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# Synthesis and evaluation of pharmacological CNS activity of α-hydroxy *O*-alkyl etheroximes

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**α-cetol etheroximes / *O*-alkyl etheroximes / analgesic activity / anticonvulsant / anti-inflammatory compound**

## Introduction

In previous studies [1–3], we have shown that some branched chain α-hydroxyoximes possessed tranquillizing properties (findings patented) [2]. Their pharmacological spectrum is benzodiazepine-like as, according to the doses given, they either have anticonvulsive, anxiolytic, sedative, myorelaxant or hypnotic properties. As an extension of this research, it seemed interesting to complete the study of this structure by substituting the oxime group by an ether group, in order to determine the effect of this structure on the pharmacological activity. In this study, we therefore report the synthesis, anticonvulsant and anti-inflammatory properties of the new title compounds.

## Chemistry

Synthesis of α-hydroxy ketones **1** was carried out from acetylenic tertiary alcohols using the mercuric hydration method [1]. The hydroxy alkyl etheroximes **3** were obtained by condensation of the appropriate hydroxy ketones **1** with *O*-alkyl hydroxylamine hydrochlorides **2** and sodium acetate in boiling absolute ethanol according to a general procedure [4] (scheme 1).

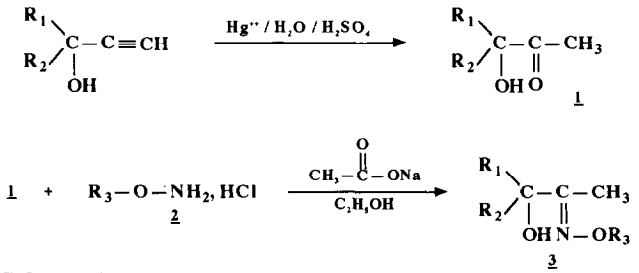
*E* and *Z* configurations were theoretically available for these compounds. However, a single but not established isomeric form was found in our experimental synthesis. The pure products **3** were characterized by their IR, $^1$H-NMR spectra (tables I, II) and elemental analysis. The structure of the majority was also confirmed by $^{13}$C-NMR spectrometry.

## Pharmacology and discussion

The pharmacological results have been presented in table III.

*Acute toxicity*

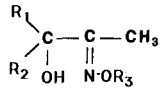
Under our experimental conditions, the tested compounds were found to be atoxic except for **3i** and **3j** which induced 33% of lethality at the highest dose (1000 mg/kg) and 0% at the lowest doses (100 and 10 mg/kg), (level 2 in the Lorke's classification) [5]. The following clinical signs: prostration, palpebral plosis, staggering then loss of turning reflex, were found for almost all of the compounds at a dose of 1000 mg/kg.




**Scheme 1.**

*Correspondence and reprints

**Table I.** Physical properties and IR spectra of compounds **3a–3k**.



| *No* | $R_1$ | $R_2$ | $R_3$ | *bp (°C)/ mmHg* | *Yield %* | *IR ν ($cm^{-1}$)* |
|---|---|---|---|---|---|---|
| **3a** | Cyclohexyl | | $CH_2$-CH = $CH_2$ | 134/15 | 31 | 3500 (OH), 1640 (C = N), 940 (= NO) |
| **3b** | Cyclohexyl | | $CH_2C_6H_5$ | 150/0.5 | 53 | 3440 (OH), 1630 (C = N), 970 (= NO) |
| **3c** | Cyclohexyl | | $CH_3$ | 105/15 | 26 | 3440 (OH), 1640 (C = N), 970 (= NO) |
| **3d** | Cyclohexyl | | $C_2H_5$ | 115/15 | 25 | 3440 (OH), 1640 (C = N), 970 (= NO) |
| **3e** | $C_3H_7$ | $C_3H_7$ | $CH_2C_6H_5$ | 126/0.7 | 79 | 3500 (OH), 1640 (C = N) 940 (= NO) |
| **3f** | $C_3H_7$ | $C_3H_7$ | $CH_3$ | 52/0.5 | 78 | 3500 (OH), 1640 (C = N), 975 (= NO) |
| **3g** | $C_3H_7$ | $C_3H_7$ | $C_2H_5$ | 69/0.7 | 79 | 3500 (OH), 1645 (C = N), 950 (= NO) |
| **3h** | $CH_3$ | $C_3H_7$ | $CH_2C_6H_5$ | 130/0.4 | 30 | 3500 (OH), 1645 (C = N), 950 (= NO) |
| **3i** | $CH_3$ | $C_3H_7$ | $CH_3$ | 58/0.4 | 36 | 3500 (OH), 1640 (C = N), 960 (= NO) |
| **3j** | $CH_3$ | $C_3H_7$ | $C_2H_5$ | 64/0.4 | 25 | 3500 (OH), 1645 (C = N), 950 (= NO) |
| **3k** | $C_2H_5$ | $C_2H_5$ | $CH_2C_6H_5$ | 120/15 | 67 | 3500 (OH), 1640 (C = N), 970 (= NO) |

*Anti-oedematous activity*

Results were expressed as a percentage of oedematous podal reduction with regard to control animals which only received 1% carrageenan. A positive sign was assigned in the case of anti-oedematous activity. Dunnett's test [6] applied to the whole assay showed significant activity ($P \leq 0.05$) for acetylsalicylic acid; compound **3k** with 32% activity was not significantly active. All the other compounds except **3d** and **3h** reduced oedema moderately but not significantly.

*Anticonvulsant activity*

Results were expressed as percentage increase (+) or decrease (–) with respect to the control animals which only received a convulsant just for 1 parameter (clonic seizures); the number of tonic seizures and deaths was expressed in %. These results were analyzed by the Newman–Keuls' test [7, 8] for the latency period before clonic seizures or by $\chi^2$ [9] for percentages of tonic seizures or dead animals.

The Newman–Keuls' test showed a significant increase ($P < 0.01$) of the latency period before clonic

**Table II.** $^{1}H$ and $^{13}C$-NMR spectra of compounds **3a**– **3k**.

| N° | $^{1}H$ NMR ($CDCl_3$): δ(ppm), J (Hz) | $^{13}C$ NMR (solvent): δ(ppm) |
|---|---|---|
| **3a** | 1.30-1.75 (m, 10H, cyclohexyl), 1.85 (s, 3H, $CH_3$), 3.40 (s, 1H, OH), 4.53 (d, 2H, $OCH_2$, J = 5.5), 5.15-5.40 (m, 2H, $=CH_2$), 5.90-6.15 (m, 1H, =CH) | (DMSO $d_6$): 9.70 ($CH_3$), 21.42, 25.32, 34.65 (cyclohexyl), 71.63 (C-OH), 73.51 (O-$CH_2$), 116.59 (CH=$\underline{C}H_2$), 134.69 (CH), 161.91 (C=N) |
| **3b** | 1.40-1.80 (m, 10H, cyclohexyl), 1.90 (s, 3H, $CH_3$), 3.15 (s, 1H, OH), 5.10 (s, 2H, $OCH_2$), 7.40 (m, 5H, $C_6H_5$) | ($CDCl_3$): 10.55 ($CH_3$), 21.43, 25.39, 35.33 (cyclohexyl), 73.44 (C-OH), 75.92 (O-$CH_2$), 127.67, 128.06, 128.27 ($CH_{arom}$), 161.42 (C=N) |
| **3c** | 1.30-1.75 (m, 10H, cyclohexyl), 1.85 (s, 3H, $CH_3$), 3.20 (s, 1H, OH), 3.85 (s, 3H, $OCH_3$) | ($CDCl_3$): 10.17 ($CH_3$), 21.36, 25.36, 35.26 (cyclohexyl), 61.55 (O-$CH_3$), 73.24 (C-OH), 160.89 (C=N) |
| **3d** | 1.25 (t, 3H, $CH_3$ ethyl, J = 7.0), 1.40-1.75 (m, 10H, cyclohexyl), 1.85 (s, 3H, $CH_3$), 3.35 (s, 1H, OH), 4.10 (q, 2H, $CH_2$ ethyl, J = 7.0) | ($CDCl_3$): 10.30 ($CH_3$), 14.70 ($CH_3$ ethyl), 21.44, 25.42, 35.39 (cyclohexyl), 69.33 ($CH_2$ ethyl), 73.34 (C-OH), 160.49 (C=N) |
| **3e** | 0.86 (t, 6H, $CH_3$ propyl, J = 6.4), 1.30-1.60 (m, 8H, $CH_2$ propyl), 1.80 (s, 3H, $CH_3$), 3.90 (s, 1H, OH), 5.13 (s, 2H, $OCH_2$), 7.34 (s, 5H, $C_6H_5$) | ($CDCl_3$): 11.17 ($CH_3$), 14.34 ($CH_3$ propyl), 16.40 ($CH_3$-$\underline{C}H_2$), 41.56 ($CH_3$-$CH_2$-$\underline{C}H_2$), 75.97 (O-$CH_2$), 76.98 (C-OH), 127.71, 127.96, 128.28 ($CH_{arom}$), 159.53 (C=N) |
| **3f** | 0.90 (t, 6H, $CH_3$ propyl, J = 6.5), 1.30-1.75 (m, 8H, $CH_2$ propyl), 1.72 (s, 3H, $CH_3$), 3.45 (s, 1H, OH), 3.90 (s, 3H, $OCH_3$) | |
| **3g** | 0.86 (t, 6H, $CH_3$ propyl, J = 6.8), 1.25 (t, 3H, $CH_3$ ethyl, J = 7.0), 1.32-1.62 (m, 8H, $CH_2$ propyl), 1.78 (s, 3H, $CH_3$), 3.18 (s, 1H, OH), 4.20 (q, 2H, $CH_2$ ethyl, J = 7.0) | |
| **3h** | 0.88 (t, 3H, $CH_3$ propyl, J = 6.9), 1.32 (s, 3H, $CH_3$), 1.50-1.65 (m, 4H, $CH_2$-propyl), 1.85 (s, 3H, $CH_3$ C=N), 3.08 (s, 1H, OH), 5.00 (s, 2H, $OCH_2$), 7.35 (s, 5H, $C_6H_5$) | |
| **3i** | 0.87 (t, 3H, $CH_3$ propyl, J = 7.0), 1.31 (s, 3H, $CH_3$), 1.50-1.62 (m, 4H, $CH_2$-propyl), 1.80 (s, 3H, $CH_3$ C=N), 2.98 (s, 1H, OH), 3.88 (s, 3H, $OCH_3$) | ($CDCl_3$): 10.52 ($CH_3$), 14.22 ($CH_3$ propyl), 16.70 ($CH_3$-$\underline{C}H_2$), 26.66 ($CH_3$-$CH_2$-$\underline{C}H_2$), 42.22 ($CH_3$-C-O), 61.71 (O-$CH_3$), 74.38 (C-OH), 159.77 (C=N) |
| **3j** | 0.90 (t, 3H, $CH_3$ propyl, J = 7.0), 1.22 (t, 3H, $CH_3$ ethyl, J = 7.0), 1.31 (s, 3H, $CH_3$), 1.50-1.63 (m, 4H, $CH_2$ propyl), 1.81 (s, 3H, $CH_3$-C=N), 3.40 (s, 1H, OH), 4.10 (q, 2H, $CH_2$ ethyl, J = 7.0) | |
| **3k** | 0.75 (t, 6H, $CH_3$ ethyl, J = 7.4), 1.51-1.68 (m, 4H, $CH_2$ ethyl), 1.80 (s, 3H, $CH_3$-C=N), 3.00 (s, 1H, OH), 5.10 (s, 2H, $OCH_2$), 7.35 (s, 5H, $C_6H_5$). | ($CDCl_3$): 7.35 ($CH_3$ ethyl), 10.70 ($CH_3$), 31.60 ($CH_2$ ethyl), 75.93 (C-OH), 76.99 (O-$CH_2$), 126.67, 127.48, 128.23 ($CH_{arom}$), 158.92 (C=N) |

**Table III.** Pharmacological results.

| Compounds | Anticonvulsant activity | | | | Antinociceptive activity | | | |
|---|---|---|---|---|---|---|---|---|
| | *Anti-oedematous activity* | *Latency period before clonic seizures* | *Tonic seizures* | *Lethality* | *Before treatment* | *Tail immersion + 1 h* | *+ 2 h* | *Koster* |
| *250 mg/kg[a] po* | *% of variation/ control* | *% of variation/ control* | *% of presence* | *% of presence* | *% of variation/ control* | *% of variation/ control* | *% of variation control* | *% of variation/ control* |
| Control | – | – | 20 | 20 | – | – | – | – |
| **3a** | +30.0 | +51.8 | 10 | 10 | –5.7 | +32.3 | +81.8 | +14.1 |
| **3b** | +10.8 | +19.1 | 50 | 40 | +8.9 | +0.1 | +50.0 | +29.9 |
| **3c** | +14.8 | +67.2 | 0 | 0 | +9.1 | +12.1 | +56.9 | +14.1 |
| **3d** | 0.0 | –26.7 | 0 | 0 | +1.8 | +26.3** | –9.3 | +10.8 |
| **3e** | +14.8 | –16.4 | 40 | 30 | –2.2 | +10.6 | +32.2 | +22.0 |
| **3f** | +22.4 | +54.9 | 0 | 0 | +14.8 | –16.2 | +12.3 | +43.2*** |
| **3g** | +23.5 | +66.1 | 10 | 10 | +4.4 | –4.0 | –4.7 | +22.8 |
| **3h** | –20.9 | +40.0 | 0 | 0 | +9.4 | +26.5 | +15.0 | +0.8 |
| **3i** | +20.6 | +190.4* | 0 | 0 | +10.8 | +6.3 | +27.4 | +26.1 |
| **3j** | +30.3 | +180.5* | 0 | 0 | +17.2 | +21.8 | +1.0 | +14.9 |
| **3k** | +31.8 | +3.6 | 20 | 20 | +0.7 | +76.7 | +32.4 | +4.6 |
| ASA | +52.0° | – | – | – | –10.3 | –7.1 | +10.4 | –22.8 |
| Diazepam[b] | – | +190.4* | 0 | 0 | – | | – | – |

[a]50 mg/kg *po*, Koster's test; [b]2.5 mg/kg *po*, Dunnett's test; °$P < 0.05$; *$P < 0.01$, Newman–Keuls' test/control; **$P < 0.03$, Mann–Whitney's *U* test/control; ***$P < 0.02$ Mann–Whitney's *U* test/control.

seizures with compounds **3i**, **3j** and diazepam, and anticonvulsive effect. This protective effect was not found by the $\chi^2$ test applied to the percentages of tonic seizures and deaths. On the other hand, compounds **3b** and **3e** increased the pentyltetrazole convulsive effect (tonic seizures and deaths for **3b**, tonic seizures for **3e**). This effect was significant with regard to most of the other tested compounds except for the control, **3a**, **3g** and **3k** groups. Compounds **3i** and **3j**, which possessed anticonvulsive activity, were those found to be without toxicity.

*Antinociceptive activity: 'tail immersion'*

Results were expressed in percentage variation with regard to control animals. They were analyzed by Kruskal–Wallis [10, 11] and Mann–Whitney's U tests [12] which showed 1 h after treatment a significant increase in the latency period before tail flick compared to the control group for compound **3d** ($P < 0.03$) with 26.3% activity. With an increase of 21.8%, compound **3j** showed borderline activity ($P < 0.07$); on the other hand, results obtained for **3a** and **3k** were not significant as the responses in that group were not standard.

*Antinociceptive activity: 'Koster'*

Results were expressed as a percentage protection per sign + with respect to control animals which received only acetic acid. They were analyzed by the Kruskal–Wallis [10, 11] and Mann–Whitney's *U* tests which showed a significant reduction in the number of writhes only for compound **3f** ($P < 0.02$) with regard to the control group. The decrease observed in all the other treated groups was not at any time significant.

## Experimental protocols

*Chemistry*

Infrared spectra were obtained as films on a Perkin–Elmer 1310 spectrophotometer. $^1$H- and $^{13}$C-NMR spectra were recorded on a Bruker AC 200 spectrometer using TMS as internal standard and $CDCl_3$ as solvent (excepted for compound **3a**, which was dissolved in DMSO–$d_6$). Elemental analysis, performed by the Service Central d'Analyses du CNRS, were within 0.4% of the theoretical values. Thin-layer chromatography was carried out on Merck precoated 0.25-mm analytical silica-gel 60 $F_{254}$ plates using the solvent system: toluene/ethyl acetate (80:20, v/v) with ultraviolet detection.

*Synthesis of O-alkyl ether oximes of α-hydroxy-oximes*

For example, the synthesis of **3d** was described. The α-hydroxyketone **1d** was prepared by mercuric hydration of the corresponding acetylenic alcohol. A mixture of **1d** (0.1 mol), *O*-ethyl hydroxylamine hydrochloride **2d** (0.109 mol) and anhydrous sodium acetate (0.109 mol) was solubilized in absolute ethanol (200–300 ml). After refluxing for 20 h, the reaction mixture was filtered. The filtrate was concentrated at reduced pressure and the residual fraction was treated by $NaHCO_3$ (pH = 7–8), then extracted with diethyl ether. The organic layer was washed with saturated NaCl (pH = 5–6), dried over anhydrous $Na_2SO_4$, and evaporated *in vacuo* to give **3d**, which was purified by distillation at reduced pressure.

*Pharmacology*

Compounds obtained (**3a–3k**) were screened for their analgesic and anti-inflammatory activities as well as their acute toxicity. All experiments were performed on male Swiss mice SPF breed, CERJ 53680 Le Genest, acclimatized to laboratory conditions for 1 week. The animals weighed ≈ 20 g and were deprived of food for 18 h prior to the experiment with water available *ad libitum*.

*Acute toxicity*
Acute toxicity was studied according to the method described by Lorke [5] in which the tested compounds were administered orally *(po)* at doses of 10–100–1000 mg/kg in 3 groups of 3 mice. Animals were kept under observation during the 15 d following treatment. The purpose of this study was to define lethality according to 10 levels: first level (no lethality for the 3 tested doses) corresponding to the lowest acute toxicity, and the 10th level, maximum acute toxicity (100% lethality for the 3 doses tested).

*Anti-oedematous activity*
The method of Lévy [13] was used for mice randomly divided into various groups, each group including 10 treated animals or 20 control animals. Thirty min after administration of the test compound or acetylsalicylic acid (ASA) (250 mg/kg *po*) or vehicle, 0.025 ml of freshly prepared suspension of carrageenan in distilled water (1%) was injected under the planter aponeurosis of the hind paw. Four h after the beginning of the experiment, all the animals were killed and the hind paws were cut at the tarsocrural articulation level and weighed.

*Anticonvulsant activity*
The method described by Krall [14] and modified by Kupferberg [15] was applied to groups of 10 mice (treated or control). Treated animals received 250 mg/kg *po* of each of the tested compounds (except the reference compound diazepam which was studied at 2.5 mg/kg po) 13 min before the sc injection of pentetrazole (PTZ) (100 mg/kg) in the scruff of the neck. The measured parameters were as follows: latency period before clonic and tonic seizures and percentage of clonic, tonic seizures and death.

*Central antinociceptive activity*
The study of 'central analgesic' properties according to the 'tail immersion' method described by Ben-Bassat [16] was applied to groups of 10 treated mice and to 1 group of 20 control mice. The treated animals received 250 mg/kg *po* acetylsalicylic acid (ASA) or each tested compound. The test consisted of measuring the latency period before tail flick following tail immersion in a water bath at 58°C .The test was applied before, then 1 and 2 h after treatment. The compounds' antinociceptive activity was measured with regard to control animals.

*Peripheral antinociceptive activity*
According to the method described by Koster [17] in the study of 'peripheral analgesic' properties, this test was applied to groups of 10 treated or control mice. Treated animals received 50 mg/kg *po* acetylsalicylic acid (ASA) or each tested compound 30 min before *ip* injection of acetic acid solution (0.5 ml per mouse 0.5% aqueous solution). Analgesic activity was evaluated by the number of writhes over a 10-min period. For the 5 study types, the tested compounds were administered *po* in suspension in a 0.1% aqueous solution of carboxymethyl cellulose under 0.5 ml/20 g body weight constant volume administration.

## Conclusion

The comparison of pharmacological activities of α-hydroxy oximes previously described [1–3] and compounds studied in this report, shows that the 2 series are slightly toxic and that the modification of the oxime group does not suppress the anticonvulsant activity because it is found again with the 2 compounds **3i** and **3j** [8]. On the contrary, compounds **3b** and **3e** increase the convulsant effect of the pentetrazole. Furthermore, analgesic activity was revealed by the Koster test for **3f**: similar activity has been previously reported [1].

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