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Synthesis and biological evaluation of C-12 triazole and oxadiazole analogs of salvinorin A

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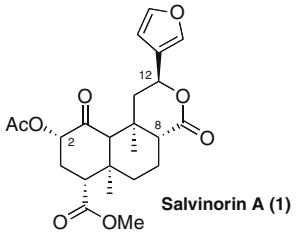
ABSTRACT

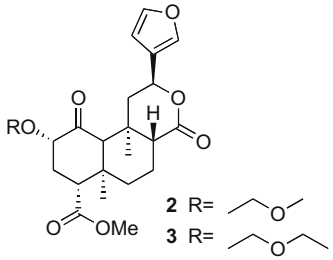
Salvinorin A (**1**), the main active ingredient of *Salvia divinorum*, is a potent and selective κ-opioid receptor (KOPR) agonist. A series of C-12 triazole analogs and the oxadiazole (**4**) analog of **1** are synthesized and screened for binding affinity at κ, μ (MOPR), or δ (DOPR). Surprisingly, all triazole analogs have shown negligible binding affinity at opioid receptors and the oxadiazole **4**, a reported MOPR and KOPR antagonist, exhibits very low affinities to opioid receptors and no antagonism in our binding assays. These results suggest that electronic factors that may affect either the electron density of hydrogen bond acceptor at C-12 or hydrophobic interactions between C-12 moiety and KOPR are critical to C-12 analog's affinity for KOPR.

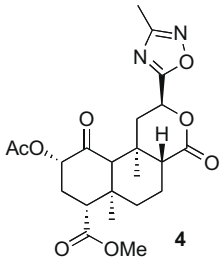
Salvinorin A (**1**), a naturally occurring hallucinogen, has attracted much attention due to its potent KOPR agonist activity. It represents the first non-alkaloid opioid subtype-selective drug.[1] The unique structural and biological features of **1** make it an attractive probe for exploring opioid pharmacology. It can also function as a potential template for development of novel psychotherapeutic drugs.



Salvinorin A (1)

Prior structure–activity relationship studies of **1** by our group and others have largely focused on its A ring.[2] These studies have led to the synthesis of methoxymethyl ether (**2**)[3] and ethoxymethyl ether (**3**).[4] Currently, **2** and **3** are the only salvinorin derivatives with a higher binding affinity and potency than **1** at KOPR. Compound **2** also exhibits longer duration of action than **1** in vivo.[5] Interestingly, prolonged use of drugs such as cocaine and amphetamine has been found to upregulate the KOPR system. When the drugs are withdrawn, the upregulated KOPR system becomes unmasked and the dysphoric states are manifested, which drives the patient's craving for the drug to normalize the mood.[6] Therefore, it is reasonable to conclude that selective KOPR antagonists can be useful relapse prevention agents for drug abuse patients. They are also potential anxiolytics and antidepressants. Moreover, small-molecule selective KOPR antagonists are invaluable tools in the understanding of pharmacological functions of KOPR.[7]





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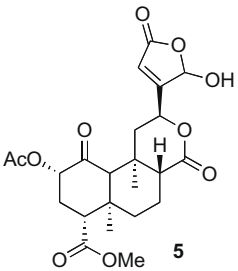

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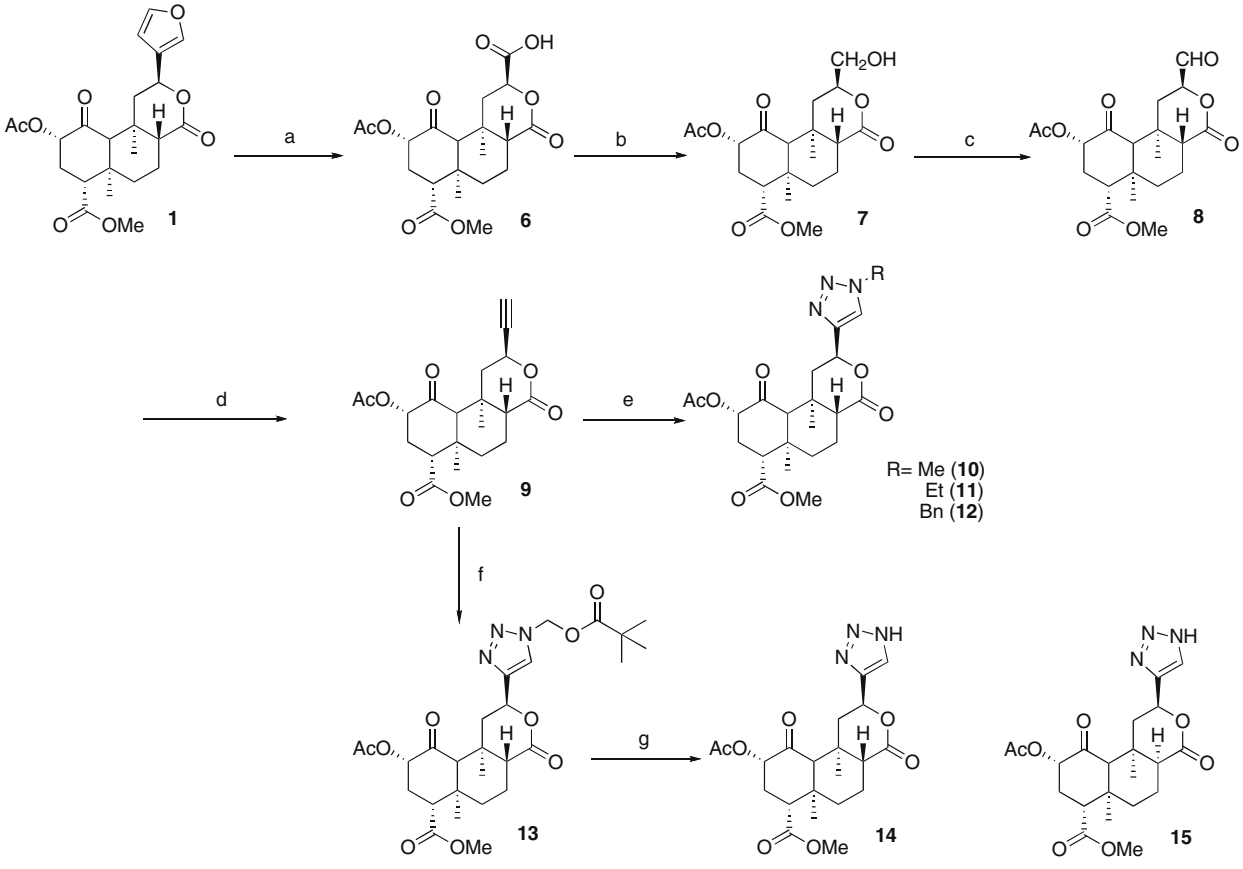
Recent studies by Prisinzano et al.[8] have shown that the furan ring modified analogs of **1**, oxadiazole (**4**) and salvidivin A (**5**), exhibit antagonist activities at MOPR and KOPR. The reduced furan ring stereochemistry of **5** may also affect its efficacy.[8] According to Roth's binding model of **1** at KOPR, the furan oxygen may act as a hydrogen bond acceptor to tyrosine moieties of the KOPR.[9] It is then conceivable that either a substitution of the furan oxygen with other hetero-atoms or a modification of the stereochemistry of the oxidized furan ring may cause a change in the binding behavior of **1** at the KOPR. This could then lead to changes in **1**'s efficacy at the receptors leading to alterations in its subsequent coupling to G proteins. Therefore, we feel that optimization of the hydrogen bond acceptor properties of the five-membered ring in **1**, especially those heterocycles that may interact with residues in the binding pocket of KOPR, might be a viable way to find novel selective KOPR antagonists. To this end, a series of C-12 modified analogs of **1** ought to be synthesized in particular the five-membered heterocycles that resemble the furan ring in **1** would have priority.

As evidenced, 1,2,3-triazole pharmacophores have displayed a broad spectrum of biological activities associated with antibacterial, antifungal and herbicidal, antiallergic, and anti-HIV.[10] We were also encouraged by the advances in click chemistry, particularly the copper (I)-catalyzed 1,3-dipolar cycloaddition of organic azides and alkynes which allows efficient construction of the triazole framework under mild conditions.[11] Furthermore, replacing of the furan ring of **1** with a triazole ring would increase its drug-like property if considering that the average nitrogen content per molecule in natural products is lower than in drug molecules, and the opposite is observed for oxygen content.[12] Here, we report the synthesis and biological evaluation of a series of C-12 triazole analogs of **1**. To provide a direct in vitro comparison of the triazoles with the known KOPR antagonist, **4** is synthesized by following the reported procedure and isolated with negligible amount of C-8 epimer.[8b]

Salvinorin A is isolated from *Salvia divinorum* as previously described.[13] It is degraded by ruthenium-catalyzed oxidation to acid (**6**),[14] which provides an ideal template for further modification at C-12 position (Scheme 1). Based on our previous work in chemoselective reduction of the C-4 acid to alcohol,[15] **6** is reduced with borane to primary alcohol (**7**) at 55 °C. Notably, forcing conditions, for example, excess borane or higher temperature, have no effect in improving the yield of **7** or leading to C-8 epimerization that normally observed under basic conditions. Attempts to oxidize **7** with Swern or PCC conditions form a complex mixture of products. Alternatively, it is treated with Dess–Martin periodinane at 0 °C to provide the desired aldehyde (**8**) in good yield. We find quenching the reaction with sodium bicarbonate significantly lowers the yield of **8**, presumably due to the hydrolysis of C-2 acetate. To elaborate the synthesis of the five-membered triazole analogs, **8** is converted to terminal alkyne (**9**) by Ohira–Bestmann alkynylation,[16] which sets the stage for 1,3-dipolar cycloaddition click reaction. Interestingly, basic Ohira–Bestmann condition is well tolerated in the homologation and no C-8 *epi* alkyne or C-2 deacetylation product is isolated. Subsequent coupling of **9** with various azides under the copper (I)-catalyzed 'click reaction' condition affords 1,4-disubstituted-1,2,3-triazoles (**10**), (**11**), and (**12**) in high yields. *N*-unsubstituted 1,2,3-triazole heterocycles are found to have



Scheme 1. Reagents and conditions: (a) Ref. 14, 65%; (b) BH_3, THF, 55 °C, 3 h, 82%; (c) DMP, CH_2Cl_2, 0 °C, 2 h, 43%; (d) Ohira–Bestmann reagent, 61%; (e) RN_3,$CuSO_4$ (cat.), Na asorbate, *t*-BuOH/H_2O (1:1), 88%; (f) azidomethyl pivalate, $CuSO_4$ (cat.), Na asorbate, *t*-BuOH/H_2O (1:1), 88%; (g) i—NaOH(2.2 equiv) MeOH/H_2O (1:1); ii—1 M HCl (2.2 equiv); iii—Ac_2O, Pyr, 54% for 3 steps 14:15 (4:6).

significant nevertheless different biological properties from their substituted analogs.[17] This prompts us to synthesize the *N*-unsubstituted triazole analog of **1**. Compound **9** undergoes facile cycloaddition with azidomethyl pivalate to give the pivalyl triazole (**13**). Treatment of **13** with 1 N NaOH at room temperature removes both *N*-pivalyl group and the C-2 acetyl group[18] and also leads to C-8 epimerization. Acetylation of the epimeric mixture followed by preparative TLC separation furnishes two epimeric *N*-unsubstituted triazoles (**14**) and (**15**) with a ratio of 4:6.

Compounds **4** and **10**–**15** are evaluated for their affinities for MOPR, DOPR, and KOPR by competitive inhibition of [^{3}H]diprenorphine (~0.3 nM) binding to membranes prepared from Chinese hamster ovary (CHO) cell lines stably transfected with rat MOPR, FLAG-mouse DOPR, and human KOPR. The non-selective opioid receptor agonist, etorphine, is used as the reference compound in the binding assays. The binding data for compounds **10**–**15** is listed in Table 1. Surprisingly, none of the C-12 triazole analogs of **1** shows significant binding affinities for μ, δ, and κ-opioid receptors (K_i > 1000 nM). Roth's binding model of salvinorin A-KOPR three point interactions suggests that KOPR interacts with tyrosines 320 or 119 via hydrogen bonding with the C-12 furanyl substituent of **1** and the hydrogen bond between furan oxygen and tyrosines is weaker than a normal hydrogen bond due to the delocalization of the lone-pair electrons of the oxygen atom in the furan ring.[9] Despite the triazole moiety of analogs **10–15** offers two nitrogen sp^2 lone-pairs for hydrogen bonding, inductive effects of the three vincinal nitrogens decrease the electron density of the lone-pairs and make triazole moiety a much weaker hydrogen bond acceptor than furan moiety, which might explain the loss of binding affinity at KOPR for compounds **10–15**. A more recent model by Kane et al.[19] suggests the tyrosines from TM II and TM VII stabilize salvinorin A through hydrophobic effects, either by *pi*-stacking or other electronic effects. In this case, substitution of the furan with triazole would not significantly affect *pi*-stacking, however, the introduction of the triazole group changes the electronics of the environment from neutral to basic, which does not favor hydrophobic interaction in the KOPR binding pocket that this group seems to occupy. For *N*-unsubstituted triazoles **14** and **15**, rapid NH tautomerization could be an additional unfavorable electronic factor for binding. Interestingly, the oxadiazole analog **4**, reported to have antagonist activities at MOPR and KOPR, shows negligible affinities for MOPR, DOPR, and KOPR in our binding assays (K_i > 1000 nM for all three). Compound **4** at 3 μM only induces about 50% inhibition on [^{3}H]diprenorphine binding to the hKOPR (Fig. 1).

The discrepancy may result from differences in radioligand and binding buffer used. While Simpson et al.[8a] used [^{125}I] IOXY in 50 mM Tris–HCl buffer, pH 7.4, containing a protease inhibitor cocktail [bacitracin (100 μg/mL), bestatin (10 μg/mL), leupeptin (4 μg/mL) and chymostatin (2 μg/mL) in characterizing binding properties of the **4**, we have used [^{3}H]diprenorphine and 50 mM Tris–HCl buffer containing 1 mM EGTA (pH 7.4) for our binding assay.

Table 1
K_i values (in nM) of compounds **4** and **10**–**15** binding to the rMOPR, mDOPR, and hKOPR stably expressed in CHO cells[a]

Compound	rMOPR	mDOPR	hKOPR
4	>1000	>1000	>1000
10	>1000	>1000	>1000
11	>1000	>1000	>1000
12	>1000	>1000	>1000
13	>1000	>1000	>1000
14	>1000	>1000	>1000
15	>1000	>1000	>1000
Etorphine	0.14 ± 0.02	2.9 ± 0.6	0.5 ± 0.05

[a] Competitive inhibition by **4** and **10**–**15** of [^{3}H]diprenorphine (~0.3 nM) binding to MOPR, DOPR, and KOPR is conducted and their K_i values are determined by the program Prism. Etorphine is used as the reference compound.

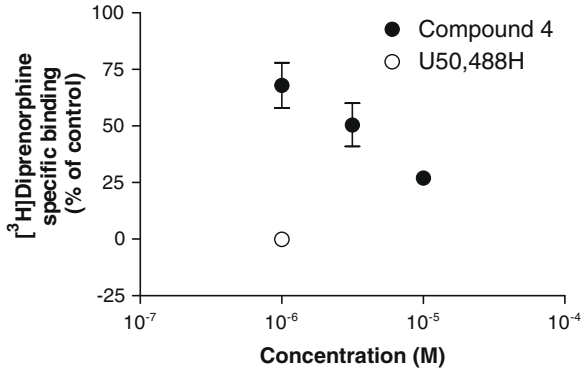


Figure 1. Effect of the compound **4** on [^{3}H]diprenorphine binding to the membranes prepared from Chinese hamster ovary cells (CHO) stably transfected with the human KOPR (hKOPR).

To examine the possibility that EGTA in our buffer may induce protonation of the oxadiazole group of **4** by its acidic hydrogens, we have evaluated inhibition of [^{3}H]diprenorphine binding to KOPR by **4** in Tris buffer (50 mM, pH 7.4) in the presence or absence of EGTA. Compound **4** at 3 μM induces the same extent of inhibition (about 50%, K_i > 1000 nM) with and without EGTA, indicating that EGTA does not affect affinity of **4** for the KOPR.

As [^{3}H]diprenorphine is an antagonist, we have also evaluated inhibition of [^{3}H]DAMGO (2 nM), an agonist, binding to MOPR by **4** in Tris buffer (50 mM, pH 7.4). Compound **4** at 3 μM induces only 8% inhibition of [^{3}H]DAMGO binding, compared with 99% inhibition by the reference compound etorphine (1 μM), indicating that **4** does not bind to MOPR (K_i > 1000 nM).

Although Simpson et al.[8a] reported **4** to be a weak KOPR antagonist in antagonizing agonist-induced increase of [^{35}S]GTPγS binding as (K_e = 360 nM), they did not examine the effect of **4** alone. Here we find that compound **4** increases KOPR-mediated [^{35}S]GTPγS binding in a dose-dependent manner with an EC_{50} value of >1 μM (Fig. 2). Its maximal response is similar to that of U50,488H, a prototypical KOPR full agonist. These results indicate that compound **4** is a full agonist with very weak potency, but not an antagonist, of KOPR. It is not surprising **4** has low affinities at KOPR given that oxadiazole group is weakly basic and the electron density of the three sp^2 lone-pairs are delocalized due to inductive effect, which is in keeping with that found in triazole analogs.

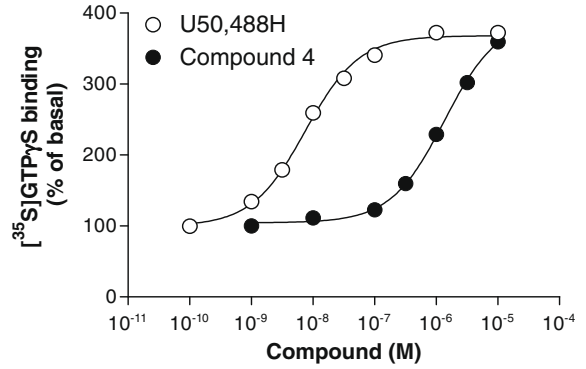


Figure 2. Agonist-induced increase in [^{35}S]GTPγS binding. Reference compound U50,488 is a full agonist of KOPR. Compound **4** showed to possess KOPR agonist properties with much weaker potency and similar efficacy as U50,488H.

In conclusion, C-12 triazole analogs **10–15** have been synthesized with facile copper (I)-catalyzed 1,3-dipolar cycloaddition reaction, which represents the first example of introducing another pharmacophore to the salvinorin A template by 'click chemistry'. Binding assay results indicate that replacement of furan with the triazoles result in loss of affinity at KOPR. Similar binding affinity result has been obtained on oxadiazole analog **4**. These results suggest that either electron density of the hetero-atom acting as hydrogen bond acceptor on C-12 or hydrophobic interactions between C-12 moiety and KOPR is critical to salvinorin A templated analog's binding affinity at KOPR. Efforts in optimizing the hydrogen bond accepting capability and hydrophobic properties of C-12 moiety are underway and will be reported elsewhere.

Acknowledgments

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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.2009.01.078.

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