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1-(4-Amino-phenyl)-pyrrolidin-3-yl-amine and 6-(3-amino-pyrrolidin-1-yl)-pyridin-3-yl-amine derivatives as melanin-concentrating hormone receptor-1 antagonists

Charles Q. Huang,[a] Tracy Baker,[a] David Schwarz,[b] Jun Fan,[b] Christopher E. Heise,[c] Mingzhu Zhang,[a] Val S. Goodfellow,[a] Stacy Markison,[d] Kathleen R. Gogas,[d] Takung Chen,[e] Xiao-Chuan Wang[a] and Yun-Fei Zhu[a,*]

[a]*Department of Medicinal Chemistry, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*
[b]*Department of Molecular Biology, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*
[c]*Department of Pharmacology, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*
[d]*Department of Neurosciences, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*
[e]*Department of Pre-clinic Development, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*

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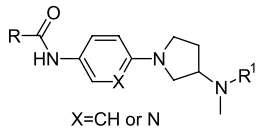
Abstract—Derivatives of 1-(4-amino-phenyl)-pyrrolidin-3-yl-amine and 6-(3-amino-pyrrolidin-1-yl)-pyridin-3-yl-amine were identified as potent and functionally active MCH-R1 antagonists. One compound with K_i = 2.3 nM demonstrated good oral bioavailability (32%) and in vivo efficacy in rats.

Melanin-concentrating hormone (MCH) is a cyclic neuropeptide produced in the lateral hypothalamus and zona incerta of the mammalian brain. These regions have been implicated in the control of a variety of biological functions, including the control of food intake and body weight. Alterations of MCH expression suggest that this peptide plays a pivotal role in modulating this feeding behavior. For instance, administration of exogenous MCH to rodents stimulates feeding[1] and body weight gain[2,3] in a dose-dependent manner, while elimination of the gene encoding the peptide precursor results in animals that are hypophagic and lean.[4]

In humans, two independent receptors have been identified that bind MCH with high-affinity: MCH-R1[5,6] and MCH-R2.[7] While both receptors share a broad—and often overlapping—pattern of expression within the brain,[7–9] it is MCH-R1 that appears to be primarily responsible for mediating the orexigenic effects of the peptide. First and foremost, rodents appear to express only a single receptor for MCH, and that receptor shares greatest homology with human MCH-R1. Furthermore, elimination of the MCH-R1 locus in mice results in animals that are lean and resistant to diet-induced obesity.[10] Finally, these data have stimulated the development of several small-molecule antagonists for MCH-R1 that have been shown to reduce both food intake and body weight gain when administered to rodents.[11–15]



I

Herein, we report the development of a series of small-molecule MCH-R1 antagonists (**I**) using aminoaryl-substituted 3-aminopyrrolidine as the central core. The structure–activity relationship (SAR) studies resulted in a series of potent and orally bioavailable MCH-R1 antagonists. One of the molecules was also proved to be efficacious in animal feed models.

* Corresponding author. Tel.: +1 858 617 7745; fax: +1 858 617 7619; e-mail: fzhu@neurocrine.com

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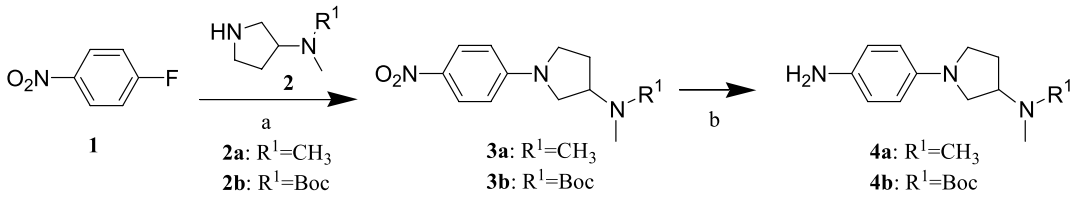
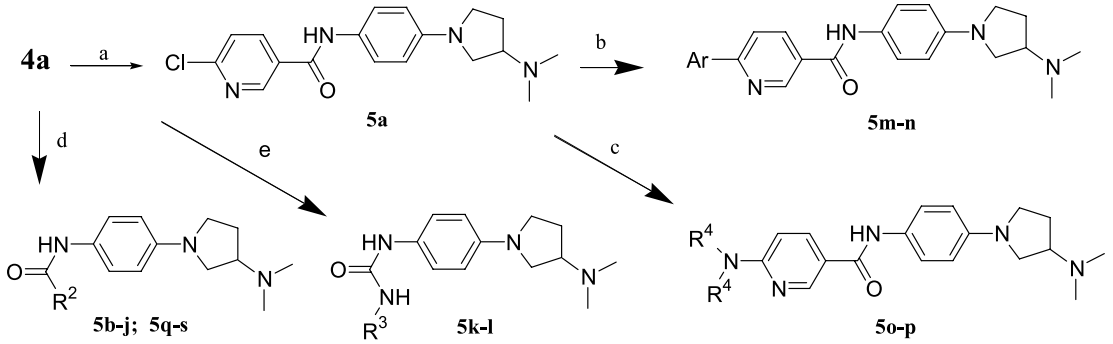


Scheme 1. Reagents and conditions: (a) DMSO, rt, 1 h, 95–100% and (b) $Na_2S_2O_4$, THF–H_2O, 0.5 h, 97% for **4a**; H_2, Pd–C, THF, 5 h, 100% for **4b**.

Scheme 1 outlines the synthesis of compounds **4a**, **b**, which served as common intermediates for further derivatization. 4-Fluoronitrobenzene (**1**) was reacted with *N,N*-disubstituted-aminopyrrolidines (**2a**, **b**) to give the intermediates **3a**, **b**. The nitro group was reduced either by hydrogenation or sodium hydrosulfite to afford the anilines **4a**, **b**. A number of selected acids , acyl chlorides, and isocyanates were coupled to **4a** to form the compounds **5b**–**j**, **5q**–**s**, and **5k**, **l** (Scheme 2, Table 1). Additionally, **4a** was reacted with 2-(6-chloro)-nicotinyl chloride to yield **5a**, which was subjected to Suzuki coupling conditions to give the desired compounds **5m**, **n**. **5a** could also be reacted with a variety of amines to generate analogs exemplified by **5o**, **p**. Variation of substituents on the basic amine was carried out according to Scheme 3. Thus, **4b** was coupled with biphenyl carbonyl chloride to give compound **6**. After TFA treatment, the resulting secondary amine (**7a**) was subjected to reductive amination to yield **7b**–**f** (Table 2). It was also acylated to form **7g**.

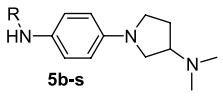
The pyridyl equivalent of compound **7a**, compound **10** (Scheme 4), was prepared in several steps from the commercially available 2-chloro-5-nitro-pyridine (**8**) by reacting with 3-(*N*-Boc-*N*-methyl)-aminopyrrolidine (**2b**). The resulting intermediate **9** was reduced by hydrogenation to the corresponding aniline, which was immediately coupled with 4-biphenylcarbonyl choride due to its unstable nature. The resulting product was deprotected, yielding the desired compound **10**. To avoid handling the unstable intermediate mentioned above, an alternative approach employing 2-amino-5-chloropyridine (**11**) as the starting material was used. Compound **11** was first acylated to form amide **12**, which yielded the same product **10** upon heating with Boc protected **(2)** 3-amino-pyrrolidine and followed by deprotection.

The SAR studies initially focused on the left side of molecule **I** by scanning a variety of amides. The biphenyl carboxamide **5b** proved to be a potent MCH-R1 antagonist possessing a receptor affinity (K_i) of 5.2 nM (Table 1).[16] Modifications targeting the biphenyl group were then explored. Both insertion of an oxygen between the two phenyl groups (**5c**) and cyclization of the biphenyl group (**5d**) resulted in complete loss of activity. One methylene extension between the biphenyl and carbonyl groups also caused a substantial loss of potency (**5e**, 4.3 μM). However, modification of the 'internal' phenyl group to a non-aromatic moiety, exemplified by **5f**–**l**, led to a moderate success. Among these analogs, 4-(4-chloro-phenyl)-cyclohexylcarboxamide (**5h**) and 4-(4-chlorophenyl)-4-oxo-butyramide (**5j**), were only about 2-fold less potent than **5b**. Urea analogs (**5k**, **5l**) were a few folds less potent than the similar amides. Surprisingly, replacement of the internal phenyl group with a pyridyl group (**5m**) led to a 20-fold loss of affinity (K_i = 110 nM). Interestingly, after incorporation of a 4-methoxyl group on the outside phenyl group, the activity was almost restored (**5n**, 16 nM). Enhancement of the binding affinity by 4-substitution of the phenyl group was observed also in the case of **5j** versus **5i**. On the other hand, attempts to replace the 'outside' phenyl group with piperidine and substituted piperazine resulted in very low to total loss of receptor affinity (**5o**, **p**). Thus a less radical modification strategy was employed by replacing the outside phenyl group with a heteroaromatic group, which is represented by the three compounds **5q**–**s**. The results showed that only the thiophene **5q**, the non-polar aromatic still maintained a reasonable binding affinity (34 nM), where the compounds with polar aromatic substituents (imidazole **5r** and oxazole **5s**) showed a substantial loss of activity. These results suggested that the outside phenyl group

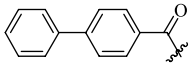
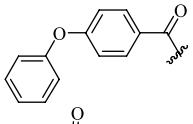
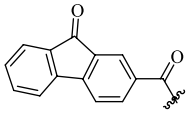
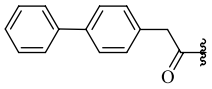
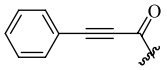
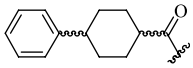
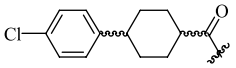
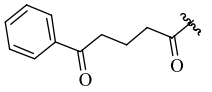
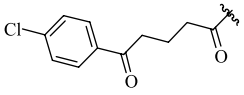
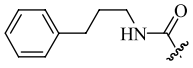
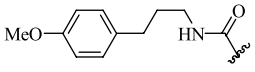
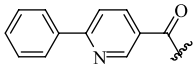
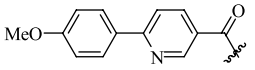
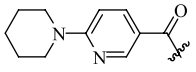
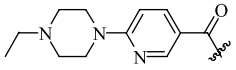
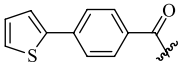
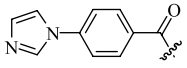
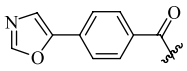


Scheme 2. Reagents and conditions: (a) 6-Chloronicotinyl chloride, DCM, Et_3N, 1 h, 30%; (b) $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, toluene, water, 100 °C, 24 h, 60–70%; (c) $(R^4)_2NH$, DMSO, 100 °C, 24 h, 61–99%; (d) R^2COCl, Et_3N or R^2CO_2H, EDC.HCl, HOBt, THF, 1–5 h, 30–70%; and (e) R^3NCO, THF, 2 h, 50%.

Table 1. Binding affinities of compounds **5b–s** on human MCH-R1



5b-s

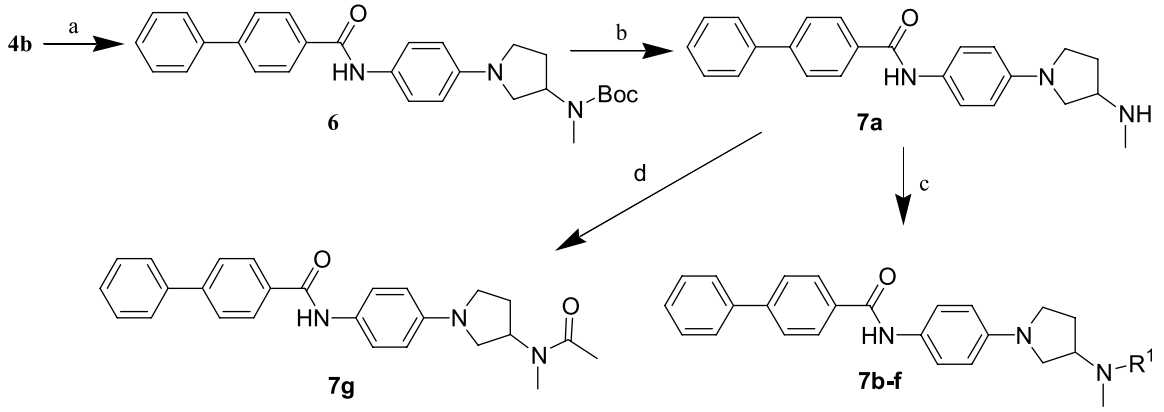
Compounds	R	K_i (nM ± SEM)
5b		5.2 ± 1.0
5c		>10,000
5d		>10,000
5e		4300 ± 780
5f		>10,000
5g		14 ± 3.8
5h		12 ± 3.6
5i		250 ± 2.8
5j		13 ± 0.4
5k		43 ± 2.5
5l		52 ± 12
5m		110 ± 4.1
5n		16 ± 3.3
5o		2400 ± 320
5p		>10,000
5q		34 ± 1.5
5r		>10,000
5s		>10,000

is one of the key interaction sites with MCH-R1, while the internal phenyl group might have dual roles. It could serve as a spacer to control the orientation of the outside phenyl group and also as a lipophilic site to interact with the receptor. The phenyl group might prove to be one of the best spacers, it was replaceable by both rigid and flexible, but relatively liophilic groups. On the other hand, the potency was less dependent on the substitution of the basic nitrogen, as evidenced by compounds **7a**–**7c**, **7f**, where substitution even as large as a phenylpropyl exhibited similar binding profiles. The basic nitrogen might be one of the key features for the receptor binding since the non-basic intermediate **6** was not active and the acetamide **7g** was only a weak binder.

Although the calculated log *P* for compound **7a** indicated that it was not a highly lipophilic molecule (clog *P* = 3.2),[17] its solubility as a hydrogen chloride salt in water was poor (approx. 0.25 mg/ml) based on a simple measurement.[18] Because of the potential experimental difficulty for in vivo efficacy studies due to the poor water solubility for this series, the SAR was expanded by using the more hydrophilic template, 6-(3-aminopyrrolidin-1-yl)-pyridin-3-yl-amine template (molecule **I** where X = N). The binding affinities of this series of compounds were found to be comparable to the corresponding 1-(4-amino-phenyl)-pyrrolidin-3-yl-amine series. For example, compound **10** has a binding affinity of 2.3 nM (K_i), which is similar to compound **7a** (3.1 nM). As expected, its solubility in water was indeed much improved. The solubility of pyridine **10** as a hydrogen chloride salt[19] was measured at 25 versus 0.25 mg/ml of phenyl analog **7a** in water.

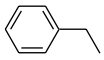
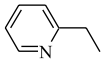
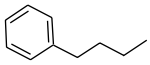
The functional antagonism of such compounds was initially confirmed based on their ability to inhibit the MCH-stimulated Ca^{2+} influx in a dose-dependent manner. For example, compounds **7a** and **10** had a IC_{50} of 166 and 122 nM in this assay. Functional activity of compound **10** was further established by the GTPγS assay (IC_{50} = 61 nM).[20] Compound **10** as a HCl salt also showed promising pharmacokinetic properties. In rats, after the dosing at 10 mg/kg for both iv and po administrations, it exhibited 32% oral bioavailability with a terminal half-life of 2.7 h.[21]

In vivo efficacy of compound **10** (as a HCl salt) was investigated in two feeding models. In the first study, food-deprived male Wistar rats (221 ± 10 g; n = 7–8/group) were given compound **10** (3–30 mg/kg, po), and 45 min later were allowed to eat. Orally administered compound **10** significantly suppressed food intakes at all doses out to 6 h post-dose ($p < 0.01$; Fig. 1). We also examined compound **10** in a chronic feeding model. In this study, Wistar rats (217 ± 9 g; n = 6–8/group) were implanted with a 7-day osmotic pump that contained compound **10** (10 mg/kg/day) in 50% DMSO or vehicle alone. In addition to ad libitum fed vehicle-treated animals, we included a vehicle-treated pair-fed group to examine the potential effects of MCH antagonism on metabolism. Rats in the pair-fed group were fed the same amount of food that the drug-treated animals ate. Results indicated that food intake was significantly



Scheme 3. Reagents and conditions: (a) 4-biphenylcarbonyl choride, Et_3N, DCM, 2 h, 74%; (b) TFA, DCM, 4 h, 100%; (c) aldehyde, $NaBH(OAc)_3$, MeOH, DCM, 14 h; and (d) acetic anhydride, DIPEA, DCM, 5 h, 85%.

Table 2. Binding affinities of compounds **6**, **7a**–**g**, and **10** on human MCH-R1

Compounds	R^1	K_i (nM ± SEM)
6	—	>10,000
7a	—	3.1 ± 0.7
7b	Ethyl	5.9 ± 0.3
7c		5.5 ± 0.6
7d		31 ± 4.7
7e		130 ± 16
7f		8.6 ± 0.3
7g	—	3000[a]
10	—	2.3 ± 0.3

[a] Only assayed once.

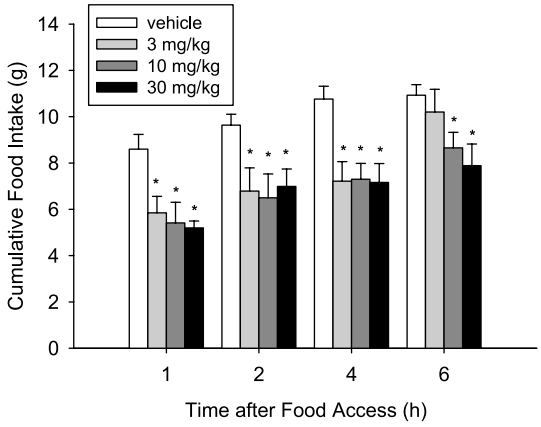
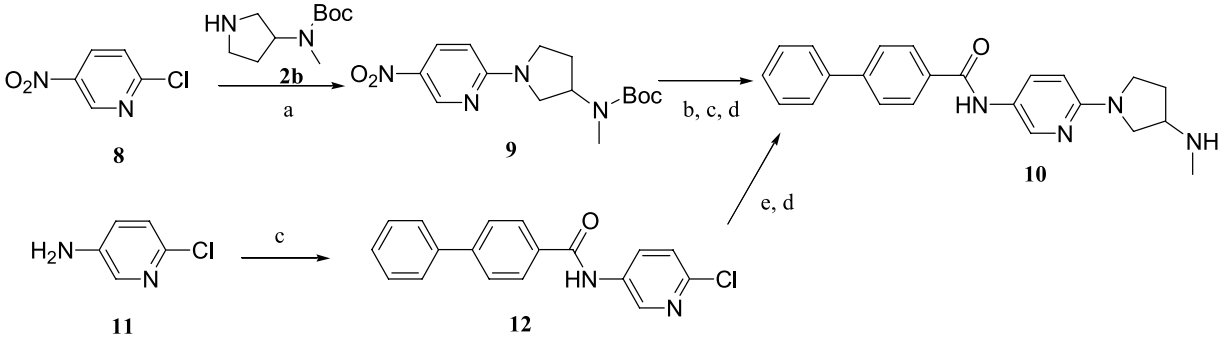


Figure 1. Effect of orally administered compound **10** (3–30 mg/kg, po, dosed in 0.25% methylcellulose) on deprivation-induced feeding in rats. Cumulative food intake was significantly suppressed out to 6 h at all doses tested (*$p < 0.01$). Values are means ± SEM.

decreased in drug-treated animals (and in the pair-fed group) relative to vehicle-controls ($p < 0.001$; Fig. 2A). Body weight was also significantly suppressed in both groups ($p < 0.001$). Interestingly, although the pair-fed and drug-treated groups ate the same amount of food, rats given compound **10** lost significantly more weight than their pair-fed counterparts ($p < 0.001$; Fig. 2B). This finding suggests that in addition to anorectic effects, antagonism of the MCH1-R can result in metabolic changes.

In summary, SAR around 1-(4-amino-phenyl)-pyrrolidin-3-yl-amine and its closely related analog 6-(3-amino-pyrrolidin-1-yl)-pyridin-3-yl-amine yielded potent



Scheme 4. Reagents and conditions: (a) CH_3CN, rt, 4 h, 80%; (b) H_2, Pd/C, THF, 1 h, 81%; (c) 4-biphenylcarbonyl chloride, Et_3N, DCM, 3 h, 42–97%; (d) HCl, ether, 5 h, 89%; and (e) **2b** in DMSO, 100 °C, 72 h, 12%.

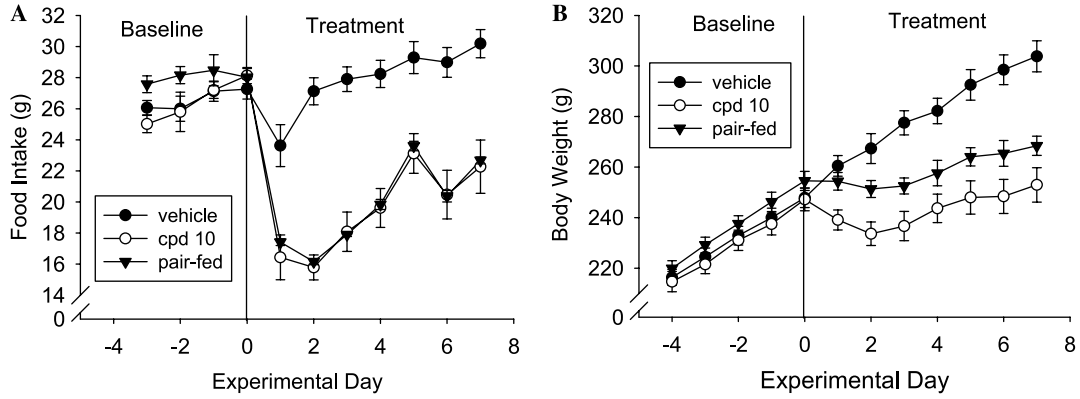


Figure 2. Effect of compound **10** delivered via osmotic pump (10 mg/kg/day) over 7 days on food intake (A) and body weight (B) in rats. Both food intake and body weight were significantly suppressed in compound **10**-treated and pair-fed animals relative to controls (p's < 0.001). Furthermore, body weight was significantly lower in rats given compound **10** compared to pair-fed animals ($p < 0.001$). Values are means ± SEM.

MCH-R1 antagonists.[22] Variation of the substitutions on the carboxamide showed that a bi-aryl group was preferred with the outside phenyl group as the critical receptor-binding component. At the other end of the molecule, a basic amine was required to give high potency, although substitution on the basic amine was not crucial for the binding. One of the potent compounds demonstrated good oral bioavailability and in vivo efficacy in rats. The SAR knowledge from this work provided the starting point for design and optimization of the next generation MCH-R1 antagonists.

Acknowledgments

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16. All compounds described here were assayed for their ability to competitively displace radiolabeled [^{125}I-Tyr^{13}]-MCH from HEK 293 cells expressing human MCH1-R1 that has been modified for optimal expression. K_i values are averages of at least three determinations.
17. ACDLabs8.0 was used for the calculation.
18. The measurement of water solubility was performed empirically as follows: 25 mg of a compound as its salt form was initially stirred with a minimum volume of DI

water (such as 0.5 ml) for at least 10 min, then additional water may be added with stirring until the solid completely dissolved. Solubility was then calculated by 25 mg/volume of water. The pH was not controlled during the measurement.
19. Elemental analysis indicated that the salt contained two molecules of hydrogen chloride.
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21. po dose (10 mg/kg): C_{max} = 82.6 ng/ml, AUC (0–24 h) = 393 ng h/ml; iv dose(10 mg/kg): Cl = 139.3 ml/min kg; Vd = 32 L/kg, AUC (0–24 h) = 1236 ng h/ml; brain AUC (0–24 h) = 8905 ng h/ml (from iv dose).
22. Sometime after this work was completed, a patent application (WO2004072025) claiming molecules related to this two series has appeared.