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Synthesis and Analgesic Activity of Hydrochlorides and Quaternary Ammoniums of Epibatidine Incorporated with Amino Acid Ester

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Abstract—Hydrochloride derivatives **5a–c** and quaternary ammonium derivatives **6a–c** of epibatidine incorporated with amino acid ester were synthesized and evaluated for their in vivo analgesic activity and toxicity. Among all tested compounds, compound **6c** has the most potent analgesic activity. The quaternary ammonium salts **6a** and **6c** showed better analgesic activity than the corresponding hydrochlorides **5a** and **5c**. Both **5a–c** and **6a–c** showed significantly lower toxicity than epibatidine itself.

Introduction

Epibatidine (**1**), isolated from the skin of the Ecuadoran poisonous frog *Epipedobates tricolor*, exhibits powerful analgesic activity and high binding selectivity to nicotinic acetylcholine receptors (nAChRs) but not to opioid receptors.[1,2] However, the therapeutic potential of epibatidine is limited due to its toxic dose only slightly higher than its effective analgesic dose.[3–6] Even though, its remarkable biological activity and unique 7-azabicyclo[2.2.1]heptane ring system have brought a great interest for the medicinal chemists. To date, numerous structure–activity relationship studies based upon the novel structure of epibatidine have been performed in search of high efficient analgesic agents with lower toxicity.[7–18] The research has resulted in the discovery of a series of active compounds, *N*-arylalkyl-7-azabicyclo[2.2.1]heptanes (**2**), one of which showed much high activity ($n=1$, Ar = 3-pyridyl, $K_i = 98 \pm 9$ nM).[19]

More recently, N^1,N^1-dimethyl-N^4-phenylpiperazinium iodide (**DMPP**, **3**), a well-known nicotinic agonist,[20–22] also received much attention[23–27] due to its peculiar structure which is unique among nicotinic ligands and does not fit any proposed model. This quaternary ammonium salt does not cross the blood–brain barrier (BBB) as required for the drugs useful to treat neurodegenerative diseases. However, it presents a $K_i = 250$ nM at a nicotinic receptor of the rat brain labeled by [^{3}H]-cytisine (thought to be represented mainly by the $\alpha_4\beta_2$ subtype). A more detailed investigation by Romanelli et al. showed that most of quaternary ammonium analogues of DMPP exhibited stronger affinity for the nicotinic receptor than the corresponding tertiary bases.[23] For example, the binding affinity of **DMPP** [$K_i = 250(30)$ nM] is about 40 times higher than that of **3a** ($K_i > 10{,}000$ nM).

We have engaged in the synthesis and biological activity study of quaternary piperazinium salts for many years, and have found several kinds of piperazinium compounds with high analgesic activity.[28] For instance, compound **4** with double quaternary ammonium salts in the molecule showed very good analgesic activity with 80.3% writhing inhibition on mice using the acetic acid writhing model at dose 20 mg/kg.[28b]

In order to develop new neuronal nAChRs ligands with better analgisic activity and lower toxicity and also inspired by the information described above, we prepared some epibatidine analogues **5a–c** with different amino acid ester group in position 3 of epibatidine, and their corresponding quaternary ammonium salts **6a–c**.

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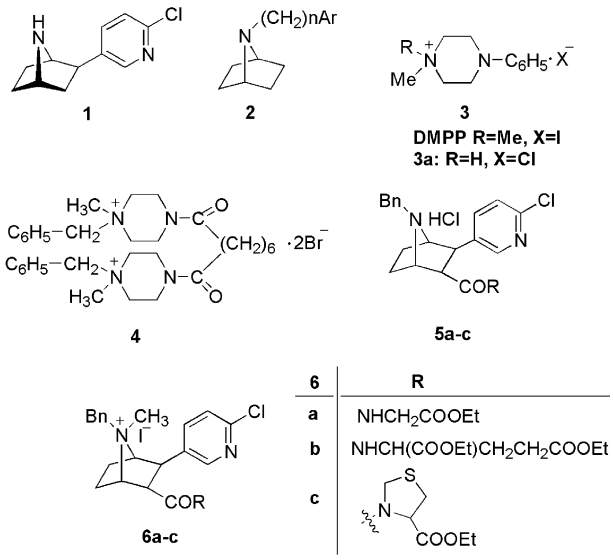
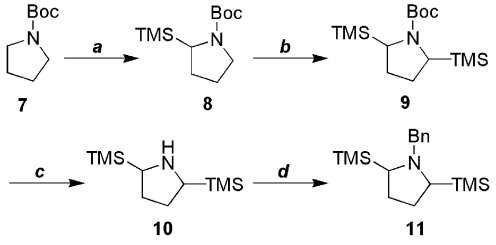
An incorporation of an amino acid ester moiety in these compounds is trying to improve the lipophilicity and increase the ability for the compound to penetrate the blood–brain barrier. Herein, we report the synthesis and in vivo analgesic activity of these new compounds.



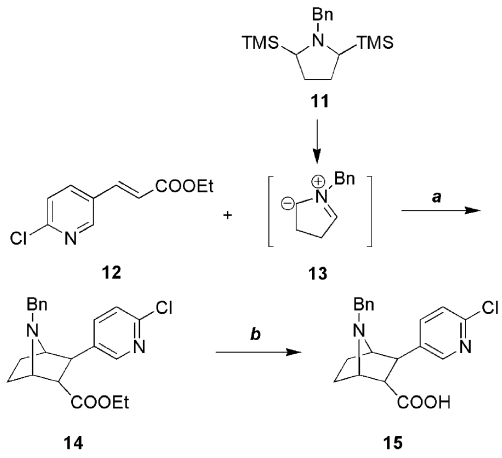
Chemistry

As shown in Scheme 1, the precursor **11** of azomethine ylide **13** was prepared from *N*-Boc-pyrrolidine through four steps in 71% yield following our improved procedure.[29] The skeleton of 7-azabicyclo[2.2.1]heptane **15** was constructed through [3+2] cycloaddition reaction of azomethine ylide **13** with ethyl 3-(6-chloro-pyridin-3-yl)-acrylate **12** according to the literature procedure[30] (Scheme 2). The ^{1}H NMR and ^{13}C NMR spectra of **14** are in complete agreement with those reported.[30]

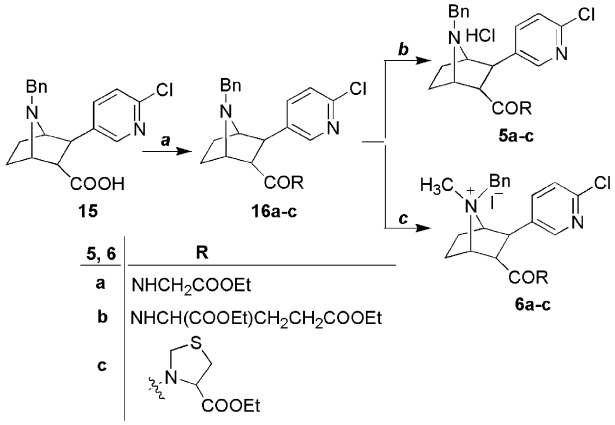
The synthetic route of compounds **5a–c** and **6a–c** was outlined in Scheme 3. Condensation of the key intermediate **15** with a variety of amino acid esters in the presence of 1,3-dicyclohexylcarbodiimide (DCC) gave the compounds **16a–c** in moderate to good yields. Treatment of compounds **16a–c** with hydrochloric acid in methanol provided the target compounds **5a–c** in quantitative yields. Quaternization of compounds **16a–c** with methyl iodide in acetone yielded the target quaternary ammonium salts **6a–c** (Scheme 3).



Scheme 1. Synthesis of compound **11**. Reagents and conditions: (a) TMEDA, *sec*-BuLi (1.1 equiv), ether, −78 °C, 2 h, TMSCl; (b) TMEDA, *sec*-BuLi (1.1 equiv), ether, −30 °C, 0.5 h, TMSCl; (c) TFA, DCM, rt 2 h; (d) BnBr, K_2CO_3, CH_3CN, reflux.



Scheme 2. Synthesis of compound **15**. Reagents and conditions: (a) AgI, DMF, rt, 4 h; (b) LiOH, MeOH–H_2O (3:1).



Scheme 3. Synthesis of compounds **5a–c** and **6a–c**. Reagents and conditions: (a) RNH_2, DCC, DCM, rt, 24 h; (b) 3 M HCl/MeOH, rt, 0.5 h; (c) MeI, acetone, rt, 6 h.

Pharmacology

All newly synthesized compounds **5a–c** and **6a–c** were evaluated for their in vivo analgesic activity and toxicity according to our reported method.[28c] The results are summarized in Table 1.

Results and Discussion

As shown in Table 1, the quaternary ammonium salts **6a** and **6c** possess obviously higher analgesic activity than the corresponding hydrochloride derivatives **5a** and **5c**. Among all tested compounds, **6c** has the most potent analgesic activity (5 mg/kg, 49.8%; 10 mg/kg, 99.3%). This result demonstrated that the formation of quaternary ammonium salt in the skeleton of epibatidine could contribute the increase of the analgesic activity.

Toxicity assays showed that no rat died at the doses of 20 mg/kg for all tested compounds except compound **6c**. Though **6c** (10 mg/kg, 80% rate of death; 5 mg/kg, no rat died) showed higher toxicity than the other tested compounds, its toxicity is still far below that of epibatidine. It is suggested from this result that the toxicity of

Table 1. The biological activities of compounds **5a–c** and **6a–c**

Compd	Dose (mg/kg sc)	Inhibitory rate[a] (%)[b]	Rate of death (%)
NS[c]	—	—	0
5a	20	26.1	0
5b	20	27.1	0
5c	20	12.3	0
6a	20	66.6	0
6b	20	1.0	0
	20	—	100
	10	99.3	80
6c	5	49.8	0
	1	9.1	0

[a]Acetic acid writhing test was used on mice.
[b]% Inhibition = 100(A/B × 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.
[c]Physiological brine.

the epibatidine could be lowered because of the formation of quaternary ammonium or introduction of amino acid ester into the skeleton of epibatidine.

Unfortunately, with the decease of the toxicity, the analgesic activity of this kind of compounds decreased also. Therefore, a further improvement is needed to lower the toxicity without lowering its activity.

In summary, hydrochloride derivatives **5a–c** and quaternary ammonium derivatives **6a–c** of epibatidine incorporated with amino acid ester were synthesized and evaluated for their in vivo analgesic activity and toxicity. The quaternary ammonium derivatives **6a** and **6c** showed better analgesic activity than the corresponding hydrochloride derivatives **5a** and **5c**. Compound **6c** is the most potent among tested compounds. Both **5a–c** and **6a–c** showed significantly lower toxicity than epibatidine itself.

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References and Notes

1. (a) Spande, T. F.; Garraffo, H. M.; Edwards, M. W.; Yeh, H. J. C.; Pannell, L.; Daly, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 3475. (b) Daly, J. W. *J. Nat. Prod.* **1998**, *6*, 162.
2. (a) Li, T. C.; Qian, C. G.; Eckman, J.; Huang, D. F.; Shen, T. Y. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2759. (b) Qian, C. G.; Li, T. C.; Shen, T. Y.; Libertinegarahan, L.; Eckman, J.; Biftu, T.; Ip, S. *Eur. J. Pharm.* **1993**, *250*, R13. (c) Fisher, M.; Huangfu, D.; Shen, T. Y.; Guyenet, P. G. *J. Pharmacol. Exp. Ther.* **1994**, *270*, 702. (d) Badio, B.; Daly, J. W. *Mol. Pharm.* **1994**, *45*, 563.
3. Sullivan, J. P.; Decker, M. W.; Brioni, J. D.; Donnelly-Roberts, D.; Anderson, D. J.; Bannon, A. W.; Kang, C.-H.; Adams, P.; Piattoni-Kaplan, M.; Buckley, M. J.; Gopalakrishnan, M.; Williams, M.; Arneric, S. P. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 624.
4. Bonhaus, D. W.; Bley, K. R.; Broka, C. A.; Fontana, D. J.; Leung, E.; Lewis, R.; Shieh, A.; Wong, E. H. F. *J. Pharmacol. Exp. Ther.* **1995**, *272*, 1199.
5. Rao, T. S.; Correa, L. D.; Reid, R. T.; Lloyd, G. K. *Neuropharmacology* **1996**, *35*, 393.
6. Molina, P. E.; Ding, Y.-S.; Carroll, F. I.; Liang, F.; Volkow, N. D.; Pappas, N.; Kuhar, M.; Abumrad, N.; Gatley, S. J.; Fowler, J. S. *Nucl. Med. Biol.* **1997**, *24*, 743.
7. Xu, R.; Bai, D.; Chu, G.; Tao, J.; Zhu, X. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 279.
8. Malpass, J. R.; Hemmings, D. A.; Wallis, A. L. *Tetrahedron Lett.* **1996**, *37*, 3911.
9. Bai, D.; Xu, R.; Chu, G.; Zhu, X. *J. Org. Chem.* **1996**, *61*, 4600.
10. Zhang, C.; Gyermek, L.; Trudell, M. L. *Tetrahedron Lett.* **1997**, *38*, 5619.
11. Wright, E.; Gallagher, T.; Sharples, C. G. V.; Wonnacott, S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2867.
12. Bencherif, M.; Caldwell, W. S.; Dull, G. M.; Lippiello, P. M. US Patent 5583140, 1996.
13. Krow, G. R.; Cheung, O. H.; Hu, Z.; Huang, Q.; Hutchinson, J.; Liu, N.; Nguyen, K. T.; Ulrich, S.; Yuan, J.; Xiao, Y.; Wypij, D. M.; Zuo, F.; Carroll, P. J. *Tetrahedron* **1999**, *55*, 7747.
14. Holladay, M. W.; Dart, M. J.; Lynch, J. K. *J. Med. Chem.* **1997**, *40*, 4169.
15. Seerden, J.-P. G.; Tulp, M.; Th, M.; Sheerren, H. W.; Kruse, C. G. *Bioorg. Med. Chem.* **1998**, *6*, 2103.
16. Rádl, S.; Hezký, P.; Hafner, W.; Buděšínský, M.; Hejnová, L. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 55.
17. Che, D.; Wegge, T.; Stubbs, M. T.; Sertz, G.; Meier, H.; Methfessel, C. *J. Med. Chem.* **2001**, *44*, 47.
18. Brieddy, L. E.; Mascarella, S. W.; Navarro, H. A.; Atkinson, R. N.; Damaj, M. I.; Martin, B. R.; Carroll, F. I. *Tetrahedron Lett.* **2001**, *42*, 3795.
19. Cheng, J.; Zhang, C.; Stevens, E. D.; Izenwasser, S.; Wade, D.; Chen, S.; Paul, D.; Trudell, M. L. *J. Med. Chem.* **2002**, *45*, 3041.
20. Kizawa, Y.; Takayanagi, I. *Gen. Pharmacol.* **1984**, *15*, 149.
21. Lippiello, P. M.; Fernandes, K. G. *Mol. Pharmacol.* **1986**, *29*, 448.
22. De Fiebre, C. M.; Meyer, E. M.; Henry, J. C.; Muraskin, S. I.; Kem, W. R.; Papke, R. L. *Mol. Pharmacol.* **1995**, *47*, 164.
23. Romanelli, M. N.; Manetti, D.; Scapecchi, S.; Borea, P. A.; Dei, S.; Bartolini, A.; Ghelardini, C.; Gualtieri, F.; Guandalini, L.; Varani, K. *J. Med. Chem.* **2001**, *44*, 3946.
24. Lin, N. H.; Meyer, M. D. *Exp. Opin. Ther. Pat.* **1998**, *8*, 991.
25. Dwoskin, L. P.; Xu, R.; Ayers, J. T.; Crooks, P. A. *Exp. Opin. Ther. Pat.* **2000**, *10*, 1561.
26. Boksa, P.; Quirion, R. *Eur. J. Pharmacol.* **1987**, *139*, 323.
27. Manetti, D.; Bartolini, A.; Borea, P. A.; Bellucci, C.; Dei, S.; Ghelardini, C.; Gualtieri, F.; Romanelli, M. N.; Scapecchi, S.; Teodori, E.; Varani, K. *Bioorg. Med. Chem.* **1999**, *7*, 457.
28. (a) Li, R. T.; Cao, S. L.; Chen, H. C.; Yang, J. Z.; Cai, M. S. *Acta Pharm. Sin.* **1996**, *31*, 757. (b) Li, R. T.; Cui, J. L.; Cai, M. S. *Acta Pharm. Sin.* **1998**, *33*, 28. (c) Li, R. T.; Cai, J. C.; Tang, X. C.; Cai, M. S. *Arch. Pharm. Pharm. Med. Chem.* **1999**, *332*, 179. (d) Wang, X.; Gao, F. L.; Piao, H. B.; Cheng, T. M.; Li, R. T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1729. (e) Gao, F. L.; Wang, X.; Zhang, H. M.; Cheng, T. M.; Li, R. T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1535.
29. Dong, J. C.; Li, R. T.; Cheng, T. M. *Chinese. Chem. Lett.* **2001**, *12*, 1061.
30. Pandey, G.; Bagul, T. D.; Sahoo, A. K. *J. Org. Chem.* **1998**, *63*, 760.