Bioorganic & Medicinal Chemistry Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Muscarinic acetylcholine receptor binding affinities of pethidine analogs

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ARTICLE INFO

Article history:
Received 1 September 2015
Revised 7 October 2015
Accepted 12 October 2015
Available online xxxx

Keywords:
Pethidine
Muscarinic acetylcholine receptors
[^{3}H]NMS binding affinity
Drug abuse

ABSTRACT

A series of pethidine analogs were synthesized and their affinities for the [^{3}H]*N*-methyl-scopolamine (NMS) binding site on muscarinic acetylcholine receptors (mAChRs) were determined using M_1, M_3 or M_5 human mAChRs expressed by Chinese hamster ovary (CHO) cell membranes. Compound **6b** showed the highest binding affinities at M_1, M_3 and M_5 mAChRs (K_i = 0.67, 0.37, and 0.38 μM, respectively).

Many drugs of abuse including cocaine, amphetamine, methamphetamine, and morphine increase extracellular dopamine (DA) in the nucleus accumbens (NAc) at doses that produce rewarding effects.[1] Indeed, bilateral microinjection of 6-hydroxydopamine which produces dopaminergic neuron damage in NAc inhibited initiation of amphetamine self-administration in rats when 6-hydroxydopamine was administered before self-administration training, and disrupted responding during maintenance of amphetamine self-administration.[2] DA containing ventral tegmental area (VTA) neurons project to NAc, and are important for drug seeking behavior. Electrical stimulation of acetylcholine-containing laterodorsal tegmental nucleus (LDT) neurons, which innervate VTA dopaminergic neurons, results in an increase in extracellular DA concentrations in NAc.[3,4] The five subtypes of mAChRs (M_1–M_5) are separated into two groups depending on their G_α protein coupling functionality. M_1, M_3, and M_5 mAChR subtypes preferentially activate $G_{\alpha q/11}$ proteins, leading to an increase in cytosolic calcium ion concentration. M_2 and M_4 mAChR subtypes preferentially couple with $G_{\alpha i/o}$ protein, inhibiting the conversion of adenosine triphosphate to cyclic adenosine monophosphate in the cytosol.[5] Important to the current study, M_5 mAChRs are highly expressed on the postsynaptic DA neurons in VTA.[6–9] In M_5 mAChRs knockout (KO) mice, LDT stimulation and morphine-induced DA release in NAc is reduced relative to wild-type mice.[10,11] M_5 KO mice also have decreased cocaine self-administration and cocaine- or morphine-induced conditioned place preference when compared to wild-type controls.[11,12] Microinfusion into VTA of scopolamine, a mAChR antagonist, robustly decreased cocaine-seeking behavior during withdrawal in rats.[13] Together, these findings led us to hypothesize that selective antagonism of M_5 mAChRs represents a novel target for the treatment of drug abuse.

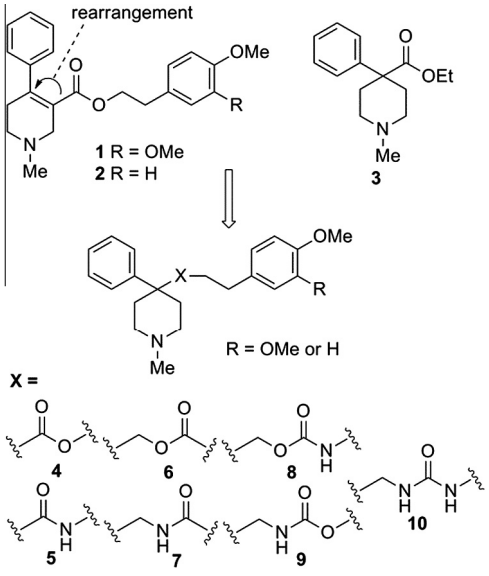

Figure 1. Structure of compounds **1** and **2**, pethidine (**3**), and design of pethidine analogs **4**–**10** as novel mAChR ligands.

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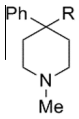
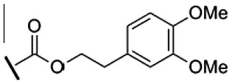
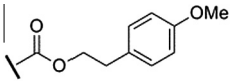
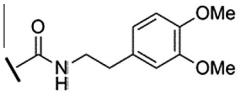
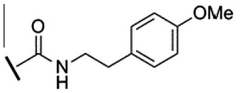
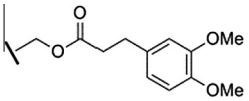
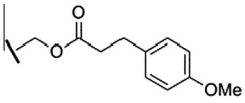
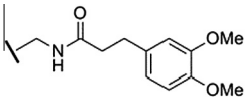
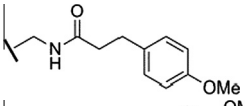
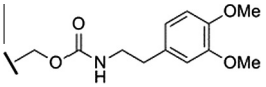
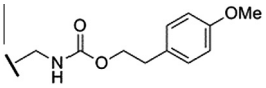
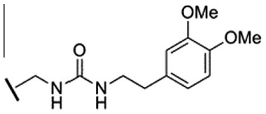
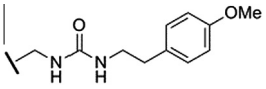
http://dx.doi.org/10.1016/j.bmcl.2015.10.029
0960-894X/

Table 1
Structures and binding affinity for analogs at M_1, M_3, and M_5 mAChRs[a]

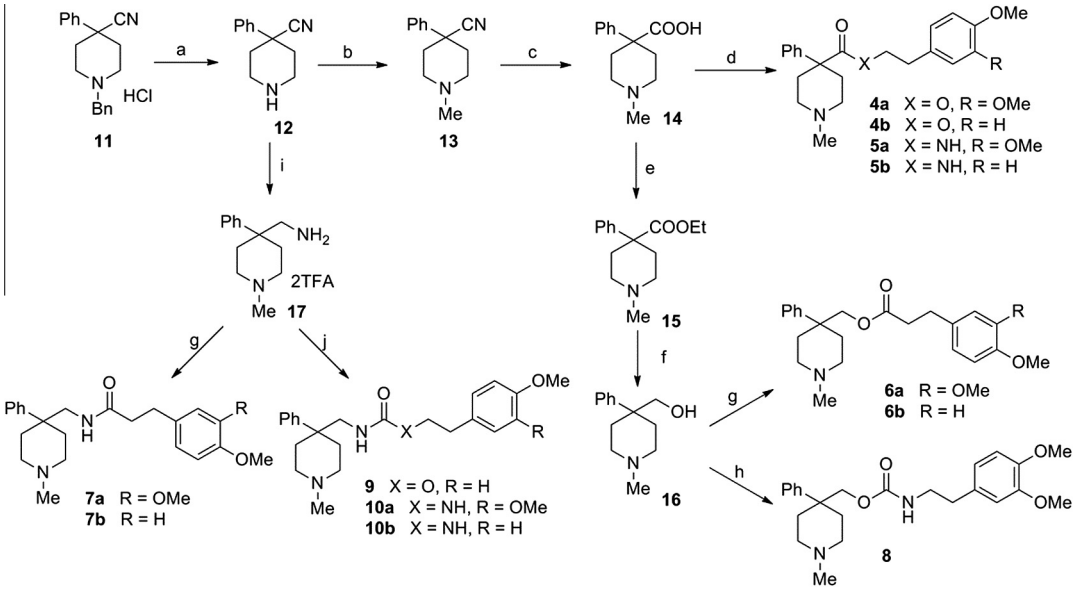


Compd	R	[^{3}H]NMS binding K_i ± SEM (μM)		
		M_1	M_3	M_5
1[b]	–	25.3	>100	2.24
2[b]	–	0.02 ± 0.002	ND[c]	0.03 ± 0.005
4a		10.8 ± 0.67	5.26 ± 0.33	6.95 ± 0.47
4b		1.20 ± 0.11	0.64 ± 0.037	0.87 ± 0.053
5a		>30	>10	>30
5b		>10	3.29 ± 0.80	6.97 ± 0.77
6a		3.63 ± 0.22	4.60 ± 0.61	2.14 ± 0.22
6b		0.67 ± 0.078	0.37 ± 0.045	0.38 ± 0.011
7a		>10	>30	>10
7b		3.44 ± 0.93	1.54 ± 0.19	1.81 ± 0.11
8		5.09 ± 0.29	4.03 ± 0.57	5.41 ± 0.59
9		2.91 ± 0.17	2.30 ± 0.22	2.05 ± 0.30
10a		>10	>10	>10
10b		5.40 ± 1.22	3.55 ± 0.11	4.30 ± 0.29

[a] Three independent experiments, each experiment included duplicate samples, were performed to obtain K_i values (mean ± SEM).
[b] Data from Ref. 14.
[c] Not determined.

We recently reported on a class of M_5-preferring orthosteric antagonists based on the scaffold of 1,2,5,6-tetrahydropyridine-3-carboxylic acid.[14] Compound **1** (Fig. 1, Table 1) was identified as the most selective M_5 mAChRs antagonist in this series. Interestingly, removal of the meta-methoxy group in **1** (compound **2**) significantly increased binding affinities at both M_1 and M_5 receptors, but resulted in a complete loss in selectivity for M_5 over M_1. To further explore the structure–activity relationship (SAR), we planned to reposition the carboxylate group in **1** and **2** from C-3 to C-4 of the piperidine ring. New analogs resulted from such rearrangement resembling pethidine (**3**, Fig. 1), a once popular analgesic. Interestingly, pethidine has been identified as an antagonist at mAChRs in guinea-pig ileum assays.[15] Thus, analogs based on the pethidine scaffold may afford interesting SAR at mAChRs. In addition to ester containing analogs (**4** and **6**), we also planned to evaluate amides (**5** and **7**), carbamates (**8** and **9**), and carbamides (**10**). Herein, we describe the synthesis of these novel analogs and the evaluation of their binding affinity at mAChRs.

Please cite this article in press as: Lee, N.-R.; et al. *Bioorg. Med. Chem. Lett.* (2015), http://dx.doi.org/10.1016/j.bmcl.2015.10.029



Scheme 1. Reagents and conditions: (a) $Pd(OH)_2$, H_2, MeOH; (b) HCHO (37% aqueous), $NaBH(OAc)_3$, THF; (c) 6 N HCl, reflux; (d) (1) $SOCl_2$, DCM, reflux; (2) alcohol or amine, TEA, DCM; (e) EtOH, $SOCl_2$, reflux; (f) LAH, THF, 0 °C–rt; (g) 3-(3,4-dimethoxy or 4-methoxy)phenylpropanoyl chloride, TEA, DCM; (h) (1) 4-nitrophenyl chloroformate, Na_2CO_3, THF; (2) 2-(3,4-dimethoxyphenyl)ethanamine Na_2CO_3, THF; (i) (1) LAH, THF, 0 °C–rt; (2) Boc_2O, Na_2CO_3, THF/H_2O; (3) TFA/DCM; (j) (1) 4-nitrophenyl chloroformate, Na_2CO_3, THF; (2) alcohol or amine, Na_2CO_3, THF.

Compounds **4a**, **4b**, **5a**, and **5b** were synthesized by converting 1-methyl-4-phenylpiperidine-4-carboxylic acid (pethidinic acid, **14**) to the corresponding carbonyl chloride in the presence of $SOCl_2$, followed by reacting with a phenyl ring substituted 2-phenylethanol or 2-phenylethanamine (Scheme 1). Compound **14** was synthesized by initial catalytic hydrogenolysis of compound **11** under $Pd(OH)_2$ to afford **12**, followed by N-methylation to form compound **13** and hydrolysis of the cyano group. Conversion of **14** to alcohol **16** was achieved by a standard two-step procedure. Esterification between phenyl ring substituted 3-phenylpropanoyl chloride and **16** afforded compounds **6a** and **6b**. Treatment of **16** with 4-nitrophenyl chloroformate followed by reaction with 2-(3,4-dimethoxyphenyl)ethanamine provided carbamate **8**. Similarly, amides **7a** and **7b**, carbamate **9**, and carbamides **10a** and **10b** were synthesized from amine intermediate **17**. A three-step process of an initial LAH reduction of the cyano group in **12** to amino group, followed by Boc protection to reduce polarity for column purification and de-Boc afforded **17**.

Subtype selectivity for M_5 over M_1 and M_3 mAChRs is particularly difficult to achieve because M_5 exhibit the high amino acid sequence identity with M_3 and M_1 mAChRs (85%, 79%, 73%, and 68% with M_3, M_1, M_4, and M_2 mAChRs, respectively).[16] In addition, all three subtypes prefer to bind $G_{\alpha q/11}$. Thus, in this study, we evaluated binding affinity of our analogs at M_1, M_3, and M_5 mAChR subtypes as a first approach, and then compared selectivity of analogs at M_5 over M_1 and M_3 mAChRs. Analog affinities for M_1, M_3 and M_5 mAChRs were determined by measuring inhibition of [^{3}H]*N*-methylscopolamine (NMS) binding to Chinese hamster ovary (CHO) cell membranes expressing M_1, M_3, or M_5 recombinant human mAChRs. CHO cells stably expressing each of the human mAChRs were obtained from Dr. Tom Bonner of National Institute of Mental Health (NIMH). Detailed materials and methods for cell culture and cell membrane preparation were described previously.[14,17] IC_{50} values were obtained and K_i values were calculated using the equation of Cheng and Prusoff.[18] Results are summarized in Table 1.

Similar to the SAR generated for the parent compounds **1** and **2**, mono-methoxy substituted analogs (**4b**, **5b**, **6b**, **7b**, and **10b**) consistently exhibited higher affinity (2 to 9-fold at M_1; 3 to 19-fold at M_3; 2 to 8-fold at M_5) when compared to their corresponding di-methoxy substituted analogs (**4a**, **5a**, **6a**, **7a**, and **10a**, respectively). The corresponding carboxylate moiety repositioned in molecule **4a** exhibited 2- and 19-fold higher affinity at M_1 and M_3 mAChRs, respectively, compared with compound **1**. However, the affinity of **4a** at M_5 mAChRs was decreased by 30%. Thus, compound **4a** was not subtype selective. It is unclear why reposition of the carboxylate group in 1 resulted in a complete loss of subtype selectivity.

In addition, replacement of the ester link in **4a/b** or **6a/b** with an amide link (**5a/b** and **7a/b**, respectively) resulted in a loss of affinity at all three mAChR subtypes (**4a** vs **5a**, 2 to 4-fold; **4b** vs **5b**, 5 to 8-fold; **6a** vs **7a**, 3 to 7-fold; **6b** vs **7b**, 4 to 5-fold). In general, analogs with carbamate and carbamide linkers exhibited up to an 8-fold lower affinity compared to the esters. Furthermore, the reverse ester of **4a** (i.e., **6a**) exhibited a moderate 1 to 3-fold increase in affinity at all three mAChRs. A similar increase in affinity was observed for the other reverse ester/amide series, that is, **4b** versus **6b** and **5b** versus **7b**. Analog **6b** was identified as the most potent compound at M_5 in this series.

In summary, a series of pethidine analogs was synthesized and evaluated to determine binding affinity for the [^{3}H]NMS binding site on M_1, M_3, and M_5 human mAChRs expressed by CHO cell membranes. Compound **6b** showed the highest binding affinity at M_1, M_3 and M_5 mAChRs (K_i = 0.67, 0.37, and 0.38 μM, respectively). However, this series of new analogs did not exhibit selectivity for M_5 mAChRs over M_1 and M_3 subtypes. Further SAR and pharmacological evaluations are needed to identify potent and selective M_5 mAChR antagonists. Additionally, pethidine has been reported to have weak μ-opioid receptor agonist activity.[19] Thus, in future studies, once analogs are demonstrated to have high affinity and selectivity for M_5 mAChRs, they will be evaluated also for μ-opioid receptor affinity to assure selectivity at the M_5 mAChR target.

Acknowledgment

This work was supported by funding from the National Institutes of Health (DA030667 and UL1 TR000117).

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16. Bonner, T. I.; Young, A. C.; Brann, M. R.; Buckley, N. J. *Neuron* **1988**, *1*, 403.
17. [^{3}H]NMS binding affinity method: [^{3}H]NMS binding assays were performed using 96-well plates. Membrane aliquots containing 10 μg of protein per well for M_1 or M_5 subtypes and 3 μg of protein per well for M_3 subtype were added to wells containing 1 nM to 100 μM of test analog, 0.3 nM [^{3}H]NMS (specific activity 84.2 Ci/mmol; Perkin-Elmer/NEN, Boston, MA), and buffer (50 mM Tris–HCl, pH 7.4), and then incubated for 2 h at 25 °C. Nonspecific binding of [^{3}H]NMS was determined in the presence of 1 μM atropine. Reactions were terminated by rapid filtration onto GF/B filters using a Filtermate harvester (PerkinElmer Life and Analytical Sciences, Boston, MA) and washed three times with buffer (50 mM Tris–HCl, pH 7.4). Subsequently, 40 μL of MicroScint 20 (PerkinElmer Life and Analytical Sciences, Waltham, MA) was added to each well and radioactivity bound determined using liquid scintillation spectrometry.
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