3. 6-Membered heterocycles in 4-position.



11

comp	R	K_i σ_I[a] (*h*, nM)
11a	Pyridin-3-yl	926
11b	2-Methylpyridin-3-yl	732
11c	2-Methoxypyridin-5-yl	38293
11d	4-Methylpyridin-3-yl	707
11e	5-Methylpyridin-3-yl	447
11f	2-Methoxypyridin-3-yl	112
11g	Isoquinolin-4-yl	666
11h	Pyridin-2-yl	634
11i	6-Methylpyridin-2-yl	709
11j	Pyridin-4-yl	1717

[a]See footnote of Table 1.

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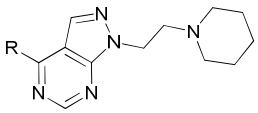
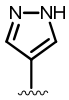
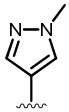
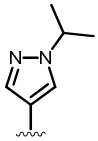
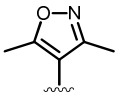
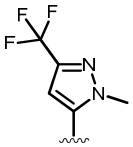
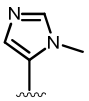
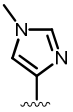
Table 4. 5-Membered heterocycles in 4-position.



12

comp	R	K_i σ_1[a] (*h*, nM)	% 1 μM σ_2[b] (gp)	cLog*P*
12a		475	40	1.5
12b		802	---	-0.2
12c		9460	---	1.1
12d		2915	---	1.9
12e		72	76	1.0
12f		41	>1000 nM[c]	1.3
12g		137	72	2.3
12h		31	78	2.2
12i		715	---	1.0
12j		11843	---	1.0

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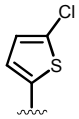
12k		35	75	3.5
12l		23832	---	0.7
12m		1834	---	1.4

[a,b]See footnotes of Table 1. [c]K_i value

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Table 5. *In vivo* activity of **12f** in comparison to **1**.

	Formalin[a] 40 mg/kg i.p. ***Phase II***	**Formalin[a] 80 mg/kg p.o.** ***Phase II***	**Capsaicin[b] 80 mg/kg p.o.**	**PSNL[c] 80 mg/kg p.o.**
1	50 ± 13	66 ± 9	48 ± 7	62 ± 17
12f	67 ± 8	54 ± 15	43 ± 2	46 ± 21

[a]Percentage of antinociception in the formalin test evaluated as the licking-biting time in drug-treated animals *vs* vehicle. [b]Percentage reduction of mechanical hypersensitivity in the capsaicin test evaluated as the latency time to the paw withdrawal response to upward pressure by von Frey filament stimulation in drug-treated animals *vs* vehicle. [c]Percentage reduction of mechanical hypersensitivity induced by partial sciatic nerve ligation evaluated as the pressure threshold (grams) required to elicit the paw withdrawal response following von Frey filament stimulation in drug-treated animals after compound administration *vs* response 8 days after surgery (pain condition, vehicle treatment) and before nerve ligation (baseline normal conditions).

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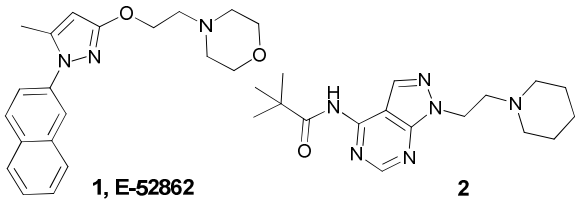

Figure 1. Reference compound **1** and lead compound **2**.

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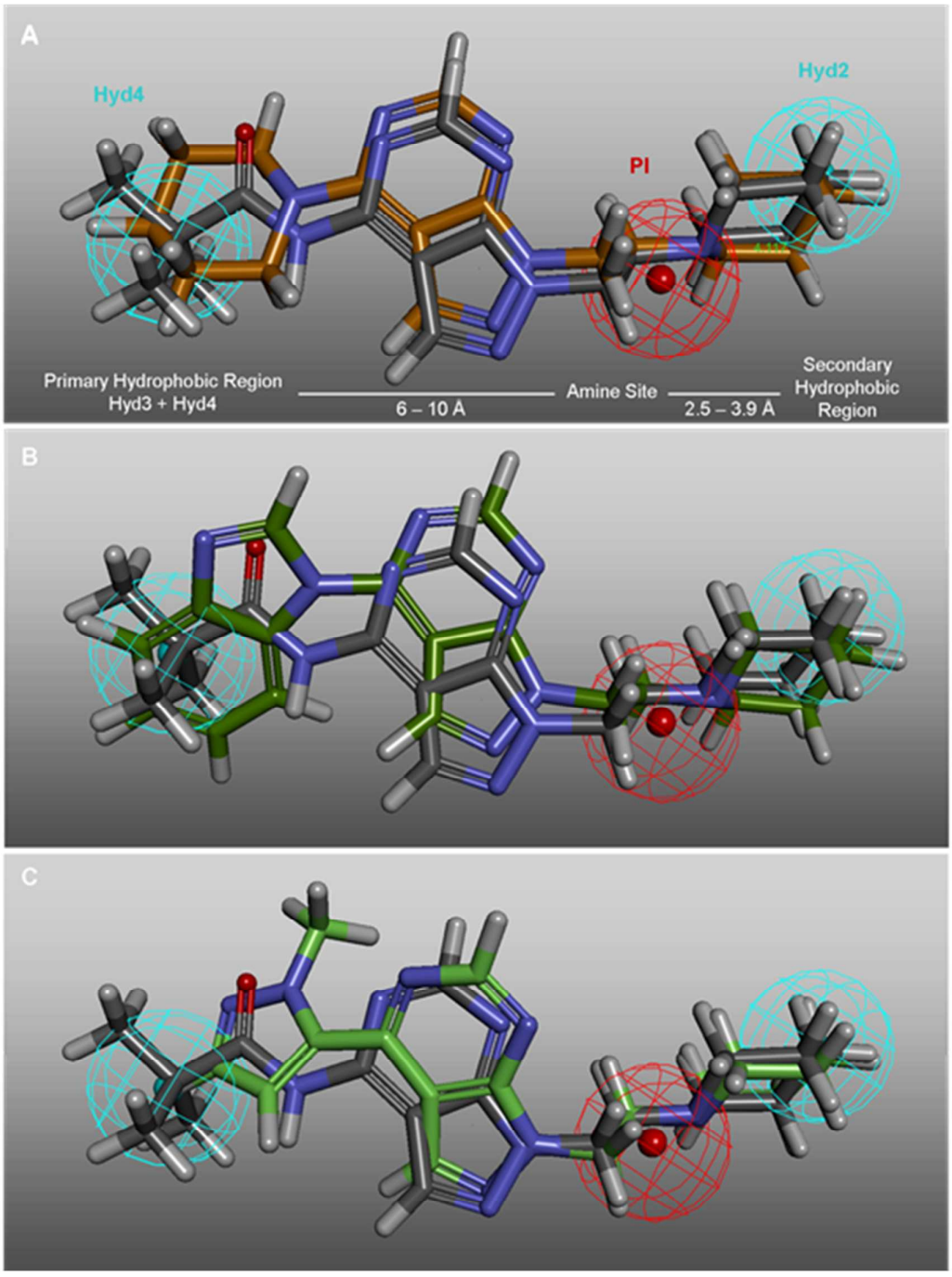

Figure 2. 3D superposition of compound **2** with ligands **9a** (A), **9b** (B) and **12f** (C) correlated with the Laggner model

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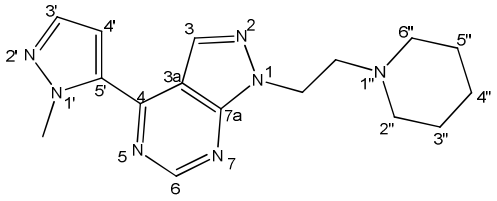

12f
MW = 311.4
tPSA = 59.16
cLog*P* = 1.3
LLE = 6.1
Ther solubility = 1.5 mg/mL

Figure 3. Physicochemical properties of compound **12f**.

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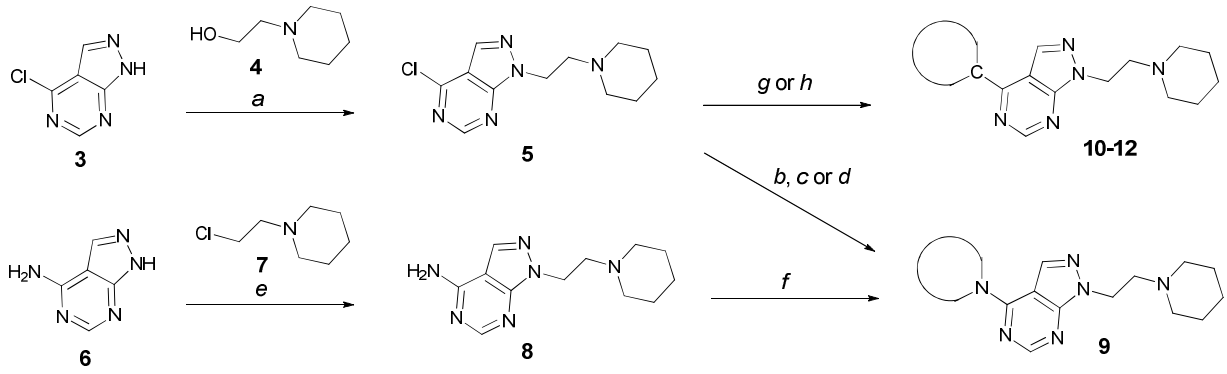
Scheme 1



Reagents and conditions: a) Diisopropylazodicarboxylate, PPh_3, THF, 4 ºC, 18 h; b) Method A: K_2CO_3, ACN, rt; c) Method B: Amine, $Pd(OAc)_2$, Xantphos, Cs_2CO_3, Tol, MW, 150 ºC, 30 min; d) Method C: Amine, Bu^tOK, ACN, rt; e) NaH, DMF, rt, 18 h; f) Method D: Hexane-2,5-dione, MW; g) Method E: $ArylB(OH)_2$ (or ester), $Pd(PPh_3)_4$, K_2CO_3, Tol, MW, 150 ºC, 30 min; h) Method F: $ArylSnBu_3$, $Pd(PPh_3)_4$, K_2CO_3, Tol, MW, 150 ºC, 30 min.

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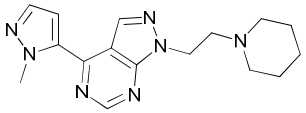

12f
K_i, σ_1 = 41 nM
K_i, σ_2 >1000 nM
MW = 311.4
tPSA = 59.16
cLog*P* = 1.3
LLE = 6.1
Ther solubility = 1.5 mg/mL

A SAR study identified the 4-(1-methylpyrazol-5-yl) derivative, **12f**, as a selective σ_1R antagonist with a good ADMET profile and potent antinociception.