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New benzomorphan derivatives of MPCB as MOP and KOP receptor ligands

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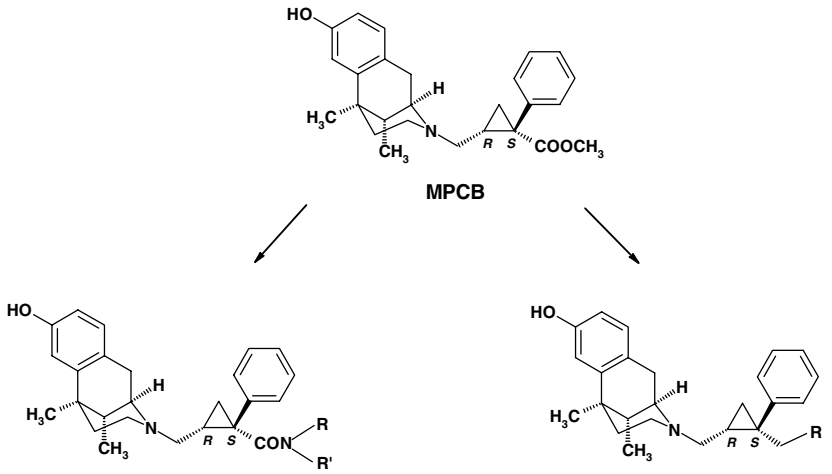
There is considerable interest in developing KOP Opioid receptor ligands as clinically useful analgesics. Moreover, compounds with mixed KOP receptor and mu-opioid peptide (MOP) receptor agonist/antagonist properties could have a better therapeutic potential. The benzomorphan-based synthetic ligands MPCB and CCB have been shown to bind KOP receptors with high affinity and selectivity. We report here a series of compounds synthesized to perform structure-affinity relationship (SAR) studies on MPCB. The aim of this study was to optimise KOP receptor-ligand interaction and to modulate MOP receptor selectivity. In the benzylamide analogue of MPCB (compound **9**) the presence of a third aromatic nucleus, at an appropriate distance and conformation with respect to aromatic pharmacophoric residues, increased KOP receptor affinity by about 6-fold compared to MPCB ($K_i = 35$ nM and $K_i = 240$ nM, respectively). Instead, compound **28** with a tertiary amino group in the nitrogen substituent displayed a comparable KOP receptor affinity ($K_i = 179$ nM) but also high MOP receptor affinity ($K_i = 45$ nM). Thus, the present study shows that in benzomorphan-based ligands the presence of different functional groups in the nitrogen substituent, ranging from a positive charged amine to an additional aromatic ring, is able to promote the correct aligment of aromatic pharmacophoric residues with MOP and KOP receptor types. Evaluation of docking simulations of compounds **9** and **28** into the KOP and MOP receptor displayed selective ligand interactions with the important amino acid residues Tyr320 (TMVII) and Trp318 (TMVII), respectively.

1. Introduction

The biological effects of opioid analgesics are mediated through three major types of opioid receptors, identified as MOP, KOP and delta-opioid peptide (DOP) receptors (Dhawan et al. 1996). The availability of selective ligands has played an important role in the cloning and pharmacological characterization of these opioid receptor types (Raynor et al. 1994). Most of opioid analgesics used in clinical treatment of pain act preferably via MOR MOP receptor. Therefore, their use in chronic pain is limited by severe side effects (including respiratory depression, tolerance and physical dependence) that are caused by MOP receptor agonists in addition to the analgesic effect. Since KOP receptor ligands have a lower tendency to cause respiratory depression and physical dependence than MOP receptor ligands (Millan 1990), there is a considerable interest in developing KOP receptor ligands as clinically useful analgesics. Interest has been shown in the development of KOP receptor agonists because some studies have demonstrated that KOP receptor agonists are involved in the antagonism of some effects of cocaine abuse through dopamine level modulation (Werling et al. 1988; Margolis et al. 2003). In the "dopamine hypothesis" the increase of dopamine seems important in mediating the abuse effects (Kuhar et al. 1991), thus KOP receptor agonists could be useful in treatment of cocaine abuse, too (Prisinzano et al. 2005). Because KOP receptor agonists produce some side effects such as sedation and vomiting, the use of compounds with KOP receptor agonist and MOP receptor agonist/antagonist properties has been proposed. In fact, it is supposed that these mixed-action compounds could be used in abuse treatment with a minor presence of side effects (Neumeyer et al. 2001; Zhang et al. 2004).

The benzomorphan-based synthetic ligand MPCB and its p-chlorophenyl analogue (CCB), synthesized by Ronsisvalle and co-workers, have shown to bind KOP receptor with high affinity and selectivity (Ronsisvalle et al. 1993; Ronsisvalle et al. 1995). MPCB was also used in modelling approaches to understand different ligand binding modes and to provide insights into the design of new synthetic compounds (Lavecchia et al. 2000). In MPCB the cylopropylmethyl-normetazocine nucleus represents a scaf-

Scheme 1



Compd.	R	R′	Compd.	R
8	$CH-(CH_3)-C_6H_5$	H	**25**	$OCOCH_3$
9	$CH_2-C_6H_5$	H	**26**	OH
10	C_2H_5	C_2H_5	**27**	Pyrrolidin-1-yl
13	CH_3	H	**28**	Piperidin-1-yl
14	C_2H_5	H	**29**	Morpholin-1-yl
16	H	H		
17	CH_3	CH_3		
18	C_6H_5	H		
19	$-(CH_2)_4-$			
20	$-(CH_2)_5-$			
21	$-(CH_2)-O-(CH_2)_2-$			

fold to support the phenolic ring, the basic nitrogen and the phenyl ring in a conformation mimicking the N-terminal fragment of dynorphin A, the endogenous ligand for KOP receptor (Ronsisvalle et al. 1993). In fact, in peptide and nonpeptide opioid ligands the location and the relative orientation of the aromatic pharmacophoric residues are important structural features for receptor binding (Tömboly et al. 2004; Fujita et al. 2004; Grieco et al. 2005). The fact that the C-terminal residues dictate the overall conformational preferences (Balboni et al. 2005) in peptide ligands has been well demonstrated, therefore the objective of this study was to optimise KOP receptor-ligand interaction and to modulate MOP receptor selectivity by replacing the ester group of MPCB. The synthesis of MPCB analogues could demonstrate the importance of the ester group and of other functional groups in the overall conformation of relative ligands and in the capability to promote the correct aligment of their pharmacophoric groups with the KOP receptor. Thus, we report here a series of compounds synthesized to perform SAR studies on MPCB (Ronsisvalle et al. 2001). By modifying the ester functionality amides (**8**–**10**, **13**, **14** and **16**–**21**), amines (**27**–**29**), reversed ester (**25**) and alcohol (**26**) were synthesized as shown in Scheme 1.

2. Investigations and results

2.1. Synthesis of the compounds

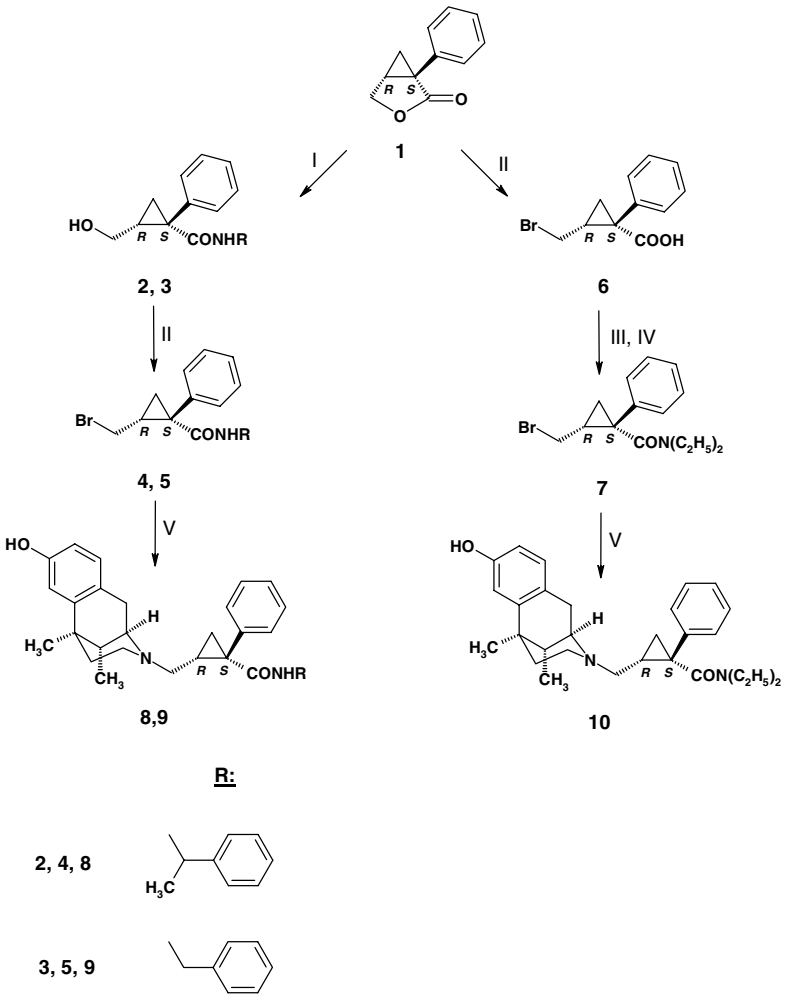
The MPCB analogues were obtained from *cis*-(−)-(1*R*,5*R*,9*R*)-*N*-normetazocine that was separated from a racemic mixture as previously reported (Brine et al. 1990).

Synthesis of the amides **8**–**10**, **13**, **14** and **16**–**21** was performed in different ways with the aim of optimizing the yields. Amides **8**–**10** were prepared by alkylation of *cis*-(−)-(1*R*,5*R*,9*R*)-*N*-normetazocine with the respective *cis*-bromo amides in methyl ethyl ketone at 60 °C using $NaHCO_3$ as shown in Scheme 2. The *cis*-bromo amides were obtained from lactone **1**, following procedures reported in the literature (Shuto et al. 1996; Bonnaud et al. 1987). Alkylation of *cis*-(−)-(1*R*,5*R*,9*R*)-*N*-normetazocine with (−)-methyl 2-(bromomethyl)-1-phenylcyclopropanecarboxylate (Marrazzo et al. 2002) gave the methyl ester **12** which was used for the preparation of the other amides (Scheme 3). Amides **13** and **14** were prepared by treatment of the methyl ester **12** with the respective aqueous amine solutions. The hydrolysis of the methyl ester **12** gave the free acid **15** that provided the compounds **16**–**21** by treatment with $SOCl_2$ followed by condensation of the unpurified product with the respective amines in anhydrous THF at 0 °C.

Compounds **25** and **26** were synthesized according to Scheme 4. Selective reduction of the (−)-*cis*-chloro-methyl ester **22**, obtained from lactone **1** by general procedures (Casadio et al. 1978), with alane-*N*,*N*-dimethylethylamine complex in anhydrous THF at 0 °C gave compound **23** with 76% yield (Ronsisvalle et al. 2000a). Acetylation of compound **23** with acetic anhydride in anhydrous THF provided compound **24**. Alkylation of *cis*-(−)-(1*R*,5*R*,9*R*)-*N*-normetazocine with compounds **23** and **24** gave the respective final compounds **25** and **26**.

Finally, reduction of the amides **18**, **19** and **20** with $LiAlH_4$ in anhydrous THF provided the amines **27**, **28** and **29** (Scheme 5).

Scheme 2



(I) RNH_2, 2-hydroxypyridine, toluene, reflux; (II) HBr/CH_3COOH, 80 °C; (III) $SOCl_2$, C_6H_6, reflux, 2 h; (IV) diethylamine, THF, r.t., overnight; (V) *cis*-(−)-(1*R*,5*R*,9*R*)-*N*-normetazocine, methyl ethyl ketone, $NaHCO_3$, KI, 60 °C, 18 h

2.2. Pharmacology

All compounds were evaluated for their binding affinity and selectivity at KOP, MOP and DOP receptors using the selective radioligands [^{3}H]U-69,593, [^{3}H]DAMGO, and [^{3}H]DADLE, respectively. These data in comparison with the standard compound MPCB are summarized in Tables 1 and 2.

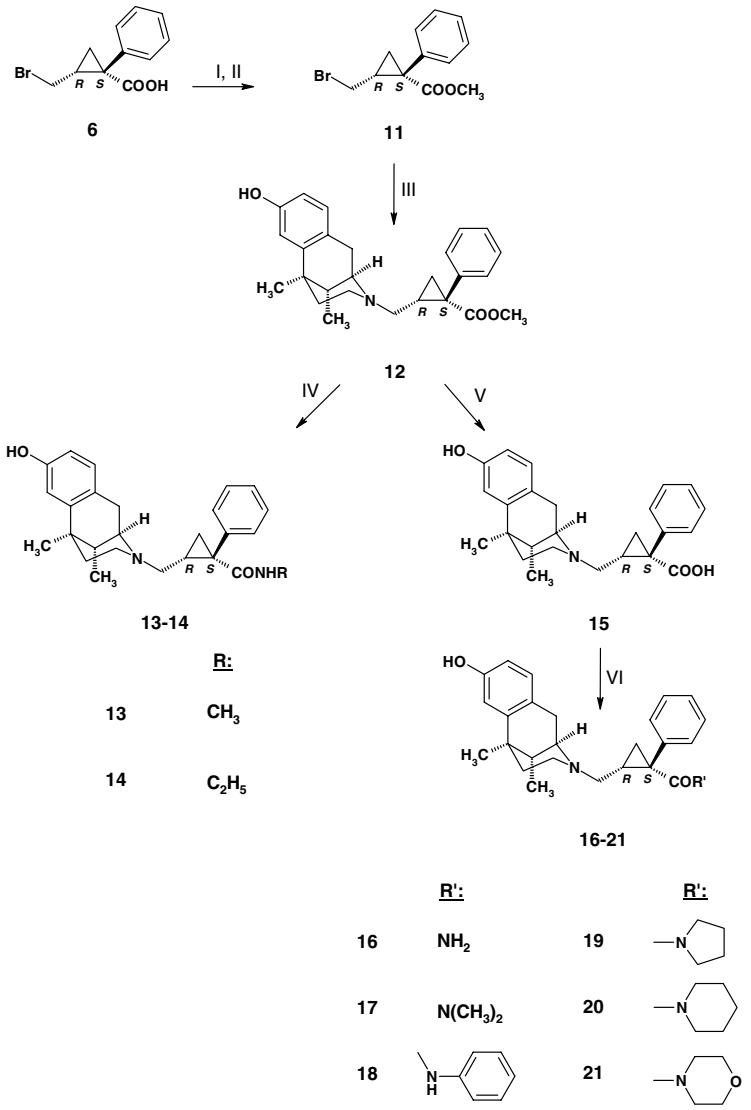
As shown in Table 1, with the exception of compound **9**, all amide MPCB analogues (**8**–**10**, **13**, **14** and **16**–**21**) have binding affinities to the KOP receptor lower than that of MPCB. In this series only compound **9**, a benzylamide derivative, displayed a significative KOP receptor affinity ($K_i = 35$ nM). Compound **25**, with a reversed ester functionality, maintained significant KOP receptor binding affinity ($K_i = 228$ nM) and selectivity MOP receptor ($K_i = 897$ nM, DOP receptor $K_i = >5{,}000$ nM), while the introduction of the alcohol function (compound **26**) further decreased KOP affinity ($K_i = 396$ nM) relative to that of MPCB (Table 2). Comparison of MPCB with compounds **27**–**29** showed that the substitution of ester function with various tertiary amines did not significantly affect KOP receptor affinity (Table 2).

Comparing MOP receptor affinity of MPCB with the amide compounds, it is evident that they show higher affinities (Table 1). Moreover, an examination of the affinities of the amine compounds **27**–**28** reveals that they display high binding affinity to the MOP receptors. In particular, compound **28** displayed the highest MOP receptor affinity ($K_i = 45$ nM). As regards DOP receptor binding, a negligible affinity was shown by all MPCB analogues.

3. Discussion

This study on benzomorphan derivatives shows that the presence of primary, secondary and tertiary amides has detrimental effects on KOP receptor binding affinities of MPCB analogues. It is possible that the introduction of an amide functionality prevents the correct aligment of aromatic pharmacophoric residues with the KOP receptor hindering H-bonding and π-stacking between carbonyl oxygen and the phenyl ring present in the nitrogen substituent of MPCB and the side chain of Tyr312 (TMVII) (Lavecchia et al. 2000). Only the benzylamide derivative (**9**), which possesses a third aromatic nucleus at an appropriate distance and with an appropriate conformation with respect to aromatic pharmacophoric residues, increases KOP receptor affinity by about 6-fold and thus seems to compensate for the presence of the amide group. This additional interaction has stringent stereochemical require-

Scheme 3

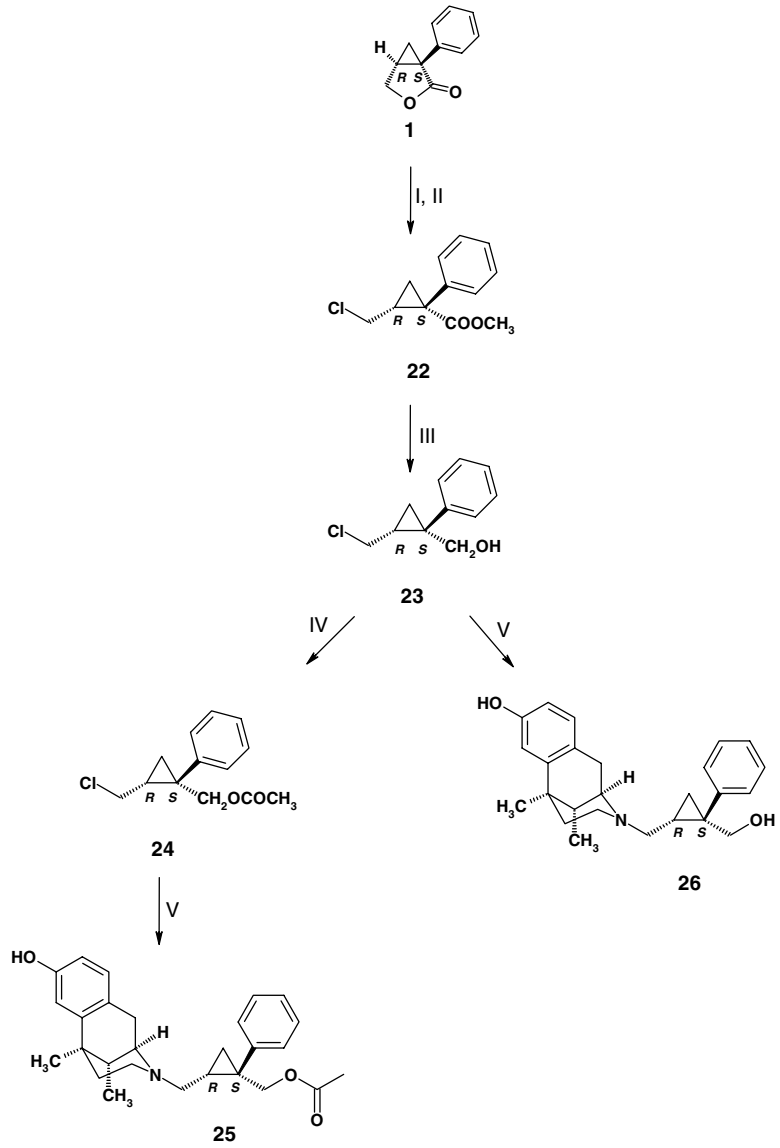


(I) $SOCl_2$, C_6H_6, reflux, 5 h; (II) CH_3OH/3N HCl, r.t., overnight; (III) *cis*-(−)-(1*R*,5*R*,9*R*)-N-normetazocine, methyl ethyl ketone, $NaHCO_3$, KI, 60 °C, 18 h; (IV) RNH_2 aqueous solution, CH_3OH, sealed tube, 70 °C, 48 h; (V) 1 N NaOH, reflux, 5 h; aqueous HCl; (VI) for product **16**: $SOCl_2$, r.t., overnight; NH_3, diethyl ether, 0 °C, 0.5 h; for products **17**–**21**: $SOCl_2$, r.t., overnight; R′H, THF, 0 °C, 0.5 h

ments as is demonstrated by compounds **8** and **18**. In fact, these compounds, although they possess an additional aromatic nucleus in the benzomorphan nitrogen substituent, have negligible KOP receptor binding affinities.

In order to find structural explanations for ligand-receptor binding results by application of modeling studies, docking simulations of the benzylamide derivative (**9**) of MPCB into the KOP receptor and MOP receptor were performed using the automated docking program GOLD 3.0 and the ChemScore fitness function to rank the compounds on the basis of their ability to form favorable interactions with the active site. In KOP and MOP receptors the ligand is docked near the extracellular opening of the binding pocket and is located in such a way as to present the basic interaction between the protonated nitrogen of the benzomorphan part of the ligands with the carboxyl group of conserved aspartate in TMIII (Asp138 in KOP receptor and Asp147 in MOP receptor, respectively). In fact, this interaction is a key anchoring point for opioid binding and is supported by site-directed mutagenesis studies (Kong et al. 1994; Surratt et al. 1994). Figure 1 shows the proposed compound **9** KOP receptor binding mode. The best docking position on the KOP receptor shows that the key residue involved in selective binding is Tyr320 (TMVII). This amino acid residue also has great effect on the affinity of salvinorin A, a selective κ opioid agonist. In fact, site-directed mutagenesis studies revealed that Tyr320 may be involved in π-stacking or other favorable hydrophobic interactions with salvinorin A (Yan et al. 2005; Kane et al. 2006; Singh et al. 2006). Like salvinorin A, the binding of compound **9** to the KOP receptor could show a favorable effect due to the hydrophobic interaction of Tyr320 with the additional phenyl ring present in the benzomorphan nitrogen substituent. Moreover, the difference in affinity between compound **9** and the other amide derivatives of MPCB could be due to this third aromatic nucleus at an appropriate distance and with an appropriate conformation with respect to aromatic pharmacophoric residues. With respect to the MOP receptor, compound **9** exhibited decreased binding affinity. This could be related

Scheme 4



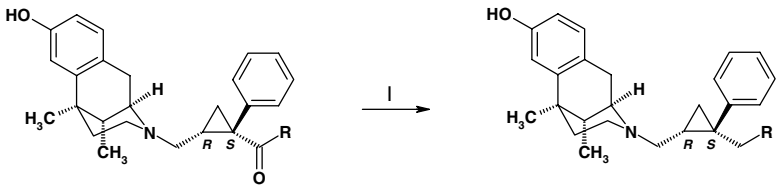
(I) $SOCl_2$, $ZnCl_2$, C_6H_6, reflux, 5 h; (II) CH_3OH/3N HCl, r.t., overnight; (III) $C_2H_5N(CH_3)_2AlH_3$, THF, 0 °C, 2.5 h; (IV) acetyl chloride, DMAP, THF, r.t., 20 h; (V) *cis*-(−)-(1*R*,5*R*,9*R*)-N-normetazocine, DMF, $NaHCO_3$, KI, 75 °C, 6 h

to the absence of key interaction between Tyr320 (TMVII) present in the KOP receptor and the additional phenyl ring of compound **9**. On the other hand, in studies on chimera KOP/MOP receptor it was demonstrated that regions of MOP receptor from TMV to TMVII prevent some typical KOP receptor interactions (Kane et al. 2006).

Among the amines, the piperidin-1-ylmethyl analogue of MPCB **28** with an additional positive charge in the benzomorphan nitrogen substituent shows an increase of MOP receptor affinity. Figure 2 shows the proposed compound **28** MOP receptor binding mode. In this case the best docking position on the MOP receptor shows that the key residue involved in selective binding is Trp318 (TMVII). The selective interaction of this receptor specific binding pocket residue with the phenyl ring present in the nitrogen substituent of MPCB could justify a better affinity of compound **28** to the MOP receptor. This interaction was shown in MOP receptor selective agonists such as JOM6 and fentanyl derivatives (Pogozheva et al. 2005; Dosen-Micovic et al. 2006). Figure 3 shows the proposed compound **28** KOP receptor binding mode. Like compound **9**, binding of compound **28** to the KOP receptor shows a favorable hydrophobic interaction between the piperidine ring present in the benzomorphan nitrogen substituent and Tyr320 (TMVII). The difference in affinity observed in compound **9** and compound **28** may be the result of a π-stacking interaction between Tyr320 and the additional phenyl ring present in the benzomorphan nitrogen substituent of compound **9**. In salvinorin A the importance of this interaction was demonstrated by site-directed mutagenesis studies (Kane et al. 2006).

In conclusion, the present study demonstates that in the benzomorphan-based ligands the presence in the nitrogen substituent of different functional groups, varying from a positive charged amine to an additional aromatic ring, is able to promote the correct aligment of aromatic pharmacophoric residues with MOP and KOP receptors. For example, compound **9** with a strictly oriented third aromatic nucleus indicates additional interactions with KOP recep-

Scheme 5



R:

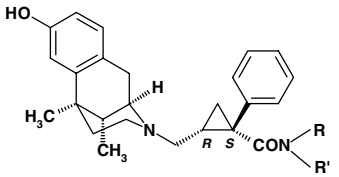
19, 27 —N

20, 28 —N

21, 29 —N O

(I) $LiAlH_4$, THF anh., reflux, 12 h

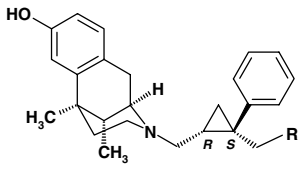
Table 1: Binding affinity of amide analogues of MPCB toward MOP, KOP and DOP receptors



Compd.	R	R′	$K_i \pm$ SEM (nM)[a,b]		
			KOP	MOP	DOP
8	$CH-(CH_3)-C_6H_5$	H	>5000	565 ± 21	>5000
9	$CH_2-C_6H_5$	H	35 ± 3	885 ± 61	>5000
10	C_2H_5	C_2H_5	>5000	1294 ± 70	>5000
13	CH_3	H	>5000	1559 ± 65	>5000
14	C_2H_5	H	>5000	>5000	>5000
16	H	H	>5000	705 ± 28	>5000
17	CH_3	CH_3	>5000	360 ± 14	>5000
18	C_6H_5	H	2355 ± 90	>5000	>5000
19		$-(CH_2)_4-$	>5000	478 ± 23	>5000
20		$-(CH_2)_5-$	>5000	421 ± 23	>5000
21		$-(CH_2)_2-O-(CH_2)_2-$	>5000	>5000	>5000
MPCB[c]			240 ± 39	>25000	>25000

[a] Values are the mean of three separate experiments each carried out in duplicate. [b] K_i values were obtained as [^{3}H]U-69593 displacement for KOP receptors, [^{3}H]DAMGO displacement for MOP receptors and [^{3}H]DADLE displacement for DOP receptors. [c] Ronsisvalle et al. 1993

Table 2: Binding affinity of amine, alcohol and reversed ester analogues of MPCB toward MOP, KOP and DOP receptors



Compd.	R	$K_i \pm$ SEM (nM)[a,b]		
		KOP	MOP	DOP
25	$OCOCH_3$	228 ± 6	897 ± 36	>5000
26	OH	396 ± 22	481 ± 22	>5000
27	Pyrrolidin-1-yl	170 ± 7	261 ± 8	>5000
28	Piperidin-1-yl	179 ± 8	45 ± 4	>5000
29	Morpholin-1-yl	3700 ± 150	558 ± 18	>5000
MPCB[c]		240 ± 39	>25000	>25000

[a] Values are the mean of three separate experiments each carried out in duplicate. [b] K_i values were obtained as [^{3}H]U-69,593 displacement for KOP receptors, [^{3}H]DAMGO displacement for MOP receptors and [^{3}H]DADLE displacement for DOP receptors. [c] Ronsisvalle et al. 1993

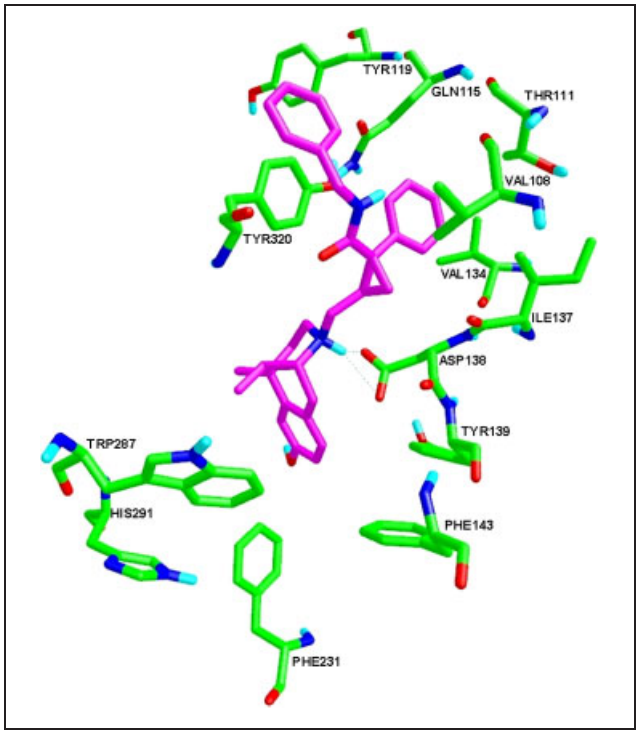


Fig. 1: Compound **9** in the binding pocket of the agonist-bound conformation of KOP receptor. Compound **9** and key amino acid residues are shown. Hydrogen bond is shown as dotted lines

tor while new analogues of compound **28** could permit us to study the structural determinants that can shift the KOP receptor affinity of MPCB to MOP receptor affinity with the aim of developing new mixed-active ligands.

4. Experimental

4.1. Chemistry

All commercial chemicals were used as received from Aldrich Chemical Co. unless otherwise specified. *Cis*-(±)-*N*-normetazocine was obtained from Fabbrica Italiana Sintetici (Vicenza, Italy). Melting points were determined in open capillary tubes with a Büchi 530 apparatus and are uncorrected. Optical rotations were determined in a CH_3OH solution with a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a 1600 FT-IR Perkin-Elmer instrument. ^1H and ^{13}C NMR spectra were recorded on a Varian Inova-200 spectrometer in DMSO-d_6 or $CDCl_3$ solution; chemical shifts (δ) are expressed in ppm with reference to TMS as an internal standard and coupling constants (J) are in hertz. Molmass was determined on a Kratos 2S RFA spectrometer using a Tektronix 4205 computer system. Elemental analyses were carried out by an elemental analyzer, Model 1106, Carlo Erba instrument. Purity of the compounds was checked on silica gel 60 F_{254} aluminum sheets (Merck) using iodine as visualizing agent. All the elemental analyses results were within 0.4% of the theoretical values. All described compounds showed NMR, IR and MS spectral data consistent with those of the assigned structure. Flash column chromatography was carried out on Merck silica gel 60 (230–400 mesh).

4.1.1. (1S,2R)-2-(Hydroxymethyl)-1-phenyl-N-[(1R)-1-phenylethyl]cyclopropanecarboxamide (**2**)

To a solution of (1*S*,5*R*)-*cis*-1-phenyl-3-oxabicyclo[3.1.0]hexan-2-one (**1**, 700 mg, 4.01 mmol) in anh. toluene (3.5 ml), (*R*)-α-methylbenzylamine (973 mg, 8.03 mmol) and 2-hydroxypyridine (382.14 mg, 4.01 mmol) were added. The reaction mixture was refluxed for 15 h and was then concentrated. The residue was dissolved in CH_2Cl_2 and the solution was washed with 0.5N HCl and brine, and dried over anh. Na_2SO_4. Solvent was removed *in vacuo* and the crude product was crystallized from diethyl ether. Yield: 76.5%; m.p. 101–102 °C; IR (KBr): $\bar{\nu}$ (C=O) 1650 cm^{-1}; $[\alpha]_D^{20}$ – 44 (c 1.0, CH_3OH); MS (m/z): 295 [M^+]; ^1H NMR ($CDCl_3$) δ: 1.35 (dd, 1 H, J = 4.5, 8.8 Hz), 1.44 (d, 3 H, J = 6.8), 1.72 (dd, 1 H, J = 4.5, 7.2 Hz), 1.85–1.95 (m, 1 H), 3.60 (dd, 1 H, J = 7.5, 11.0 Hz), 3.68 (dd, 1 H, J = 5.6, 11.0 Hz), 5.14 (q, 1 H, J = 6.8), 5.55 (bs, 2 H), 7.00–7.65 (m, 10 H); ^{13}C NMR ($CDCl_3$) δ: 20.71, 22.95, 34.15, 35.21, 51.35, 65.78, 121.67, 125.44, 127.32, 129.10, 129.35, 130.45. 142.68, 144.90, 172.36. $C_{19}H_{21}NO_2$ (295.4)

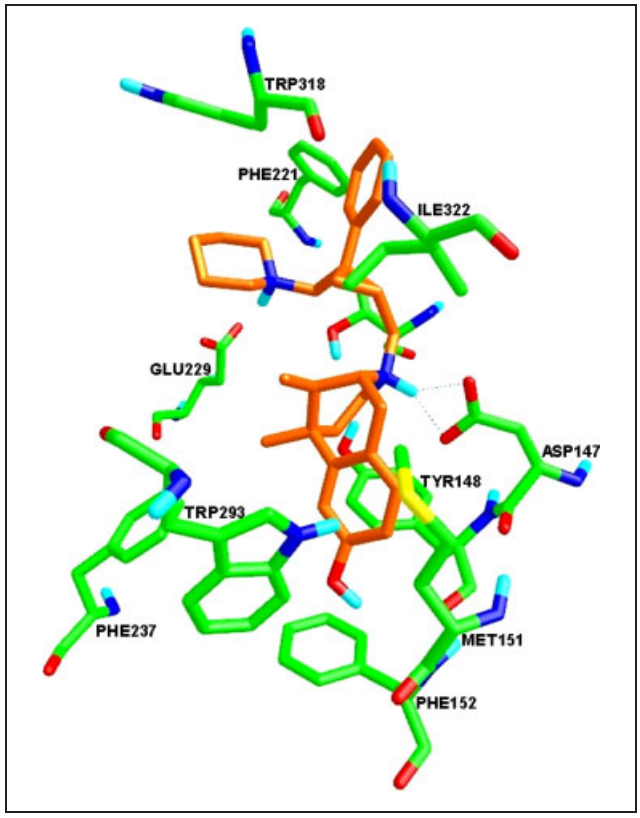


Fig. 2: Compound **28** in the binding pocket of the agonist-bound conformation of MOP receptor. Compound **28** carbon atom and key amino acid residues are shown. Hydrogen bond is shown as dotted lines

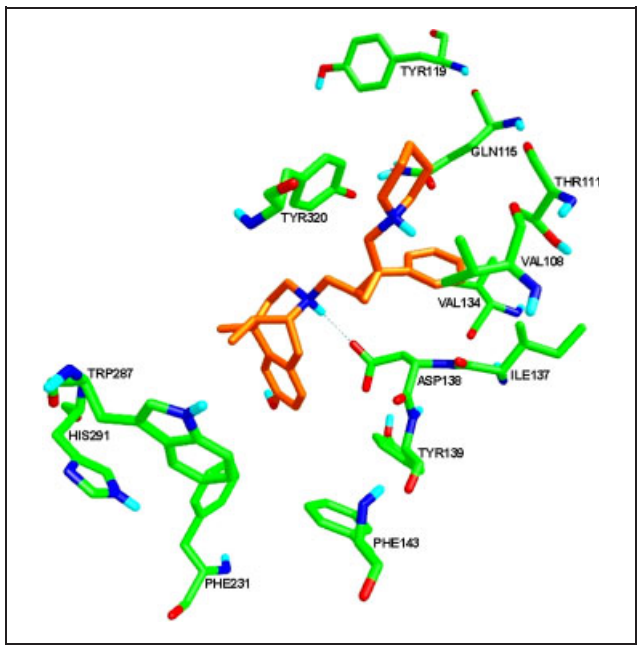


Fig. 3: Compound **28** in the binding pocket of the agonist-bound conformation of KOP receptor. Compound **28** and key amino acid residues are shown. Hydrogen bond is shown as dotted lines

4.1.2. (1S,2R)-N-Benzyl-2-(hydroxymethyl)-1-phenylcyclopropanecarboxamide (**3**)

To a solution of (1*S*,5*R*)-*cis*-1-phenyl-3-oxabicyclo[3.1.0]hexan-2-one (**1**, 700 mg, 4.01 mmol) in anh. toluene (3.5 ml), benzylamine (868 mg, 8.03 mmol) and 2-hydroxypyridine (382.14 mg, 4.01 mmol) were added. The reaction mixture was refluxed for 6 h and was then concentrated. The residue was dissolved in CH_2Cl_2 and the solution was washed with 0.5N

HCl and brine, and dried over anh. Na_2SO_4. Solvent was removed *in vacuo* and the crude product was crystallized from diethyl ether. Yield: 79.5%; m.p. 90 °C; IR (KBr): $\bar{\nu}$ (C=O) 1640 cm^{-1}; $[\alpha]_D^{20}$ – 58 (c 1.0, CH_3OH); MS (m/z): 281 [M^+]; ^1H NMR ($CDCl_3$) δ: 1.30 (dd, 1 H, J = 5.5, 8.0 Hz), 1.50 (dd, 1 H, J = 6.1, 8.0), 1.82–1.91 (m, 1 H), 3.62 (dd, 1 H, J = 7.3, 10.7 Hz), 3.72 (dd, 1 H, J = 5.3, 10.7 Hz), 4.60 (s, 2 H), 5.32 (bs, 2 H), 7.03–7.62 (m, 10 H); ^{13}C NMR ($CDCl_3$) δ: 19.24, 33.25, 35.78, 45.76, 65.23, 121.67, 125.44, 126.21, 128,25, 128.63, 129.21, 137.25, 144.90, 172.36.
$C_{18}H_{19}NO_2$ (281.3)

4.1.3. (1S,2R)-2-(Bromomethyl)-1-phenyl-N-[(1R)-1-phenylethyl]cyclopropanecarboxamide (**4**)

To a solution of HBr in 33% acetic acid (4 ml) compound **2** (500 mg, 1.69 mmol) was added and the reaction mixture was heated at 80 °C for 1 h. After cooling, was poured into an ice-water mixture. The aqueous phase was extracted with $CHCl_3$ and the combined organic layers were dried over anh. Na_2SO_4, evaporated and purified by flash column chromatography (petroleum ether 30–60° : ethyl acetate, 90 : 10). The product was then crystallized from *n*-hexane. Yield: 50.8%; m.p. 110–112 °C; IR (KBr): $\bar{\nu}$ (C=O) 1650 cm^{-1}; $[\alpha]_D^{20}$ – 39 (c 1.0, CH_3OH); MS (m/z): 358 [M^+]; ^1H NMR ($CDCl_3$) δ: 1.43 (d, 3 H, J = 7.0), 1.78 (dd, 1 H, J = 5.45, 7.8 Hz), 1.85–1.95 (m, 2 H), 3.20–3.80 (m, 2 H), 5.07 (q, 1 H, J = 7.0), 6.55 (bs, 1 H), 7.00–7.80 (m, 10 H); ^{13}C NMR ($CDCl_3$) δ: 20.93, 24.75, 33.37, 38.11, 41.36, 51.00, 120.95, 125.32, 127.66, 128.13, 128.33, 129.10, 143.11, 144.88, 174.27.
$C_{19}H_{20}BrNO$ (358.3)

4.1.4. (1S,2R)-N-Benzyl-2-(bromomethyl)-1-phenylcyclopropanecarboxamide (**5**)

The brome derivative **5** was prepared from compound **3** (400 mg, 1.42 mmol) using the procedure described above. The product was crystallized from *n*-hexane. Yield: 51%; m.p. 99–100 °C; IR (KBr): $\bar{\nu}$ (C=O) 1680 cm^{-1}; $[\alpha]_D^{20}$ – 50 (c 1.0, CH_3OH); MS (m/z): 344 [M^+]; ^1H NMR ($CDCl_3$) δ: 1.60 (dd, 1 H, J = 5.6, 7.8 Hz), 1.93 (dd, 1 H, J = 6.5, 7.8), 1.98–2.30 (m, 1 H), 3.32–3.72 (m, 2 H), 4.48 (s, 2 H), 6.54 (bs, 1 H), 7.10–7.98 (m, 10 H); ^{13}C NMR ($CDCl_3$) δ: 23.77, 34.00, 36.97, 39.97, 44.68, 121.95, 125.32, 126.68, 128,35, 128.47, 129.03, 136.44, 143.88, 174.54.
$C_{18}H_{18}BrNO$ (344.3)

4.1.5. (1S,2R)-2-(Bromomethyl)-1-phenylcyclopropanecarboxylic acid (**6**)

To a solution of HBr in 33% acetic acid (4 ml) (1*S*,5*R*)-*cis*-1-phenyl-3-oxabicyclo[3.1.0]hexan-2-one (**1**, 300 mg, 1.72 mmol) was added and the reaction mixture was heated at 80 °C for 2 h. After cooling, the reaction mixture was poured into an ice-water mixture The resulting precipitate was collected by filtration and dried. Yield: 96.6%; m.p. 147–148 °C; IR (KBr): $\bar{\nu}$ (C=O) 1685 cm^{-1}; $[\alpha]_D^{20}$ – 51 (c 1.0, CH_3OH); MS (m/z): 255 [M^+]; ^1H NMR ($CDCl_3$) δ: 1.66 (dd, 1 H, J = 4.9, 8.2 Hz), 1.88 (dd, 1 H, J = 5.2, 8.2 Hz), 2.25–2.35 (m, 1 H), 3.62 (dd, 1 H, J = 9.8, 11.4 Hz), 3.85 (dd, 1 H, J = 4.7, 11.4 Hz), 7.20–7.55 (m, 5 H), 8.55 (bs, 1 H); ^{13}C NMR ($CDCl_3$) δ: 23.62, 28.45, 38.43, 40.91, 120.67, 125.98, 128.44, 139.62, 178.27.
$C_{11}H_{11}BrO_2$ (255.1)

4.1.6. (1S,2R)-2-(Bromomethyl)-N,N-diethyl-1-phenylcyclopropanecarboxamide (**7**)

Thionyl chloride (1.5 ml, 15.68 mmol) was added dropwise to a solution of (1*S*,2*R*)-2-(bromomethyl)-1-phenylcyclopropanecarboxylic acid (**6**, 400 mg, 1.56 mmol) in C_6H_6 with vigorous stirring at 0 °C. The reaction mixture was refluxed for 2 h and then evaporated *in vacuo*. The resulting residue was dissolved in THF (4 ml) and added dropwise to a solution of diethylamine (229.33 mg, 3.13 mmol) in THF (6 ml) at −10 °C. The reaction mixture was stirred overnight at room temperature and the residue was then filtered off and the solvent was removed *in vacuo* to provide the crude product. Yield: 92.5%; IR (KBr): $\bar{\nu}$ (C=O) 1630 cm^{-1}; $[\alpha]_D^{20}$ – 53 (c 1.0, CH_3OH); ^1H NMR ($CDCl_3$) δ: 1.25 (t, 6 H, J = 7.0 Hz), 1.86 (dd, 1 H, J = 6.9, 7.8 Hz), 1.98 (dd, 1 H, J = 5.3, 7.8 Hz), 2.35–2.45 (m, 1 H), 3.30 (q, 4 H, 7.0 Hz) 3.42–3.73 (m, 2 H), 7.35–7.85 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ: 14.88, 22.02, 26.67, 38.67, 42.03, 42.78, 123.01, 125.67, 127.99, 140.94, 177.45.

4.1.7. Preparation of compounds **8**,**9** *and* **10** *(general procedure)*

To *cis*-(−)-(1*R*,5*R*,9*R*)-*N*-normetazocine (187 mg, 0.85 mmol) in methyl ethyl ketone (6 ml), the amides **4**, **5** or **7** (0.85 mmol), $NaHCO_3$ (109 mg, 1.28 mmol), and a catalytic amount of KI were added. The reaction mixture was stirred at 60 °C for 18 h, then filtered and concentrated. The residue obtained was purified by flash column chromatography ($CHCl_3 : C_6H_{12} : CH_3OH$, 50 : 40 : 10) and was then dissolved in THF, treated with a solution of $H_2C_2O_4 \cdot 2\,H_2O$ in THF to give the oxalate salt as a white solid. The analytically pure sample was obtained by re-crystallization.

4.1.7.1. (−)-(1*S*,2*R*)-2-{[(2*R*,6*R*,11*R*)-8-Hydroxy-6,11-dimethyl-1,4,5,6-tetrahydro-2,6-methano-3-benzazocin-3(2*H*)-yl]methyl}-1-phenyl-*N*-[(1*R*)-1-phenylethyl]cyclopropanecarboxamide oxalate (**8**)

According to the general procedure, compound **8** was prepared starting from (1*S*,2*R*)-2-(bromomethyl)-1-phenyl-*N*-[(1*R*)-1-phenylethyl]cyclopropanecarboxamide (**4**) Yield: 40%; m.p. 148–150 °C; $[\alpha]_D^{20}$ – 28 (c 1.0, CH_3OH); MS (m/z): 494 [M^+]; ^1H NMR (DMSO-d_6) δ: 0.77 (d, 3 H J = 6.4 Hz), 1.28 (s, 3 H), 1.32 (d, 3 H J = 6.8 Hz), 1.35 (dd, 1 H J = 5.3, 8.0 Hz), 1.60–2.22 (m, 4 H), 2.39–3.25 (m, 7 H), 3.30–3.70 (m, 1 H), 4.90 (q, 1 H, J = 6.8 Hz) 5.40 (s br, 4 H), 6.45–7.80 (m, 13 H), 8.33 (bs, 1 H); ^{13}C NMR (DMSO-d_6) δ: 12.90, 19.71, 19.73, 21.25, 24.24, 30.21, 34.80, 37.16, 38.88, 43.13, 45.43, 50.16, 54.31, 58.34, 111.95, 113.86, 122.90, 125.86, 126.68, 127.13, 1228.26, 128.43 140.12, 140.27, 144.54, 156.30, 163.86, 168.88.
$C_{33}H_{38}N_2O_2 \cdot H_2C_2O_4 \cdot H_2O$ (602.7)

4.1.7.2. (−)-(1*S*,2*R*)-*N*-Benzyl-2-{[(2*R*,6*R*,11*R*)-8-hydroxy-6,11-dimethyl-1,4,5,6-tetrahydro-2,6-methano-3-benzazocin-3(2*H*)-yl]methyl}-1-phenylcyclopropanecarboxamide oxalate (**9**)

According to the general procedure, compound **9** was prepared starting from (1*S*,2*R*)-*N*-Benzyl-2-(bromomethyl)-1-phenylcyclopropanecarboxamide (**5**). Yield: 45%; m.p. 203-204 °C; $[\alpha]_D^{20}$ – 90 (c 1.0, CH_3OH); MS (m/z): 480 [M^+]; ^1H NMR ($CDCl_3$) (free base) δ: 0.71 (d, 3 H J = 7.0 Hz), 1.24 (s, 3 H), 1.27–1.42 (m, 3 H), 1.44–2.25 (m, 5 H), 2.40–2.95 (m, 5 H), 2.98–3.18 (m, 1 H), 4.34 (dd, 1 H J = 5.4, 15.2 Hz), 4.46 (dd 1 H J = 6.0, 15.2 Hz), 6.50–7.50 (m, 13 H), 7.80 (bs, 1 H); ^{13}C NMR ($CDCl_3$) (free base) δ: 14.07, 19.05, 23.80, 24.99, 25.21, 34.78, 36.29, 41.38, 41.96, 43.94, 44.31, 54.53, 59.12, 112.24, 113.08, 122.47, 127.18, 127.28, 127.40, 1228.21, 128.60, 128.84, 129.42, 138.61, 142.91, 143.89, 154.33, 171.87.
$C_{32}H_{36}N_2O_2 \cdot H_2C_2O_4 \cdot H_2O$ (588.7)

4.1.7.3. (−)-(1*S*,2*R*)-*N*,*N*-Diethyl-2-{[(2*R*,6*R*,11*R*)-8-hydroxy-6,11-dimethyl-1,4,5,6-tetrahydro-2,6-methano-3-benzazocin-3(2*H*)-yl]methyl}-1-phenylcyclopropanecarboxamide (**10**)

According to the general procedure, compound **10** was prepared starting from (1*S*,2*R*)-2-(Bromomethyl)-*N*,*N*-diethyl-1-phenylcyclopropanecarboxamide (**7**). Yield: 41%; m.p. 233–234 °C; $[\alpha]_D^{20}$ – 8 (c 1.0, CH_3OH); MS (m/z): 446 [M^+]; ^1H NMR (DMSO-d_6) δ: 0.70–0.97 (m, 6 H), 1.04 (t, 3 H J = 6.8), 1.03 (s, 3 H,), 1.40–2.20 (m, 5 H), 2.85–3.90 (m, 13 H), 6.40–7.45 (m, 8 H), 9.60 (bs, 2 H); ^{13}C NMR (DMSO-d_6) δ: 12.19, 12.57, 12.99, 19.47, 21.83, 22.15, 24.20, 34.24, 34.80, 37.80, 41.47, 44.84, 54.79, 58.66, 111.93, 113.83, 123.05, 125.69, 126.94, 128.66, 128.85, 139.52, 140.06, 156.22, 169.10, 174.02.
$C_{29}H_{38}N_2O_2 \cdot H_2C_2O_4 \cdot H_2O$ (554.7)

4.1.8. Methyl (1S,2R)-2-(bromomethyl)-1-phenylcyclopropanecarboxylate (**11**)

Thionyl chloride (0.6 ml, 8.75 mmol) was added dropwise to a solution of (1*S*,2*R*)-2-(bromomethyl)-1-phenylcyclopropanecarboxylic acid (**6**, 320 mg, 1.25 mmol) in C_6H_6 with vigorous stirring at 0 °C. The reaction mixture was refluxed for 5 h, cooled to 0 °C and a solution of 3N CH_3OH/HCl (1.5 ml) was added dropwise. The reaction mixture was stirred overnight at room temperature. The reaction mixture was then concentrated *in vacuo* and the residue was dissolved in $CHCl_3$, washed with a solution of 4% $NaHCO_3$ and brine, and dried over anh. Na_2SO_4. Solvent was evaporated *in vacuo* to obtain the desired product. Yield: 95%; m.p. 53–55 °C; IR (KBr): $\bar{\nu}$ (C=O) 1715 cm^{-1}; $[\alpha]_D^{20}$ – 36 (c 1.0, CH_3OH); MS (m/z): 269 [M^+]; ^1H NMR ($CDCl_3$) δ: 1.52 (dd, 1 H, J = 4.6, 8.5 Hz); 1.78 (dd, 1 H, J = 4.6, 7.5 Hz); 2.05–2.12 (m, 1 H); 3.72 (dd, 1 H, J–10, 11.2 Hz); 3.80 (s, 3 H); 3.89 (dd, 1H, J = 5.6, 11.2 Hz); 7.29–7.50 (m, 5H); ^{13}C NMR (DMSO-d_6) δ: 22.34, 27.57, 38.38, 38.93, 50.54, 118.2, 125.33, 128.67, 138.42.
$C_{12}H_{13}BrO_2$ (269.1)

4.1.9. Methyl (−)-(1S,2R)-2-{[(2R,6R,11R)-8-hydroxy-6,11-dimethyl-1,4,5,6-tetrahydro-2,6-methano-3-benzazocin-3(2H)-yl]methyl}-1-phenylcyclopropanecarboxylate (**12**)

To a solution of *cis*-(−)-(1*R*,5*R*,9*R*)-*N*-normetazocine (187 mg, 0.85 mmol) in methyl ethyl ketone (6 ml), methyl (1*S*,2*R*)-2-(bromomethyl)-1-phenylcyclopropane carboxylate (**11**, 229 mg, 0.85 mmol), $NaHCO_3$ (109 mg, 1.28 mmol), and a catalytic amount of KI were added. The reaction mixture was stirred at 60 °C for 18 h, then filtered and concentrated. The residue was purified by flash column chromatography ($CHCl_3 : C_6H_{12} : CH_3OH$, 50 : 40 : 10). Yield: 75%; m.p. 181–184 °C; $[\alpha]_D^{20}$ – 131 (c 1.0, CH_3OH); MS (m/z): 405 [M^+]; ^1H NMR ($CDCl_3$) (free base) δ: 0.75 (d, 3 H J = 7.0 Hz), 1.05 (dd, 1 H J = 4.9, 8.2 Hz), 1.27 (s, 3 H), 1.28–2.10 (m,

5 H), 2.15–3.05 (m, 7 H), 3.54 (s, 3 H), 6.50–7.45 (m, 8 H), 7.70 (bs, 1 H); ^{13}C NMR ($CDCl_3$) (free base) δ: 13.80, 18.57, 23.34, 25.20, 26.45, 35.44, 35.87, 37.61, 39.80, 40.22, 44.75, 53.99, 57.78, 111.80, 113.02, 126.80, 127.85, 127.90, 128.43, 141.80, 142.20, 155.58, 170.60.
$C_{26}H_{31}NO_3$ (405.5)

4.1.10. (−)-(1S,2R)-2-{[(2R,6R,11R)-8-Hydroxy-6,11-dimethyl-1,4,5,6-tetrahydro-2,6-methano-3-benzazocin-3(2H)-yl]methyl}-N-methyl-1-phenylcyclopropanecarboxamide (**13**)

To a solution of methyl (−)-(1*S*,2*R*)-2-{[(2*R*,6*R*,11*R*)-8-hydroxy-6,11-dimethyl-1,4,5,6-tetrahydro-2,6-methano-3-benzazocin-3(2H)-yl]methyl}-1-phenylcyclopropanecarboxylate (**12**, 230 mg, 0.56 mmol) in CH_3OH a 40% aqueous methylamine solution (6 ml) was added. The reaction mixture was stirred at 70 °C for 48 h, then filtered and concentrated. The residue was purified by flash column chromatography ($CHCl_3 : C_6H_{12} : CH_3OH$, 60/39/1). Yield: 23%; m.p. 205–207 °C; $[\alpha]_D^{20}$ − 122 (c 1.0, CH_3OH); MS (m/z): 404 [M$^+$]; ^{1}H NMR ($CDCl_3$) (free base) δ: 0.73 (d, 3 H J = 6.9 Hz), 1.06 (dd, 1 H J = 5.2, 8.0 Hz), 1.26 (s, 3 H), 1.30–2.13 (m, 5 H), 2.15–3.05 (m, 7 H), 3.33 (s, 3 H), 6.40–7.43 (m, 8 H), 7.60 (bs, 1 H), 9.00 (s, 1 H); ^{13}C NMR ($CDCl_3$) (free base) δ: 13.74, 18.51, 23.28, 25.15, 26.40, 35.39, 35.81, 37.61, 39.83, 40.31, 44.80, 54.87, 60.01, 110.97, 113.45, 126.78, 127.84, 127.89, 128.46, 140.85, 141.20, 155.68, 171.53.
$C_{26}H_{32}N_2O_2 \cdot 1.5\ H_2O$ (431.6)

4.1.11. (−)-(1S,2R)-N-Ethyl-2-{[(2R,6R,11R)-8-hydroxy-6,11-dimethyl-1,4,5,6-tetrahydro-2,6-methano-3-benzazocin-3(2H)-yl]methyl}-1-phenylcyclopropanecarboxamide (**14**)

Compound **14** was prepared from a 70% aqueous ethylamine solution using the above procedure. Yield: 22%; m.p. 193–195 °C; $[\alpha]_D^{20}$ − 106 (c 1.0, CH_3OH); MS (m/z): 418 [M$^+$]; ^{1}H NMR ($CDCl_3$) (free base) δ: 0.75 (d, 3 H J = 7.0 Hz), 1.04 (t, 3 H, *J* = 7.2 Hz), 1.12–1.30 (m, 2 H), 1.35 (s, 3 H), 1.37–1.68 (m, 2 H), 1.80–2.40 (m, 4 H), 2.60–3.40 (m, 8 H), 6.29 (bs, 1 H), 6.50–7.44 (m, 8 H); ^{13}C NMR ($CDCl_3$) (free base) δ: 13.93, 14.79, 19.55, 24.12, 25.19, 25.37, 29.69, 34.96, 35.53, 36.16, 38.45, 44.84, 53.61, 59.40, 112.31, 113.46, 127.71, 128.36, 129.00, 129.90, 131.33, 141.50, 142.63, 154.89, 171.70.
$C_{27}H_{34}N_2O_2 \cdot 1.5\ H_2O$ (445.6)

4.1.12. (−)-(1S,2R)-2-{[(2R,6R,11R)-8-Hydroxy-6,11-dimethyl-1,4,5,6-tetrahydro-2,6-methano-3-benzazocin-3(2H)-yl]methyl}-1-phenylcyclopropanecarboxylic acid (**15**)

To compound **12** (1.2 g, 2.95 mmol) 9 ml of a solution of 1 N NaOH (9 mmol) was added and the reaction mixture was refluxed and stirred for 5 h. After neutralization of the solution with diluted HCl, the resulting acid was collected by filtration. Yield: 91%; m.p. 210–212 °C; $[\alpha]_D^{20}$ − 116 (c 1.0, CH_3OH); MS (m/z): 391 [M$^+$]; ^{1}H NMR (DMSO-d_6) δ: 0.78 (d, 3H J = 7.1 Hz), 1.30 (s, 3 H), 1.35 (dd, 1 H J = 5.2, 7.9 Hz), 1.38–2.08 (m, 5 H), 2.13–3.30 (m, 7 H), 6.54–7.55 (m, 8 H), 7.65 (bs, 2 H); ^{13}C NMR (DMSO-d_6) δ: 15.60, 18.33, 23.41, 23.84, 28.45, 35.21, 38.71, 41.00, 43.21, 44.75, 56.98, 60.91, 115.83, 116,27, 126.71, 127.88, 127.92, 128.11, 128.67, 141.02, 142.00, 155.44, 173.36.
$C_{25}H_{29}NO_3 \cdot H_2O$ (409.5)

4.1.13. (−)-(1S,2R)-2-{[(2R,6R,11R)-8-Hydroxy-6,11-dimethyl-1,4,5,6-tetrahydro-2,6-methano-3-benzazocin-3(2H)-yl]methyl}-1-phenylcyclopropanecarboxamide oxalate (**16**)

Thionyl chloride (0.3 ml, 3 mmol) was added dropwise to a solution in THF of (−)-(1*S*,2*R*)-2-{[(2*R*,6*R*,11*R*)-8-hydroxy-6,11-dimethyl-1,4,5,6-tetrahydro-2,6-methano-3-benzazocin-3(2*H*)-yl]methyl}-1-phenylcyclopropanecarboxylic acid (**15**, 250 mg, 0.58 mmol) with vigorous stirring at 0 °C. The reaction mixture was stirred at room temperature overnight and the volatiles were evaporated *in vacuo*. The resulting acyl chloride was dissolved in diethyl ether (2 ml) and anh. gaseous NH_3 was bubbled into this solution at 0 °C for 0.5 h. The crystalline product was filtered off and dried. The solid was then dissolved in THF, treated with a solution of $H_2C_2O_4 \cdot 2H_2O$ in THF to give the oxalate salt as a white solid. The analytically pure sample was obtained by re-crystallization. Yield: 82%; m.p. 164–166 °C; $[\alpha]_D^{20}$ − 96 (c 1.0, CH_3OH); MS (m/z): 390 [M$^+$]; ^{1}H NMR (DMSO-d_6) δ: 0.80 (d, 3 H J = 7.0 Hz), 1.55 (dd, 1 H J = 5.3, 7.6 Hz), 1.32 (s, 3 H), 1.38–2.15 (m, 5 H), 2.80–3.70 (m, 7 H), 4.76 (bs, 3 H), 6.55–7.45 (m, 8 H), 7.50 (s, 2 H); ^{13}C NMR (DMSO-d_6) δ: 13.01, 15.19, 18.74, 20.63, 24.34, 34.88, 35.62, 38.00, 43.12, 45.60, 57.98, 64.94, 111.93, 11384, 123.15, 127.29, 128.45, 128.55, 129.16, 140.23, 140.51 156.26, 164,50, 171.93.
$C_{25}H_{30}N_2O_2 \cdot H_2C_2O_4$ (498.6)

4.1.14. Preparation of compounds **17**, **18**, **19**, **20** *and* **21** *(general procedure)*

Thionyl chloride (0.4 ml, 3.6 mmol) was added dropwise to a solution in THF of the (−)-(1*S*,2*R*)-2-{[(2*R*,6*R*,11*R*)-8-hydroxy-6,11-dimethyl-1,4,5,6-tetrahydro-2,6-methano-3-benzazocin-3(2*H*)-yl]methyl}-1-phenylcyclopropanecarboxylic acid (**15**, 310 mg, 0.72 mmol) with vigorous stirring at 0 °C. The reaction mixture was stirred at room temperature overnight and the volatiles were evaporated *in vacuo*. The resulting acyl chloride was dissolved in THF (2 ml) and added dropwise to a solution of the appropriate amine (2.88 mmol) in THF (6 ml) at −10 °C. The reaction mixture was stirred at 0 °C for 0.5 h and was then concentrated *in vacuo*. The residue was dissolved in $CHCl_3$, washed with a solution of 4% $NaHCO_3$ and brine, and dried over anh. Na_2SO_4. The solvent was evaporated *in vacuo* and the residue was purified by flash column chromatography ($CHCl_3 : C_6H_{12} : CH_3OH$, 60 : 39 : 1). The solid was then dissolved in THF, treated with a solution of $H_2C_2O_4 \cdot 2\ H_2O$ in THF to give the oxalate salt as a white solid. The analytically pure sample was obtained by re-crystallization.

4.1.14.1. (−)-(1*S*,2*R*)-2-{[(2*R*,6*R*,11*R*)-8-Hydroxy-6,11-dimethyl-1,4,5,6-tetrahydro-2,6-methano-3-benzazocin-3(2*H*)-yl]methyl}-*N*,*N*-dimethyl-1-phenylcyclopropanecarboxamide (**17**)

According to the general procedure, compound **17** was prepared using dimethylamine. Yield: 45%; m.p. 200–202 °C; $[\alpha]_D^{20}$ − 2 (c 1.0, CH_3OH); MS (m/z): 418 [M$^+$]; ^{1}H NMR (DMSO-d_6) δ: 0.78 (d, 3 H J = 6.0 Hz), 1.22 (s, 3 H), 1.35–2.15 (m, 6 H), 2.85 (s, 3 H), 2.87 (s, 3 H), 2.90–3.95 (m, 7 H), 6.20 (bs, 3 H) 6.43–7.45 (m, 8 H); ^{13}C NMR (DMSO-d_6) δ: 13.00, 20.54, 22.39, 24.27, 33.67, 34.87, 35.22, 35.43, 36.48, 37.35, 42.27, 44.62, 54.43, 58.28, 111.90, 113.71, 123.30, 125.47, 126.69, 128.50, 128.77, 139.66, 140.27, 156.19, 164.46, 169.21.
$C_{27}H_{34}N_2O_2 \cdot H_2C_2O_4 \cdot H_2O$ (526.6)

4.1.14.2. (−)-(1*S*,2*R*)-2-{[(2*R*,6*R*,11*R*)-8-Hydroxy-6,11-dimethyl-1,4,5,6-tetrahydro-2,6-methano-3-benzazocin-3(2*H*)-yl]methyl}-*N*,1-diphenylcyclopropanecarboxamide oxalate (**18**)

According to the general procedure, compound **18** was prepared using aniline. Yield: 82%; m.p. 202–204 °C; $[\alpha]_D^{20}$ − 48 (c 1.0, CH_3OH); MS (m/z): 466 [M$^+$]; ^{1}H NMR (DMSO-d_6) δ: 0.80 (d, 3 H J = 6.2 Hz), 1.29 (s, 3 H), 1.35 (dd, 1 H J = 5.0, 7.7 Hz), 1.45 (dd, 1 H J = 5.0, 8.0 Hz), 1.60–1.85 (m, 1 H), 1.90–2.55 (m, 3 H), 2,85–3.70 (m, 7 H), 5.00 (s br, 4 H), 6.45–7.80 (m, 13 H), 10.02 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ: 12.91, 19.90, 20.15, 22.17, 24.29, 34.80, 37.16, 37.88, 37.99, 45.47, 54.16, 58.20, 111.91, 113.82, 119.92, 120.45, 123.07, 123.72, 127.17, 127.81, 128.55, 129.09, 138.92, 140.13, 140.39, 156.26, 163.33, 168.04.
$C_{31}H_{34}N_2O_2 \cdot H_2C_2O_4 \cdot H_2O$ (574.7)

4.1.14.3. (−)-(2*R*,6*R*,11*R*)-6,11-Dimethyl-3-{[(1*R*,2*S*)-2-phenyl-2-(pyrrolidin-1-ylcarbonyl)-cyclopropyl]methyl}-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol oxalate (**19**)

According to the general procedure, compound **19** was prepared using pyrrolidine. Yield: 82%; m.p. 165–167 °C; $[\alpha]_D^{20}$ − 4 (c 1.0, CH_3OH); MS (m/z): 444 [M$^+$]; ^{1}H NMR (DMSO-d_6) δ: 0.83 (d, 3 H, J = 7.0), 1.10 (dd 1 H, J = 5.2, 7.5), 1.18 (s, 3 H,), 1.35–2.30 (m, 9 H), 2.45–3.80 (m, 11 H), 6.55–7.55 (m, 8 H), 8.60 (bs, 3 H); ^{13}C NMR (DMSO-d_6) δ: 12.31, 22.47, 23.55, 23.78, 24.22, 28.01, 31.23, 36.12, 36.70, 39.01, 42.27, 46.12, 46.78, 47.21, 55.88, 63.21, 111.88, 112.45, 121.01, 125.32, 127.12, 128.64, 129.35, 144.09, 145.32, 156.04, 169.23, 171.44.
$C_{29}H_{36}N_2O_2 \cdot H_2C_2O_4 \cdot H_2O$ (552.7)

4.1.14.4. (−)-(2*R*,6*R*,11*R*)-6,11-Dimethyl-3-{[(1*R*,2*S*)-2-phenyl-2-(piperidin-1-ylcarbonyl)-cyclopropyl]methyl}-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol oxalate (**20**)

According to the general procedure, compound **20** was prepared using piperidine. Yield: 46%; m.p. 180–182 °C; $[\alpha]_D^{20}$ − 6 (c 1.0, CH_3OH); MS (m/z): 458 [M$^+$]; ^{1}H NMR (DMSO-d_6) δ: 0.81 (d, 3 H, J = 5.6), 0.88–1.25 (m 2 H), 1.31 (s, 3 H,), 1.33–2.30 (m, 10 H), 2.50–3.83 (m, 11 H), 5.93 (bs, 3 H) 6.50–7.55 (m, 8 H); ^{13}C NMR (DMSO-d_6) δ: 12.93, 19.96, 22.11, 23.73, 24.21, 25.00, 33.71, 34.79, 39.77, 42.51, 44.73, 46.35, 54.45, 58.16, 111.93, 113.825, 123.07, 125.65, 126.82, 128.60, 128.76, 139.67, 140.11, 156.26, 163.67, 167.61.
$C_{30}H_{38}N_2O_2 \cdot H_2C_2O_4 \cdot H_2O$ (566.7)

4.1.14.5. (−)-(2*R*,6*R*,11*R*)-6,11-Dimethyl-3-{[(1*R*,2*S*)-2-(morpholin-4-ylcarbonyl)-2-phenylcyclopropyl]methyl}-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol oxalate (**21**)

According to the general procedure, compound **21** was prepared using morpholine. Yield: 81%; m.p. 169–171 °C; $[\alpha]_D^{20}$ − 8 (c 1.0, CH_3OH); MS (m/z): 460 [M$^+$]; ^{1}H NMR (DMSO-d_6) δ: 0.88 (dd, 1 H J = 5.1, 5.9 Hz), 0.95 (dd, 1 H J = 5.1, 5.2, Hz), 1.20 (d, 3 H, J = 6.55), 1.21–1.55 (m, 1 H), 1.68 (s, 3 H), 1.73–1.98 (m, 3 H), 2.25–2.95 (m, 9 H), 3.03–3.80 (m, 8 H), 6.15 (s br, 7 H), 6.45–7.47 (m, 8 H); ^{13}C NMR (DMSO-d_6) δ: 12.97, 17.94, 19.51, 24.30, 26.88, 34.87, 40.40, 45.03, 52.28, 52.82, 57.36, 58.04, 60.89, 63.85, 64.88, 112.03, 113.90, 123.10, 126.66, 128.35, 128.57, 128.87, 140.20, 143.57, 156.35, 162.73.
$C_{29}H_{36}N_2O_3 \cdot H_2C_2O_4 \cdot H_2O$ (568.7)

4.1.15. Methyl (1S,2R)-2-(chloromethyl)-1-phenylcyclopropanecarboxylate (**22**)

Thionyl chloride (0.7 ml, 8.5 mmol) was added dropwise to a solution of (1*S*,5*R*)-*cis*-1-phenyl-3-oxabicyclo[3.1.0]hexan-2-one (**1**, 500 mg, 2.87 mmol) and a catalytic amount of $ZnCl_2$ in anh. C_6H_6 (2.5 ml) at 0 °C. After refluxing and stirring for 5 h, the reaction mixture was cooled to 0 °C and a solution of 3 N HCl in CH_3OH (0.7 ml) was added dropwise and stirred overnight at room temperature. The mixture was concentrated *in vacuo* and the residue was dissolved in $CHCl_3$, washed with a solution of 4% $NaHCO_3$ and brine, and dried over anh. Na_2SO_4. The solvent was evaporated *in vacuo* and the residue was purified by flash column chromatography (C_6H_{12} : toluene, 60 : 40). Yield: 83%; m.p. 46–47 °C; IR (KBr): $\bar{\nu}$ (C=O) 1715 cm^{-1}; $[\alpha]_D^{20}$ − 50 (c 1.0, CH_3OH); MS (m/z): 224 [M^+]; ^{1}H NMR ($CDCl_3$) δ: 1.52 (dd, 1 H J = 4.6, 8.5 Hz), 1.78 (dd, 1 H, J = 4.6, 7.5 Hz), 2.05–2.12 (m, 1 H), 3.72 (dd, 1 H, J = 10.0, 11.2 Hz), 3.80 (s, 3 H), 3.89 (dd, 1 H, J = 5.6, 11.2 Hz), 7.29–7.50 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ: 22.32, 27.54, 38.36, 38.91, 50.51, 118.30, 125.32, 128.65, 138.41, 173.61.
$C_{12}H_{13}ClO_2$ (224.7)

4.1.16. [(1S,2R)-2-(Chloromethyl)-1-phenylcyclopropyl]methanol (**23**)

A solution of methyl (1*S*,2*R*)-2-(chloromethyl)-1-phenylcyclopropanecarboxylate (**22**, 500 mg, 2.22 mmol) in THF (5 ml) was added dropwise to a solution of the alane-*N,N*-dimethylethylamine complex (4.5 ml, 2.24 mmol) in THF (5 ml) at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred for 2.5 h and quenched with an H_2O-THF (1 : 1) solution to give a white precipitate. The mixture was dissolved in 1N HCl and extracted with diethyl ether. The organic extracts were dried with anh. Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash column chromatography (C_6H_{12} : ethyl acetate, 90 : 10). Yield: 76%; m.p. 32–33 °C; IR (KBr): $\bar{\nu}$ (OH) 3405 cm^{-1}; $[\alpha]_D^{20}$ − 45 (c 1.0, CH_3OH); MS (m/z): 196 [M^+]; ^{1}H NMR ($CDCl_3$) δ: 0.92 (dd, 1 H, J = 7.2, 8.3 Hz), 1.15 (dd, 1 H, J = 7.7, 8.3 Hz), 1.34–1.63 (m, 1 H), 3.40–3.46 (m, 2 H), 3.98–4.15 (m, 2 H), 4.51 (br s, 1 H), 7.29–7.52 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ: 18.22, 25.54, 37.46, 48.05, 68.45, 121.72, 125.35, 128.32, 142.91.
$C_{11}H_{13}ClO$ (196.7)

4.1.17. [(1S,2R)-2-(Chloromethyl)-1-phenylcyclopropyl]methyl acetate (**24**)

A solution of [(1*S*,2*R*)-2-(chloromethyl)-1-phenylcyclopropyl]methanol (**23**, 520 mg, 2.64 mmol) and 4-dimethylaminopyridine (DMAP) (150 mg, 3.96 mmol) in anh. THF was added under vigorous stirring to acetic anhydride (2ml, 2.12 mmol) at 0 °C. After stirring for 1.5 h at 0 °C, the reaction mixture was quenched with a 4% solution of $NaHCO_3$, extracted with diethyl ether and dried over anh. Na_2SO_4. The solvent was removed *in vacuo* and the residual oil was purified by silica gel flash column chromatography (C_6H_{12} : ethyl acetate, 97 : 3). Yield: 79%; m.p. 68–70 °C; IR (KBr): $\bar{\nu}$ (C=O) 1740 cm^{-1} ; $[\alpha]_D^{20}$ − 45 (c 1.0, CH_3OH); MS (m/z): 238 [M^+]; ^{1}H NMR ($CDCl_3$) δ: 0.89 (dd, 1 H, J = 7.4, 8.3 Hz), 1.02 (dd, 1 H, J = 7.8, 8.3 Hz), 1.31–1.40 (m, 1 H), 2.2 (s, 3 H), 3.38–3.45 (m, 2 H), 4.48–4.70 (m, 2 H), 7.15–7.55 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ: 18.33, 22.35, 27.03, 35.21, 47.32, 70.22, 122.12, 124.88, 128.66, 141.12, 169.89.
$C_{13}H_{15}ClO_2$ (238.7)

4.1.18. Preparation of compounds **25** *and* **26** *(general procedure)*

To *cis*-(−)-(1*R*,5*R*,9*R*)-*N*-normetazocine (205 mg, 0.94 mmol) in DMF (5 ml), the compounds **23** or **24** (0.94 mmol), $NaHCO_3$ (120 mg, 1.41 mmol), and a catalytic amount of KI were added. The reaction mixture was stirred at 75 °C for 6 h, then filtered and concentrated. The residue was purified by flash column chromatography ($CHCl_3$: C_6H_{12} : CH_3OH, 50 : 49 : 1) and was then dissolved in THF, treated with a solution of $H_2C_2O_4 \cdot 2\,H_2O$ in THF to give the oxalate salt as a white solid. The analytically pure sample was obtained by re-crystallization.

4.1.18.1. (−)-((1*S*,2*R*)-2-{[(2*R*,6*R*,11*R*)-8-Hydroxy-6,11-dimethyl-1,4,5,6-tetrahydro-2,6-methano-3-benzazocin-3(2*H*)-yl]methyl}-1-phenylcyclopropyl)methyl acetate oxalate (**25**)

According to the general procedure, compound **25** was prepared starting from [(1*S*,2*R*)-2-(chloromethyl)-1-phenylcyclopropyl]methyl acetate (**24**). Yield: 50%; m.p. 180–181 °C; $[\alpha]_D^{20}$ − 80 (c 1.0, CH_3OH); MS (m/z): 419 [M^+]; ^{1}H NMR (DMSO-d_6) δ: 0.79 (d, 3 H J = 6.6 Hz), 1.03 (dd, 1 H J = 5.1, 8.0 Hz), 1.22 (dd, 1 H J = 5.3, 8.0 Hz), 1.30 (s, 3 H), 1.35–1.73 (m, 2 H), 1.75–1.90 (m, 2 H), 1.90 (s, 3 H), 2,85–3.45 (m, 4 H) 3.55–3.82 (m, 3 H), 4.15 (d, 1 H, J = 12.0 Hz), 4.48 (d, 1 H, J = 12.0 Hz), 5.58 (s br, 4 H), 6.42–7.40 (m, 8 H); ^{13}C NMR (DMSO-d_6) δ: 13.11, 18.16, 19.68, 20.88, 22.33, 24.40, 28.67, 31.98, 34.98, 40.27, 44.95, 55.32, 58.44, 67.45, 112.03, 113.93, 123.40, 126.91, 128.25, 128.49, 128.76, 140.39, 142.80, 156.24, 164.92, 170.49.
$C_{27}H_{33}NO_3 \cdot H_2C_2O_4 \cdot H_2O$ (527.6)

4.1.18.2. (−)-(2*R*,6*R*,11*R*)-3-{[(1*R*,2*S*)-2-(Hydroxymethyl)-2-phenylcyclopropyl]methyl}-6,11-dimethyl-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol oxalate (**26**)

According to the general procedure, compound **26** was prepared starting from [(1*S*,2*R*)-2-(chloromethyl)-1-phenylcyclopropyl]methanol (**23**). Yield: 63%; m.p. 140–142 °C; $[\alpha]_D^{20}$ − 76 (c 1.0, CH_3OH); MS (m/z): 377 [M^+]; ^{1}H NMR (DMSO-d_6) δ: 0.81 (d, 3 H J = 6.2 Hz), 0.84 (dd, 1 H J = 5.2, 7.8 Hz), 0.95–1.73 (m, 2 H), 1.30 (s, 3 H), 2,80–3.50 (m, 3 H) 3.55–3.82 (m, 7 H), 3.92 (d, 2 H, J = 7.8 Hz), 6.10 (s br, 6 H), 6.52–7.45 (m, 8 H); ^{13}C NMR (DMSO-d_6) δ: 12.96, 17.45, 19.08, 24.26, 25.81, 31.77, 34.84, 39.76, 44.71, 53.14, 64.52, 111.94, 113.83, 123.14, 126.21, 126.65, 128.27, 128.50, 140.17, 144.05, 156.27, 163.71.
$C_{25}H_{31}NO_2 \cdot H_2C_2O_4 \cdot H_2O$ (485.6)

4.1.19. Preparation of compounds **27**, **28** *and* **29** *(general procedure)*

To a mixture of $LiAlH_4$ (76 mg 1.9 mmol) in THF, cooled to 0 °C and under nitrogen atmosphere, a solution of the appropriate amides **18**–**20** (0.20 mmol) in THF (2 ml) was slowly added. The reaction mixture was refluxed for 12 h and then quenched with an aqueous Na_2SO_4 saturated solution at 0 °C. The precipitate was removed by filtration and the organic layer was washed twice with brine and dried over anh. Na_2SO_4. The solvent was evaporated *in vacuo* and the residue was purified by flash chromatography ($CHCl_3$: CH_3OH, 98 : 2). The purified compound was then dissolved in THF, treated with a solution of $H_2C_2O_4 \cdot 2\,H_2O$ in THF to give the oxalate salt as a white solid. The analytically pure sample was obtained by re-crystallization.

4.1.19.1. (−)-(2*R*,6*R*,11*R*)-6,11-Dimethyl-3-{[(1*R*,2*S*)-2-phenyl-2-(pyrrolidin-1-ylmethyl)cyclopropyl]methyl}-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol oxalate (**27**)

According to the general procedure, compound **27** was prepared starting from (−)-(2*R*,6*R*,11*R*)-6,11-dimethyl-3-{[(1*R*,2*S*)-2-phenyl-2-(pyrrolidin-1-ylcarbonyl)-cyclopropyl]methyl}-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol (**19**). Yield: 63%; m.p. 175–177 °C; $[\alpha]_D^{20}$ − 60 (c 1.0, CH_3OH); MS (m/z): 431 [M^+]; ^{1}H NMR (DMSO-d_6) δ: 0.82 (d, 3 H J = 5.4 Hz), 0.88 (dd, 1 H J = 5.1, 5.3 Hz), 1.29 (s, 3 H), 1.32–1.73 (m, 6 H), 1.65–2.25 (m, 3 H), 2,40–2.60 (m, 2 H) 2.85–4.10 (m, 11 H), 6.00 (s br, 7 H), 6.50–7.55 (m, 8 H); ^{13}C NMR (DMSO-d_6) δ: 13.03, 17.49, 20.68, 22.41, 24.31, 27.43, 34.86, 41.32, 43.25, 44.92, 52.01, 54.26, 58.12, 58.58, 112.00, 113.85, 123.15, 127.49, 128.48, 128.75, 129.03, 140.24, 141.62, 156.31, 163.81.
$C_{29}H_{38}N_2O \cdot 2H_2C_2O_4 \cdot H_2O$ (628.7)

4.1.19.2. (−)-(2*R*,6*R*,11*R*)-6,11-Dimethyl-3-{[(1*R*,2*S*)-2-phenyl-2-(piperidin-1-ylmethyl)cyclopropyl]methyl}-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol oxalate (**28**)

According to the general procedure, compound **28** was prepared starting from (−)-(2*R*,6*R*,11*R*)-6,11-dimethyl-3-{[(1*R*,2*S*)-2-phenyl-2-(piperidin-1-ylcarbonyl)-cyclopropyl]methyl}-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol (**20**). Yield: 64%; m.p. 163–165 °C; $[\alpha]_D^{20}$ − 66 (c 1.0, CH_3OH); MS (m/z): 445 [M^+]; ^{1}H NMR (DMSO-d_6) δ: 0.80 (d, 3 H J = 5.8 Hz), 0.88 (dd, 1 H J = 5.0, 5.3 Hz), 1.30 (s, 3 H), 1.33–1.75 (m, 11 H), 1.80–2.42 (m, 3 H), 2.75–3.12 (m, 5 H), 3.15–3.75 (m, 5 H), 5.75 (s br, 7 H), 6.45–7.47 (m, 8 H); ^{13}C NMR (DMSO-d_6) δ: 12.99, 17.47, 21.02, 21.49, 22.17, 24.28, 26.04, 34.87, 39.75, 40.20, 41.02, 44.91, 51.95, 52.81, 57.96, 59.62, 111.98, 113.82, 123.16, 127.22, 128.46, 128.61, 128.69, 140.25, 142.16, 156.27, 163.94.
$C_{30}H_{40}N_2O \cdot 2H_2C_2O_4 \cdot H_2O$ (642.7)

4.1.19.3. (−)-(2*R*,6*R*,11*R*)-6,11-Dimethyl-3-{[(1*R*,2*S*)-2-(morpholin-4-ylmethyl)-2-phenylcyclopropyl]methyl}-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol oxalate (**29**)

According to the general procedure, compound **29** was prepared starting from (−)-(2*R*,6*R*,11*R*)-6,11-dimethyl-3-{[(1*R*,2*S*)-2-(morpholin-4-ylcarbonyl)-2-phenylcyclopropyl]methyl}-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol (**21**). Yield: 60%; m.p. 152–153 °C; $[\alpha]_D^{20}$ − 48 (c 1.0, CH_3OH); MS (m/z): 447 [M^+]; ^{1}H NMR (DMSO-d_6) δ: 0.88 (dd, 1 H J = 5.1, 5.9 Hz), 0.95 (dd, 1 H J = 5.1, 5.2, Hz), 1.20 (d, 3 H, J = 6.55), 1.21–1.55 (m, 1 H), 1.68 (s, 3 H), 1.73–1.98 (m, 3 H), 2.25–2.95 (m, 9H), 3.03–3.80 (m, 8 H), 6.15 (s br, 7 H), 6.45–7.47 (m, 8 H); ^{13}C NMR (DMSO-d_6) δ: 12.97, 17.94, 19.51, 24.30, 26.88, 34.87, 40.40, 45.03, 52.28, 52.82, 57.36, 58.04, 60.89, 63.85, 64.88, 112.03, 113.90, 123.10, 126.66, 128.35, 128.57, 128.87, 140.20, 143.57, 156.35, 162.73.
$C_{29}H_{38}N_2O_2 \cdot 2H_2C_2O_4 \cdot H_2O$ (644.7)

4.2. Computational method

Automated docking studies were performed using CCDC's GOLD (Genetic Optimization for Ligand Docking) software package version 3.0 (Jones et al. 1997). Structures of the complexes formed by KOP receptor in the

active state (residues 55-348) and pentapeptide 10, Tyr-c[D-Cys-Phe-D-Pen]-NH_2 (S-Et-S bridge), and MOP receptor in the active state (residues 64-354) and JOM6 Tyr-c[D-Cys-Phe-Phe-D-Cys]-NH_2 (SS bridge) were retrieved from http://www.mosberglab.phar.umich.edu/resources/. For the docking calculation, the ligands were removed from the complexes and all hydrogen atoms were added by using Sybyl 7.0 (the Biopolymer module) (SYBYL 6.9, Tripos Inc., 1699 South Hanley Road, St. Louis, Missouri, 63144, USA). The structures of the molecules **9** and **28** were constructed using standard bond lengths and angles from the Sybyl 7.0 fragment library and fully optimized by the semiempirical quantum mechanical method AM1. Ligands were modeled in their nitrogen-protonated form. ChemScore score was chosen as fitness function, the standard default settings were used in all the calculations and 100 individual GA runs were performed. A 10.0 Å radius active site was defined centered on the α-carbon atom of residue Asp138 in the KOP receptor and Asp147 in the MOP receptor. A hydrogen bond constraint was set: the hydrogen of the protonate nitrogen of the benzomorphan of the ligands and carboxylate oxygen atom of the residue Asp138 and of the residue Asp147 for the KOP receptor and the MOP receptor, respectively. The best docking-complexes were then energy-minimized using 1,000 steps of steepest descent minimization until RMS = 0.5 and 1000 steps of conjugated gradients algorithm until RMS = 0.5.

4.3. Opioid receptor binding assays

Membranes were prepared from male Hartley guinea pigs (250–300 g) and Sprague-Dawley rats (200–250 g) purchased from Charles River (Como Italy).

Binding to MOP and DOP receptor was carried out on rat membranes obtained from whole brain minus the cerebellum as previously reported (Ronsisvalle et al. 2000b). MOP receptor-binding assay was carried out as reported by Furness et al. (2000). Briefly, membranes (1 mg/ml) were incubated in 50 mM Tris-HCl, pH 7.4, containing 100 mM choline chloride, 3 mM $MnCl_2$ and 100 nM DPDPE to eliminate binding to DOP receptor, and a protease inhibitor cocktail, bacitracin (100 μg/ml), bestatin (10 μg/ml), leupeptin (4 μg/ml), and chymostatin (2 μg/ml). Incubation proceeded for 2 h at 25 °C with 2 nM [^{3}H]DAMGO. Nonspecific binding was defined in the presence of 20 μM unlabeled levallorphan. DOP receptor-binding sites were labeled with 2 nM [^{3}H]DADLE and rat brain membranes as above reported. Aliquots of homogenates (1 mg/ml) were incubated under the same conditions with the exception of DAMGO in place of DPDPE to eliminate binding to MOP receptor. Nonspecific binding was defined in the presence of 20 μM unlabeled levallorphan.

The KOP receptor-binding assay was performed on membranes obtained from guinea pig cerebella prepared as previously described (Ronsisvalle et al. 1993). Aliquots of homogenates (1 mg/ml) were incubated in 50 mM Tris-HCl, pH 7.4, containing a protease inhibitor cocktail, 100 nM DPDPE and 100 nM DAMGO, to eliminate binding to DOP and MOP receptor, respectively. Incubations proceeded for 1 h at 25 °C with 2 nM [^{3}H]U-69,593. Nonspecific binding was defined in the presence of 10 μM U-50,488.

The incubation was terminated by rapid filtration through Whatman GF/C glass filters which were presoaked in a 0.1% poly(ethyleneimine) solution for 1 h. Filters were washed twice with 4 ml of ice-cold 50 mM Tris-HCl buffer. After overnight incubation in 4 ml of Filter Count Cocktail (Packard), radioactivity retained on the filters was measured by liquid scintillation spectrometry using a 1414 Winspectral PerkinElmer Wallac. Competition inhibition constant (K_i) values were calculated with the EBDA/LIGAND program (McPherson 1985) purchased from Elsevier/Biosoft.

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BOOK REVIEWS

Kompendium der Immunologie

Von Miroslav Ferencik, Jozef Rovensky, Vladimir Mat'ha, Manfred Herold: Wien 2006, Springer-Verlag, 39,80 Euro, ISBN 3-211-25536-2

In den letzten Jahrzehnten gehört die Immunologie zusammen mit der Genetik und der Molekularbiologie zu den wichtigsten Wissenschaftsbereichen. Dokumentiert wird diese Bedeutung in den Nobelpreisen: Zwischen 1901 und 2005 wurden 95 Preise für Medizin und Physiologie an insgesamt 187 Laureaten vergeben, von denen allein 80 Immunologen waren. 42 der 95 Preise wurden für Arbeiten vergeben, die entweder direkt oder wenigstens in sehr engem Zusammenhang mit der Immunologie standen. Kein Wunder also, dass immer mehr Bücher auf den Markt kommen, die sich mit verschiedenen Aspekten dieser Wissenschaft befassen.
Das vorliegende Buch ergäänzt das Repertoire an Immunologie-Büchern und stellt „Grundlagen und Klinik" in den Mittelpunkt.
Insgesamt gliedert sich das Buch in 14 Kapitel: Nach einer kurzen Einleitung wird die Entstehung des Lebens und der Informationssysteme beschrieben bevor das Immunsystem mit den Zellen, Geweben und Molekülen und die Mechanismen der angeborenen bzw. erworbenen Immunitäät vorgestellt werden.
Die anschließenden Kapitel beschäftigen sich mit verschiedenen immunologischen Krankheiten bzw. Krankheitsprozessen, angefangen bei den Entzündungen, den Abwehrmechanismen gegen Mikroorganismen und Tumoren, über Immundefizienzen, Autoimmunerkrankungen, Allergische Erkrankungen, bis hin zu Prionerkrankungen im Kontext mit dem Immunsystem. Die letzten drei Kapitel haben das Immunsystem im Alter, Immunmodulation und das Zusammenspiel von Immun- und neuroendokrinem System zum Thema.
Das Buch ist recht gut verstäändlich geschrieben. Es näähert sich dem Immunsystem eher von der Krankheitsseite an und erkläärt dann die Symptome durch die biologischen Zusammenhänge. Im Vergleich zu den sonst üblichen Lehrbüchern zu dem Thema überrascht es ein wenig wegen des einfachen Erscheinungsbildes mit vergleichsweise wenigen Bildern, die überwiegend in schwarz/weiß gehalten sind. Sehr positiv zu bewerten sind die in den Texten hervorgehobenen Begriffe, die dem Leser eine schnelle Orientierung über den Inhalt der verschiedenen Kapitel ermöglichen.
Alles in allem ist es ein sehr informatives Buch zu einem verhältnismääßig günstigen Preis.

Zündorf (Frankfurt)

Pulmonary Drug Delivery

edited by K. Bechtold-Peters and H. Luessen. Aulendorf 2007: ECV Editio Cantor Verlag; 412 pp., € 126,–; ISBN 978-3-87193-322-6

Pulmonary drug delivery is gaining more and more interest since knowledge about pulmonary absorption, drug metabolism and requirements for controlled lung targeting has been constantly improved. Inhalative drug administration has serious advantages compared to the "routine" routes of drug application i.e. avoiding painful injections and the first pass metabolism, moreover offering the option for local as well as systemic therapy. In 6 principal chapters, comprising 19 subheadings the book discusses a broad field of relevant aspects for pulmonary drug delivery.
Starting with chapter I, the book deals with structure, function and physiology of the lung followed by contemporary aerosol therapy for COPD and Asthma. Since therapeutic benefit is highly correlated to efficient and reproducible drug deposition the chapter also introduces the reader to the biophysics of aerosolized particles and inhalation technology.
Chapter II covers *in vitro* and *in vivo* test models yet introduced to investigate cell-particle interaction and drug absorption from lung epithelium. Decisive and determining experimental parameters, analytical limitations for the different models and current limitations for aerosol delivery systems are discussed from a clinician's perspective.
Chapter III focuses on application devices, the field in aerosol research which has experienced probably most rapid development in recent years. The design of modern, technologically improved inhalation devices considering changes in pulmonary target regions and needs in therapeutic regimen are presented at great length.
Following on device design, chapter IV deals with formulation and production of different aerosol preparations. Since only few excipients are so far approved for pulmonary use, it is of greatest importance to know about influence of preparation composition and manufacturing parameters on aerosol performance. Latest results in sugar glass technology and future perspectives regarding delivery of instable proteins or lipophilic small organic molecules reflects ongoing pharmaceutical research not only in the field of pulmonary delivery.
Chapter V comprises as well regulatory as aerosol specific issues. The authors allude to differences between EU and US regulations regarding classification and control of pulmonary products, thus concluding that initiatives regarding international harmonization will be a future challenge. The second part of the chapter focuses on methodology for physico-chemical characterization of aerosols and discusses "routine" as well as "new" methods e.g. atomic force microscopy for use in particle characterization.
In the last part of the book, chapter VI focuses on future aspects and trends of pulmonary drug delivery systems. Application of Microparticles and Liposomes shall become options for as well systemic as local therapy in the nearby future due to a better understanding of lung physiology and inflammatory response.
The recurring discussion on many aspects of pulmonary drug delivery reveals continued interest as well as uncertainty. Starting with Igor Gonda's introduction about "Visions, Expectations and Achievements" the book discusses a broad range of issues on pulmonary drug delivery. The authors intention to close a knowledge gap furthermore giving an outlook on future development is certainly accomplished by this book. Even though the book in general is easy-to-read, also experts will find comprehensive details on materials, processing and application of therapeutic aerosols. Numerous references to relevant literature will guide the reader to more detailed information according to his area of interest. Therefore, all in all, the book can be recommended to both, novices and specialists in the field of pulmonary drug delivery, particularly when they come from a mainly pharmaceutical background.

Henning (Saarbrücken)