Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 2675–2678
Bioorganic & Medicinal Chemistry Letters

Alkyne–quinuclidine derivatives as potent and selective muscarinic antagonists for the treatment of COPD

Jean-Philippe Starck, Laurent Provins,* Bernard Christophe, Michel Gillard, Sophie Jadot, Patrick Lo Brutto, Luc Quéré, Patrice Talaga and Michel Guyaux

UCB, R&D, Chemin du Foriest, B-1420 Braine-L'Alleud, Belgium

Received 24 January 2008; revised 5 March 2008; accepted 6 March 2008
Available online 18 March 2008

Abstract—SAR around alkyne–quinuclidine derivatives allowed the discovery of highly potent muscarinic antagonists displaying interesting preferential slow off-rates from the M3 receptor.

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide. This pathology is foreseen to become the third leading cause of death and the fifth leading cause of morbidity over the next 10 years. Current treatments are essentially palliative, focusing on relieving symptoms, preventing acute disease exacerbations and improving quality of life. The understanding of the molecular and cellular mechanisms involved in COPD allowed the identification of many potential therapeutic approaches based on a variety of biological targets. For example, the β2-adrenergic or the cholinergic system, several phosphodiesterases or the chemokines-interleukines pathways have been extensively investigated.[1–3]

Anticholinergics drugs alone or in combination with β2 agonists are reported to be the preferred choice for the management of COPD, at all stages of the disease. Vagally-mediated reversible bronchoconstriction is an important component of airway obstruction in COPD patients. Three muscarinic receptors subtypes (M1R, M2R and M3R) have been identified in the respiratory system with each of these having been experimentally involved in specific effects and physiological responses. The M1 receptor is thought to facilitate the cholinergic neurotransmission in parasympathetic ganglia, the M2 receptor provides negative feedback modulation on acetylcholine release on postganglionic nerves whilst the M3 receptor mediates the contractile response in airways smooth muscles as well as secretion from submucosal glands.[4]

The M1 and M3 receptors are the main receptor subtypes present in the human lungs.[5] The M2 receptor is also postulated to exist in bronchi[6] although it has not been autoradiographically visualized in human lungs.[5]

Current anticholinergic drugs are non-selective muscarinic antagonists. Their bronchorelaxing effects are thought to be mainly mediated by the blockade of M3 receptors on lung smooth muscles although an additional contribution of M1 receptors is not excluded.

A long duration of action is an important feature to treat chronic illnesses such as COPD. The measurement of kinetics of drug interaction to the target protein offers another approach to improve the duration of action of a drug besides pharmacokinetic factors.[7]

This strategy has been used to discover Tiotropium bromide (Spiriva®), the current gold standard anticholinergic drug for the treatment of COPD. Contrary to Ipratropium bromide (Atrovent®), Tiotropium is characterized by a slow dissociation rate from the M3 ($t_{1/2off}$ = 3308 min) and M1 receptors ($t_{1/2off}$ = 876 min). Thanks to this remarkable property, this drug displays a very long duration of action allowing a once-a-day administration as an inhaled dry powder.[8,9]

Interestingly, Tiotropium bromide also demonstrates a receptor-subtype kinetic selectivity with a far shorter

Keywords: Muscarinic receptor(s); COPD; Quinuclidine.
* Corresponding author. Tel.: +32 2 3863217; fax: +32 2 3863669; e-mail: laurent.provins@ucb-group.com

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.03.024

dissociation from the M2 receptor ($t_{1/2off}$ = 88 min). Even so the clinical impact of this latest property has not been fully demonstrated, it is nevertheless seen as a potential advantage to avoid a putative increased release of acetylcholine in airways but also to reduce the risk of cardiovascular adverse effects (mainly tachycardia) associated to blockade of M2 receptors on the heart (Fig. 1).

During the course of a work directed towards the identification of a potent M3 receptor antagonist as a potential new drug for the treatment of overactive bladder, we have surprisingly discovered a new class of compounds displaying a dissociation rate profile quite similar to the one of tiotropium bromide.

In this article, we wish to report the structure–activity relationships around these compounds focusing on the modulation of R^1, R^2 and R^3 groups that confer either binding equilibrium or kinetic selectivity for the M3 receptor (Fig. 2). It has to be interestingly noticed that close scaffold elements are also found in other reported long-acting muscarinic receptor antagonists.[10]

The general method of synthesis of compounds listed in Table 1 is outlined in Scheme 1. The majority of the quinuclidine derivatives reported in this manuscript were prepared according to the synthetic methodology described in a previous paper.[11]

Briefly, the alkyne derivative **C** is coupled with selected ketones (Scheme 1) yielding **D**, obtained as a mixture of diastereoisomers or as pure products after separation by chiral chromatography.

The replacement of the hydroxyl group by different chemical moieties has also been explored. Thus, compounds **E** and **F** bearing a methyl or a cyano group were prepared starting from the corresponding primary alkynes **G**[12–14] and 3-quinuclidinone **A** in three steps, first involving nitrogen protection (via an amino-borane complex), followed by methylation and finally deprotection of the quinuclidine moiety.

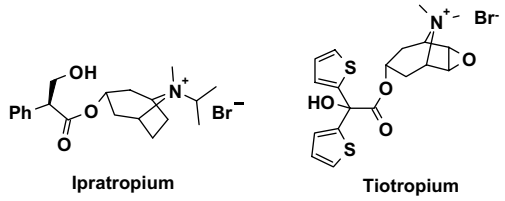


Figure 1. Reference anticholinergic agents.

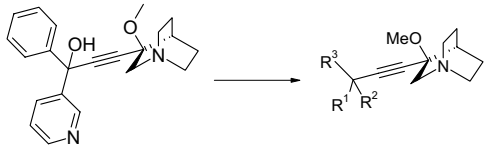


Figure 2. Alkyne–quinuclidine scaffold.

Compounds **H** and **I** bearing, respectively, a fluorine or a hydrogen atom instead of the hydroxyl group, were obtained starting from **D**. Introduction of fluorine was performed using DAST as the fluorinating agent, while introduction of the hydrogen atom was carried out under reductive conditions in the presence of Et_3SiH and $BF_3{\cdot}OEt_2$.[15]

Finally, the *bis*-*O*-methylated analogue **J** was obtained by methylation of the amino-borane complex, followed by the deprotection of the nitrogen under acidic conditions.

All compounds were tested for their binding affinity[16] and dissociation rates ($t_{1/2off}$) from the M2 and M3 muscarinic receptors.[17]

This investigation was prompted by the observation that compound **1** showed a markedly slower dissociation rate from M3 receptor compared to M2 subtype. In order to try to increase this selectivity within this series of molecules, several analogues with different substituents at R^1/R^2/R^3 were prepared (Table 1).

On the basis of this initial result, we speculated that the lipophilic moiety could contribute to modulate the dissociation rate from the muscarinic receptor subtypes. First, the introduction of small cycloalkyl groups (cyclobutyl **2** or cyclopentyl **3**) at the R^3 position yielded compounds displaying rather short $t_{1/2off}$ values, comparable to the one of the corresponding diphenyl derivative **1**. Nevertheless, the replacement of one of the two aromatic rings by a cyclohexyl ring yielded **4** exhibiting more than 15-fold $t_{1/2off}$ −M3/M2 selectivity.

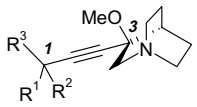
This result prompted us to perform further modifications by introducing larger cycloalkyl and/or bulkier substituents at the same position. The cycloheptyl derivative **7** dissociated 50 times more slowly from the M3 receptors than from the M2 receptors. An even better profile was obtained with the cyclooctyl analogue **10**.

The stereochemistry proved to have a large impact on both the binding affinities (about 10-fold difference) and the dissociation rates from the M3 receptor. In all cases, compounds characterized by the (1*R*, 3*R*) stereochemistry displayed the highest $t_{1/2off}$ values and, as expected, the best affinity (compare **5** and **6**, **8** and **9**, **11** and **12**).

However, modulating the bulkiness of the R^3 substituent by introducing a linear (*n*-butyl, **14**), a branched alkyl moiety (**15**) or an adamantyl group (**13**) resulted in a faster dissociation from the M3 receptor while keeping a rather good affinity. Of interest, the introduction of a sulfur-containing cycloalkyl group (**16**) resulted in a somewhat lower binding affinity and quite reduced dissociation rates.

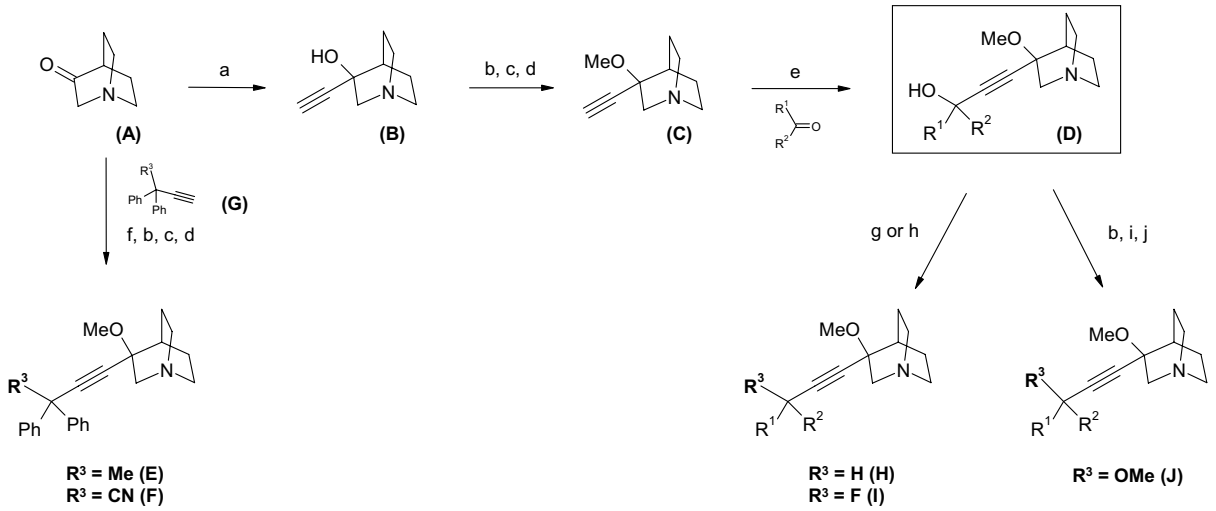
Next, we evaluated the impact of the nature of the group at the R^1 position. Thus, replacement of the hydroxyl group by a hydrogen atom (**21**), by a methyl (**19**), or by a nitrile moiety (**18**) reduced the $t_{1/2off}$ value in all

Table 1. Binding affinities and dissociation constant ($t_{1/2off}$) for M3 and M2 muscarinic receptors



Compound	Stereochemistry	R^1	R^2	R^3	M3		M2	
					pK_i	$t_{1/2off}$ (min)	pK_i	$t_{1/2off}$ (min)
1	3*R*	OH	Ph	Ph	9.9	58	9.1	6
2	Mixt.	OH	Ph	Cyclobutyl	9.0	11	7.9	<1
3	Mixt.	OH	Ph	Cyclopentyl	9.6	44	8.2	<1
4	Mixt.	OH	Ph	Cyclohexyl	9.3	147	8.2	9
5	1*R*, 3*R*	OH	Ph	Cyclohexyl	9.7	210	8.8	10
6	1*S*, 3*R*	OH	Ph	Cyclohexyl	8.4	25	7.6	2
7	Mixt.	OH	Ph	Cycloheptyl	9.4	404	8.4	8
8[£]	1*R*, 3*R*	OH	Ph	Cycloheptyl	9.5	830	8.6	5
9	1*S*, 3*R*	OH	Ph	Cycloheptyl	8.7	12	7.7	<1
10	Mixt.	OH	Ph	Cyclooctyl	8.8	444	7.6	10
11[$]	1*R*, 3*R*	OH	Ph	Cyclooctyl	9.5	1418	8.5	20
12	1*S*, 3*R*	OH	Ph	Cyclooctyl	8.6	58	7.5	5
13	Mixt.	OH	Ph	1-Adamantyl	8.5	51	7.5	5
14	Mixt.	OH	Ph	*n*-Butyl	8.7	10	7.3	<1
15	Mixt.	OH	Ph		9.4	42	8.2	3
16	Mixt.	OH	Ph		8.9	42	7.4	<1
17	3*R*	OMe	Ph	Ph	8.0	7.4	6.8	<1
18	Racemate	CN	Ph	Ph	8.6	21	8.2	<1
19	Racemate	Me	Ph	Ph	7.8	10	7.4	5
20	Mixt.	F	Ph	Cyclohexyl	8.8	147	8.2	9
21	Mixt.	H	Ph	Cyclohexyl	7.9	32	7.6	14
22		Ipratropium bromide			9.5	62	8.9	3.1
23		Tiotropium bromide			9.7	3308	9.5	88

Except racemates, all compounds possess the 3*R* configuration. Mixt.: 1:1 mixture of 1*R*, 3*R* and 1*S*, 3*R*.
£: Compound **8**: pA_2 M_3 = 8.2, pA_2 M_2 = 8.3.
$: Compound **11**: pA_2 M_3 = 8.2, pA_2 M_2 = 7.5.



Scheme 1. Synthesis of compounds. Reagents and conditions: (a) Li acetylide (ethylene diamine complex), THF; (b) BH_3·THF, THF, −10 °C; (c) NaH, NBu_4I, MeI, THF, rt; (d) 5 M HCl, acetone/Et_2O, rt; (e) *n*-BuLi, ketone, THF, −78 °C to rt; (f) *n*-BuLi, alkyne, THF, −70 °C; (g) DAST, CH_2Cl_2, −30 °C to −10 °C; (h) Et_3SiH, BF_3·OEt_2, CH_2Cl_2, −40 °C; (i) NaH, MeI, THF; (j) TFA, acetone/Et_2O.

cases. Replacement of the hydroxyl group by fluorine (**20**) was however tolerated, yielding a compound with a $t_{1/2off}$ of 147 min. Methylation of the hydroxyl group (**17**) also dramatically reduced the dissociation rate for both muscarinic receptor subtypes.

The antagonistic properties of all the compounds have been confirmed (see Table 1, data for compounds **8** and **11**) using the isolated guinea pig trachea or left atrium both stimulated by carbachol as functionally integrated M3 and M2 assays, respectively. These compounds also induced a very slowly reversible inhibition (recovery of 20% of the maximal effect >280 min) of the twitch contraction induced by electrical field stimulation of the isolated guinea pig trachea, possibly confirming the long $t_{1/2off}$ values.

In summary, this series of quinuclidine derivatives led to potent muscarinic antagonists, displaying interesting preferential slow off-rate from the muscarinic M3 receptor, versus the M2 subtype. The reasons for that behaviour remain unclear though a link to lipophilicity of the R^3 substituent may be evoked. We are working towards the elucidation of these findings.

Compounds **8** and **11** displaying dissociation rates and selectivity comparable to tiotropium bromide have been selected for extensive biological and pharmacological evaluation, as potential therapeutic candidates for the treatment of COPD. These results will be reported in due course.

Acknowledgements

The authors thank Dr Véronique Pinilla and her team for chiral chromatography, Dr Corinne Audouin and Dr Christophe Genicot for careful reading of the manuscript.

References and notes

1. Barnes, P. J. *Pharmacol. Rev.* **2004**, *56*, 515.
2. Barnes, P. J.; Hansel, T. T. *Lancet* **2004**, *364*, 985.
3. Krishna, G.; Sankaranarayanan, V.; Chitkara, R. K. *Expert Opin. Investig. Drugs* **2004**, *13*, 255.
4. Barnes, P. J. *Life Sci.* **1993**, *52*, 521.
5. Mak, J. C.; Barnes, P. J. *Am. Rev. Respir. Dis.* **1990**, *141*, 1559.
6. Minette, P. A.; Barnes, P. J. *J. Appl. Physiol.* **1988**, *64*, 2532.
7. Dowling, M. R.; Charlton, S. J. *Br. J. Pharmacol.* **2006**, *148*, 927.
8. Panning, C. A.; DeBisschop, M. *Pharmacotherapy* **2003**, *23*, 183.
9. Hvizdos, K. M.; Goa, K. L. *Drugs* **2002**, *62*, 1195.
10. See for example: Norman, P. *Expert Opin. Ther. Patents* **2006**, *16*, 1315, and references cited therein.
11. Starck, J.-P.; Talaga, P.; Quéré, L.; Collart, P.; Christophe, B.; Chimmanamada, D.; Zanda, M.; Wagner, A.; Mioskowski, C.; Guyaux, M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 373.
12. Dehmlow, E. *Tetrahedron Lett.* **1971**, 563.
13. Arumugam, S.; Verkade, J. G. *J. Org. Chem.* **1997**, *62*, 4827.
14. Norman, R. O. C.; Thomas, C. B. *J. Chem. Soc. B* **1967**, 598.
15. Batt, D. G.; Maynard, G. D.; Petraitis, J. J.; Shaw, J. E.; Galbraith, W.; Harris, R. R. *J. Med. Chem.* **1990**, *33*, 360.
16. Affinities for the M3 and M2 muscarinic receptors were determined by competition binding experiments with [^{3}H]-*N*-methylscopolamine performed in CHO cells membranes expressing recombinant human receptors.
17. K_{off} on both receptors are measured following 1 h association at 25 °C followed by dissociation by atropine (10^{-6}M). $t_{1/2off}$ are calculated according to the equation: $t_{1/2off} = \mathrm{Ln}\,2/K_{off}$.