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# Synthesis of novel triplet drugs with 1,3,5-trioxazatriquinane skeletons and their pharmacologies. 3: Synthesis of novel triplet drugs with the bis(epoxymethano) or bis(dimethylepoxymethano) structure (double-capped triplet)

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ABSTRACT

Novel double-capped triplet drugs, which have one pharmacophore unit and two epoxymethano or dimethylepoxymethano structures (termed cap or diMe-cap structures, respectively) were synthesized. Key intermediate oxazoline **16** derived from acetone enabled the effective synthesis of double-capped triplets. SYK-134 (**7a**) and SYK-135 (**8a**) with *N*-cyclopropylmethyl substituent and cap structures showed selectivities for the $\kappa$ opioid receptor. On the other hand, the *N*-Me series exhibited selectivities for the $\mu$ opioid receptor. The double-capped triplet drugs with diMe-cap structures preferred the $\mu$ receptor independently of their *N*-substituents. SYK-385 (**19b**), one of the $\mu$-selective double-capped triplet drugs, showed the highest selectivity for the $\mu$ receptor among the reported $\mu$-selective nonpeptide ligands.


Many twin drugs have been reported.[1,2] Symmetrical twin drugs can simultaneously fit into the symmetrical binding sites of a protein complex to afford increased activity, whereas nonsymmetrical twin drugs may bind to each individual binding site to give dual action.[3] However, twin drugs can act in only one mode, either with an increase of activity or with a dual action. The advent of a rigid triplet drug (trimer drug) containing three pharmacophore units in a single molecule is expected to provide the ability to deliver both increased activity and dual actions. Recently, we have reported a synthetic method for rigid symmetrical and nonsymmetrical triplet drugs **1** with the 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 drug 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. We also synthesized capped homotriplet drugs **2**, which have two identical pharmacophore units and the epoxymethano structure (termed 'cap structure').[6] One of the synthesized capped triplet drugs, KNT-123 ($R^3$, $R^4$ = OH, $R^5$ = Me) showed the highest selectivity for the $\mu$ opioid receptor over the $\kappa$ opioid receptor among the reported $\mu$-selective nonpeptide ligands.[6] For the comparison with pharmacological effects induced by triplet drugs **1** or capped triplet drugs **2**, we considered the importance of the synthesis of the corresponding double-capped triplet drugs **7** and **8** (Scheme 1), which have a pharmacophore unit and two cap structures. Double-capped triplet drugs **7** and stereoisomer **8** were expected to provide insights into the effect of the

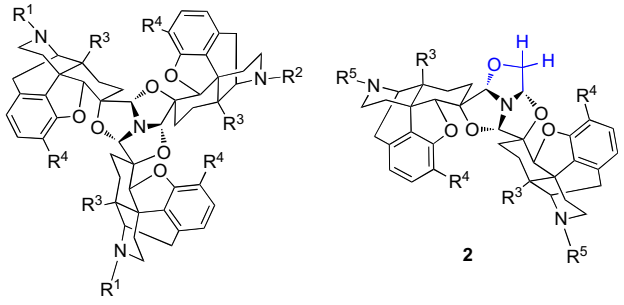


**Figure 1.** Structures of symmetrical or nonsymmetrical triplet drugs **1** and capped homotriplets **2**. The cap structure is depicted in blue. CPM: cyclopropylmethyl, CBM: cyclobutylmethyl

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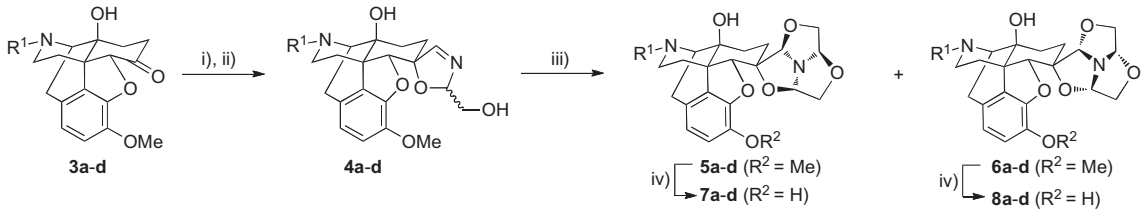



**Scheme 1.** Reagents and conditions: (i) TosMIC, $K_2CO_3$, MeOH, rt; (ii) 2 M HCl, 60 °C, then glycolaldehyde dimer, HMDS, $NH_4Cl$, AcONa, MeOH, rt, **4a**: 50% from **3a**, **4b**: 67% from **3b**, **4c**: 86% from **3c**, **4d**: 45% from **3d**; (iii) glycolaldehyde dimer, CSA, $CHCl_3$, rt, **5a**: 18%, **6a**: 45%, **5b**: 21%, **6b**: 21%, **5c**: 21%, **6c**: 20%, **5d**: 27%, **6d**: 37%; (iv) *n*-PrSH, *t*-BuOK, DMF, 130 °C, **7a**: 60%, **8a**: 62%, **7b**: 81%, **8b**: 69%, **7c**: 86%, **8c**: 36%, **7d**: 71%, **8d**: 70%. CPM: cyclopropylmethyl, TosMIC: *p*-toluenesulfonylmethyl isocyanide, HMDS: hexamethyldisilazane, CSA: camphorsulfonic acid.

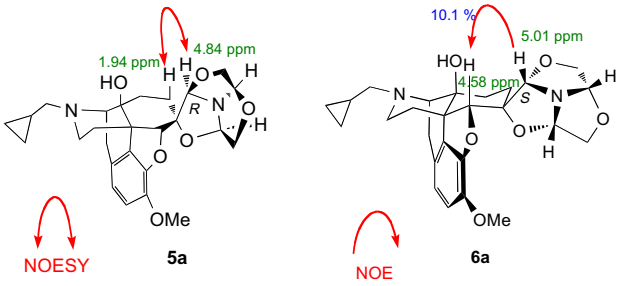
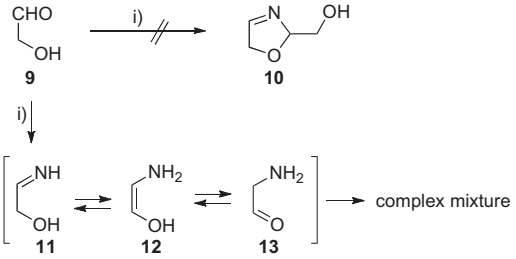


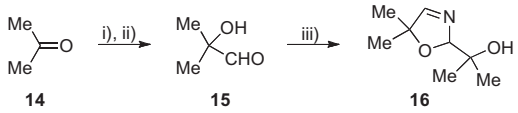

**Figure 2.** Observed NOESY and NOE in compounds **5a** and **6a**, respectively.

1,3,5-trioxazatriquinane skeleton on the pharmacological properties. Herein, we report the synthesis of double-capped triplet drugs **7** and **8** and their pharmacologies.

Synthesis of double-capped triplet drugs **7** and **8** stemmed from naltrexone methyl ether (**3a**) or the related compounds **3b**–**d** (Scheme 1). Naltrexone methyl ether (**3a**) was treated with *p*-toluenesulfonylmethyl isocyanide (TosMIC) in the presence of $K_2CO_3$, and then 2 M HCl.[6] The thus obtained α-hydroxyaldehyde was converted into oxazoline **4a** by the treatment with glycolaldehyde dimer in 50% yield from **3a**. The reaction of oxazoline **4a** with glycolaldehyde dimer afforded the mixture of double-capped triplets **5a** and **6a** in 18% and 45% yield, respectively. NOESY and NOE experiments determined that the configuration of the methine moiety of 1,3,5-trioxazatriquinane skeleton in **5a** and **6a** was *R* and *S*, respectively (Fig. 2). *O*-Demethylation of **5a** and **6a** was conducted by treatment with *n*-PrSH and *t*-BuOK to give the corresponding phenolic compounds **7a** (SYK-134) and **8a** (SYK-135) in respective 60% and 62% yields. The other double-capped triplet drugs **7b**–**d** and **8b**–**d** with *N*-Me, *i*-Bu, or Bn substituents were prepared from the corresponding compounds **3b**–**d** in the same manner (Scheme 1).



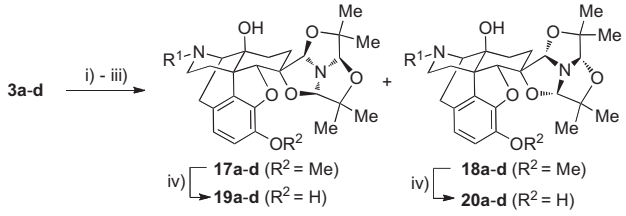

**Scheme 2.** Reagents and conditions: (i) glycolaldehyde dimer, $NH_4Cl$, AcONa, MeOH, rt.




**Scheme 3.** Reagents and conditions: (i) TosMIC, $K_2CO_3$, MeOH, rt; (ii) 2 M HCl, THF, rt; (iii) $NH_4Cl$, AcONa, MeOH, reflux, 71% from **14**.

As shown in Scheme 1, we achieved the synthesis of the double-capped triplet drugs **7** and **8** with various N-substituents. However, this method required the stepwise synthesis of each oxazoline **4** for the respective double-capped triplet drugs **7** and **8**. Therefore, we attempted to develop a more efficient one-pot synthesis using oxazoline **10** composed from two glycolaldehyde units. The reaction of oxazoline **10** with α-hydroxyaldehyde derived from **3** would then directly provide double-capped triplets **5** and **6**. However, our attempt to synthesize oxazoline **10** furnished a complex product mixture (Scheme 2). This outcome may have resulted from labile intermediate **11**. Iminoalcohol **11** with hydrogens at the α-position of the hydroxy group would be in equilibrium among **11**, aminoenol **12**, and aminoaldehyde **13** (Scheme 2). Inspired with this result, we planned to synthesize new targets **19** and **20**. For the purpose, we attempted to synthesize α-hydroxyaldehyde **15** which lacked α-protons (Scheme 3). Acetone (**14**) was converted into α-hydroxyaldehyde **15** by the procedure described above. The treatment of **15** with $NH_4Cl$ in the presence of AcONa provided oxazoline **16** in 71% yield from **14**.

With oxazoline **16** in hand, we then reacted **16** with α-hydroxyaldehyde derived from naltrexone methyl ether (**3a**) to afford the other double-capped triplets **17a** and **18a** in 15% and 21% yield, respectively (Scheme 4). The obtained double-capped triplets **17a** and **18a** have two dimethylepoxymethano structures (hereafter termed 'dimethylcap (diMe-cap) structure'). The configuration of the methine moiety of the 1,3,5-trioxazatriquinane




**Scheme 4.** Reagents and conditions: (i) TosMIC, $K_2CO_3$, MeOH, rt; (ii) 2 M HCl, 60 °C; (iii) oxazoline **16**, CSA, $CHCl_3$, reflux, **17a**: 15% from **3a**, **18a**: 21% from **3a**, **17b**: 17% from **3b**, **18b**: 27% from **3b**, **17c**: 22% from **3c**, **18c**: 48% from **3c**, **17d**: 35% from **3d**, **18d**: 48% from **3d**; (iv) $BBr_3$, $CH_2Cl_2$, −78 °C to rt, **19a**: 86%, **20a**: 93%, **19b**: quant., **20b**: 83%, **19c**: quant., **20c**: 91%, **19d**: 94%, **20d**: 63%.

**Table 1**
Binding affinities of synthesized double-capped triplet drugs for opioid receptors[a]

| Compound | N-Substituent | Configuration | $K_i$ (nM) | | | κ Selectivity | | μ Selectivity | |
|---|---|---|---|---|---|---|---|---|---|
| | | | μ[b] | δ[c] | κ[d] | μ/κ | δ/κ | δ/μ | κ/μ |
| **7a** (SYK-134) | CPM | R | 8.65 | 99.8 | 3.86 | 2.24 | 25.9 | 11.5 | 0.446 |
| **8a** (SYK-135) | | S | 6.85 | 78.6 | 5.95 | 1.15 | 13.2 | 11.5 | 0.869 |
| **19a** (SYK-342) | | R | 2.14 | 71.8 | 7.89 | 0.271 | 9.10 | 33.6 | 3.69 |
| **20a** (SYK-341) | | S | 5.35 | 22.0 | 24.2 | 0.221 | 0.909 | 4.11 | 4.52 |
| **7b** (SYK-379) | Me | R | 2.34 | 171 | 109 | 0.0214 | 1.57 | 73.1 | 46.6 |
| **8b** (SYK-380) | | S | 2.38 | 133 | >1000 | <0.0024 | <0.133 | 55.9 | >420 |
| **19b** (SYK-385) | | R | 1.85 | 79.1 | >1000 | <0.0019 | <0.079 | 42.8 | >541 |
| **20b** (SYK-386) | | S | 2.67 | 9.78 | >1000 | <0.0027 | <0.0098 | 3.66 | >375 |
| **7c** (SYK-381) | *i*-Bu | R | 99.1 | 79.1 | >1000 | <0.099 | <0.079 | 0.798 | >10.1 |
| **8c** (SYK-382) | | S | 85.7 | 848 | >1000 | <0.086 | <0.848 | 9.89 | >11.7 |
| **19c** (SYK-394) | | R | 88.1 | >1000 | >1000 | <0.088 | —[e] | >11.4 | >11.4 |
| **20c** (SYK-395) | | S | 134 | 541 | >1000 | <0.134 | <0.541 | 4.04 | >7.46 |
| **7d** (SYK-368) | Bn | R | >1000 | >1000 | >1000 | —[e] | —[e] | —[e] | —[e] |
| **8d** (SYK-369) | | S | 581 | 84.0 | >1000 | <0.581 | <0.084 | 0.145 | >1.72 |
| **19d** (SYK-396) | | R | 450 | >1000 | >1000 | <0.450 | —[e] | >2.22 | >2.22 |
| **20d** (SYK-397) | | S | >1000 | >1000 | >1000 | —[e] | —[e] | —[e] | —[e] |

[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.
[e] Selectivity was not calculated as both $K_i$ values were over 1000 nM.

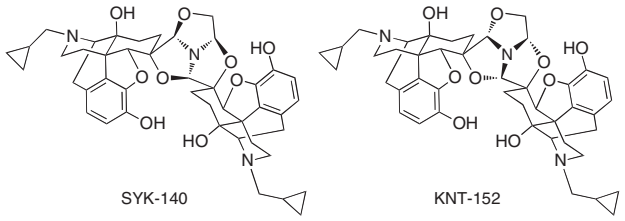



**Figure 3.** Structures of capped homotriplet drugs SYK-140 and KNT-152.

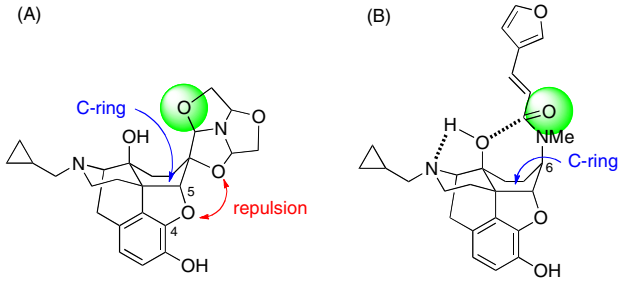



**Figure 4.** Proposed active conformation of SYK-134 (**7a**) and SYK-135 (**8a**) (A) and nalfurafine (B) binding to the κ receptor. The green sphere indicates a hydrogen bond accepting pharmacophore.

skeleton in **17a** and **18a** was determined by NOE and NOESY experiments. Double-capped triplets **17a** and **18a** were demethylated with $BBr_3$ to give the corresponding phenolic compounds **19a** and **20a** in 86% and 93% yield, respectively. The other double-capped triplet drugs **19b–d** and **20b–d** with diMe-cap structures were prepared in the same manner (Scheme 4).

The binding affinities of the prepared double-capped triplet drugs for the opioid receptors were evaluated with competitive binding assays (Table 1). The assays were performed by a previously reported procedure.[7] In the capped homotriplet drugs with *N*-cyclopropylmethyl (CPM) substituents, the configuration of the methine moiety of 1,3,5-trioxazatriquinane skeleton had an extreme effect on the binding affinities: the affinities ($K_i$ (μ) = 38.33 nM, $K_i$ (δ) = >1000 nM, $K_i$ (κ) = 247.2 nM) of SYK-140 (*R*-isomer, Fig. 3) were much worse than those ($K_i$ (μ) = 0.480 nM, $K_i$ (δ) = 16.15 nM, $K_i$ (κ) = 2.752 nM) of KNT-152 (*S*-isomer, Fig. 3).[6] In contrast, double-capped triplet drugs with the *N*-CPM group demonstrated sufficient binding to each type of opioid receptor independently of their configurations.

SYK-134 (**7a**) and SYK-135 (**8a**) with cap structures showed κ selectivities, whereas SYK-341 (**20a**) and SYK-342 (**19a**) with diMe-cap structures exhibited μ selectivities. SYK-134 (**7a**) (*R*-isomer) was more selective for the κ receptor than SYK-135 (**8a**) (*S*-isomer), while SYK-342 (**19a**) (*R*-isomer) was more selective for the μ receptor over the δ receptor than SYK-341 (**20a**) (*S*-isomer). On the other hand, the affinities for the κ receptor of compounds with an *N*-Me substituent were extremely decreased, whereas their affinities for the μ receptor were increased compared to the compounds with *N*-CPM group. As a result, double-capped triplet drugs with the *N*-Me substituent showed μ selectivity. This substituent effect was also observed in other morphinan derivatives.[8–11] Among compounds with *N*-Me substituent, SYK-385 (**19b**) was the most selective for the μ receptor and was more selective than KNT-123,[6] which was a capped homotriplet drug with an *N*-Me group and was reported to show the highest selectivity for the μ receptor over the κ receptor among the reported μ-selective nonpeptide ligands.[12–15] The affinities of compounds with bulky substituents (*i*-Bu or Bn group) on the nitrogen were very low.

SYK-134 (**7a**) and SYK-385 (**19b**), which were the most selective compounds for the κ and μ receptor, respectively, were assessed for functional activity in the [$^{35}$S]GTPγS-binding assay in human receptor transfected CHO cells. Procedures similar to those previously reported[16] were used. Both compounds showed agonist activities: SYK-134 (**7a**): $EC_{50}$ (κ) = 25.0 nM, $E_{max}$ (κ)[17] = 70.0%; SYK-385 (**19b**): $EC_{50}$ (μ) = 6.0 nM, $E_{max}$ (μ)[17] = 79.1%.

The κ selectivity of SYK-134 (**7a**) and SYK-135 (**8a**) can be interpreted in terms of their possible conformation: the dipole-dipole repulsion between 4,5-epoxy moiety and the cap structure would raise the 1,3,5-trioxazatriquinane structure to the upper side of the C-ring (Fig. 4A), which would resemble the active conformation of nalfurafine[18–21] (Fig. 4B) for binding to the κ receptor. According to our 3D-pharmacophore model of κ agonists,[20,21] the 6-amide

side chain of nalfurafine is one of the important pharmacophores and functions as a hydrogen bond acceptor. The oxygen of the cap structure in SYK-134 (**7a**) and SYK-135 (**8a**) may also function as a hydrogen bond acceptor. As a result, SYK-134 (**7a**) and SYK-135 (**8a**) would exert $\kappa$ selectivity and $\kappa$ agonist activity. It is interesting that the diMe-cap structure increased and decreased the affinities for the $\mu$ and $\kappa$ receptor, respectively, to exhibit $\mu$ selectivity in both the *N*-CPM and *N*-Me series. These phenomena indicate that the $\kappa$ receptor binding site, with which the cap or diMe-cap structures would interact, might be less spacious and more restricted compared to the $\mu$ receptor.

Concerning the symmetrical triplet drugs with three identical pharmacophore units, the 1,3,5-trioxazatriquinane skeleton seemed to mainly function as a spacer because the binding profiles of the triplets resembled that of each pharmacophore unit. On the other hand, the 1,3,5-trioxazatriquinane skeleton in the double-capped triplets effected the binding profiles of the triplets; the selectivity for the $\kappa$ receptor was provided to SYK-134 (**7a**) and SYK-135 (**8a**), while SYK-385 (**19b**) was obtained the selectivity the $\mu$ receptor.

In conclusion, novel double-capped triplet drugs with cap or diMe-cap structures were synthesized. Key intermediate oxazoline **16** enabled the effective synthesis of double-capped triplets. SYK-134 (**7a**) and SYK-135 (**8a**), which had the *N*-CPM substituent and cap structures, showed $\kappa$ selectivities. On the other hand, the *N*-Me series exhibited $\mu$ selectivities. The double-capped triplet drugs with diMe-cap structures preferred the $\mu$ receptor independently of their *N*-substituents. SYK-385 (**19b**), one of the $\mu$-selective double-capped triplet drugs, showed the highest selectivity for the $\mu$ receptor among the reported $\mu$-selective nonpeptide ligands.

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