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Unique spirocyclopiperazinium salt. Part 4: Modification of dispirocyclopiperazinium (DSPZ) salts as analgesics

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Abstract—In order to improve the analgesic activity of lead compound **7a**, two series of dispirocyclopiperazinium (DSPZ) salts **9a**–**h**, **10a**–**e** and compounds **14**, **15** were synthesized and evaluated for their in vivo analgesic activity both by acetic acid induced writhing test and hot plate test. Compounds **9h**, **14**, and **15** exhibited better analgesic activities than **7a**. Several important structure–activity relationships were revealed from this study: (1) the introduction of aryl group would obviously improve the activity; (2) it was favorable to enhance the analgesic activity and reduce the toxicity to incorporate alkyl group with suitable length in the molecule; (3) carbamate analogues displayed lower toxicity than carboxylic ester analogues; (4) hydroxylation and chlorination of lead compound could increase the analgesic activity in hot plate test.

Pain is a pervasive public health problem. The chief treatments of pain are agents that bind at opioid receptors and inhibitors of cyclooxygenase.[1] However, both general classes of agents have undesirable side effects associated with their use,[2] and this has prompted a search for mechanistically different analgesic agents. Over the past few years, considerable efforts have been directed toward the identification of ligands selective for subtypes of nAChR and several high affinity compounds have been reported.[3]

During our study on the synthesis and biological activity of quaternary piperazinium salts,[4,5] we found a significant analgesic piperazinium salt **1**, whose structure is similar to a well-known nicotinic agonist N^1,N^1dimethyl-N^4-phenylpiperazinium iodide (DMPP, **2**). DMPP is considered to represent a unique ligand among hundreds of nicotinic agonists,[6] because it does not fit any proposed pharmacophore for nicotinic binding, yet it presents a $K_i = 250$ nM as a nicotinic receptor of the rat brain labeled by [^{3}H]cytisine (thought to be represented mainly by the α_4 β_2 subtype). The structural similarity of the two analgesic compounds encouraged us to further study piperazinium salts as analgesics. Hence, we synthesized several more classes of piperazinium salts,[7–12] including nonspirocyclopiperazinium salts (NSPZ) (**3** and **4**), monospirocyclopiperazinium (MSPZ) (**5** and **6**), dispirocyclopiperazinium (DSPZ) salts (**7** and **8**), and several compounds were found to own good analgesic activity (Fig. 1).[11,12]

However, due to the poor lipophilicity of piperazinium salts, those compounds could not cross the blood–brain barrier easily, the pharmacological experiments of compound **3** (R = methyl, R′ = phenethyl, $n = 6$) proved that its analgesic effective dose of subcutaneous injection was 4000 times higher than that of intracerebroventricular injection,[13] which hinted that improving CNS permeability of piperazinium salts may be a valid method to increase the analgesic activity of quaternary ammonium salts. Therefore, we selected a promising compound **7a** (80.97%, 0.04 mmol/kg, sc, in writhing test) as the lead compound to improve its lipophilicity. Ester formation is the most commonly employed approach for increasing lipophilicity of highly polar parent compounds.[14] Besides, carbamates were usually used in cholinocepter agonists and acetylcholinesterase

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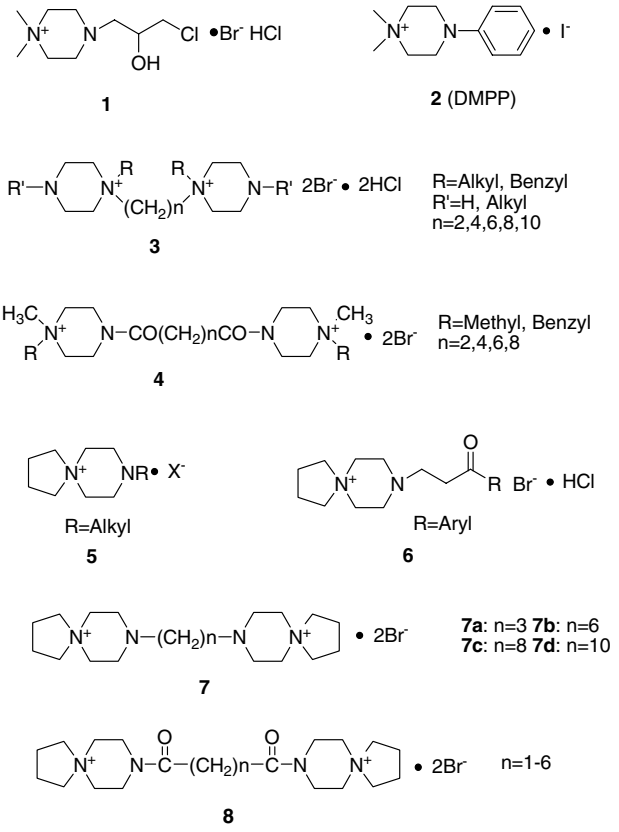
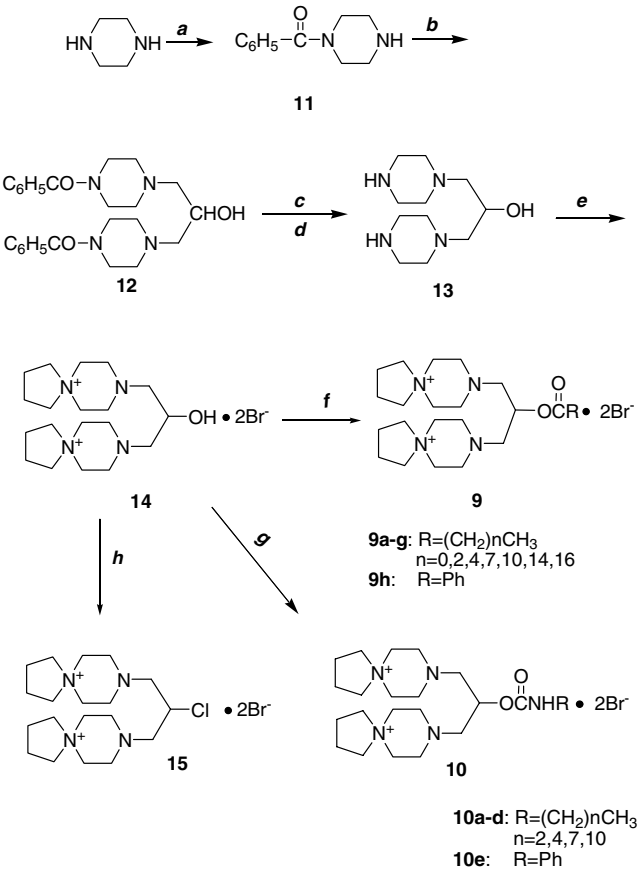


Figure 1. Known quaternary ammoniums with analgesics.



Scheme 1. Synthesis of compounds **9**, **10**, and **15**. Reagents and conditions: (a) C_6H_5COCl, AcOH, 56%; (b) $ClCH_2CH(OH)CH_2Cl$ (0.5 equiv), K_2CO_3, EtOH, reflux, 61%; (c) 10% HCl; (d) NaOH, 85% overall; (e) $Br(CH_2)_4Br$ (2 equiv), $NaHCO_3$, EtOH, reflux, 76%; (f) $(RCO)_2O$, DMAP, DMF, 70–100 °C, 50–70%; (g) RNCO, TEA, DMF, 70–100 °C, 40–60%; (h) $SOCl_2$, DMF, rt, 29%.

inhibitors.[15,16] Hence, two series of DSPZ salt conjugates carboxylic esters **9** and carbamates **10** were designed (Fig. 2).

The designed compounds (**9** and **10**) were synthesized as outlined in Scheme 1. The reaction of two equivalents of 1-benzoyl piperazine **11** with one equivalent of 1,3-dichloro-2-propanol in the presence of potassium carbonate provided **12**. Deprotection of **12** in 10% hydrochloric acid, followed by neutralization with aqueous sodium hydroxide, gave the 1,3-di(1-piperazyl)-2-propanol **13**. One equivalent of **13** was reacted with two equivalents of 1,4-dibromobutane to yield the key intermediate dispirocyclopiperazinium bromide **14**. Treatment of compound **14** with $SOCl_2$ afforded the compound **15**.

Compounds **9** were synthesized from **14**. Because of its poor solubility and steric blocks, quaternary ammonium salts **9** were not obtained from conventional acylation reagents such as acid chloride and carboxylic acid. After trying several different methods, we found that **14** was treated with 20 equivalents of corresponding acid anhydrides in DMF with 4-dimethylaminopyridine (DMAP) as catalyst for 3h at 70–100 °C affording the expected products **9** in 50–70% yields. The compounds **9** were purified by column chromatography on neutral aluminum oxide using methanol/ethyl acetate as the eluents.

Compounds **10** were obtained from the reaction of **14** with corresponding isocyanates which were prepared according to the literature.[17] The reaction was carried out in DMF with triethylamine (TEA) as base for 7 h at 70–100 °C giving the products in 40–60% yields. The purification of products **10** was similar to that of series **9**.

All newly synthesized compounds were tested for their in vivo analgesic activity by acetic acid induced writhing test and hot plate test using reported method.[13] Generally, we chose 0.04 mmol/kg as the dose of subcutaneous injection. If causing death of mice, the dose would be reduced. The results are summarized in Table 1.

As shown in Table 1, the analgesic activity data have an approximate normal distribution for the compounds **9a–g**. For instance, when $n = 4$, 7, and 10, compounds in series

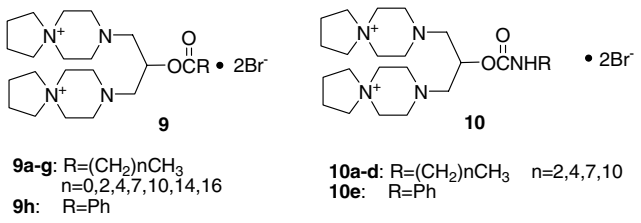
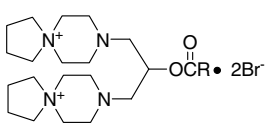
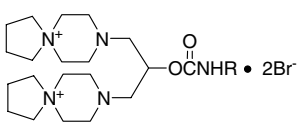


Figure 2. Structures of compounds **9** and **10**.

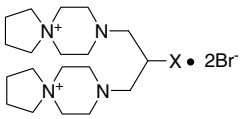
Table 1. Analgesic activity of compounds **7a**, **9**, **10**, **14**, **15** by acetic acid induced writhing test and hot plate test in mice



9



10



7a: X=H
14: X=OH
15: X=Cl

Compound	R(X)	Writhing test		Hot plate	
		Dose (mmol/kg)	Analgesic activity[a] (%)	Dose (mmol/kg)	Analgesic activity[b] (%)
7a		0.04	81.0	0.04	64.2
9a	Me	0.005[c]	32.1	0.01[c]	35.7
9b	$Me(CH_2)_2$–	0.005[c]	36.4	0.01[c]	36.4
9c	$Me(CH_2)_4$–	0.005[c]	69.5	0.01[c]	−13.4
9d	$Me(CH_2)_7$–	0.02[c]	57.8	0.04	62.9
9e	$Me(CH_2)_{10}$–	0.04	55.5	0.04	79.6
9f	$Me(CH_2)_{14}$–	0.04	25.3	0.04	−10.3
9g	$Me(CH_2)_{16}$–	0.04	0	0.04	−10.3
9h	Ph–	0.02[c]	44.5	0.04	117.0
10a	$Me(CH_2)_2$–	0.04	70.9	0.04	26.4
10b	$Me(CH_2)_4$–	0.04	75.2	0.04	−17.9
10c	$Me(CH_2)_7$–	0.04	36.4	0.04	4.6
10d	$Me(CH_2)_{10}$–	0.04	39.0	0.04	−5.2
10e	Ph–	0.02[c]	79.7	0.04	42.8
14	OH	0.04	88.9	0.04	91.8
15	Cl	0.02[c]	51.9	0.02[c]	98.4

[a] % Inhibition = $100 - (A/B \times 100)$, where A = incidence of writhing in the treated group and B = incidence of writhing in the control group, occurring from the 5th to 10th min after administration of the noxious agents.

[b] % Elevation of pain threshold = $100 \times (A - B)/B$, where A = latency time of treated group and B = latency time of control group. The time interval from placing animals on the surface of the hot plate to a licking of the hind paws or jumping was defined as hot plate latency.

[c] Toxic at higher dose.

9 showed higher analgesic activity (**9c**: $n = 4$, 69.5%, dose 0.005 mmol/kg in writhing test; **9d**: $n = 7$, 57.8%, dose 0.02 mmol/kg in writhing test; 62.9%, dose 0.04 mmol/kg in hot plate; **9e**: $n = 10$, 55.5% in writhing test and 79.6% in hot plate, dose 0.04 mmol/kg). These results demonstrate that substituents with suitable length will maintain definite analgesic effects. Similarly, when $n = 4$, compound in series **10** exhibited the definite analgesic activity (**10b**: $n = 4$, 75.2%, writhing test).

It is also found from Table 1 that the carbamate analogues **10** displayed lower toxicity than carboxylic ester analogues **9**. Meantime, carbamates **10** only showed remarkable analgesic activity in writhing test. These results suggest that introduction of carbamoyl groups may be favorable to decrease the toxicity and enhance the selectivity.

Compounds **9h** (R = Ph) and **10e** (R = Ph) were designed to investigate the influence of aromatic group on the pharmacological activity. The results showed that the compounds **9h** (117.0%, dose 0.04 mmol/kg in hot plate) and **10e** (R = Ph, 79.7%, dose 0.02 mmol/kg in writhing test) exhibited the highest analgesic activity in corresponding analogues. These results give us an inspiration that the introduction of aryl group may be an available route for the modification of this kind of compounds.

Compound **14** is the key intermediate for the synthesis of compounds **9** and **10**. In comparison, we tested its analgesic activity as well. Surprisingly, compound **14** showed excellent analgesic activity in both tests (88.9% in writhing test, 91.8% in hot plate, dose 0.04 mmol/kg), which encouraged us to further synthesize the chloride derivative **15**. Differing from compound **14**, the analgesic activity of compound **15** was notably different in both tests. Its hot plate test gave significant analgesic activity (98.4%, dose 0.02 mmol/kg in hot plate test), whereas writhing test only showed moderate analgesic activity (51.9%, dose 0.04 mmol/kg). Therefore, introduction of hydroxyl or chloride on the link between two spirocyclopiperiziniums may benefit the analgesic activity.

To explore whether these two series compounds were hydrolyzed by esterase and released the parent compound **14** after subcutaneous injection, we selected compounds **9a**, **9e**, **9g**, and **10d** to study their hydrolytic ability in buffer, 10% mice blood, and 10% mice brain homogenate using reported method.[18] TLC was used to monitor the result. It was found that all tested compounds were stable within 3 h whether in buffer or in biological matrices. Therefore, these compounds may act as their original forms. This result can explain the reason that only derivatives with medium length of side chain had higher activity, and coincides with our previous conclusion too.[12]

In summary, two series of dispirocyclopiperazinium salts were synthesized and evaluated for their in vivo analgesic activity in both acetic acid writhing test and

hot plate test. Some of them showed analgesic effects, especially, compounds **9h**, **14**, and **15**, with excellent analgesic activity in hot plate test. Several important structure–activity relationships were revealed from this study: (1) the introduction of aryl group can obviously improve the activity; (2) it is favorable to enhance the analgesic activity to incorporate alkyl group with suitable length in the molecule; (3) carbamate analogues displayed lower toxicity than carboxylic ester analogues; (4) both hydroxylation and chlorination of lead compound could increase the analgesic activity. These results will provide the inspiration for the further modification of this kind of compounds.

Acknowledgment

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