Bioorganic & Medicinal Chemistry 22 (2014) 1838–1844

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Evaluation of 1,2,5-thiadiazoles as modulators of M_1/M_5 muscarinic receptor subtypes

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ARTICLE INFO

Article history:
Received 21 October 2013
Revised 20 January 2014
Accepted 30 January 2014
Available online 7 February 2014

Keywords:
Muscarinic receptor
M_5 antagonist
Drug abuse

ABSTRACT

Studies have demonstrated the presence of allosteric binding sites on each of the muscarinic acetylcholine receptor (mAChR) subtypes. Since most drugs targeting muscarinic receptors bind to the highly conserved orthosteric binding site, they fail to achieve appreciable subtype selectivity. Targeting non-conserved allosteric sites may provide a new way of enhancing selectivity for individual subtypes of muscarinic receptor. Tetra(ethyleneglycol)(3-methoxy-1,2,5-thiadiazol-4-yl)[3-(1-methyl-1,2,5,6-tetrahydropyrid-3-yl)-1,2,5-thiadiazol-4-yl] ether, CDD-0304 (**10**), was found to be a $M_{1/2/4}$ selective muscarinic agonist and might prove useful in treating the symptoms associated with schizophrenia (*J. Med. Chem.* **2003**, *46*, 4273). It was hypothesized that the observed subtype selectivity demonstrated by **10** may be due to its ability to function as a bitopic ligand (*J. Med. Chem.* **2006**, *49,* 7518). To further investigate this possibility, a novel series of compounds was synthesized using a 1,2,5-thiadiazole moiety along with varying lengths of a polyethylene glycol linker and terminal groups, for evaluation as potential allosteric modulators of muscarinic receptors. Preliminary biological studies were performed using carbachol to stimulate M_1 and M_5 receptors. No significant agonist activity was observed at either M_1 or M_5 receptors for any of the compounds. Compound **18**, 2-(4-methoxy-1,2,5-thiadiazol-3-yloxy)-*N*,*N*-dimethylethanamine fumarate (CDD-0361F) was found to block the effects of carbachol at M_5 muscarinic receptors.

1. Introduction

Muscarinic receptors contain a classical acetylcholine binding site (orthosteric binding site), with high sequence homology among receptor subtypes, and allosteric binding sites which are topographically distinct from the orthosteric binding site, and less highly conserved between muscarinic receptor subtypes.[3] The orthosteric site is responsible for acetylcholine (ACh) binding and most drugs that bind to the highly conserved orthosteric site of muscarinic receptors fail to achieve subtype selectivity. Most reportedly selective muscarinic agonists exhibit some binding affinity for all five muscarinic receptor subtypes, but show functional selectivity in activating one or more subtypes preferentially.

In contrast, the binding of an allosteric ligand to an allosteric binding site may alter the conformation of the orthosteric site on muscarinic receptors and may result in either an increase (positive allosterism) or a decrease (negative allosterism) in the affinity of the receptor for muscarinic agonists, such as ACh, and/or classical antagonists. With neutral cooperativity, allosteric compounds bind to the receptor but have no effect on the binding of a primary ligand at any concentration. Two likely sites for allosteric modulation have been identified with at least one site located on the extracellular surface of muscarinic receptors near the orthosteric binding site.[4,5] The influence of allosteric binding on the function of the classical binding site can be measured by determining changes in the association, dissociation, and equilibrium binding of muscarinic agonists and competitive antagonists,[6] or by measuring the biochemical responses produced by muscarinic agonists in the absence and presence of the putative allosteric ligand. Allosteric modulation can be helpful in increasing subtype-specificity of an agonist as well.[7,8] Allosteric muscarinic drugs might be more efficacious and have fewer side effects than classic muscarinic agonists and antagonists.

Several novel monovalent muscarinic agents that bind to their target receptors through allosteric mechanisms are reported in the literature (Fig. 1). N-heterocyclic derivatives of quinolone

Abbreviations: ACh, acetylcholine; CCh, carbachol.

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http://dx.doi.org/10.1016/j.bmc.2014.01.049
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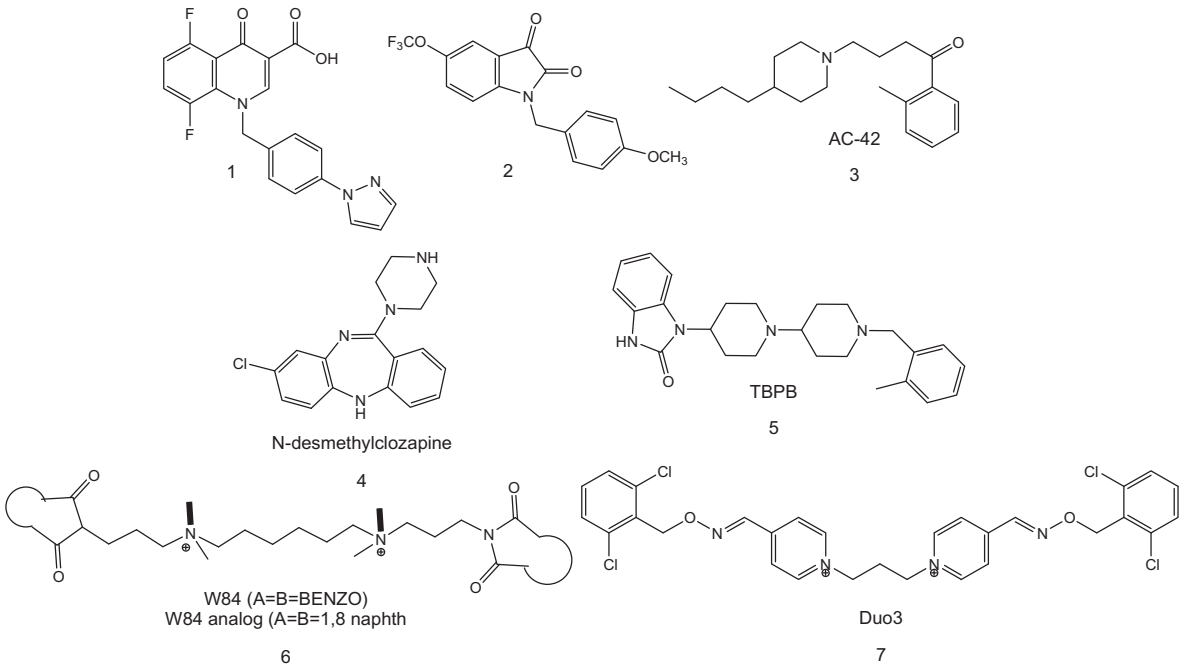


Figure 1. Selective muscarinic agonists: allosteric compounds.

carboxylic acid (**1**) recently were reported to be M_1 selective positive allosteric modulators.[9] VU0238429 (**2**) provides >30 fold selectivity for M_5 versus the other four receptor subtypes and represents the first highly selective M_5 positive allosteric modulator.[10] AC-42 (4-*n*-butyl-1-[4-(2-methylphenyl)-4-oxo-1-butyl]-piperidine hydrogen chloride) (**3**) and *N*-desmethylclozapine (**4**) activate M_1 mAChRs with a considerable degree of selectivity by interacting with non-orthosteric receptor binding sites.[11,12] TBPB (1,3-dihydro-1-[1′-[(2-methylphenyl)methyl][1,4′-bipiperidin]-4-yl]-2*H*-benzimidazol-2-one) (**5**) was found to be a selective allosteric activator for M_1 receptors. It displayed antipsychotic like activity in rodent models and also decreased the production of Aβ in vitro by increasing non-amyloidogenic processing of APP.[13] The bisbenzyl bispyridinium (DUO series) and W-84 have been shown to be potent allosteric modulators of muscarinic M_2 muscarinic receptors[14–19] (compounds **6** and **7**). Hence novel selective allosteric compounds provide a new way of enhancing selectivity for individual muscarinic receptor subtypes and reducing the potential for adverse effects.

Xanomeline (**8**) is reported to display two different modes of binding at M_1 muscarinic receptors, with the first a reversible binding through interaction with the orthosteric site and the second a wash-resistant and very stable binding that takes place at a different position which is accompanied by marked allosteric modulation of competitive ligands with the receptor[7] (Fig. 2). Aceclidine analogs carrying a 1,2,5-thiadiazole moiety have also been identified as potent M_1 receptor agonists.[20] Another selective M_1 agonist is the bivalent compound tetra(ethylene glycol) di[3-(1-methyl-1,2,5,6-tetrahydropyrid-3-yl)-1,2,5-thiadiazol-4-yl] ether, CDD-0273 (**9**), which contains two xanomeline pharmacophores connected through a tetraethylene glycol linker[21] (Fig. 2). Binding of one agonist pharmacophore increases the affinity for the other at M_1 receptors and hence results in high selectivity and activity at M_1 receptors. It may be possible for both **9** (CDD-0273) and **10** (CDD-0304) to behave as bitopic ligands, which contain separate orthosteric and allosteric moieties separated by a tetraethylene glycol linker, and can, theoretically, bind to both the orthosteric and allosteric sites (Fig. 2). For these molecules, interactions with the allosteric site at M_1 receptors may help in achieving subtype selectivity and interactions at orthosteric sites may promote receptor activation.

Receptor binding studies at wild-type and mutant (Thr192Ala) M_1 receptors studies were helpful for identifying the interaction of muscarinic agonists with amino acid residues present in the transmembrane domain of M_1 receptors. Thr192 was found to be an important amino acid residue involved in the binding of the muscarinic agonists xanomeline[22] and **10**. Since xanomeline shares many structural features with compounds **9** and **10**, the data suggest that the pharmacophore common between these compounds might be interacting with transmembrane domains of M_1 receptors. Chimeric receptor data and site directed mutagenesis studies suggest that amino acids found in the second and third extracellular loops of the M_1 receptor are critical for binding and activity of **10**.[2] In particular Glu170 and Gln185 present in second extracellular loop were identified as important residues contributing to the potency and activity of **10** at M_1 receptors as compared with M_5 receptors (Fig. 3). The tetraethylene glycol linker present in **10** may position the 3-methoxy-1,2,5-thiadiazole to allow interactions with amino acids present in loop regions.

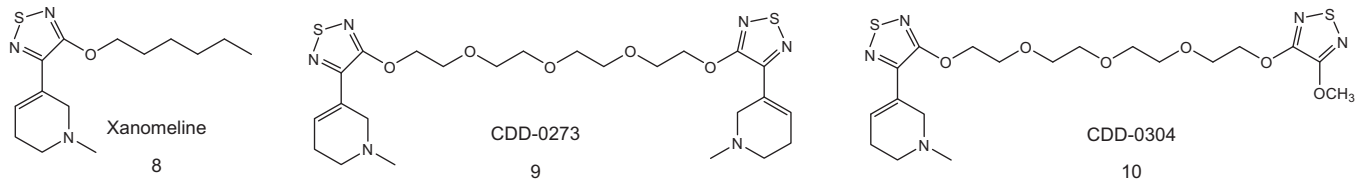


Figure 2. Selective muscarinic agonists: bitopic ligands.

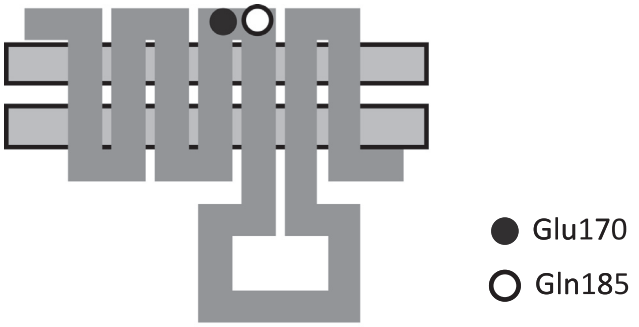


Figure 3. Schematic depiction of the M_1 muscarinic acetylcholine receptor.

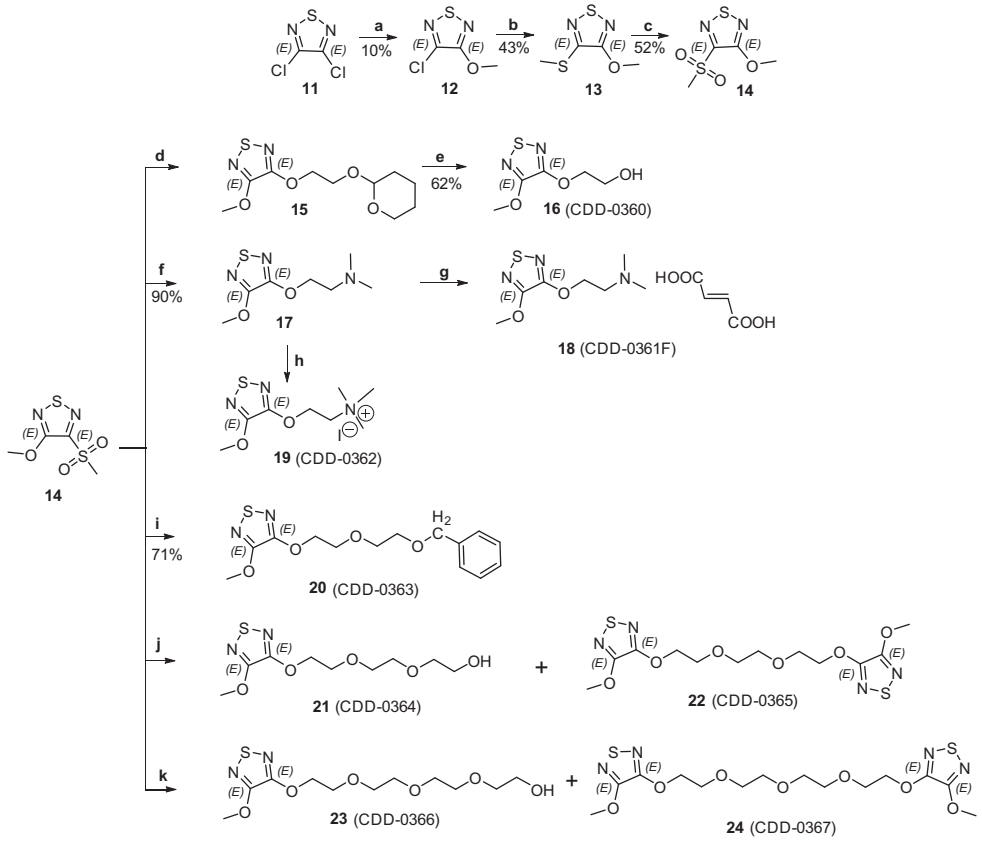
To explore the possibility that this interaction is allosteric, several novel analogs of 3-methoxy-1,2,5-thiadiazole containing varying lengths of a polyethylene glycol linker were synthesized. If this interaction is allosteric then these compounds could modulate the binding of ligands that interact only with the orthosteric site of muscarinic receptors, like carbachol.

2. Materials and methods

2.1. Chemistry

As shown in Scheme 1, compound **12**[1] was synthesized in 10% yield and converted to **14** using established methods.[23] 3-Methylsulfonyl-4-methoxy-1,2,5-thiadiazole offered two advantages over 3-chloro-4-methoxy-1,2,5-thiadiazole (CMT). Firstly, it has a melting point of 110.5 °C and hence is more compatible than CMT (which sublimates at 52.5 °C) for use at high temperatures. Additionally, sulfone is a much better leaving group than the chloro group and hence can be more easily replaced by other nucleophiles.

Compound **14** was converted to compound **15** using 2-(tetrahydro-2*H*-pyran-2-yloxy)-ethanol and then the tetrahydropyranyl group was removed under mild acidic conditions to provide **16** (CDD-0360). Compound **17** was synthesized from compound **14** using commercially available *N*,*N*-dimethylethanolamine and then converted to the fumarate salt **18** (CDD-0361F). Compound **17** also was converted to the quaternary amine **19** (CDD-0362), using methyl iodide in quantitative yield. Compound **20** (CDD-0363) was synthesized from compound **14** using di(ethylene glycol) benzyl ether and potassium *tert* butoxide. The benzyl group could not be removed under hydrogenation even using high pressures. It could be removed however, under 1,2-dichloro-4,5-dicyanobenzoquinone (DDQ)/acetonitrile/80°C[24] but the reaction was not clean and the resulting compound could not be purified. Compound **14** was treated with triethylene glycol and potassium *tert* butoxide to yield **21** (CDD-0364) as the major product in 45% yield and **22** (CDD-0365) as a minor product in 15% yield. Similarly, compound **14** was converted to **23** (CDD-0366) as the major product in 41% yield and **24** (CDD-0367) as a minor product in 8% yield using tetraethylene glycol. The ^1H and ^{13}C spectra (D_2O) of **19** were comparable with commercially available acetylcholine chloride due to high structural resemblance.[25]



Scheme 1. Synthesis of compounds **16** through **24**. Reagents and conditions: (a) Methanol, sodium hydride, THF, overnight; (b) sodium hydrosulfide hydrate, iodomethane, DMF, rt; (c) potassium peroxymonosulfate, water, 5 h; (d) 2-(tetrahydro-2*H*-pyran-2-yloxy)-ethanol, potassium *tert*-butoxide, THF, rt, overnight; (e) *p*-toluene sulfonic acid, methanol, overnight; (f) *N*,*N*-dimethylethanolamine, potassium *tert*-butoxide, THF, rt, overnight; (g) fumaric acid, ethanol; (h) iodomethane, acetone, 5 h; (i) di(ethylene glycol) benzyl ether, potassium *tert*-butoxide, THF, rt, overnight; (j) potassium *tert*-butoxide, triethylene glycol, THF, rt, overnight; (k) potassium *tert*-butoxide, tetraethylene glycol, THF, rt, overnight.

2.2. Pharmacology

For in vitro biological evaluations, a phosphatidylinositol (PI) turnover assay (for G_q coupled receptors) was employed to explore the potential allosteric activity of novel compounds. The procedure for determining levels of inositol phosphates following the stimulation of muscarinic receptors has been described previously.[1,2] In brief, A9 L cell lines stably expressing human muscarinic receptors (M_1 or M_5 receptors through plasmids obtained from Missouri S&T cDNA Resource Center) were seeded in 96-well tissue culture plates (100 μl with approximately 30,000 cells).

Cells were incubated for 24 h at 37 °C in an incubator conditioned with 5% CO_2. The following day, the plates were rinsed twice with 200 μl PBS. To radiolabel the cells, 100 μl of inositol free (IF) DMEM supplemented with D-glucose (25 mM), L-glutamine (4 mM), BSA (0.6%) and [^{3}H]-inositol (10 μCi/ml) was dispensed into each well. The labeled cells were incubated overnight at 37 °C with 5% CO_2.

The next day, test ligand dilutions were prepared in HBSS buffer supplemented with LiCl (10 mM) and HEPES (20 mM). Receptor activation was initiated by addition of 100 μl of the appropriate concentration of test ligands (with buffer serving to measure basal [control] levels) to each well in triplicate sets. The cells were incubated subsequently at 37 °C (5% CO_2) for 1 h. Receptor stimulation was terminated by rapid removal of all media. Then, 100 μl of ice-cold formic acid (50 mM) was added to each well prior to incubation at room temperature for 20 min.

During this incubation, 80 μl of YSi-SPA beads (1 mg/80 μl water) was added to a 96 well white plate. After incubation, the plate containing cells was tapped on all sides to obtain a homogeneous cell lysate. 20 μl of the cell extract in formic acid was added to corresponding wells containing SPA beads (white plate). In order to get a blank reading corresponding to formic acid, 20 μl of formic acid also was assessed for activity. The 96 well (white) plates were sealed using TopSeal A and contents were mixed via shaking for 1 h at 4 °C at 200 rpm using an orbital shaker. After standing at 4 °C for 2 h or overnight, radioactivity in counts per minute (CPM) was determined using the Topcount NXT system. Activity was presented either as the percentage activation above basal levels (CPM-CNTRL/CNTRL-BLANK * 100) or counts per minute. Experiments were repeated three times to confirm the activity of all the tested compounds. Carbachol served as a positive control for muscarinic receptor activation in each assay. Two factor analysis of variance (ANOVA) was used to assess the impact of varying concentrations of carbachol and synthesized compounds on phosphoinositide metabolism, with α set at 0.05.

3. Results and discussion

Compound **18** (Fig. 4) did not elicit a pharmacological response at either M_1 or M_5 receptors. At M_1 muscarinic receptors, the carbachol response was not significantly different from the response produced by carbachol in the presence of 1 μM and 100 μM **18** (P >0.05). However, at M_5 muscarinic receptors, **18** inhibited the

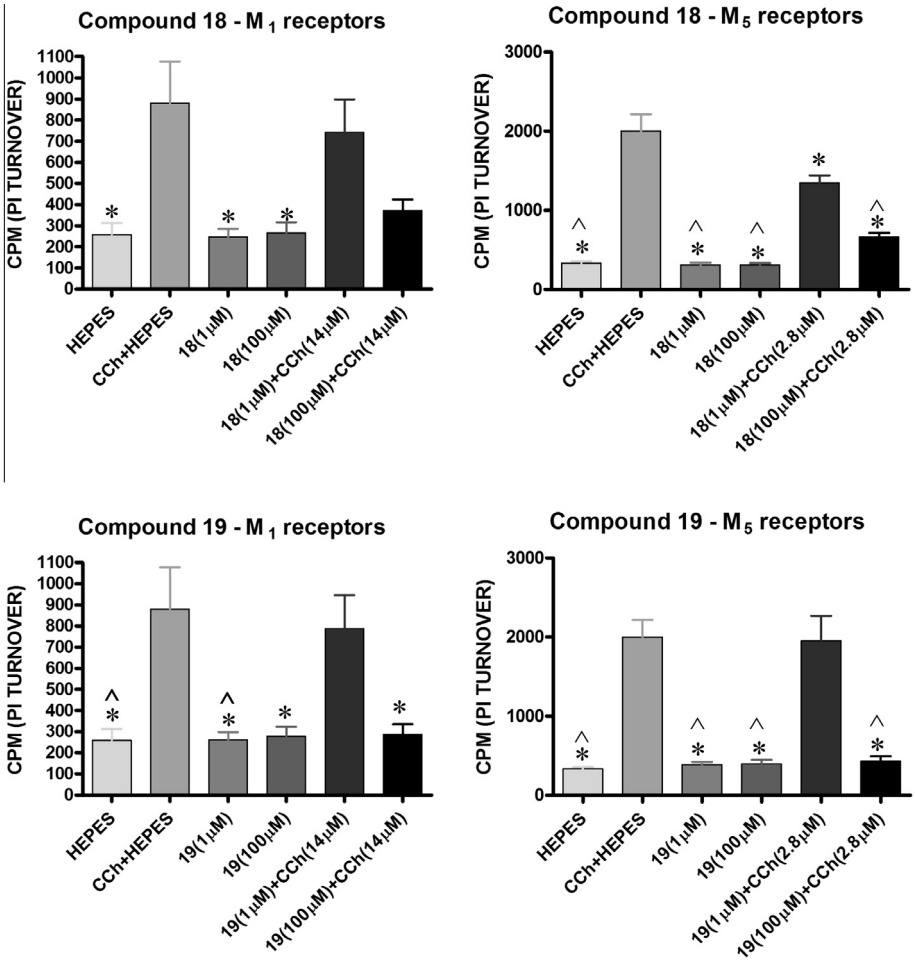


Figure 4. Stimulation of phosphoinositide metabolism by carbachol, **18** and **19** (separately and combined) at M_1 and M_5 muscarinic receptors expressed in A9 L cells. Data represent the mean (±SEM) of three experiments each performed in triplicate. *Significantly different from carbachol + HEPES values (P <0.05); ^significantly different from compound (1 μM) + carbachol values (P <0.05); #significantly different from compound (100 μM) + CCh values (P <0.05).

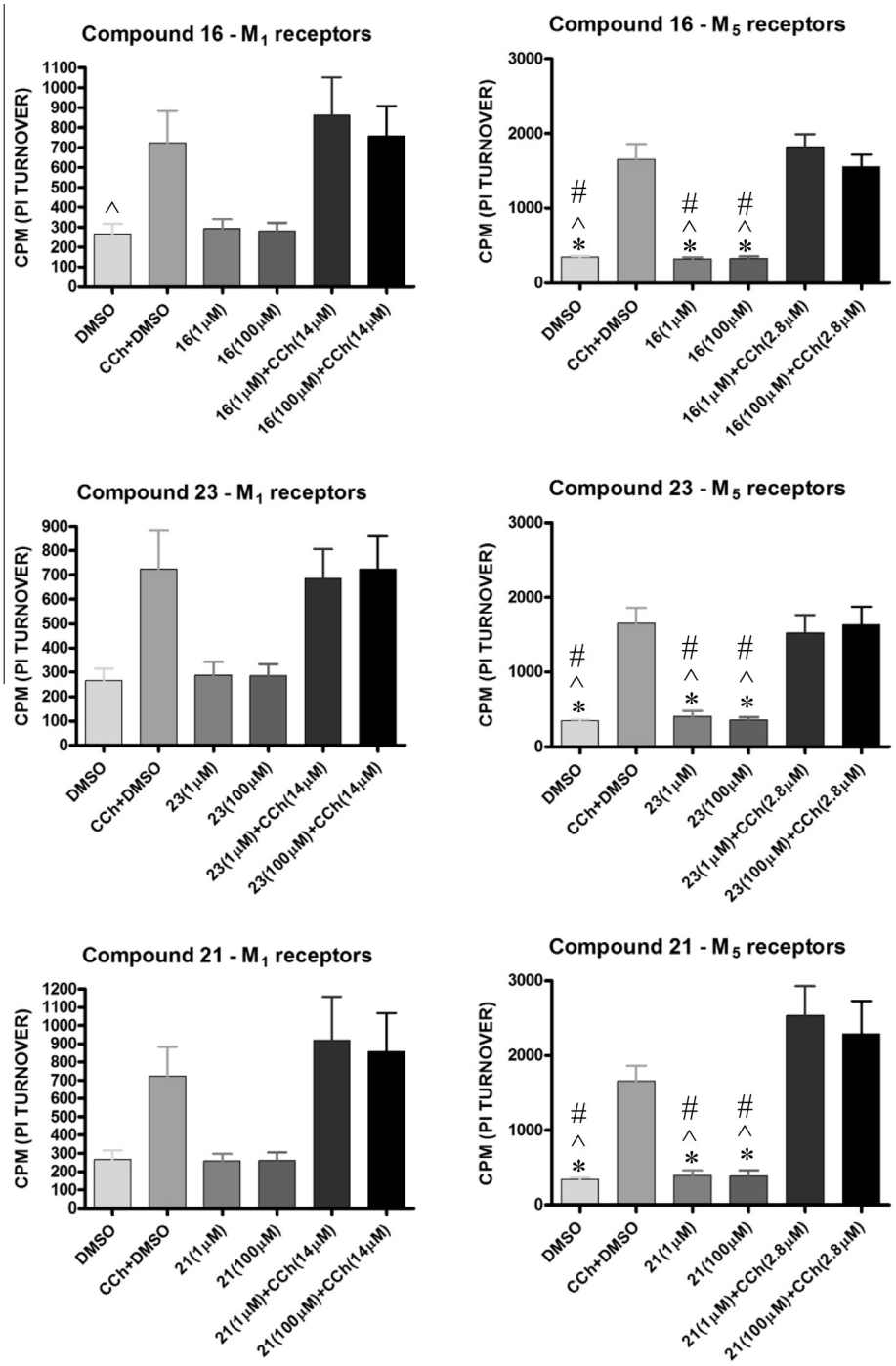


Figure 5. Stimulation of phosphoinositide metabolism by carbachol, **16**, **21** and **23** (separately and combined) at M_1 and M_5 muscarinic receptors expressed in A9 L cells. Data represent the mean (±SEM) of three experiments each performed in triplicate. *Significantly different from CCh + DMSO values (P <0.05); ^significantly different from compound (1 μM) + CCh values (P <0.05); #significantly different from compound (100 μM) + CCh values (P <0.05).

response of carbachol at both concentrations (P <0.05) consistent with potential antagonist activity.

Compound **19** (Fig. 4) was inactive at M_1 and M_5 receptors by itself. When tested along with carbachol at both M_1 and M_5 receptors, it inhibited the response of carbachol at the 100 μM concentration, suggesting it may act as an antagonist. The statistical analysis indicated that the (drug + carbachol) response was significantly different from that of carbachol itself at 100 μM of **19** (P <0.05).

Compounds **16** and **23** (Fig. 5), on the other hand, were unable to stimulate M_1 or M_5 receptors. Moreover, **16** and **23** did not alter the response of carbachol at either the 1 μM or 100 μM concentration (P >0.05).

Compound **21** (Fig. 5) did not possess any intrinsic activity at M_1 and M_5 receptors, and did not significantly alter carbachol responses at either concentration of **21** (P >0.05). Compound **20** along with the bivalent ligands **22** and **24** exhibited poor solubility, even in a 50% DMSO solution, and hence could not be tested for activity at muscarinic receptors.

To further understand the mode of antagonism (competitive or allosteric) for **18**, a Schild regression analysis was performed at M_1 and M_5 receptors. This analysis is based on the principle that with

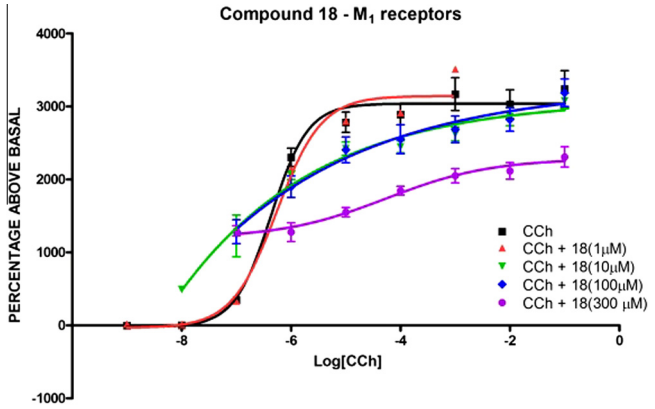


Figure 6. The dose–response curve for carbachol in the absence and presence of **18** (1 μM, 10 μM, 100 μM and 300 μM) at M_1 muscarinic receptors.

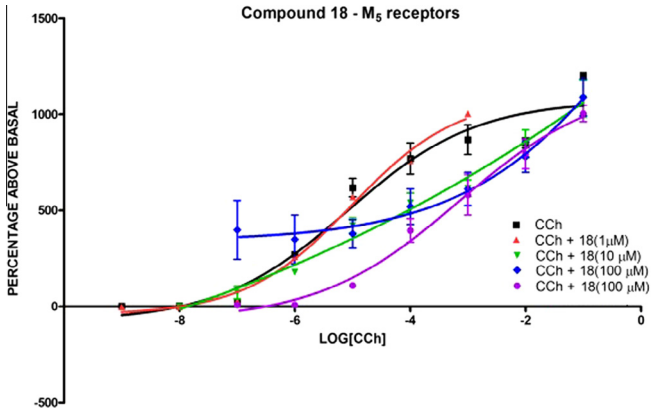


Figure 7. The dose–response curve for carbachol in the absence and presence of **18** (1 μM, 10 μM, 100 μM and 300 μM) at M_5 muscarinic receptors.

increasing concentrations of an antagonist, the dose–response curve of the agonist will shift to the right in a parallel manner for competitive antagonism. That is, a higher concentration of agonist would be required to achieve the same level of activation in the presence of an antagonist. Dose–response curves for carbachol were obtained in the absence and presence of fixed concentrations of **18** (1 μM, 10 μM, 100 μM or 300 μM). The response was measured using the well-established PI turnover assay.

At M_1 receptors (Fig. 6), **18** did not produce a shift in the carbachol response at the 1 μM concentration. However, at 10 μM and 100 μM, **18** did produce a shift along with an increase in the baseline value. Similarly, at the 300 μM concentration of **18**, the dose–response curve for carbachol further shifted towards right but the baseline increased dramatically to give almost a straight line. These observations rendered calculations for two data points unfit for the logarithmic plot, thereby limiting the usefulness of the data for a Schild analysis.

At M_5 muscarinic receptors (Fig. 7), low concentrations of **18** shifted the carbachol response curve towards the right in a dose dependent manner suggesting competitive inhibition. However, at the 100 μM concentration, compound **18** elevated the responses produced by low concentrations of carbachol suggesting a more complex interaction with M_5 receptors. Therefore, similar to the results from the interaction of compound **18** at M_1 receptors, the logarithmic plot did not permit an evaluation of the type of interaction that compound **18** exerts at M_5 receptors in the presence of carbachol.

Overall, the synthesized analogs of 3-methoxy-1,2,5-thiadiazole, carrying varying lengths of a polyethylene glycol linker, did not activate either M_1 or M_5 muscarinic acetylcholine receptors. On the other hand, compounds such as **18** and **19** possessed antagonist activities which, given their resemblance in structure to carbachol, could be attributed to possible interactions with the orthosteric site. Increasing concentrations of compound **18** did not shift the dose response curves for carbachol to the right in a parallel manner, however, suggesting that compound **18** is not a competitive antagonist. Further studies, including radioligand binding assays, are needed to evaluate the nature of the interaction of compound **18** with muscarinic receptors.

4. Conclusions

Out of five compounds (**16**, **18**, **19**, **21** and **23**) tested so far, **18** and **19** were found to possess antagonistic properties at the M_5 receptor subtype. By themselves, both these compounds were inactive at M_1 and M_5 muscarinic receptors. The data suggest that **18** and **19** behave as antagonists at M_5 receptors. These compounds might interact with the orthosteric binding site rather than at allosteric sites. The structural similarity of these compounds with carbachol might allow them to compete with carbachol for the orthosteric binding site and hence behave as antagonists at muscarinic receptors. Since the logarithmic plot was not linear in the Schild regression analysis on either M_1 or M_5 receptors, it was not possible to define the mode of antagonism. Further evaluation of these compounds at other receptor subtypes (M_2, M_3, and M_4 receptors) will help determine their selectivity. In this regard, studies focusing on assessing the binding affinity of these compounds for muscarinic receptors subtypes would be insightful. In addition, future studies will assess the ability of the compounds to modulate acetylcholine activity at M_5 receptors.

Acknowledgment

The authors would like to thank Karen Papadakis for providing secretarial assistance.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.01.049.

References and notes

1. Cao, Y.; Zhang, M.; Wu, C.; Lee, S.; Wroblewski, M. E.; Whipple, T.; Nagy, P. I.; Takacs-Novak, K.; Balazs, A.; Toros, S.; Messer, W. S., Jr. *J. Med. Chem.* **2003**, *46*, 4273.
2. Tejada, F. R.; Nagy, P. I.; Xu, M.; Wu, C.; Katz, T.; Dorsey, J.; Rieman, M.; Lawlor, E.; Warrier, M.; Messer, W. S., Jr. *J. Med. Chem.* **2006**, *49*, 7518.
3. May, L. T.; Leach, K.; Sexton, P. M.; Christopoulos, A. *Annu. Rev. Pharmacol. Toxicol.* **2007**, *47*, 1.
4. Lanzafame, A. A.; Sexton, P. M.; Christopoulos, A. *Mol. Pharmacol.* **2006**, *70*, 736.
5. Lazareno, S.; Popham, A.; Birdsall, N. J. *Mol. Pharmacol.* **2000**, *58*, 194.
6. Jakubik, J.; Bacakova, L.; Lisa, V.; el-Fakahany, E. E.; Tucek, S. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8705.
7. Jakubik, J.; Tucek, S.; El-Fakahany, E. E. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 1033.
8. Voigtlander, U.; Johren, K.; Mohr, M.; Raasch, A.; Trankle, C.; Buller, S.; Ellis, J.; Holtje, H. D.; Mohr, K. *Mol. Pharmacol.* **2003**, *64*, 21.
9. Kuduk, S. D.; Di Marco, C. N.; Cofre, V.; Pitts, D. R.; Ray, W. J.; Ma, L.; Wittmann, M.; Veng, L.; Seager, M. A.; Koeplinger, K.; Thompson, C. D.; Hartman, G. D.; Bilodeau, M. T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1334.
10. Bridges, T. M.; Marlo, J. E.; Niswender, C. M.; Jones, C. K.; Jadhav, S. B.; Gentry, P. R.; Plumley, H. C.; Weaver, C. D.; Conn, P. J.; Lindsley, C. W. *J. Med. Chem.* **2009**, *52*, 3445.
11. May, L. T.; Avlani, V. A.; Langmead, C. J.; Herdon, H. J.; Wood, M. D.; Sexton, P. M.; Christopoulos, A. *Mol. Pharmacol.* **2007**, *72*, 463.
12. Wess, J.; Eglen, R. M.; Gautam, D. *Nat. Rev. Drug Disc.* **2007**, *6*, 721–733.

13. Jones, C. K.; Brady, A. E.; Davis, A. A.; Xiang, Z.; Bubser, M.; Tantawy, M. N.; Kane, A. S.; Bridges, T. M.; Kennedy, J. P.; Bradley, S. R.; Peterson, T. E.; Ansari, M. S.; Baldwin, R. M.; Kessler, R. M.; Deutch, A. Y.; Lah, J. J.; Levey, A. I.; Lindsley, C. W.; Conn, P. J. *J. Neurosci.* **2008**, *28*, 10422.
14. Gilsbach, R.; Grossmuller, M.; Alptuzun, V.; Erciyas, E.; Trankle, C.; Holzgrabe, U.; Mohr, K. *Neurochem. Res.* **2003**, *28*, 667.
15. Holzgrabe, U.; Wagener, M.; Gasteiger, J. *J. Mol. Graph.* **1996**, *14*, 185. 217–221.
16. Kostenis, E.; Botero Cid, H. M.; Holzgrabe, Y.; Mohr, K. *Eur. J. Pharmacol.* **1996**, *314*, 385.
17. Nassif-Makki, T.; Trankle, C.; Zlotos, D.; Bejeuhr, G.; Cambareri, A.; Pfletschinger, C.; Kostenis, E.; Mohr, K.; Holzgrabe, U. *J. Med. Chem.* **1999**, *42*, 849.
18. Trankle, C.; Kostenis, E.; Burgmer, U.; Mohr, K. *J. Pharmacol. Exp. Ther.* **1996**, *279*, 926.
19. Trankle, C.; Mies-Klomfass, E.; Cid, M. H.; Holzgrabe, U.; Mohr, K. *Mol. Pharmacol.* **1998**, *54*, 139.
20. Ward, J. S.; Merritt, L.; Calligaro, D. O.; Bymaster, F. P.; Shannon, H. E.; Mitch, C. H.; Whitesitt, C.; Brunsting, D.; Sheardown, M. J.; Olesen, P. H.; Swedberg, M. D. B.; Jeppesen, L.; Sauerberg, P. *J. Med. Chem.* **1998**, *41*, 379.
21. Rajeswaran, W. G.; Cao, Y.; Huang, X. P.; Wroblewski, M. E.; Colclough, T.; Lee, S.; Liu, F.; Nagy, P. I.; Ellis, J.; Levine, B. A.; Nocka, K. H.; Messer, W. S., Jr. *J. Med. Chem.* **2001**, *44*, 4563.
22. Huang, X. P.; Nagy, P. I.; Williams, F. E.; Peseckis, S. M.; Messer, W. S., Jr. *Br. J. Pharmacol.* **1999**, *126*, 735.
23. N. C. B. Bodick, F. P.; Offen, W. W.; Shannon, H. E., United States (1998).
24. Wright, J. A.; Yu, J. Q.; Spencer, J. B. *Tetrahedron Lett.* **2001**, *42*, 4033.
25. Al-Badr, A. A.; El-Obeid, H. A. *Profiles Drug Subst. Excip. Relat. Methodol.* **2005**, *31*, 1.