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Synthesis and structure–analgesic activity relationships of a novel series of monospirocyclopiperazinium salts (MSPZ)

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ABSTRACT

A series of monospirocyclopiperazinium salts were designed and synthesized to search for a peripherally-acting analgesic drug with low side effects. Extensive SAR studies revealed that a suitable NR^2R^3 was critical for the analgesic activity, which might be beneficial to expose the cationic nitrogen to bind to the receptor, and possibly interact with the receptor via π–π interaction. Introduction of substituting group on the N^4-phenyl ring could improve the activity, and the best position was the 4-position. Compound **14n** showed more potent analgesic activity (63%, 20 μM/kg, sc) and holds promise for development as a mechanically new analgesic drug.

Pain is a pervasive public health problem. Current analgesics fall into two major classes: opioid agents and non-steroidal anti-inflammatory drugs (NSAIDs).[1] However, both general classes of agents have undesirable side effects associated with their use,[1] and this has prompted a search for mechanistically different analgesic agents.[2] Recently, considerable attention has been focused on the identification of ligands for nicotinic acetylcholine receptors (nAChR) and several compounds with high affinity have been reported.[1,3] Nevertheless, most of them still have significant side effects due to their unselective action on the other subpopulations of nAChRs. For example, tebanicline (previously ABT-594), a famous α4β2 agonist with significant antinociceptive effects, failed in phase II trials because of gastrointestinal adverse effects presumably caused by the α3β4 nAChR component of the compound.[4] Analyzing the papers on the nAChR ligands, most of them are concentrated on the development of central nAChR ligands, except a few reports on the peripheral nAChR ligands.

Generally, the quaternary ammonium salts cannot easily pass the blood–brain barrier (BBB). However, some of them exhibit significant affinity for nAChRs (Fig. 1). For example, N^1,N^1-dimethyl-N^4-phenylpiperazinium iodide (**DMPP**) is a well-known nicotinic agonist.[5] Gualtieri and co-workers found that most of the quaternary ammonium analogues (**1**) of **DMPP** showed excellent affinity for α4β2 nAChR after systematically studying the structure–activity relationships of DMPP.[6] Glennon and co-workers reported that quaternary ammonium analogues (**3**) of nicotinic ligands (**2**) could retain the antinociceptive effects of

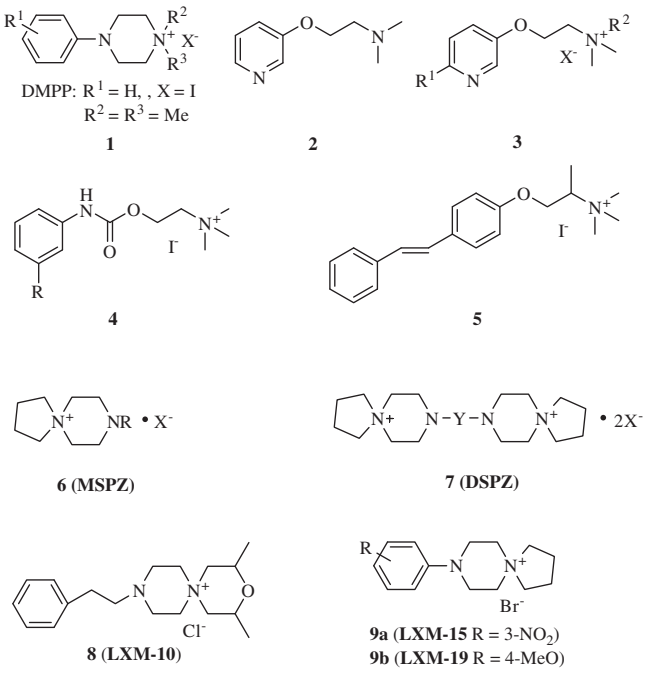

Figure 1. Structure of compounds **1**–**9**.

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the tertiaryamine counterparts.[7] Gündisch et al. showed that the phenylcarbamate derivatives (**4**) have significantly higher affinity for α7 nAChR than for α4β2 nAChR.[8] Gotti et al. also reported that choline ether (**5**) is a α7-selective antagonist (α7 K_i = 0.057 μM) with little affinity (K_i = 39 μM) for β2-containing nACh receptors.[9] Moreover, Wang and co-workers reported that activation of peripheral α7 nAChR by choline elicits antinociceptive effects in inflammatory pain after iv treatment.[10] These results suggest that quaternary ammonium nAChR ligands might result in a novel class of peripherally-acting analgesic agents with decreased side effects due to the reduced ability to penetrate the blood–brain barrier.

Since 1996, our group has done a lot of work on the development of quaternary ammonium salts as analgesics (Fig. 1).[11] A series of novel monospirocyclopiperazinium salts **6** (**MSPZ**)[12] and dispirocyclopiperazinium salts **7** (**DSPZ**)[13] have been synthesized and compounds **8** (**LXM–10**), **9a** (**LXM–15**), and **9b** (**LXM–19**) were identified to show significant in vivo analgesic activities without obvious side effects. Pharmacological studies revealed that this kind of compounds could selectively bind to peripheral α7 nAChR and M4 mAChR.[14] As a result they produced neither central side effects nor typical side effects associated with AChR agonists. These results further demonstrate that quaternary ammonium compounds hold promise for development as a peripherally-acting analgesic drug with low side effects. In continuing our long-term research on piperaziniums as analgesics, we herein reported the synthesis and structure–activity relationships of **9b** analogues (**14a–14v**).

The synthesis of compounds **14a–14v** is outlined in Scheme 1. Reaction of substituted aniline (**10a–10h**) with 2-chloroethanol in the presence of KI gave *N*,*N*-bis(2-hydroxyethyl)anilines **11a–11h**.[15] Compound **11i** was obtained via the reaction of 4-chloronitrobenene and diethanolamine.[15] Among them, compounds **11b** and **11c** were without purification and directly chlorinated with $SOCl_2$ in chloroform to afford the corresponding *N*,*N*-bis(2-chloroethyl)anilines **13b** and **13c** in good yields. However, the similar procedure was unsuitable for the preparation of compounds **13a** and **13d–13i**, which were actually prepared via chlorination of the methanesulfonates **12** according to reference.[15] Intermediates **13a–13g** reacted with a variety of secondary amines in the presence of $NaHCO_3$ to give the corresponding quaternary ammonium chlorides (**14a–14o**, **14r**, **14t–14v**). Compound **14s** was obtained via the catalytic hydrogenation of its precursor **14s′**. In addition, compounds **14p** and **14q** were not chlorides as we expected but were methanesulfonates, because the reaction of *N*,*N*-bis(2-chloroethyl)-3-nitroaniline (**13h**) and *N*,*N*-bis(2-chloroethyl)-4-nitroaniline (**13i**) with indoline was so difficult that we had to use more active intermediates **12h** and **12i**.

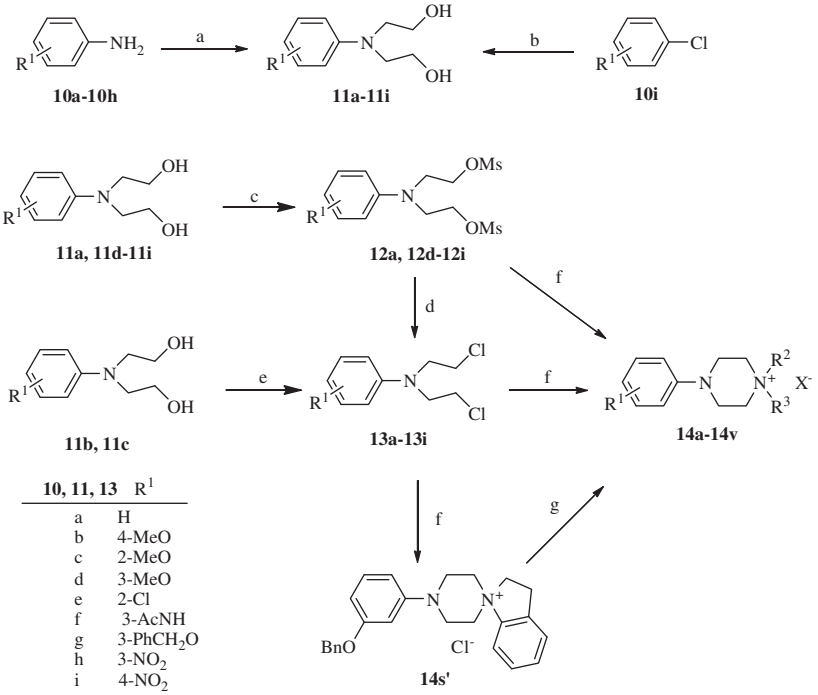
On the bases of our previous experimental results, the preliminary analgesic activities of compounds **14a–14v** have been assessed at doses of 20 μM/kg, sc by acetic acid writhing test,[16] and the results were listed in Table 1.

For the acyclic piperaziniums **14a–14c**, the activities decreased from 56% to 43% when the bulk of R^2 and R^3 increased. This indicated that the steric hindrance of NR^2R^3 is unfavorable for the analgesic activity of the acyclic piperaziniums.

Compounds **14d–14f** are spirocyclopiperaziniums. It is clear that the analgesic activity obviously dropped when enlarging the ring of NR^2R^3, such as, compound **14d** (five-member ring, 41% analgesic activity) and **14e** (six-member ring, 26% analgesic activity). The possible explanation is the influence of the size of cycle on the conformation. Therefore, an appropriate conformation was critical for the interaction between cationic nitrogen and receptor.

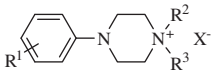
Comparing the analgesic activities of **14e**, **14g**, and **14h**, it is found that a heteroatom at the 4-position of the six-member NR^2R^3 ring is beneficial for the analgesic activity and steric hindrance could weaken the effect. It was also observed that compound **14i** (HNR^2R^3 = piperazine) showed almost no activity but **14j** (HNR^2R^3 = 1-methylpiperazine) had an analgesic activity of 32%. These results indicated that the heteroatom at the 4-position of the six-member NR^2R^3 ring might bind to the receptor as H-bond acceptor.

Considering the 2-phenylethyl group and 4-methoxylphenyl group as key pharmacophore in **LXM-10** and **LXM-19**, respectively,



Scheme 1. Synthesis of **14a–14v**. Reagents and conditions: (a) $ClCH_2CH_2OH$, $CaCO_3$, KI, H_2O, reflux for 8 h; (b) $HN(CH_2CH_2OH)_2$, 120 °C for 6 h; (c) MsCl, EtOAc, rt for 1 h; (d) NaCl, DMF, reflux for 20 min; (e) $SOCl_2$, $CHCl_3$, reflux for 1 h; (f) HNR^2R^3, $NaHCO_3$, EtOH, reflux for 10 h; (g) H_2, Pd/C, EtOH.

Table 1
The analgesic activities of compounds **14a–14v** assessed by acetic acid writhing test



14a-v

Compd	R^1	HNR^2R^3	X	Analgesic activities[a,b,c] (%)
14a	4-CH_3O	Dimethylamine	Cl	56
14b	4-CH_3O	Dibutylamine	Cl	54
14c	4-CH_3O	*N*-Methylbenzylamine	Cl	43
14d	4-CH_3O	Pyrrolidine	Cl	41
14e	4-CH_3O	Piperidine	Cl	26
14f	4-CH_3O	Hexamethyleneimine	Cl	28
14g	4-CH_3O	Morpholine	Cl	41
14h	4-CH_3O	2,6-Dimethyl morpholine	Cl	21
14i	4-CH_3O	Piperazine	Cl	5
14j	4-CH_3O	1-Methylpiperazine	Cl	32
14k	4-CH_3O	1-(2-Phenylethyl)piperazine	Cl	27
14l	4-CH_3O	1-(4-Methoxyphenyl) piperazine	Cl	17
14m	4-CH_3O	1-Benzoyl piperazine	Cl	50
14n	4-CH_3O	Indoline	Cl	63
14o	H	Indoline	Cl	28
14p	4-NO_2	Indoline	MsO	54
14q	3-NO_2	Indoline	MsO	39
14r	3-CH_3O	Indoline	Cl	37
14s	3-OH	Indoline	Cl	34
14t	3-AcNH	Indoline	Cl	39
14u	2-Cl	Indoline	Cl	44
14v	2-CH_3O	Indoline	Cl	39

[a] Drugs were dissolved in isotonic saline solution (NaCl 0.9%) immediately before use. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 10 mL/kg. Mice were administered with drugs (20 μmol/kg, sc) 30 min prior to injection of 0.6% acetic acid (10 mL/kg, ip). The number of stretching movements was recorded for 10 min, starting 5 min after acetic acid injection.
[b] Percent analgesia was expressed by following formula: % Inhibition = $100 - (A/B \times 100)$, where A = incidence of writhing in the treated group and B = incidence of writhing in the control group.
[c] $P < 0.05$.

compounds **14k** and **14l** were synthesized. The results revealed that a large substituent at the 4-position of the six-member NR^2R^3 ring is not preferred. However, **14m** with 1-benzoyl piperazine for NR^2R^3 exhibited 50% analgesic activity. Since the C=O bond is sp^2 hybridized, **14m** may have a special conformation to bind to the receptor. Inspired by this result, we introduced indoline and obtained a rather good result of 63% analgesic activity (**14n**, 63%). The results of **14m** and **14n** hint us that a phenyl ring at a proper position of NR^2R^3 was preferred, which might contribute to affinity with a suitable conformation and/or π–π interaction.

Furthermore, we explored the influence of the substituted groups of the N^4-aromatic ring on analgesic activity. Compared to compound **14o** (28%) without any substituted group, both electron-donating group (**14n**, 4-CH_3O, 63%) and electron-withdrawing group (**14p**, 4-NO_2, 54%) at the 4-position were beneficial for the analgesic activity, while similar substituted groups at the 3-position afforded only slight enhancement in activity (**14r**, 3-CH_3O, 37%; **14q**, 3-NO_2, 39%). In addition, groups such as 3-OH (**14s**, 34%) and 3-AcNH (**14t**, 39%), which are both H-bond donors and acceptors, did not further improve the analgesic activity. Considering the special conformation of the spirocyclopiperazinium structure, substituted groups at the 4-position of the N^4-aromatic ring might bind more tightly to the receptor than those at the 3-position. Furthermore, compounds **14u** and **14v** were also a little more effective than **14o**. This result is opposite to traditional agonists, whose substituents at the 2-position weaken the activity significantly. It may confirm the effect of the special spirocyclopiperazinium conformation.

In summary, selecting monospirocyclopiperazinium salt **9b** as the lead compound, a series of monospirocyclopiperazinium salts were designed and synthesized to search for a peripherally-acting analgesic drug with low side effects. Extensive SAR studies revealed that a suitable NR^2R^3 was critical for the analgesic activity, which might be beneficial to expose the cationic nitrogen to bind to the receptor, and possibly interact with the receptor via π-π interaction; introduction of substituting group on the N^4-phenyl ring could improve the activity, and the best position was the 4-position. More importantly, compound **14n** with a new kind of monospirocyclopiperazinium salt was identified to show potent analgesic activity, which would be very valuable for the further investigation of this program.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.052.

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