Bioorganic & Medicinal Chemistry Letters 21 (2011) 6198–6202

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of novel triplet drugs with 1,3,5-trioxazatriquinane skeletons and their pharmacologies. Part 2: Synthesis of novel triplet drugs with the epoxymethano structure (capped homotriplet)

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ARTICLE INFO

Article history:
Received 30 May 2011
Revised 16 July 2011
Accepted 16 July 2011
Available online 26 July 2011

Keywords:
Cap structure
Capped homotriplet
p-Toluenesulfonylmethyl isocyanide
Opioid
Antinociception

ABSTRACT

An improved synthetic method for triplet drugs with the 1,3,5-trioxazatriquinane skeleton was developed that used *p*-toluenesulfonylmethyl isocyanide (TosMIC) instead of 1,3-dithiane. Using the improved method, we synthesized compounds with two identical pharmacophore units and an epoxymethano group, that is, capped homotriplets. Among the synthesized capped homotriplets, KNT-123 showed high selectivity for the μ receptor over the κ receptor, and the μ selectivity was the highest among the reported μ selective nonpeptide ligands. KNT-123 administered subcutaneously induced a dose-dependent analgesic effect in the acetic acid writhing assay, and its potency was 11-fold more potent than that of morphine. KNT-123 may serve as a useful tool for the study of the pharmacological actions mediated specifically via the μ receptor.

Many twin drugs have been reported.[1,2] Symmetrical twin drugs can simultaneously fit into symmetrical binding sites of a protein complex to afford increased activity, while nonsymmetrical twin drugs may bind to individual relevant binding sites to provide dual action.[3] However, twin drugs can only play one role, either an increase of activity or a dual action. The advent of a rigid triplet drug (trimer drug) containing three pharmacophore units in a single molecule might provide the ability to deliver both increased activity and dual actions. Recently, we reported a method for synthesizing the rigid symmetrical and nonsymmetrical triplet drug **1** with a 1,3,5-trioxazatriquinane skeleton that, depending on the substituents, carried three identical and nonidentical morphinan units, respectively (Fig. 1).[4,5] Among the synthesized triplet drugs, the symmetrical triplet KNT-93 ($R^1 = R^2 = Me$, $R^3 = R^4 = OH$) showed about 56-fold more potent analgesic effects than did morphine in an acetic acid writhing test,[5] indicating that triplet drugs would be useful tools for pharmacological investigation. For the comparison with pharmacological effects induced by triplet drugs, we considered the importance of the synthesis of the corresponding compounds **2**, which have two identical pharmacophore units and the epoxymethano structure (we term the structure 'cap structure'). Moreover, the compound **2** is expected to be useful for investigation of the dimerization phenomena of G protein-coupled receptors.[6–11] Herein, we report the synthesis of novel compound **2** with the cap structure (which we named 'capped homotriplets') and their pharmacologies. We also report the improved synthesis of triplet drugs.

In the previous synthesis of triplet drugs (Scheme 1), air- and moisture-sensitive *n*-BuLi and malodorous 1,3-dithiane were used.

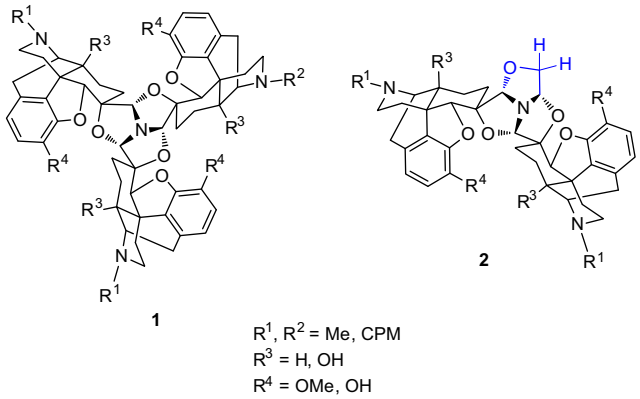

Figure 1. Structures of symmetrical or nonsymmetrical triplet drugs **1** and capped homotriplets **2**. The cap structure is depicted in blue. CPM: cyclopropylmethyl.

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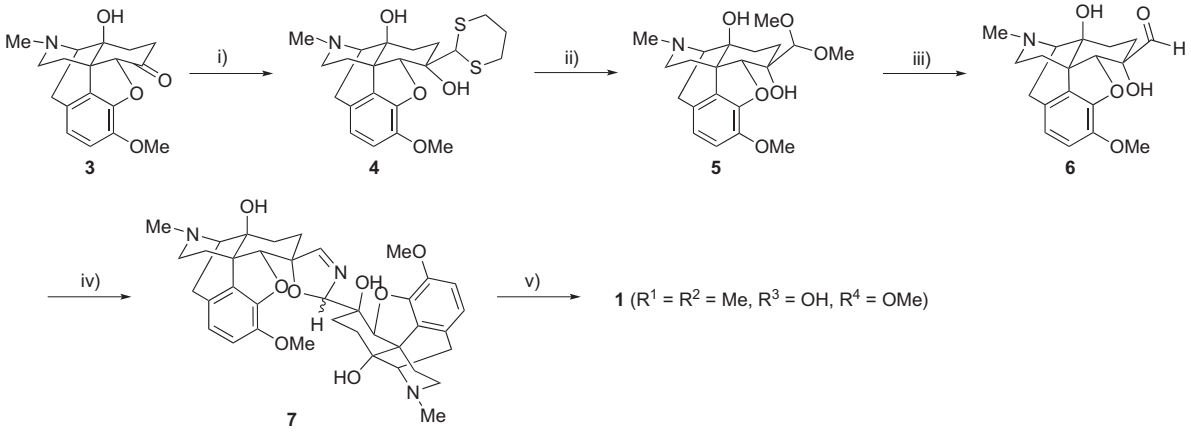
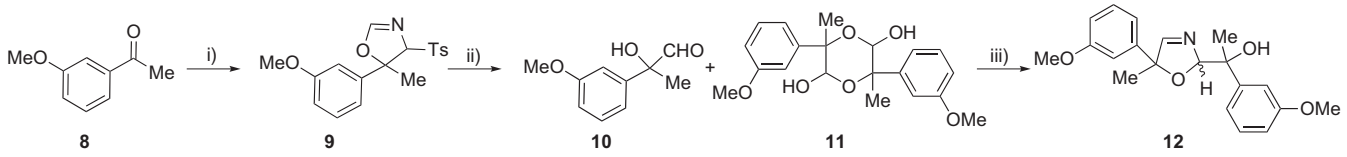
doi:10.1016/j.bmcl.2011.07.065



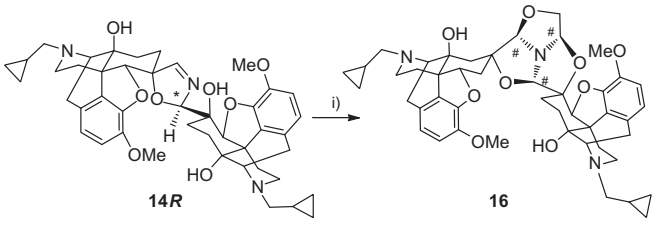
Scheme 1. Previous synthesis of triplet **1** ($R^1 = R^2 = Me$, $R^3 = OH$, $R^4 = OMe$). Reagents and conditions: (i) *n*-BuLi, 1,3-dithiane, DME, −78 °C, 65%; (ii) $CuCl_2{\cdot}2H_2O$, *p*-TsOH·H_2O, $CH(OMe)_3$, MeOH, reflux, 38%; (iii) 2 M HCl, reflux; (iv) NH_4Cl, AcONa, MeOH, reflux, 75% from **5**; (v) CSA, $CHCl_3$, reflux, 29%.



Scheme 2. New synthesis of the key intermediate oxazoline dimer **12**. Reagents and conditions: (i) TosMIC, K_2CO_3, MeOH, rt; (ii) 2 M HCl, THF, rt; (iii) NH_4Cl, AcONa, MeOH, reflux, 64% from **8**.

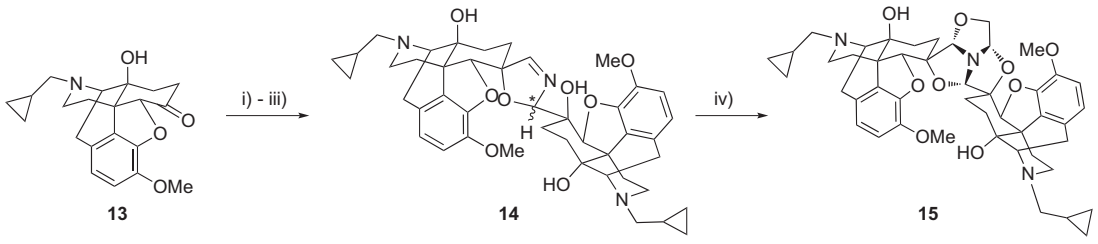
Moreover, the low reaction temperature (−78 °C) was required.[4,5] Therefore, we sought reaction conditions that were more practical and concise. After investigations using model compound **8**, we found a new synthetic method for the key oxazoline dimer intermediates **12** that used *p*-toluenesulfonylmethyl isocyanide (TosMIC).[12,13] Ketone **8** was treated with TosMIC at rt in the presence of K_2CO_3 to give tosyloxazoline intermediate **9**, followed by hydrolysis of **9** with 2 M HCl to afford a mixture of α-hydroxyaldehyde **10** and hemiacetal dimer **11**. The resulting mixture was treated with NH_4Cl in the presence of AcONa in MeOH to provide the oxazoline dimers **12** in 64% from **8** (Scheme 2).

With the milder new synthetic method for preparation of the oxazoline dimer intermediate in hand, we attempted to apply the method to the synthesis of capped homotriplet **15**. The oxazoline dimer intermediates **14**, prepared from naltrexone methyl ether (**13**) by the same method as shown in Scheme 2, was treated with the glycolaldehyde dimer in the presence of camphorsulfonic acid (CSA) at rt to give capped homotriplet **15** in 62% yield from **13** (Scheme 3). The *R*-isomer **14***R* of the oxazoline dimer **14** with the *R*-configuration at the ∗-position was reported to be the kinetically controlled product and to be converted into the corresponding trimer with *S*-configuration at all methine carbons in the trioxazatriquinan moiety via the thermodynamically controlled



Scheme 4. Reagents and conditions: (i) glycolaldehyde dimer, TMSOTf, $CHCl_3$, rt, 63%.

oxazoline dimer **14***S* with the *S*-configuration at the ∗-position.[4] Therefore, the mixture of oxazoline dimers **14***R* + **14***S* could be converted into capped homotriplet **15** under the reaction conditions described in Scheme 3. It is interesting that the treatment of the *R*-oxazoline dimer **14***R*, which was obtained by silica gel column chromatographic purification from a mixture of **14***R* and **14***S*, in the presence of TMSOTf instead of CSA provided capped homotriplet **16** with *R*-configuration at all the #-positions (Scheme 4). The configurations of the 1,3,5-trioxazatriquinane skeleton moieties in capped homotrimers **15** and **16** were determined by NOE and ROESY experiments (Fig. 2).



Scheme 3. Reagents and conditions: (i) TosMIC, K_2CO_3, MeOH, rt; (ii) 2 M HCl, THF, rt; (iii) NH_4Cl, AcONa, MeOH, reflux; (iv) glycolaldehyde dimer, CSA, $CHCl_3$, rt, 62% from **13**.

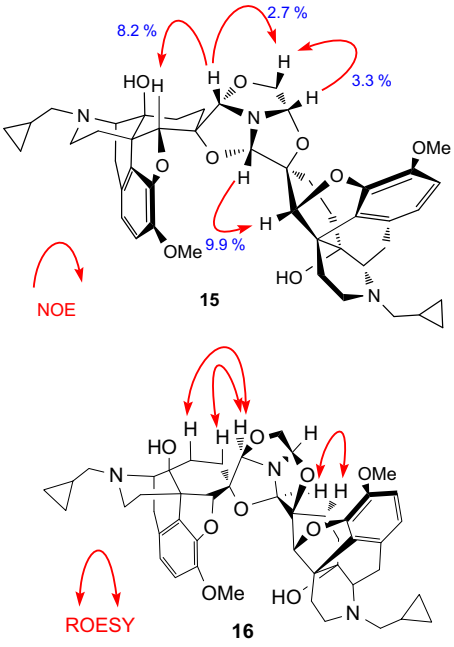
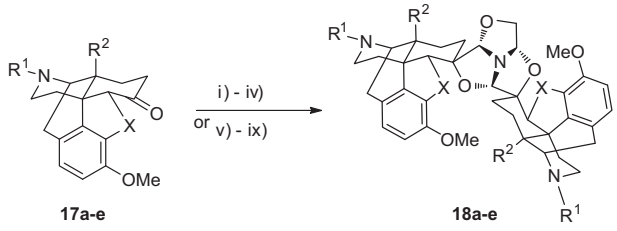
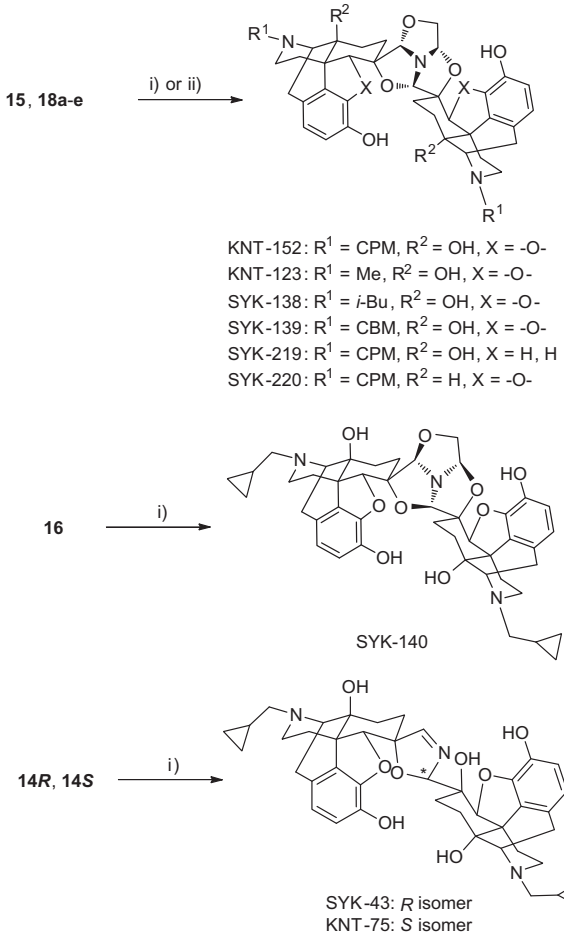


Figure 2. Observed NOEs and ROESYs in compounds **15** and **16**, respectively.



a: R^1 = Me, R^2 = OH, X = -O-
b: R^1 = *i*-Bu, R^2 = OH, X = -O-
c: R^1 = CBM, R^2 = OH, X = -O-
d: R^1 = CPM, R^2 = OH, X = H, H
e: R^1 = CPM, R^2 = H, X = -O-

Scheme 5. Reagents and conditions: (i) TosMIC, K_2CO_3, MeOH, rt; (ii) 2 M HCl, THF, rt; (iii) NH_4Cl, AcONa, MeOH, reflux; (iv) glycolaldehyde dimer, CSA, $CHCl_3$, rt, 12–19% from **17**; (v) *n*-BuLi, 1,3-dithiane, DME, −78 °C, 65–90%; (vi) $CuCl_2{\cdot}2H_2O$, *p*-TsOH·H_2O, $CH(OMe)_3$, MeOH, reflux; (vii) 2 M HCl, reflux; (viii) NH_4Cl, AcONa, MeOH, reflux; (ix) glycolaldehyde dimer, CSA, $CHCl_3$, rt, 9–12% from **17**.



Scheme 6. Reagents and conditions: (i) 1-PrSH, *t*-BuOK, DMF, 130 °C, 34–86%; (ii) BBr_3, CH_2Cl_2, 0 °C, 83%.

Capped homotriplets **18** with various *N*-substituents or without 4,5-epoxy bridges[14] or angular hydroxy groups were synthesized from the corresponding ketones **17** by the new (TosMIC method) or by the previous synthetic method using dithiane (Scheme 5). Obtained capped homotriplets **15**, **16**, and **18** and intermediate oxazoline dimers **14***R* and **14***S* were demethylated with 1-PrSH/*t*-BuOK or BBr_3 (Scheme 6).

The binding affinities of the prepared capped homotriplets and oxazoline dimer derivatives for the opioid receptors were evaluated with a competitive binding assay. The results were shown in Table 1 with the affinities of naltrexone for comparison. The assays were performed by a modification of a previously reported procedure.[15] The binding affinities of SYK-138 and SYK-140 for all types of opioid receptors were much lower than those of the other test compounds. The low affinities of SYK-138 may arise from both the weak electron donating ability and steric hindrance of *N*-isobutyl groups. Although the methyl group is a weaker electron donor than the isobutyl group, the affinities of KNT-123 with *N*-methyl groups was stronger than those of SYK-138. These results

Table 1
Binding affinity of triplet drugs for the opioid receptors[a]

Compound	K_i (μ)[b] (nM)	K_i (δ)[c] (nM)	K_i (κ)[d] (nM)	Selectivity	
				κ/μ	δ/μ
Naltrexone	0.265	12.27	0.702	2.64	46.23
KNT-152	0.480	16.15	2.752	5.73	33.62
KNT-123	2.093	52.86	337.4	161.2	25.26
SYK-138	25.27	>1000	118.7	4.70	—
SYK-139	2.600	49.96	176.1	67.73	19.22
SYK-219	0.771	5.979	8.670	11.25	7.76
SYK-220	0.719	7.797	6.355	8.84	10.85
SYK-140	38.22	>1000	247.2	6.47	—
SYK-43	1.031	39.62	1.656	1.61	38.43
KNT-75	1.900	53.74	1.164	0.61	28.28

[a] Binding assays were carried out in duplicate (κ: cerebellum of guinea pig, μ and δ: whole brain without cerebellum of mouse).
[b] [^{3}H] DAMGO was used.
[c] [^{3}H] DPDPE was used.
[d] [^{3}H] U-69,593 was used.

may be due to reduced steric hindrance of the methyl group in comparison to the isobutyl group and/or the high contribution of methyl group to the affinity for the μ receptor.[5,16] In contrast to the low affinity of SYK-140, its stereoisomer, KNT-152 showed sufficient affinity for each type of opioid receptor. The difference of affinities between the two stereoisomers SYK-140 and KNT-152 may stem from the different configurations of the trioxazatriquinane skeletons in the two isomers. The absence of the 4,5-epoxy bridges or the angular hydroxy groups (as seen in SYK-219 or

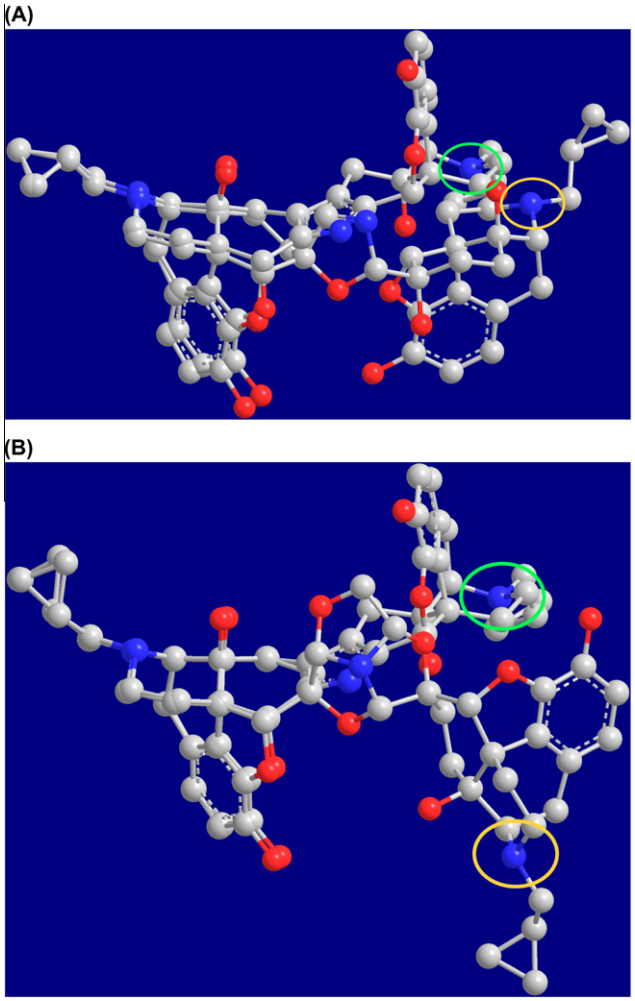


Figure 3. Superimposition of the 3D-alignment of nor-BNI onto that of KNT-75 (A) or KNT-152 (B). The 17′-nitrogen, which was reported to important to exert κ selectivity,[26] and the corresponding nitrogen in nor-BNI and KNT-75 or KNT-152 were indicated by green and yellow circle, respectively.

SYK-220) afforded only a small effect on their affinities. Interestingly, the capped homotriplet KNT-152 showed μ selectivity, whereas its precursor, the oxazoline dimer KNT-75, exhibited κ selectivity. The relative spatial locations of the two 4,5-epoxymorphinan units in KNT-75 appeared to be similar to that of the selective κ antagonist, nor-BNI,[17,18] while those of KNT-152 were different (Fig. 3). It was noteworthy that KNT-123 showed

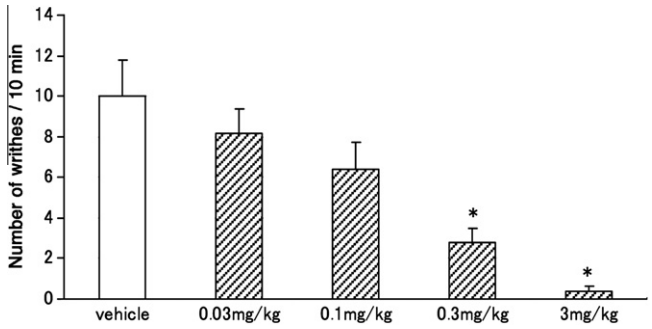


Figure 4. Analgesic effects induced by KNT-123 in the mouse acetic acid writhing test. The statistical significance of differences between the groups was assessed with one-tailed nonparametric Williams' test. *p <0.025 versus saline treated mice.

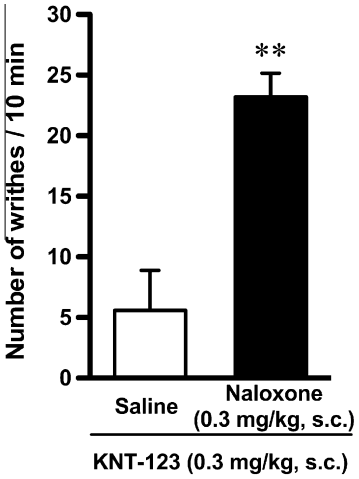


Figure 5. Effects of naloxone on the antinociception induced by KNT-123 in the mouse acetic acid writhing test. The statistical significance of differences between the groups was assessed with Student's t-test. **p <0.01 versus saline treated mice.

higher selectivity for the μ receptor over the κ receptor. A general trend for the nonpeptide ligands has been low selectivity for the μ receptor type, especially with respect to the κ receptor.[19–22] To the best of our knowledge, the μ selectivity of KNT-123 over the κ receptor appears to be the highest of the reported μ selective nonpeptide ligands. The particular pharmacological effects mediated through the μ receptor including side effects have not yet been clarified because no nonpeptide ligands with sufficient selectivity for the μ receptor have been available. Therefore, KNT-123 is expected to be a useful tool for the investigation of pharmacologies via the μ receptor.

We next evaluated the analgesic effects induced by KNT-123 in the acetic acid writhing test.[23,24] Subcutaneous administration of KNT-123 exhibited dose-dependent antinociception (Fig. 4) and the effect was significantly reduced by a μ antagonist naloxone (Fig. 5). The analgesic effect of KNT-123 (ED_{50} = 0.14 mg/kg) was nearly fourfold more potent than that of morphine, a representative μ agonist (ED_{50} = 0.6 mg/kg), but about fourfold less potent than that symmetrical triplet KNT-93 (ED_{50} = 0.037 mg/kg).[5] When the ED_{50} values were converted from gram units to molar units,[25] the potency of KNT-123 in antinociception (ED_{50} = 0.18 μmol/kg) was 11-fold higher than that of morphine (ED_{50} = 1.9 μmol/kg), but fivefold lower than that of KNT-93 (ED_{50} = 0.034 μmol/kg).

In conclusion, we have developed an improved synthetic method for oxazoline dimers, which were the key intermediates for the synthesis of triplet drugs with the 1,3,5-trioxazatriquinane skeleton. Using the improved method, we synthesized compounds with two identical pharmacophore units and an epoxymethano group, that is, capped homotriplets. Among the synthesized capped homotriplets, KNT-123 showed high selectivity for the μ receptor over the κ receptor, and the μ selectivity was the highest among the reported μ selective nonpeptide ligands. KNT-123 administered subcutaneously induced dose-dependent analgesic effects in the acetic acid writhing assay, and its potency was 11-fold more potent than that of morphine. KNT-123 is expected to be a useful tool for the study of the specific pharmacological actions via the μ receptor.

Acknowledgments

We acknowledge the financial supports from Shorai Foundation for Science and Uehara Memorial Foundation. We also acknowledge the Institute of Instrumental Analysis of Kitasato University, School of Pharmacy for its facilities.

Supplementary data

Supplementary data (new synthesis of the oxazoline dimmers **12**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.065.

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