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Diazabicyclononanones, a potent class of kappa opioid analgesics

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Abstract

The 1,5-dimethyl 3,7-diaza-3,7-dimethyl-9-oxo-2,4-di-2-pyridine-bicyclo[3.3.1]nonane-1,5-dicarboxylate, **HZ2**, has a high and selective affinity for the kappa opioid receptor and an antinociceptive activity comparable to morphine. In addition, it is characterized by a long duration of action and a high oral bioavailability. QSAR studies within series of kappa agonists revealed a chair-boat conformation of a double protonated **HZ2** characterized by an almost parallel orientation of the C9 carbonyl group and the N7-H group and at least one aromatic ring to be the pharmacophoric arrangement. Structural variations showed that the pyridine rings in 2 and 4 position can be replaced with *p*-methoxy-, *m*-hydroxy- and *m*-fluoro-substituted phenyl rings. However, all other substituents have to be kept the same for a high affinity to the kappa receptor.

Keywords: Diazabicyclononanones; Analgesics; Opiates

1. Introduction

Opiates have been one of the most investigated class of natural compounds. The most prominent opiate, morphine, has had a long history of analgesia [1]. However, the strong analgesic potency of morphine is associated with side effects, such as respiratory depression, constipation, tolerance and dependence. Thus, the search for compounds which can effectively treat strong pain, caused by cancer or surgery, remains a challenge in drug development. Beside a wide variety of emerging targets, connected with analgesia [2–4], the three subtypes of the opioid receptors, MOR (formerly μ-opioid receptor [5]), DOR (δ) and KOR (κ), are still an important focus of efforts identifying subtype-selective ligands [6]. KOR-agonists were originally believed to be free of dependence, tolerance, and respiratory depression [7]. Thus, a great number of compounds were developed as strong analgesics. Ketocyclazocin, the first non-peptide KOR-agonist derived from the benzomorphane group, gave the name to the κ-receptor. It shows only a slight preference for the KOR [8]. In the beginning of the seventieth of the last century, the arylacetamide derivatives were found to have a high affinity to the KOR, combined with a low affinity to the other opioid receptor subtypes, indicating a high selectivity. E.g. U-50,488, one of the first arylacetamides, did not show respiratory depression, constipation and tolerance [9]. However, the first compounds of this type in clinical trials for postsurgical pain, spiradoline and enadoline, have been abandoned due to dose-limiting dysphoria [10]. In order to avoid the side effects associated with the CNS, peripherally acting KOR-agonists are at present the focus of interest for the use in inflammatory hyperalgesia, such as asimadoline, which has utility in treating rheumatoid arthritis.

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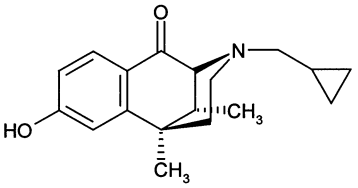
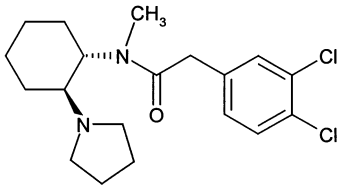


Ketocyclazocine



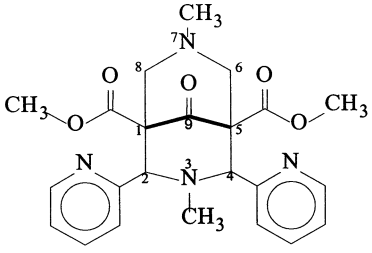
U50,488

Recently, the 2,4-di-2-pyridine substituted 3,7-dimethyl - 3,7 - diaza - 9 - oxo - bicyclo[3.3.1]nonane - 1,5-dicarboxylate, **HZ2**, was reported to have a high and selective affinity for the KOR [11,12] combined with a strong antinociceptive activity comparable to morphine and a potent action in inflammatory and persistent pain [13]. Table 1 summarizes the K_i values for **HZ2** and the references compounds. The radioligands for MOR, DOR and KOR sites were [^{3}H]naloxone, [^{3}H]Cl-DPDPE and [^{3}H]CI977, respectively. The specificity of **HZ2** is quite high, i.e. the MOR/KOR quotient is in the range of at least two orders of magnitude.

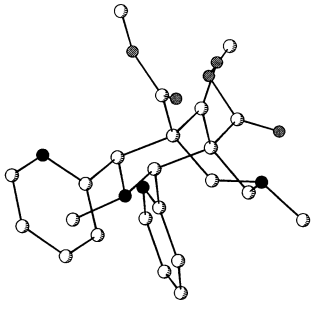
Intensive pharmacological and toxicological investigations revealed the compound to have the following profile [13]:) >

- Long duration of action.
- High oral bioavailability.
- Absence of dependence liability and respiratory depression.
- GI: Constipational potential is 3-fold less than that of morphine.
- Sedation, diuresis, saluresis induced by **HZ2** at doses close to the antinociceptive dose (expected profile of a κ-agonist).
- Persistent, dose-dependent emesis, not typical for κ-agonists.

The persistent emesis prevented **HZ2** from going into the clinical trials. This finding prompted the search for the pharmacophore and the intensive study of structure–activity relationships in order to find highly active analgesics with less side effects, especially without emesis.



HZ2



active conformation

Table 1
K_i values at opioid receptors, KOR, MOR, and DOR (μM ± SEM) in membrane preparations of the rat brain

Entity	KOR	MOR	DOR
HZ2	0.015 ± 0.004	>1	>10
U-50.488	0.0053 ± 0.001	1.6 ± 0.4	2.6 ± 0.7
U-69.593	0.0035 ± 0.0005	5.4 ± 1.0	1.4 ± 0.4
Bremazocine	0.00021 ± 0.00003	0.0006 ± 0.0001	0.00051 ± 0.00011
Morphine	0.17 ± 0.02	0.0022 ± 0.001	0.08 ± 0.0001

2. Search for the pharmacophore

In order to unravel the active conformation of the bicyclo[3.3.1]nonanones, well-known KOR-agonists such as ketocyclazocine, the arylacetamide compounds U50,488, U69,593, CI977, EMD61,753 and some analogues, were compared with **HZ2** with respect to their conformational behavior, the molecular electrostatic potential, the molecular hydrophobic potential and the hydrogen bonding potential. The analysis of the so

obtained structure–activity relationships revealed a chair–boat conformation of a protonated **HZ2** characterized by an almost parallel orientation of the C9 carbonyl group and the N7–H group (protonated) in conjunction with at least one aromatic ring to be the pharmacophoric arrangement [12].

3. Structure–activity relationships

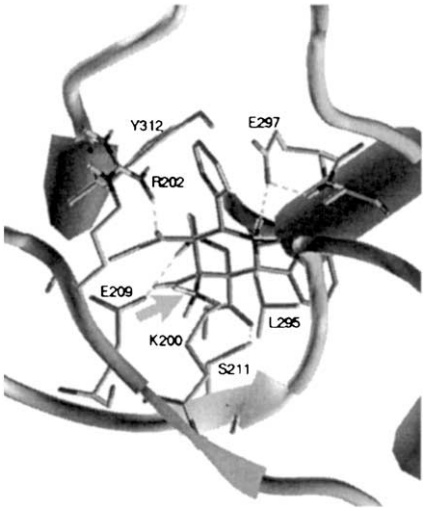
In order to study the structure–activity relationships a systematic variation of the substituents with respect to the substitution pattern of the aryl rings, and the substituents attached to the nitrogens in positions 3 and 7 was performed. The 2,4-di-aryl substituted diazabicyclo[3.3.1]nonan-9-one 1,5-dicarboxylate skeleton can be synthesized by a double Mannich reaction starting off with the condensation of 1 mole of primary amine, 2 moles of aryl aldehyde and 1 mole of dimethyl oxoglutarate to give the intermediate piperidone which can be converted to the corresponding diazabicyclononanones by refluxing 1 mole of the piperidone with 2 mole of formaldehyde and 1 mole of primary amines in methanol. Even though the bicyclic system is rather rigid, the stereochemistry has to be carefully studied because the spatial arrangement of the pharmacophoric elements governs the affinity to the KOR. In principle, boat/chair, chair/chair and chair/boat conformations as well as *cis*/*trans* and rotational isomers with respect to the aryl rings were observed [14–16]. At this point it can already be stated that all isomers which did not fulfill the aforementioned pharmacophore model of a chair/boat *cis*-substituted diazabicyclononanone did not show affinity to the KOR.

Variation of the substitution pattern resulted in the following changes of affinity: whereas the pyridine rings in 2 and 4 position can be replaced with *p*-methoxy-, *m*-hydroxy- and *m*-fluoro-substituted phenyl rings without any loss of affinity to the κ-receptor [17], the nitrogen N3 can be substituted with a hydrogen or a methyl group only [18]. Increasing the size of the substituent at this position resulted in a complete loss of affinity. Similar observations were made with increasing the bulk of the N7 substituent [16]. Both findings indicate a narrow binding pocket at the KOR. However, the double methylation of the nitrogen in position 7 gave the most active compound which will mainly bind to the peripheral KOR. Corresponding pharmacological studies are in progress.

4. Mechanism of action

In addition, **HZ2** showed an unusually long duration of action which is difficult to explain by the affinity to the receptor calculated from molecular modeling. However, the keto group in position 9 was found to have a high reactivity; in presence of protons the keto function can form a intramolecular hemiaminal using a pyridine nitrogen [19]. Correspondingly, the diazabicyclononanone may react with OH, NH or SH groups of the receptor protein. Docking studies of **HZ2** to the KOR revealed the chance that the keto function may react either with the side-chain of a cysteine, lysine or a serine residue. Docking studies of HZ2 to the extracellular binding site of KOR accompanied by semiempirical quantumchemical calculations were carried out to investigate the mechanism of the intra- and intermolecular hemiaminal formation in comparison. These calculations confirmed the principal possibility of the hemiaminal formation with the lysine residue K200. The covalent hemiaminal binding can explain the long-lasting activity of the compound. Since a hemiaminal formation is reversible, the diazabicyclononane is still able to dissociate from the receptor protein [20].

Model of the HZ2-receptor complex



5. Summary

A highly selective compound of high KOR affinity was found, the **HZ2**–methoiodide which will most likely bind to peripheral KOR. Thus, it is an interesting candidate for further testing for the utility in treating rheumatoid arthritis. The long duration of action will be advantageous in a long lasting therapy.

Acknowledgements

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References

[1] A.F. Casy, R.T. Parfitt, Opioid Analgesics—Chemistry and Receptors, Plenum Press, New York, 1986.

[2] M. Williams, E.A. Kowaluk, S.P. Arneric, Emerging molecular approaches to pain therapy, J. Med. Chem. 42 (1999) 1481–1500.
[3] T.L. Yaksh, Spinal systems and pain processing: development of novel analgesic drugs with mechanistically defined models, Trends Pharm. Sci. 20 (1999) 329–337.
[4] R.M. Eglen, J.C. Hunter, A. Dray, Ions in the fire: recent ion-channel and approaches to pain therapy, Trends Pharm. Sci. 20 (1999) 337–342.
[5] S.P.H. Alexander, A. Mathie, J.A. Peters, 2001 Nomenclature Supplement, Trends Pharm. Sci., 2001.
[6] U. Holzgrabe, C. Nachtsheim, T. Siener, S. Drosihn, W. Brandt, Opioid-Agonisten und -Antagonisten, Opioid-Rezeptoren, Pharmazie 52 (1997) 4–22.
[7] A. Barber, R. Gottschlich, Novel development with selective, non-peptidic kappa-opioid receptor agonists, Exp. Opin. Invest. Drugs 7 (1997) 1351–1368.
[8] P.E. Gilbert, W.R. Martin, The effects of morphine and nalorphine-like drugs in the nondependent, morphine-dependent and cyclazocine-dependent chronic spinal dog, J. Pharmacol. Exp. Ther. 198 (1976) 66–82.
[9] (a) J. Szmuszkovicz, U-50,488 and the κ receptor: A personalized account covering the period 1973 to 1990, Prog. Drug Res. 52 (1999) 168–195;
(b) J. Szmuszkovicz, U-50,488 and the κ receptor: part II: 1991–1998, Prog. Drug Res. 53 (1999) 3–51.
[10] E.A. Kowaluk, S.P. Arneric, Novel molecular approaches to analgesia, Ann. Rep. Med. Chem. 33 (1998) 11–20.
[11] A. Borsodi, S. Benyhe, U. Holzgrabe, A. Marki, C. Nachtsheim, Structurally novel group of ligands selective for kappa-opioid receptors, Regulatory Peptides 54 (1994) 27–28.
[12] W. Brandt, S. Drosihn, M. Haurand, U. Holzgrabe, C. Nachtsheim, Search for the pharmacophore in Kappa-agonistic diazabicyclo[3.3.1]nonan-9-one-1,5-diesters and arylacetamides, Arch. Pharm. Pharm. Med. Chem. 329 (1996) 311–323.
[13] B. Kögel, T. Christoph, E. Friderichs, H.-H. Hennies, T. Matthiesen, J. Schneider, U. Holzgrabe, HZ2, a selective κ-opioid agonist, CNS Drug Rev. 4 (1998) 54–70.
[14] T. Siener, U. Holzgrabe, S. Drosihn, W. Brandt, Semiempirical PM3 calculations to the *cis*/*trans* isomerisation of the aromatic substituents of bicyclononanones, J. Chem. Soc., Perkin 2 (1999) 1827–1834.
[15] U. Kuhl, M. Graf v. Korff, K. Baumann, C. Burschka, U. Holzgrabe, Stereochemical behaviour of κ-agonistic 2,4-dipyridine 3,7-diazabicyclo[3.3.1]nonanones – influence of the substituent in position N3, J. Chem. Soc., Perkin 2 (2001) 2037–2042.
[16] A. Cambareri, D.P. Zlotos, W. Englberger, M. Haurand, U. Holzgrabe, Stereochemistry and opioid receptor affinity of 2,4-dipyridine 3,7-diazabicyclo[3.3.1]nonanones—influence of the substituent in position N7, J. Heterocycl. Chem. (in press).
[17] T. Siener, A. Cambareri, U. Kuhl, W. Englberger, M. Haurand, B. Kögel, U. Holzgrabe, Synthesis and opioid affinity in a series of opioid-type analgesics: diazabicyclo[3.3.1]nonan-9-ones, J. Med. Chem. 43 (2000) 3746–3751.
[18] U. Kuhl, W. Englberger, M. Haurand, U. Holzgrabe, Diazabicyclo[3.3.1]nonanone-type ligands for the opioid receptors, Arch. Pharm. Pharm. Med. Chem. 333 (2000) 226–230.
[19] U. Kuhl, A. Cambareri, C. Sauber, F. Sörgel, H. Euler, R. Hartmann, A. Kirfel, U. Holzgrabe, Synthesis, X-ray analysis, and spectroscopic characterization of the hemiaminal cyclisation product from 2,4-dipyridine substituted 3,7-diazabicyclo[3.3.1]-nonanone 1,5-diester, J. Chem. Soc., Perkin 2 (1999) 2083–2088.
[20] U. Holzgrabe, W. Brandt, Mechanism of action of the diazabicyclononanone-type kappa-agonists, J. Med. Chem. (under revision).