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Convenient synthesis and in vitro pharmacological activity of 2-thioanalogs of salvinorins A and B

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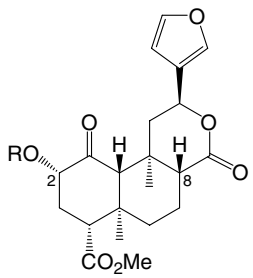
Abstract—To study drug-receptor interactions, new thio-derivatives of salvinorin A, an extremely potent natural κ-opioid receptor (KOR) agonist, were synthesized. Obtained compounds were examined for receptor binding affinity. Analogs with the same configuration at carbon atom C-2 as in natural salvinorin A showed higher affinity to KOR than their corresponding epimers.

Salvinorin A (**1**) is a neoclerodane diterpenoid with a strong hallucinogenic activity. It has been shown to have a high affinity and selectivity to kappa-opioid receptors (KOR).[1] Salvinorin A is isolated from the psychoactive plant *Salvia divinorum* as a major secondary metabolite, and makes an attractive lead compound for drug development due to its strong effects on human mood and low toxicity.

In the last three years numerous derivatives and analogs of salvinorin A were synthesized showing a broad range of KOR affinities.[2] Synthesis of new analogs of salvinorin A is important for generating new data on the structure of the receptor binding site and for possible changing of a pharmacological profile of action from agonist to antagonist. The wealth of the experimental data collected so far allows for structure-activity relationship conclusions pointing out the crucial importance of the C-2 configuration. Structural modifications of salvinorin A skeleton and functional groups in the other than C-2 positions did not provide compounds with higher affinity. In the course of our work on the molecular mechanism of interaction of salvinorin A to KOR we examined the effects of cysteine-substitution mutagenesis on the binding of salvinorin A along with analogs containing free sulfhydryl group at carbon C-2.[3] These studies prompted us to find a convenient way to synthesizes 2-thioanalogs of salvinorins A and B (Fig. 1).

Recently we reported the synthesis of sulfur analog of salvinorin A, in which the oxygen atom at the side chain at carbon atom C-2 was substituted by a sulfur atom.[2c] We now report the new method of the synthesis of sulfur analogs of salvinorin A and B epimeric at the C-2 stereogenic center. In our method salvinorin B (**2**) is transformed to 2β-chloroderivative (**3**) with 65% yield using mild condition of chlorination of alcohols with CCl_4–Ph_3P



Salvinorin A (**1**), R = Ac
Salvinorin B (**2**), R = H

Figure 1. Salvinorins A and B.

Keywords: κ-Opioid receptors agonists; Semi-synthesis; Salvinorin A and B thioderivatives.
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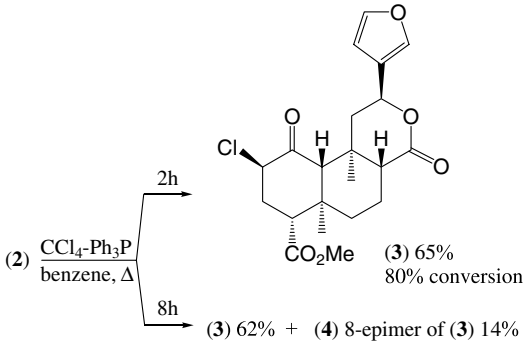
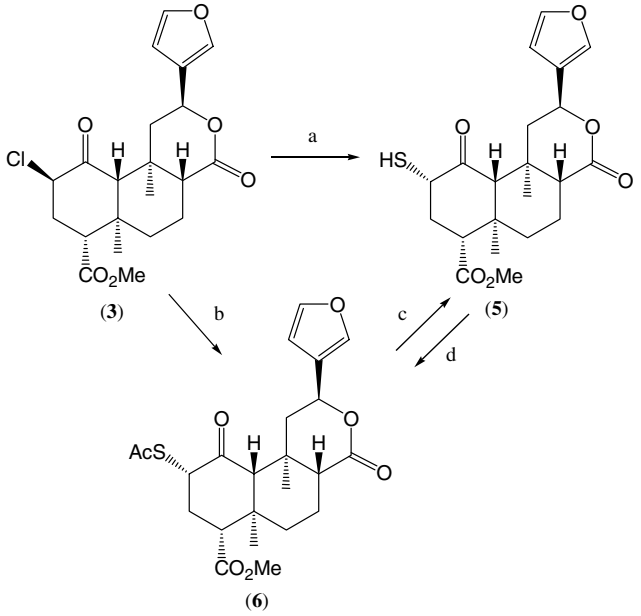
(Appel reaction). It is an improvement over previously used conditions ($SOCl_2$/Py) which give **3** with only 29% yield.[2c] After 2 h of refluxing of **2** with CCl_4-Ph_3P the only product formed was **3** with 80% conversion of starting material. The prolonged heating (6 h) moved the reaction to completion but a small amount (14%) of the product of epimerization at carbon C-8 (**4**) appeared (Scheme 1).

Epimerization at C-8 is well known in salvinorin chemistry, although it was previously observed repeatedly in basic and once in acidic conditions.[2e–n,4] The nucleophilic substitution of the chlorine atom in **3** by sulfur-containing reagents may be achieved with inversion or retention of configuration at C-2. Reaction of **3** with sodium hydrogen sulfide (NaSH) in methanol and dimethylformamide produces a thiol **5**, while the reaction with potassium thioacetate in acetone[2c] provides sulfur analog of salvinorin A (**6**) (Scheme 2).

The formation of thiol **5** from **3** is accompanied with several side products. To avoid this, the deacetylation



Scheme 1. Synthesis of 2β-chloroderivatives.



Scheme 2. 2α-Sulfur analogs of salvinorin A. Reagents and conditions: (a) NaSH, methanol, dimethylformamide, 35–40 °C, 1h (44%); (b) potassium thioacetate, acetone, Δ, 3h (71%); (c) NaSMe, methanol, CH_2Cl_2, −20 °C, 30 min (68%); (d) Ac_2O, CH_2Cl_2, 4-DMAP (75%).

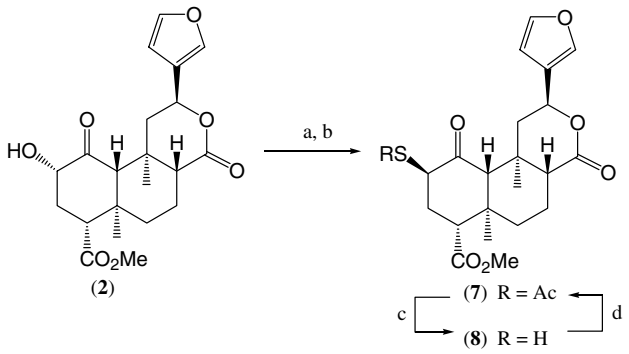
of thioacetate **6** with NaSMe was chosen as cleaner and more effective method of the synthesis of **5**. Deacetylation with sodium thiomethoxide[5a] proved to be advantageous in our case over the other known methods of hydrolysis of thioacetate group.[5b–f] The structure of compound **5** was confirmed by spectroscopic analysis (NMR) and high resolution mass spectrometry (HRMS)[6] as well as by chemical re-acetylation to thioacetate **6**. ^{1}HNMR spectrum of **5** showed the characteristic doublet of doublets of H-2 due to the coupling with two protons at C-3. The large coupling constants for H-2 and H-3 protons of **5** (J = 6 and 12 Hz) implied the β-orientation of H-2 and hence the desired α-configuration at C-2. The presence of thiol group was also confirmed by characteristic color reaction with Ellman's reagent (DTNB).

The thioacetate **7** was obtained from triflated salvinorin B by reaction with potassium thioacetate in acetone at −20 °C.[7] In these conditions the nucleophilic substitution occurred with inversion of configuration producing 2β-epimer (**7**) of compound **6**. Deacetylation of **7** in analogous conditions[8] described above for **6** yielded 2β-thiosalvinorin B (**8**) (Scheme 3).

Conducted NMR and HRMS analyses confirmed the structure of compounds **7** and **8**.[9] In this case the coupling constants between H-2 and H-3 were smaller (J = 2 Hz and 5 Hz for **7**) and corresponded well with calculated values for the dihedral angles H2α–C2–C3–H3α and H2α–C2–C3–H3β indicating the β-configuration at C-2.

Compounds **5**–**8** were evaluated for affinity to κ-opioid receptor (KOR) at the NIMH-sponsored Psychoactive Drug Screening Program at University of North Carolina at Chapel Hill using radioligand binding assays. The assays were conducted according to the procedure described earlier.[1,2o] The results are presented in Table 1.

In conclusion, we were able to obtain new sulfur analogs of salvinorins A and B with 'natural' (α) and inverted (β) configurations at carbon atom C-2. Thioanalogs with the same configuration at C-2 as in natural salvinorin A showed higher affinity to KOR than their correspond-



Scheme 3. 2β-Sulfur analogs of salvinorin A. Reagents and conditions: (a) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, −20 °C; (b) potassium thioacetate, acetone, −20 °C (75%); (c) NaSMe, methanol, CH_2Cl_2, −20 °C, 30 min (70%); (d) Ac_2O, CH_2Cl_2, 4-DMAP (78%).

Table 1. Binding affinity of compounds **1** and **5**–**8** to cloned rKOR (competitive binding in the presence of [^{3}H]U69,593) and hKOR mediated activation of intracellular calcium mobilization in HEK-293 cells

Compounds	K_i[a](nM)	EC_{50}[a,b](nM)	E_{max}[a] (%)
1	0.75 ± 0.62	2.82 ± 1.70	100
5	54.5 ± 25.7	287 ± 85	89 ± 14
6	18.4 ± 7.9	4.77 ± 2.72	107 ± 4
7	151 ± 53	123 ± 30	106 ± 1
8	546 ± 140	>2000	71 ± 12

[a] Values are means of three experiments.
[b] In vitro effective concentration.

ing epimers. Readily available 2β-chlorosalvinorin B (**3**) may serve as a convenient intermediate for the synthesis of various new analogs modified at C-2 position, retaining α-configuration of natural salvinorin A.

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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.2007.01.100.

References and notes

1. Roth, B. L.; Baner, K.; Westkaemper, R.; Siebert, D.; Rice, K. C.; Steinberg, S.; Ernsberger, P.; Rothman, R. B. *Proc. Natl. Acad. Sci.* **2002**, *99*, 11934.
2. (a) Lee, D. Y. W.; He, M.; Liu-Chen, L.-Y.; Wang, Y.; Li, J.-G.; Xu, W.; Ma, Z.; Carlezon, W. A., Jr.; Cohen, B. *Biorg. Med. Chem. Lett.* **2006**, *16*, 5498; (b) Beguin, C.; Richards, M. R.; Li, J.-G.; Wang, Y.; Xu, W.; Liu-Chen, L.-Y.; Carlezon, W. A.; Cohen, B. M. *Biorg. Med. Chem. Lett.* **2006**, *16*, 4679; (c) Stewart, D. J.; Fahmy, H.; Roth, B. L.; Yan, F.; Zjawiony, J. K. *Arzneimittel Forschung Drug Res.* **2006**, *56*, 269; (d) Tidgewell, K.; Harding, W. W.; Lozama, A.; Cobb, H.; Shah, K.; Kannan, P.; Dersch, C. M.; Parrish, D.; Deschamps, J. R.; Rothman, R. B.; Prisinzano, T. E. *J. Nat. Prod.* **2006**, *69*, 914; (e) Harding, W. W.; Schmidt, M.; Tidgewell, K.; Kannan, P.; Holden, K. G.; Dersch, C. M.; Rothman, R. B.; Prisinzano, T. E. *Biorg. Med. Chem. Lett.* **2006**, *16*, 3170; (f) Prisinzano, T. U.S. Patent Appl. 2006058264, 2006; *Chem. Abstr*. **2006**, *144*, 292904; (g) Zjawiony, J.; Fahmy, H.; Stewart, D. J.; Roth, B. PCT Int. Appl. WO 2006012643, 2006; *Chem. Abstr*. **2006**, *144*, 164284; (h) Harding, W. W.; Schmidt, M.; Tidgewell, K.; Kannan, P.; Holden, K. G.; Gilmour, B.; Navarro, H.; Rothman, R. B.; Prisinzano, T. E. *J. Nat. Prod.* **2006**, *69*, 107; (i) Beguin, C.; Carlezon, W.; Cohen, B. M.; He, M.; Lee, D. Y.-W.; Richards, M. R. PCT Int. Appl. WO 2005089745, 2005; *Chem. Abstr*. **2005**, *143*, 326476; (j) Lee, D. Y. W.; He, M.; Kondaveti, L.; Liu-Chen, L.-Y.; Ma, Z.; Wang, Y.; Chen, Y.; Li, J.-G.; Beguin, C.; Carlezon, W. A.; Cohen, B. *Biorg. Med. Chem. Lett.* **2005**, *15*, 4169; (k) Lee, D. Y. W.; Karnati, V. V. R.; He, M.; Liu-Chen, L.-Y.; Kondaveti, L.; Ma, Z.; Wang, Y.; Chen, Y.; Beguin, C.; Carlezon, W. A.; Cohen, B. *Biorg. Med. Chem. Lett.* **2005**, *15*, 3744; (l) Harding, W. W.; Tidgewell, K.; Byrd, N.; Cobb, H.; Dersch, C. M.; Butelman, E. R.; Rothman, R. B.; Prisinzano, T. E. *J. Med. Chem.* **2005**, *48*, 4765; (m) Beguin, C.; Richards, M. R.; Wang, Y.; Chen, Y.; Liu-Chen, L.-Y.; Ma, Z.; Lee, D. Y. W.; Carlezon, W. A.; Cohen, B. M. *Biorg. Med. Chem. Lett.* **2005**, *15*, 2761; (n) Munro, T. A.; Rizzacasa, M. A.; Roth, B. L.; Toth, B. A.; Yan, F. *J. Med. Chem.* **2005**, *48*, 345; (o) Chavkin, C.; Sud, S.; Jin, W.; Stewart, J.; Zjawiony, J. K.; Siebert, D. J.; Toth, B. A.; Hufeisen, S. J.; Roth, B. L. *J. Pharmacol. Exp. Ther.* **2004**, *308*, 1197; (p) Tidgewell, K.; Harding, W. W.; Schmidt, M.; Holden, K. G.; Murry, D. J.; Prisinzano, T. E. *Biorg. Med. Chem. Lett.* **2004**, *14*, 5099.
3. Yan, F.; Mosier, P. D.; Westkaemper, R. B.; Stewart, J.; Zjawiony, J. K.; Vortherms, T. A.; Sheffler, D. J.; Roth, B. L. *Biochemistry* **2005**, *44*, 8643.
4. (a) Valdez, L. J., III; Butler, W. M.; Hatfield, G. M.; Paul, A. G.; Koreeda, M. J. *J. Org. Chem.* **1984**, *49*, 4716; (b) Koreeda, M.; Brown, L.; Valdes, L. J., III *Chem. Lett.* **1990**, 2015; (c) Valdez, L. J., III; Chang, H.-M.; Visger, D. C.; Koreeda, M. *Org. Lett.* **2001**, *3*, 3935.
5. (a) Wallace, O. B.; Springer, D. M. *Tetrahedron Lett.* **1998**, *39*, 2693; (b) Yelm, K. E. *Tetrahedron Lett.* **1999**, *40*, 1101; (c) Mastalerz, H.; Zhang, G.; Kadow, J.; Fairchild, C.; Long, B.; Vyas, D. M. *Org. Lett.* **2001**, *3*, 1613; (d) Iimura, S.; Manabe, K.; Kobayashi, S. *Org. Lett.* **2003**, *5*, 101; (e) MacCoss, R. N.; Henry, D. J.; Brain, C. T.; Ley, S. V. *Synlett* **2004**, *4*, 0675; (f) Holmes, B. T.; Snow, A. W. *Tetrahedron* **2005**, *61*, 12339.
6. NMR and mass spectral data were obtained for all final products purified by preparative HPLC (C18 column, MeCN-water). 2-thiosalvinorin B (**5**): white solid, m.p. 201–203 °C, $[\alpha]_D^{25}$ −54 (c 0.05, $CHCl_3$), ^{1}H NMR (400 MHz, CD_2Cl_2): δ 1.07 (3H, s, H-19), 1.44 (3H, s, H-20), 1.56 (4H, m, H-6b, H-7b, H-11b, SH), 1.75 (1H, m, H-6a), 2.08 (2H, m, H-7a, H-8), 2.20 (1H, m, H-3b), 2.24 (1H, s, H-10), 2.47 (1H, ddd, *J* 3, 7, 14 Hz, H-3a), 2.52 (1H, dd, *J* 5, 13 Hz, H-11a), 2.79 (1H, dd, *J* 3, 13 Hz, H-4), 3.68 (3H, s, OCH_3), 3.75 (1H, dd, *J* 6, 12 Hz, H-2), 5.53 (1H, dd, *J* 5, 12 Hz, H-12), 6.42 (1H, s, H-14), 7.44 (2H, m, H-15, H-16); ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.6 (C-20), 16.9 (C-19), 18.4 (C-7), 36.0 (C-9), 37.3 (C-3), 38.4 (C-6), 42.8 (C-5), 43.9 (C-11), 49.3 (C-2), 51.6 (C-21), 52.1 (C-8), 56.0 (C-4), 66.1 (C-10), 72.1 (C-12), 108.3 (C-14), 125.3 (C-13), 139.1 (C-16), 143.5 (C-15), 170.8 (C-17), 172.4 (C-18), 202.8 (C-1); HRESIMS *m/z* $[M+H]^+$ 407.1590 (calcd. for $C_{21}H_{26}O_6S$ 406.1450).
7. Procedure for synthesis of 2-epi-2-thiosalvinorin A (**7**): To suspension of (**2**) (1 mmol) in CH_2Cl_2 (5 mL) at 0 °C were added excess pyridine (1 mL) and trifluoromethanesulfonic anhydride (1.2 mmol), the reaction mixture was stirred for 20 min. The reaction solution was washed with 1N HCl and brine, dried (Na_2SO_4), and solvents were evaporated in vacuum. A mixture of obtained extract and potassium thioacetate (5 mmol) was placed to dry acetone (10 mL) and stirred at −20 °C under argon for 1 h. The reaction mixture was allowed to warm up to room temperature,

concentrated in vacuum, diluted with water, and extracted with chloroform. The combined organic extracts were dried (Na_2SO_4), concentrated in vacuum to give semisolid product, which was purified by column chromatography (hexane/EtOAc, 2:1). Yield 75% starting from **2**, white solid, m.p. 192–194 °C, $[\alpha]_D^{25}$ −64 (c 0.05, $CHCl_3$), ^1H NMR (400 MHz, $CDCl_3$); δ 1.15 (3H, s, H-19), 1.44 (3H, s, H-20), 1.62 (4H, m, 2H-6, H-7b, H-11b), 2.09 (3H, m, H-3b, H-7a, H-8), 2.33 (1H, s, H-10), 2.38 (3H, s, $COCH_3$), 2.55 (1H, dd, *J* 5, 13 Hz, H-11a), 2.70 (2H, m, H-3a, H-4), 3.72 (3H, s, OCH_3), 4.27 (1H, dd, *J* 2, 5 Hz, H-2), 5.54 (1H, dd, *J* 5, 11, H-12), 6.40 (1H, s, H-14), 7.42 (2H, m, H-15, H-16); ^{13}C NMR (100 MHz, $CDCl_3$); δ 15.1 (C-20), 16.4 (C-19), 18.0 (C-7), 30.6 (C-22), 31.8 (C-3), 35.2 (C-9), 38.3 (C-6), 41.9 (C-5), 43.3 (C-11), 49.7 (C-2), 51.3 (C-8), 51.8 (C-23), 52.8 (C-4), 63.8 (C-10), 72.0 (C-12), 108.4 (C-14), 125.5 (C-13), 139.3 (C-16), 143.7 (C-15), 171.2 (C-17), 172.2 (C-18), 191.1 (C-21), 203.8 (C-1); HRESIMS *m/z* $[M+H]^+$ 449.1687 (calcd for $C_{23}H_{28}O_7S$ 448.1556).

8. General procedure for synthesis of 2-thiosalvinorin B (**5**) and 2-epi-2-thiosalvinorin B (**8**): To a stirred solution of thioacetate (1 mmol) in methanol (10 mL) under argon at −20 °C was added sodium thiomethoxide (1 equiv. 1 M solution in MeOH). The reaction mixture was stirred for 30 min. The solution was then added to aqueous HC1 (0.1 M, 20 mL). The aqueous solution was extracted with CH_2C1_2. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated.
9. 2-epi-2-thiosalvinorin B (**8**): white solid, m.p. 161–163 °C, $[\alpha]_D^{25}$ −160 (c 0.05, $CHCl_3$), ^1H NMR (400 MHz, MeOH): δ 1.12 (3H, s, H-20), 1.41 (3H, s, H-19), 1.70 (4H, m, 2H-6, H-7b, H-11b, SH), 2.03 (2H, m, H-7a, H-3b), 2.42 (1H, dd, *J* 3, 12 Hz, H-8), 2.52 (1H, dd, *J* 5, 13 Hz, H-11a), 2.68 (1H, ddd, *J* 6, 14, 14, H-3a), 3.08 (1H, dd, *J* 1, 6, H-4), 3.20 (1H, s, H-10), 3.57 (1H, d, *J* 6 Hz, H-2), 3.70 (3H, s, OCH_3), 5.61 (1H, dd, *J* 5, 11, H-12), 6.54 (1H, s, H-14), 7.50 (1H, s, H-15), 7.44 (1H, s, H-16); ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.5 (C-20), 16.6 (C-19), 18.4 (C-7), 31.9 (C-3), 35.3 (C-9), 38.3 (C-6), 42.4 (C-5), 43.4 (C-2), 44.2 (C-11), 50.9 (C-4), 51.6 (C-8), 52.0 (C-21), 59.1 (C-10), 72.1 (C-12), 108.4 (C-14), 125.4 (C-13), 139.3 (C-16), 143.5 (C-15), 171.1 (C-17), 172.0 (C-18), 205.9 (C-1); HRESIMS *m/z* $[M+H]^+$ 407.1597 (calcd for $C_{21}H_{26}O_6S$ 406.1450).