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# Letters

## Improving the Oral Efficacy of CNS Drug Candidates: Discovery of Highly Orally Efficacious Piperidinyl Piperidine $M_2$ Muscarinic Receptor Antagonists

Yuguang Wang,* Samuel Chackalamannil, Zhiyong Hu, William J. Greenlee, John Clader, Craig D. Boyle, James J. Kaminski, William Billard, Herbert Binch, III, Gordon Crosby, Vilma Ruperto, Ruth A. Duffy, Mary Cohen-Williams, Vicki L. Coffin, Kathleen A. Cox, Diane E. Grotz, and Jean E. Lachowicz

*Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033*

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**Abstract:** In search of a backup $M_2$ muscarinic receptor antagonist to the previously reported compound **1**, we discovered compound (+)-**14**, which showed superior oral efficacy in animal models. The improvement of oral efficacy was achieved by modulating both the molecular weight and lipophilicity of the lead compounds.

**Introduction.** The design of drug candidates with good oral efficacy is a major challenge in modern drug discovery. The oral efficacy of a CNS drug can be affected by many factors such as oral bioavailability and blood−brain barrier (BBB) permeability. Numerous publications have addressed the relationship between oral activity and the physicochemical properties of drug candidates.[1,2] For example, the rule-of-5[2] can be employed to predict the oral behavior of a drug by the physicochemical properties of molecular weight (MW), lipophilicity (logP), and hydrogen bonding. In addition, metabolic stability must be taken into account when designing orally active drugs. Herein, we would like to report the positive outcome of our efforts to optimize the oral efficacy of selective muscarinic $M_2$ receptor antagonists.

Muscarinic receptors have been shown to mediate the actions of the neurotransmitter acetylcholine (ACh) both centrally and peripherally.[3] Muscarinic autoreceptors ($M_2$), located presynaptically on cholinergic nerve terminals, are associated with the inhibitory feedback regulation of ACh release. Muscarinic $M_2$ receptor antagonists such as himbacine and AF-DX116 have been demonstrated to increase the levels of ACh release in rat striatum.[4a] Since increased levels of ACh are beneficial for the improvement of cognition,[5] we have embarked on the discovery of potent and selective muscarinic $M_2$ receptor antagonists for the treatment of neurodegenerative diseases such as Alzheimer's disease (AD). Our criteria for selection of a muscarinic $M_2$ receptor antagonist as a drug candidate required high $M_2$ receptor binding affinity ($K_i$ < 5 nM), 100-fold selectivity versus $M_1$ and $M_3$ receptor subtypes, as well as oral efficacy in animal models.[6]

To screen the synthesized muscarinic $M_2$ receptor antagonists for oral efficacy, we used the rat microdialysis and the young rat passive avoidance response (PAR) assays. In the microdialysis assay, the level of ACh released from rat striatum is measured after oral administration of a drug, thereby determining the ability of the drug to reach brain $M_2$ receptors and block the ACh negative feedback mechanism.[4] The PAR assay is a cognitive paradigm which measures whether reference memory is improved upon oral administration of a drug.[7]

**Rationale**. Compounds **1** and **2** are representatives of our first generation of muscarinic $M_2$ receptor antagonists (Figure 1).[8] These compounds were highly potent with the required subtype selectivity (Table 1). In addition, both compounds were orally active in rat microdialysis and rat PAR assays. Compound **1** was selected for phase I clinical trials based on its plasma concentration and efficacy upon oral dosing.[8a]

A common characteristic of compounds **1** and **2** was a high molecular weight (MW > 550). In the development of a backup candidate for compound **1**, we synthesized lower molecular weight analogues by truncating the left-hand portion of compound **1**. Extensive structure−activity relationship (SAR) studies led to the discovery of the lower MW analogue **3** (Figure 1).[9]

* To whom correspondence should be addressed. Phone: 908-740-3508. Fax: 908-740-7152. E-mail: yuguang.wang@spcorp.com.

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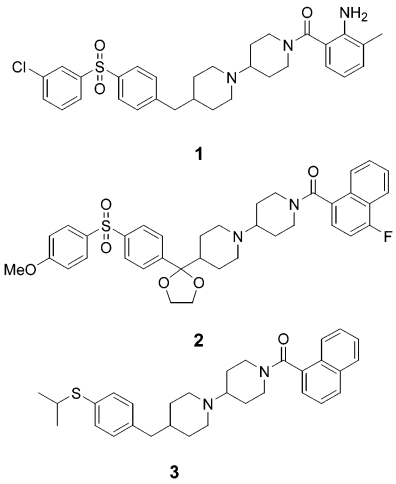


**Figure 1.** Structures of earlier $M_2$ receptor antagonists.

**Table 1.** Properties of the Earlier $M_2$ Receptor Antagonists

| compd | $M_2$[a] | $M_1/M_2$ | MD[b] | PAR[c] | MW | ClogP[d] | rat PK[e] |
|---|---|---|---|---|---|---|---|
| **1**[8a] | 0.89 | 734 | 180 ± 18 | 0.001 | 566 | 4.6 | 908 |
| **2**[8b] | 0.51 | 125 | 180 ± 25 | 0.1 | 659 | 5.0 | 603 |
| **3**[9] | 0.9 | 205 | 110 ± 15(po)<br>210 ± 21 (iv) | inact | 487 | 6.3 | nd |

[a] $K_i$, nM; all determinations were performed three times (SEM < 15%). [b] Microdialysis: %ACh release compared with baseline in rat striatum, 0 → 2 h, 10 mg/kg, po, 20% HP$\beta$CD (baseline = 100%). [c] Passive avoidance response: orally active dose (mg/kg, significant change in latency over vehicle, $n = 36$, SEM < 10%). [d] ClogP values were calculated using SYBYL version 6.6 accessed via the ClogP column type and various expression generators with a special license (Biobyte) available from Tripos, Inc. [e] AUC (high throughput single measurement): h•ng/mL, 0 → 6 h, 10 mg/kg, po, 20% HP$\beta$CD.[10]

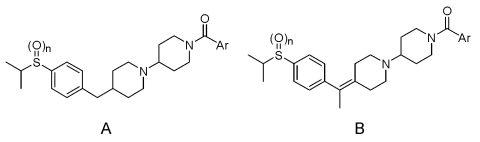
Compound **3** met our binding criteria and was subjected to the rat microdialysis assay. While it was highly active after intravenous (iv) dosing, compound **3** did not show the desired level of oral efficacy in this assay (Table 1). In addition to its low metabolic stability,[11] the high lipophilicity (ClogP = 6.3) of compound **3** also contributed to its low oral activity in the microdialysis assay. To improve the oral efficacy, we focused on modulating the lipophilicity and metabolic stability in this series of compounds. We oxidized the sulfide atom of compound **3** to a more polar and robust sulfone moiety. At the right-hand side of compound **3**, the naphthyl group was replaced with either a metabolically more stable 4-fluoronaphthyl group (**9**) or a polar 2-amino-3-methylphenyl group (**10**). However, compounds **9** and **10** showed undesirable $M_2$ receptor $K_i$ values (Table 2). Our challenge was to improve the $M_2$ receptor binding affinity of compounds **9** and **10**.

Among the known strategies to increase receptor binding affinity, we chose conformational restriction. A double bond was introduced at the benzylic position as shown to limit the degrees of freedom in the truncated analogues (Figure 2).

**Chemistry**. The synthesis of the tetrasubstituted olefins is outlined in Scheme 1.

Protection of commercially available compound **4** was followed by the displacement of fluorine atom with isopropylthiol to form sulfide/ketone **5**. Addition of methyllithium to ketone **5** followed by dehydration in refluxing TFA/$CH_2Cl_2$ (1:1) afforded amine **6** as the only olefin regioisomer. Reductive amination and sulfide oxidation transformed amine **6** to sulfoxide **7** or sulfone **8**. Moderate selectivity in the formation of sulfoxide **7** or sulfone **8** was achieved by adjusting the number of equivalents of sodium perborate during the oxidation step.[12] Both compounds **7** and **8** were treated with TFA to remove the *N*-BOC protecting group, and subsequent coupling with different aromatic acids provided the final targets.

**Table 2.** Profile of Tetrasubstituted Olefin Analogs



A B

| comp'd | Ar | | n | $M_2$[a] | $M_1/M_2$ | HMS[b] | MW | ClogP[c] |
|---|---|---|---|---|---|---|---|---|
| **9** |  | A | 2 | 221 | 5 | nd | 537 | 4.3 |
| **10** |  | A | 2 | 104 | 10 | nd | 498 | 3.1 |
| **11** |  | B | 2 | 9.6 | 73 | 74 | 549 | 4.4 |
| **12** |  | B | 2 | 32 | 8 | 80 | 510 | 2.8 |
| **13** |  | B | 1 | 2.7 | 111 | 66 | 533 | 4.4 |
| **14** |  | B | 1 | 6.6 | 45 | 64 | 494 | 2.8 |

[a] $K_i$, nM; all determinations were performed three times (SEM < 15%). [b] Human microsomal stability: % parent compound remaining after 20 min incubation with human liver microsomes. [c] ClogP values were calculated using SYBYL version 6.6 accessed via the ClogP column type and various expression generators with a special license (Biobyte) available from Tripos, Inc.

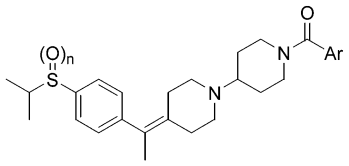
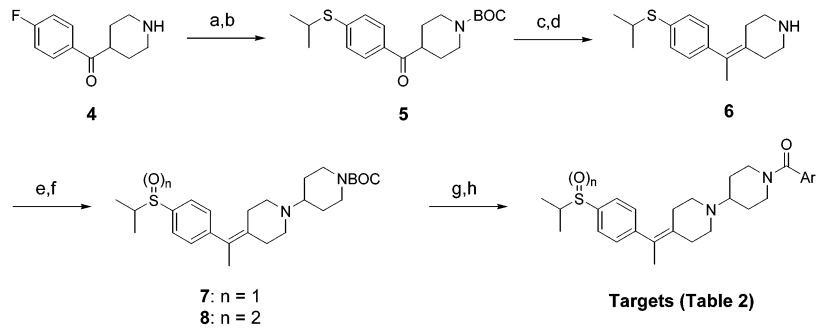


**Figure 2.** Conformational restriction by a double bond.

**Results and Discussion.** The binding affinity and selectivity of the synthesized targets against cloned human muscarinic receptors were assayed according to the reported protocol.[13] To help select the right drug candidate for clinical trials, the stability of our $M_2$ antagonists in human liver microsomes after a 20 min incubation time was assessed.[14]

A comparison of the muscarinic $M_2$ receptor binding affinities of the compounds in Table 2 indicated that the introduction of a double bond improved the binding affinity. The muscarinic $M_2$ receptor binding affinity was increased by more than 20-fold from **9** to **11** and 3-fold from **10** to **12**. More importantly, the $M_2$ receptor $K_i$ values of **13** and **14** coupled with their microsomal stability results warranted further studies of these two sulfoxides.

**Scheme 1**[a]




[a] Conditions: (a) $(BOC)_2O$, 10% NaOH/$Et_2O$, 95%; (b) NaH, DMF, isopropylthiol, 65 °C, 6 h, 92%; (c) MeLi, THF, 90%; (d) 50% TFA/$CH_2Cl_2$, reflux, 92%; (e) $NaBH(AcO)_3$, 1,2-dichloroethane, 1-*tert*-butoxycarbonyl-4-piperidone, 75%; (f) $NaBO_3$, HOAc, 65%; (g) 30% TFA/$CH_2Cl_2$, 95%; (h) ArCOOH, EDCI, DMAP, $CH_2Cl_2$, 85−96%.

**Table 3.** Binding Affinity of Chiral Sulfoxides

| compd | $M_2$[a] | $M_1/M_2$ | $M_3/M_2$ | $M_4/M_2$ | $M_5/M_2$ |
|---|---|---|---|---|---|
| (−)-**13** | 2.4 | 245 | 376 | 36 | 35 |
| (+)-**13** | 1.3 | 166 | 122 | 7 | 6 |
| (−)-**14** | 12 | 6 | nd | nd | nd |
| (+)-**14** | 0.89 | 101 | 170 | 22 | 36 |
| **1** | 0.89 | 734 | 787 | 69 | 96 |

[a] $K_i$, nM; all determinations were performed three times (SEM < 15%).

**Table 4.** Efficacy and Pharmacological Profile of Chiral Sulfoxides

| compd | MD[a] | PAR[b] | rat PK[c] | MW | ClogP[d] |
|---|---|---|---|---|---|
| (−)-**13** | 155 ± 18 | nd | nd | 533 | 4.5 |
| (+)-**13** | 179 ± 18 | nd | nd | 533 | 4.5 |
| (−)-**14** | nd | nd | nd | 494 | 2.8 |
| (+)-**14** | 225 ± 35 | 0.0001 | 5149 | 494 | 2.8 |
| **1** | 180 ± 21 | 0.001 | 908 | 566 | 4.6 |

[a] Microdialysis: %ACh release compared with baseline in rat striatum, 0 → 2 h, 10 mg/kg, po, 20% HP$\beta$CD. [b] Passive avoidance response: orally active dose (mg/kg, significant change in latency over vehicle, $n = 36$, SEM < 10%). [c] AUC (high throughput single measurement): h•ng/mL, 0 → 6 h, 10 mg/kg, po, 20% HP$\beta$CD.[10] [d] ClogP values were calculated using SYBYL version 6.6 accessed via the ClogP column type and various expression generators with a special license (Biobyte) available from Tripos, Inc.

Next, we prepared the enantiomers of sulfoxides **13** and **14**. These targets were derived from (+)-**7** and (−)-**7** which were separated by chiral HPLC.[15] Three of the enantiomerically pure compounds met our binding criteria ((−)-**13**, (+)-**13**, and (+)-**14**; Table 3) and were tested further in vivo.

Among the resolved enantiomers, compound (+)-**14** demonstrated superior oral efficacy in both the microdialysis and PAR assays (Table 4). Compared with compound **1**, the new lead (+)-**14** increased ACh release in the microdialysis assay by 45%, and showed equivalent oral efficacy in the rat PAR assay at a 10-fold lower dose. It was not surprising that the rat plasma level of compound (+)-**14** was approximately five times that of compound **1** upon oral administration. Figures 3 and 4 summarize the PAR and microdialysis[4b] data of (+)-**14**.

We attributed the excellent oral efficacy of (+)-**14** to its relatively low molecular weight (MW = 494) and optimized lipophilicity (ClogP = 2.8). We believed that the data in Table 4 provided a convincing example of the effect that modulating the physical chemical properties of a compound can have on oral efficacy. In addition, for further development of this series of compounds, we specifically examined the metabolites of (+)-**13** and (+)-**14** in human microsomal incubation studies.[16] There was no indication of metabolism of the tetrasubstituted double bond.

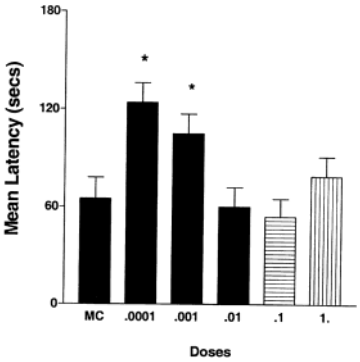



**Figure 3.** PAR data of (+)-**14**.[7] *Significant changes in latency over vehicle (4% MC) ($p < 0.05$, Dunnet's "T" test, $n = 36$).

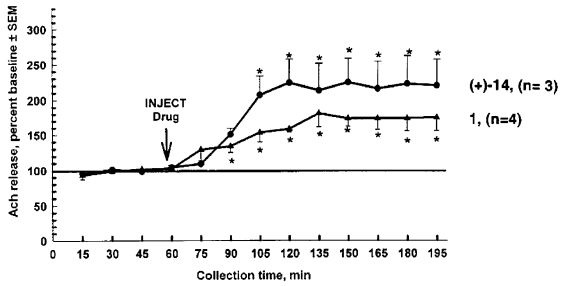



**Figure 4.** Comparison of microdialysis data of (+)-**14** and **1**. Significant stimulation over preinjection baseline ($p = 0.05$, Duncan's Multiple Range Statistic). Drug concn = 10 mg/kg, po; vehicle HP $\beta$CD.

**Conclusion.** We have successfully improved oral efficacy of the first generation muscarinic $M_2$ receptor antagonists. The discovery of (+)-**14** demonstrated the effects of molecular weight and lipophilicity on the oral efficacy of this class of CNS drug candidates. We also applied the strategy of conformational restriction to increase the muscarinic $M_2$ receptor binding affinity significantly. The excellent muscarinic $M_2$ receptor binding affinity of the tetrasubstituted double bond series made the modulation of molecular weight and lipophilicity possible.

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**Supporting Information Available:** Analytical data and spectral information available for the key compounds discussed. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) In the human microsomal stability assay, compound **3** showed only 26% parent compound left after 20 min incubation with human microsomal preparation. See ref 14.

(12) Sulfoxide was obtained as a major product (60−70%) with 0.9−1 equiv of $NaBO_3$; sulfone was obtained as a major product (80−95%) with 2.5−3 equiv of $NaBO_3$. Sulfoxide and sulfone can be easily separated with flash column chromatography. For separation details, please see the Supporting Information.

(13) $K_i$ was expressed as mean of duplicate values (SEM < 15%). All determinations were performed three times. For details, see: Lachowicz, J. E.; Lowe, D.; Duffy, R. A.; Ruperto, V.; Taylor, L. A.; Guzik, H.; Brown, J.; Berger, J. G.; Tice, M.; McQuade, R.; Kozlowski, J.; Clader, J.; Strader, C. D.; Murgolo, N. Sch 57790: A Novel $M_2$ Receptor Selective Antagonist. *Life Sci.* **1999**, *64*, 535−539.

(14) Compounds (10 $\mu$g/mL concentration) were incubated in human microsomes, and their metabolic stability was determined by the percent of the parent compound remaining after 20 min. The stability was compared to that observed for SCH 72788 (Lachowicz, J. E.; Duffy, R. A.; Ruperto, V.; Kozlowski, J.; Zhou, G.; Clader, J.; Billard, W.; Binch, H., III; Crosby, G.; Cohen-Williams, M.; Strader, C. D.; Coffin, V. Facilitation of Acetylcholine Release and Improvement in Cognition by a Selective $M_2$ Muscarinic Antagonist, SCH 72788. *Life Sci.* **2001**, *68*, 2585−2592), which was used as a reference incubated at the same time under the same conditions. A difference of 30% was considered significant, and compounds that showed stabilities 30% more than SCH 72788 were considered further. SCH 72788 typically had 15−30% parent remaining after 20 min. See the following reference for experimental details: Hecht, S. S.; Chen, C. B.; Hoffman, D. Evidence for Metabolic α Hydroxylation of N-Nitrosopyrrolidine. *Cancer Res.* **1978**, *38*, 215−218.

(15) For details of chiral HPLC separation of **7**, please see the Supporting Information.

(16) Compounds were subjected to human liver microsomal incubation for 30 min. The metabolite characterization was done by LC MS/MS of the microsomal incubation. The details will be reported by Cox, K. A., et al.


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