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Development of 2,4-diaminopyrimidine derivatives as novel SNSR4 antagonists

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ABSTRACT

2,4-Diaminopyrimidines derivatives were developed as a novel class of SNSR4 antagonists. Structure activity relationship of the diamino pyrimidine core was explored and a tool compound suitable for target validation was identified.

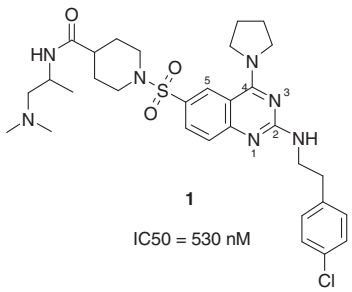
The G-protein-coupled receptor (GPCR) superfamily is the most exploited protein family for drug discovery, yet, over 50% of the members of the GPCR family remain classified as 'orphan receptors', receptors whose regulatory ligands remain unknown. Given that GPCRs are one of the most targeted protein families for therapeutic intervention, understanding the physiological roles of these receptors is of great importance.[1a–d]

Recently, the identification of a large family of GPCRs that are related to the MAS oncogene and called Mas-related genes (Mrgs), was reported.[2] These genes, also known as the sensory neuron-specific receptors (SNSRs) or dorsal root receptors (DRRs), comprise a large family of over 50 rodent and human orphan GPCRs. The restricted expression of many members of the Mrg family to sensory neurons of the dorsal root ganglion (DRG) suggests that these members of MrgX receptors may play a role in nociception. In humans, the MrgX subset of Mrg receptors is selectively expressed in DRG.[3] Interestingly, the DRG-specific members of this family show discrete patterns of expression that appear to be only partially overlapping within the sensory neurons, implying that these receptors may each play unique roles in pain sensation.[3]

Human sensory neuron specific receptor 4 (SNSR4 or MrgX1) is restricted to a subpopulation of sensory neurons in both humans and rat. These neurons are known to express other nociceptive markers such as IB4 and SNSR4 agonists such as BAM 8-22 are pro-nociceptive in rodents. SNSR4 activation might have a pro-nociceptive role in pain, and an antagonist may, therefore, have therapeutic value as an analgesic.[4a] There is also a contradictory evidence that SNSR4 agonist BAM 8-22 being analgesic.[4b] Due to this contradictory report and because the homology between human and rat SNSR4 receptors are only 60%, we decided to test the hypothesis that SNSR4 antagonists are analgesic in human using an investigational compound.

To date, there has been just one report of SNSR antagonists, the azabicyclooctanes.[5] Herein, we describe the development and structure activity relationship of 2,4-diaminopyrimidines as novel small molecule SNSR4 antagonists.

In order to find a potent (IC_{50} <50 nM) and soluble (solubility >25 μM) SNSR4 tool compound to test the hypothesis that an SNSR4 antagonist may be analgesic, the AstraZeneca compound collection was screened using a functional (FLIPR) assay,[6] which resulted in the discovery of a number of hits with a quinazoline core structure as exemplified by compound **1** (Fig. 1). Near neighbour



1

IC50 = 530 nM

Figure 1.

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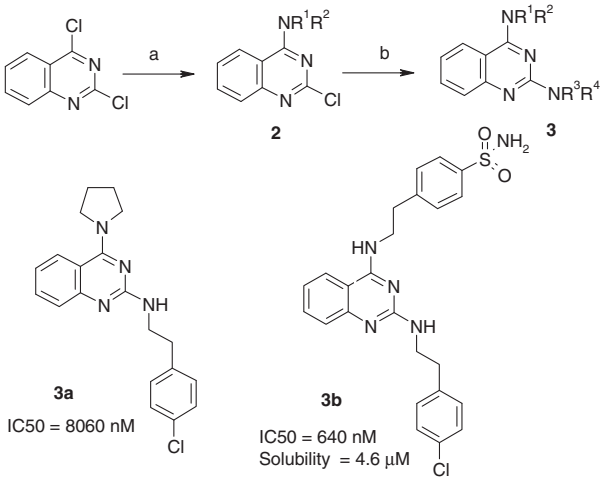
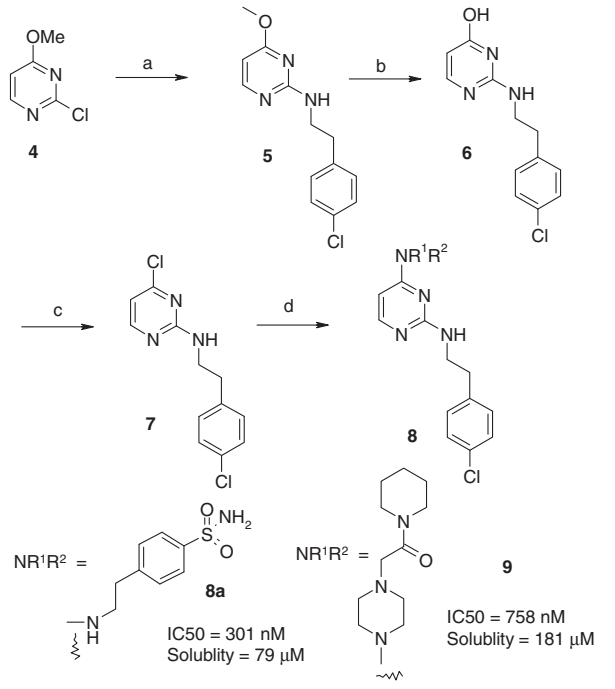
analysis of compound **1** revealed that vast majority of the near neighbours screened from the compound collection contained variations of the amino group of the sulfonamide group and contained only limited variations at 2- and 4-positions of the quinazoline core. In order to determine the significance of the sulfonamide moiety, the corresponding truncated 2,4 disubstituted quinazoline **3a** was made by two consecutive SN_{Ar} reactions of dichloroquiazoline with the corresponding amines as shown in Scheme 1. Compound **3a** was found to retain moderate micromolar activity against SNSR4. Using the same route a small directed library was prepared further to explore substitution at the 4-position of the quinazoline core and so as to improve activity at SNSR4. Compound **3b**, identified from this library, showed comparable activity to compound **1**.



Scheme 1. Reagents and conditions: (a) HNR^1R^2, NEt_3, dichloromethane, rt; (b) HNR^3R^4, NEt_3, BuOH, 120 °C.

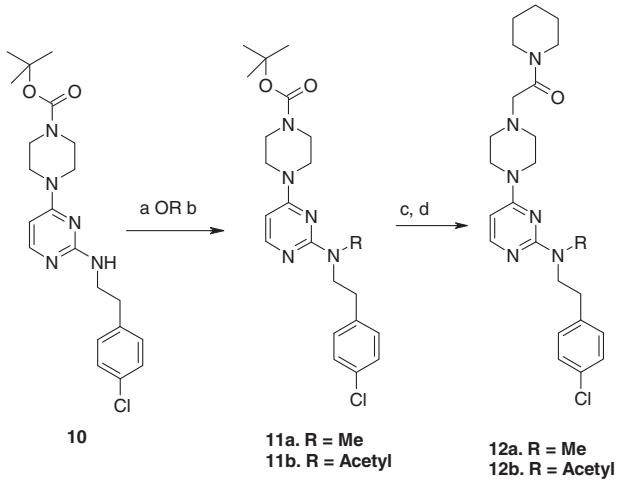


Scheme 2. Reagents and conditions: (a) *p*-chlorophenylethyl amine, NEt_3, BuOH, 120 °C; (b) 12 N HCl in AcOH; 100 °C; (c) $POCl_3$, 80 °C; (d) HNR^1R^2, NEt_3, BuOH, 120 °C.

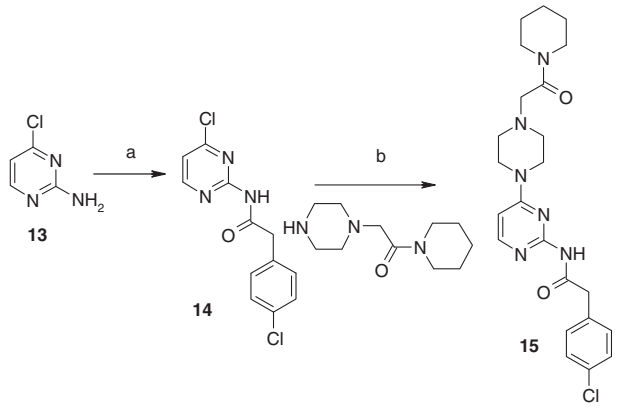
Interestingly, the simplified pyrimidine analogue, compound **8a** (Scheme 2), possessing only a 6-membered heterocyclic core showed comparable activity and improved solubility as compared with the quinazoline **3b**. The pyrimidine analogues were made by the route shown in Scheme 2 starting from 2-chloro-4-methoxy pyrimidine **4**. Displacement of the chloro group by *p*-chlorophenyl ethylamine followed by demethylation with hydrochloric acid gave the hydroxyl intermediate **6**, which was treated with $POCl_3$ to give the corresponding chloro intermediate **7**. Displacement of the chloro group with a variety of amines gave a set of 4-substituted pyrimidines **8**.

Several variations were explored by synthesizing a library of compounds varying at the 4-position of the pyrimidine core. The library was prepared by treating the 4-chloro pyrimidine intermediate **7** with a selection of structurally diverse amines under the conditions described in Scheme 2. Piperazine acetyl amide derivative **9,** obtained from this library showed further improvement in solubility and was half as potent as the sulfonamide **8a**. In addition, compound **9** provided further scope to explore SAR at the amide position.

Several substitutions were explored at the C-2 position of the pyrimidine core using similar chemistry to that shown in Scheme 2. Analogues disubstituted at the nitrogen at the 2-position of the pyrimidine were made by introducing alkyl or acetyl



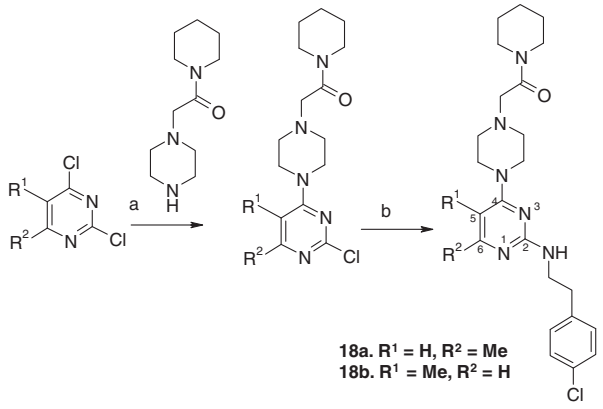
Scheme 3. Reagents and conditions: (a) NaH, MeI, DMF, rt; (b) NEt_3, AcCl, dichloromethane; (c) TFA, dichloromethane, rt; (d) NEt_3, 1-(bromoacetyl)-piperidine, DMF, rt.



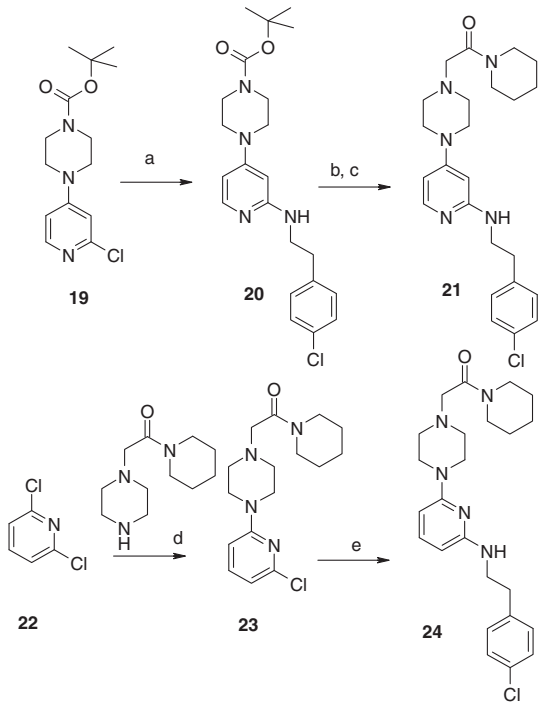
Scheme 4. Reagents and conditions: (a) NEt_3, 4-chlorophenylacetyl chloride, DMA, rt; (b) NEt_3, BuOH, 120 °C.

groups into the intermediate **10**, which was made by reacting the chloro intermediate **7** with Boc protected piperazine. Removal of the Boc group followed by alkylation with 1-(bromoacetyl)piperidine gave compounds **12a** and **12b** (Scheme 3). Compound **15** was made by the acylation of 2-amino-4-chloro pyrimidine **13** with 4-chlorophenylacetyl chloride followed by displacement of the 4-chloro group with the corresponding amine as shown in Scheme 4. However, the original 4-chlorophenyethylamino group, as in compound **9**, could not be improved upon. The *N*-acetyl amide **12b** showed a ~5-fold loss in potency and **12a** and **15** were completely inactive.

Substitution at the C-5 and C-6 positions of the pyrimidine ring were then explored. These compounds were made by two consecutive substitution reactions of the appropriately substituted



Scheme 5. Reagents and conditions: (a) NEt_3, dichloromethane, rt; (b) 4-chlorophenylethylamine, NEt_3, BuOH, 120 °C.

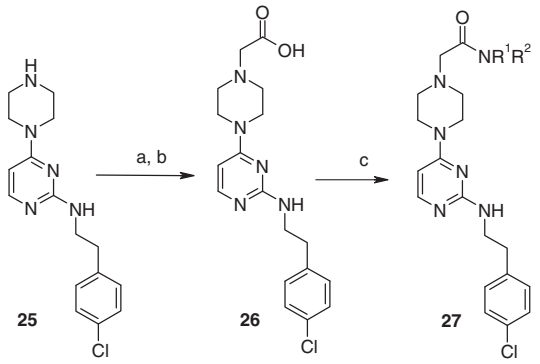


Scheme 6. Reagents and conditions: (a) 4-chlorophenylethylamine, 5 mol % $Pd_2(DBA)_3$, 5 mol % Xanphos, NaO^{t}Bu, toluene, 100 °C; (b) TFA, dichloromethane, rt; (c) NEt_3, 1-(bromoacetyl)-piperidine, DMF, rt; (d) NEt_3, BuOH, 120 °C; (e) 5 mol % $Pd_2(DBA)_3$, 5 mol % BINAP, NaO^{t}Bu, 4-chlorophenylethylamine, toluene, 80 °C.

dichloropyrimidines with amines as shown in Scheme 5. A methyl group is tolerated at the C-6 position of the pyrimidine ring as shown by compound **18a**, which showed comparable activity to compound **9** while a methyl at the C-5 position (compound **18b**) resulted in a 5–10-fold drop in activity at SNSR4 compared to compound **9**.

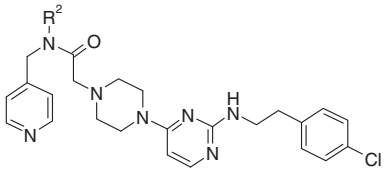
The significance of the second nitrogen atom in the pyrimidine ring was explored by making the corresponding pyridine derivatives **21** and **24** (Scheme 6). 2,4-Di-substituted pyridine analogue **21** was made starting from a known intermediate **19**.[7] Palladium catalyzed amination of the intermediate **19** with *p*-chlorophenylethylamine followed by removal of the Boc group and alkylation with the corresponding bromo acetyl amide derivative gave **21**. 2,6-Di-substituted pyridine analogue **24** was made by substitution of one of the chloro groups by substituted piperazine followed by palladium catalyzed amination at the second chloro group. 2,4-Di-substituted pyridine analogue **21** demonstrated a moderate drop (~3-fold) in potency compared to compound **9** while the 2,6-di-substituted pyridine analogue **24** had no activity.

Next, several amides on the C-4 position of the piperazine ring of compound **9** were explored in an attempt to improve potency



Scheme 7. Reagents and conditions: (a) chloroacetyl ethyl ester, NEt_3, DMF; (b) TFA, dichloromethane, rt; (c) HNR^1R^2, HATU, DIPEA, DMF, rt.

Table 1
SAR at the top amide: variation of R^2



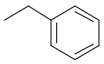
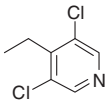
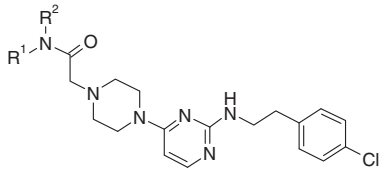
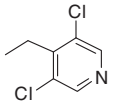
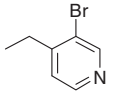
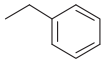
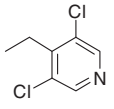
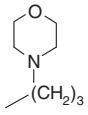
Compound	R^2	IC_{50} (nM)	Solubility (μm)
27a		20	16
27b		22	N/D
27c		24	6
27d		40	N/D
27e		88	>750
27f	Ethyl	244	1130
27g	Cyclopropyl methyl	295	104

Table 2
SAR at the top amide: variation of R^1 and R^2



Compound	R^1	R^2	IC_{50} (nM)	Solubility (μm)
27h		Ethyl	77	1.5
27i		Ethyl	108	9
27j		Ethyl	330	2
27k			14	43

and solubility. Synthesis of the amide analogues is outlined in Scheme 7. Alkylation of intermediate **25** with chloroacetyl *t*-butyl ester followed by deprotection of the *tert* butyl group gave the carboxylic acid **26**. A library of amides was made by treating the portions of the acid **26** with corresponding set of different amines in the presence of a coupling agent.

N-Ethyl-*N*-(4-pyridyl methyl) amide **27f** showed improved activity and solubility compared with compound **9**. A wide variety of ethyl amide replacements were explored varying R_2 group and keeping the *N*-(4-pyridyl methyl) group constant and the results are summarized in Table 1. Interestingly, a wide variety of groups are tolerated. Bulkier substitutions improved potency but decreased solubility. A moderate improvement in solubility was observed with the furylmethyl amide **27a**.

To improve activity and solubility further, replacements of the *N*-(4-pyridylmethyl) group were synthesized by following the route outlined in Scheme 7. The results are summarized in Table 2. Substitutions such as chloro- (compound **27h**) and bromo- (compound **27i**) at the pyridine ring improve activity but reduce solubility. The 4-pyridyl group could also be replaced with a simple phenyl group (compound **27j**), but this results in a decrease in solubility. Introduction of a morpholinopropyl group provided compound **27k** as a tool for target validation with good activity and adequate solubility.

In conclusion, 2,4-diaminopyrimidines were developed as a novel class of SNSR4 antagonists starting from a quinazoline core. Systematic exploration of the SAR led to a potent and selective SNSR4 antagonist tool compound **27k**, suitable for target validation.

Reference and notes

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4. (a) Grazzini, E.; Puma, C.; Roy, M.; Yu, X.; O'Donnell, D.; Schmidt, R.; Dautrey, S.; Ducharme, J.; Perkins, M.; Panetta, R.; Laird, J.; Ahmad, S.; Lembo, P. *Proc. Natl. Acad. Sci.* **2004**, *101*, 7175; (b) Chen, T.; Cai, Q.; Hong, Y. *Neuroscience* **2006**, *141*(2), 965.
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6. HEK293s cells (with or without co-expressed G proteins) were plated in a 384 plate at 17,000 cells/well/25 μl for 24 h in a humidified incubator (5% CO_2 and 37 °C) in DMEM supplemented with 10% FBS (also known as DMEM complete). In general, receptors expressed in HEK 293s (with or without co-expressed G proteins) cells will have to be grown at least 24 h in the absence of any selection media, that is, DMEM complete. Prior to the experiment the cell culture medium was removed from the plates by inversion. A loading solution of 30 μl of Hank's balanced salt solution (without phenol red, Gibco, catalog #: 14065-056), 20 mM Hepes with 0.1% BSA at pH 7.4, with 2 μM Fluo-3-AM (TEF labs, catalog # 0116, 1 mg/440 μl low water content DMSO, 2 mM) and 0.02% Pluronic acid F-127 (Molecular Probes, catalog #P-3000-MP, stock: 20% solution in DMSO) was added to each well. Plates were incubated at 37 °C (and 5% CO_2) for 60 min prior to start the experiment. The incubation was terminated by washing the cells four times in assay buffer (Hank's BSS, 20 mM HEPES ± 0.1% BSA, pH 7.4.), leaving a residual 25 μl buffer per well. After the washing, the cells were incubated at room temperature for 5–10 min and then transferred to the FLIPR, ready for compound additions. The day of experiment, the reference agonist and compounds were diluted in 3-fold concentration range (10 points serial dilution) for addition by FLIPR instrument. For all calcium assays, a baseline reading was taken for 30 s followed by the addition of 12.5 μl of compounds, resulting in a total well volume of 37.5 μl. Data were collected every 1.6 s for 300 s. The fluorescence emission was read using filter 1 (emission 520–545 nm) by the FLIPR on board CCD camera.
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