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Research paper

Benzimidazolone bioisosteres of potent GluN2B selective NMDA receptor antagonists

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ABSTRACT

Overactivation of the NMDA receptor is associated with excitotoxic events leading to neurodegenerative processes as observed during the development of Alzheimer's disease, ParFnson's disease, Chorea Huntington and epilepsy. Negative allosteric modulators addressing selectively the ifenprodil binding site of GluN2B subunit containing NMDA receptors are of major interest due to their neuroprotective potential accompanied by few side effects. Herein benzimidazolone bioisosteres of potent GluN2B antagonists **1**–**5** were designed and synthesized. A seven step sequence provided the central intermediate **19** in 28% yield. Elimination of water, methylation, epoxidation, epoxide rearrangement and finally reductive amination afforded the [7]annulenobenzimidazolone **30** with a 3-phenylpropylamino substituent in 6-position. Although **30** fits nicely into the pharmacophore of potent GluN2B antagonists, the gluN2B binding affinity of **30** was only moderate (K_i = 697 nM). Additionally, **30** shows low selectivity over the σ_2 receptor (K_i = 549 nM). The moderate GluN2B affinity was explained by the rigid tricyclic structure of the [7]annulenobenzimidazolone **30**.

1. Introduction

The excitatory amino acid neurotransmitter (*S*)-glutamate mediates its effects by activation of metabotropic and ionotropic glutamate receptors [1]. AMPA, kainate and NMDA receptors belong to the class of ionotropic glutamate receptors, which represent ligand gated ion channels [2]. The NMDA receptor reveals unique properties including coagonism, i.e. activation requires two agonists glutamate and glycine binding simultaneously at the receptor, a high permeability for Ca^{2+}-ions (in addition to Na^{+}- and K^{+}-ions) and voltage dependent blockade by Mg^{2+}-ions, which has to be removed before the ion channel can be opened [3–6]. Due to the Mg^{2+}-block, NMDA receptors are slower activated and deactivated than AMPA and kainate receptors [2].

The activation of the NMDA receptor causes an influx of Ca^{2+}-ions into the neuron, which activates several secondary signaling cascades leading to long lasting changes in synaptic activity [7]. The extraordinary alliance between the coagonism of glutamate and glycine and the voltage-dependent Mg^{2+}-blockade results in the development of neuronal connections [8]. Therefore it is discussed for a long time that the NMDA receptor is involved in synaptic plasticity [8], which is connected with neurogenesis [9], learning and memory [10].

Certain conditions (e.g. cerebral ischemia, stroke, epilepsy) change the glutamate homoeostasis and increase the release of glutamate resulting in overstimulation of neurons and finally in neuronal dysfunction and damage (excitotoxicity). The NMDA receptor plays a central role in this process of excitotoxicity, because the excessive amount of glutamate leads to its overactivation followed by massive influx of Ca^{2+}-ions into the neuron. The high concentration of Ca^{2+}-ions leads to uncontrolled overactivation of several enzymes inducing necrosis and apoptosis [11]. This process of excitotoxicity is associated with a number of neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Chorea Huntington and epilepsy [7]. The involvement of the NMDA receptor in the process of excitotoxicity supports the hypothesis that a blockade of the receptor results in neuroprotective effects [3]. Thus the NMDA receptor represents a promising target for the development of novel neuroprotective drugs indicated for the treatment of acute and chronic neurodamaging processes.

Three types of subunits termed GluN1-GluN3 have been identified so far [12]. Four of these subunits build up the NMDA receptor

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in a dimer of dimers fashion (heterotetramer) [13–15]. A functional NMDA receptor contains at least two GluN1 subunits [13] and, moreover, the composition of the NMDA receptor entails its pharmacology and its functional properties [16]. The GluN1 subunit existing in eight splice variants GluN1a-GluN1h is distributed ubiquitously in the central nervous system [17]. However, four genes encoding four different GluN2 subunits termed GluN2A-GluN2D have been identified. The expression of GluN2 subunits differs considerably in different regions of the central nervous system [18]. Due to this fact, it is supposed that the specific properties of the heterotetrameric ion channel are related to the type of GluN2 subunit incorporated into the receptor [18–20].

Therefore the development of NMDA receptor antagonists and allosteric modulators addressing selectively receptors containing a particular GluN2 subunit is of major interest. In particular GluN2B-selective NMDA receptor antagonists have been reported to show neuroprotective potential with little or less severe side effects [21–23]. Ifenprodil (**1**, Vadilex®, Fig. 1) was the first and is still one of the best known GluN2B-selective NMDA receptor antagonists [24]. Ifenprodil binds with high affinity (IC_{50} = 13.3 nM) [15] to GluN2B subunit containing NMDA receptors resulting in a negative allosteric modulation [25]. In addition to its high GluN2B affinity, ifenprodil originally developed as α_1 receptor antagonist shows poor selectivity over related receptors (α_1, 5-HT, σ_1, σ_2 receptors), which lead to undesirable side effects like impairment of reaction and reduced blood pressure. Moreover, the bioavailability of ifenprodil is rather low due to fast metabolism (e.g. phenol glucuronidation) [26].

Despite these unfavorable properties, ifenprodil served as lead compound for the development of different GluN2B antagonists with comparable affinity but higher selectivity. Some promising GluN2B antagonists derived from ifenprodil are displayed in Fig. 1 [8]. The basic amino functionality of ifenprodil (**1**) and Ro 25-6981 (**4**) is found in the 4-benzylpiperidine structure. However, **1** and **4** differ in the distance between the "left" benzene ring and the basic amino moiety. The additional CH_2 moiety in the flexible side chain of **4** does not influence the GluN2B affinity, considerably [27].

In order to restrict the adaptability to other receptors and thus increase selectivity, the conformational flexibility of the phenylethylamine side chain of **1** was incorporated into the 3-benzazepine ring of **2**. The phenol **2a** (K_i = 14 nM) and the methyl ether **2b** (K_i = 5.4 nM) show the same or slightly increased GluN2B affinity but considerably increased selectivity compared to ifenprodil [28,29]. A similar idea was followed during the development of the benzo[7]annulen-7-amines **3**, comprising an increased distance between the basic amino moiety and the "left" aromatic ring, which is comparable to the distance in the lead compound Ro 25-6981. The GluN2B affinity of **3a** and **3b** is in the same range as the affinity of ifenprodil and 3-benzazepines **2** [30]. The phenolic OH group of **1**, **2a** and **4** was replaced by a methoxy-group in **2b**, **3a** and **3b** without losing GluN2B affinity [28–30].

In order to get rid of the phenolic OH moiety, which is conjugated with glucuronic acid very fast [26], but retaining the H-bond donor property, the phenol of **1** was bioisosterically replaced by a benzoxazolone system as realized in besonprodil (**5**) and in the tricyclic compounds **6**. Whereas besonprodil (**5**) shows GluN2B affinity in the low nanomolar range (K_i = 30 nM) [31], the tricyclic benzoxazolones **6** were considerably less potent, which was attributed to the rather rigid structure holding the basic amino moiety and the H-bond donor in a precise orientation to each other [32]. Very recently, aminomethyl substituted benzimidazoles with high GluN2B affinity and selectivity were reported [33].

In this manuscript we report on [7]annulenobenzimidazolone derivatives **7**, which are derived from the lead compounds **1**-**6**. [7] Annulenobenzimidazolones **7a** with protons at N-atoms should be able to form H-bonds with appropriate H-bond acceptors. The corresponding methyl derivatives **7b** correspond to the methyl ethers **2b**, **3a** and **3b**, which display high GluN2B affinity. However the protonated basic amino group should be attached at the rigid system in order to allow a free orientation to an H-bond accepting group or an anionic functional group. Therefore various positions of the amino moiety were considered. The affinity of benzimidazolones **7** towards GluN2B subunit containing NMDA receptors and some related receptors (PCP binding site of the NMDA receptor, σ_1,

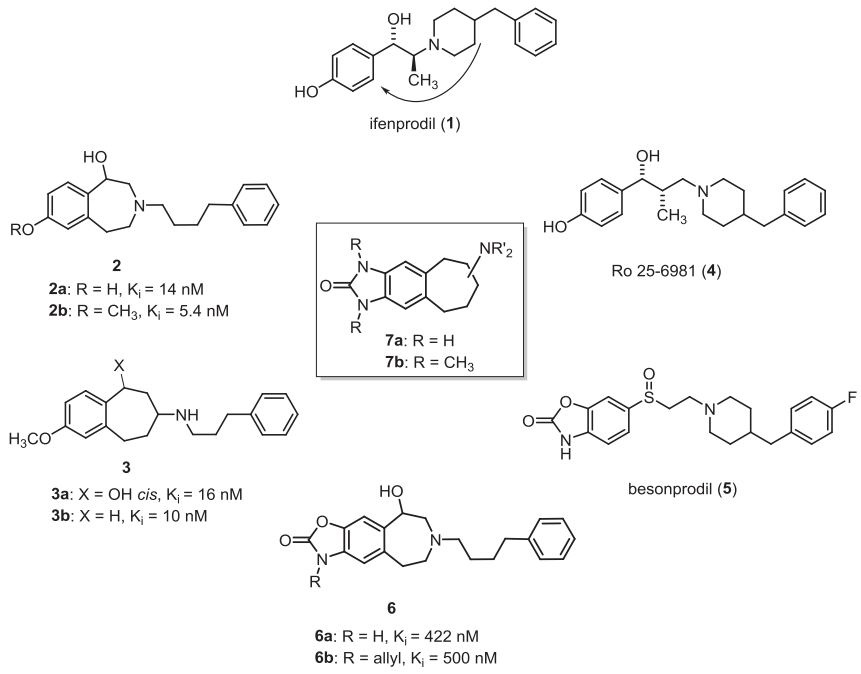


Fig. 1. Design of novel GluN2B selective NMDA receptor antagonists **7** with tricyclic benzimidazolone scaffold derived from lead compounds **1**–**6**.

σ_2 receptors) will be recorded in radioligand receptor binding studies.

2. Results and discussion

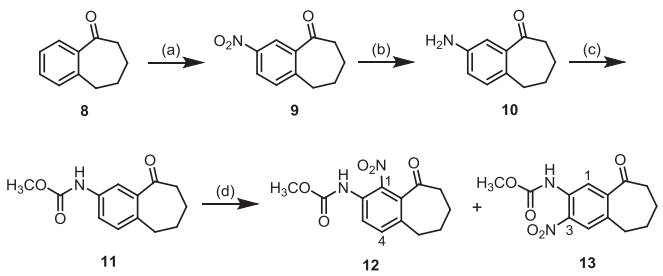
2.1. Synthesis

For the synthesis of GluN2B antagonists of type **7** the [7]annulenobenzimidazolone ring system has to be prepared. The tricyclic system should be obtained by annulation of the seven-membered carbocyclic ring at the benzimidazolone system or alternatively by establishment of the imidazolone ring at the benzo[7]annulene system.

At first a three-step synthesis was planned consisting of a Friedel–Crafts acylation of benzimidazolone with a glutaric acid derivative, Wolff-Kishner reduction and a final intramolecular Friedel–Crafts acylation. However all attempts to react benzimidazolone or 1,3-dibenzylbenzimidazolone with glutaric anhydride or glutaric acid mono methyl ester mono acid chloride in the presence of a Lewis acid failed to give the acylated benzimidazolone system.

Therefore, the second strategy, i.e. constructing the imidazolone ring at the benzo[7]annulene system, was pursued. Benzosuberone (**8**) was nitrated regioselectively with fuming HNO_3 to afford the 3-nitro derivative **9**. After reduction with H_2 and Pd/C the primary amine **10** was acylated with methyl chloroformate. Treatment of the carbamate **11** with fuming HNO_3 in glacial acetic acid resulted in a mixture of 1- and 3-nitro derivatives **12** and **13**. Although the 3-nitro derivative **13** could be isolated in 33% yield, the separation of the regioisomeric 1-nitro derivative **12** was very difficult. Moreover, slight modifications of the reaction conditions led predominantly to formation of **12**. The unexpectedly facile nitration of carbamate **11** between the keto and methoxycarbonylamino moieties was explained by a neighbor group effect. Alternatively, the nitroketone **13** was obtained by oxidation of alcohol **17** with DMP in DMSO. However, the nitroketone **13** was isolated in only 28% yield. (Scheme 1)

In order to improve the reaction sequence the nitroketone **9** was reduced first with $NaBH_4$ to yield the nitroalcohol **14** and secondly with H_2 and Pd/C to afford the aminoalcohol **15**. After conversion of **15** into the carbamate **16**, nitration with fuming HNO_3 took place with high regioselectivity providing the 3-nitro derivative **18** in 85% yield. In contrast to the nitration of the ketone **11**, nitration of the alcohol **16** did not lead to the corresponding 1-nitro derivative. It is assumed that the higher electron density and thus faster reaction of the benzene moiety of **16** leads to the selective nitration of the less hindered 3-position. The reduction of the nitro compound **18** was performed with H_2 (4 bar) in the presence of Pd/C affording the primary amine in 77% yield. (Scheme 2)



Scheme 1. First approach for the synthesis of annulated benzimidazolones by nitration of carbamate **11**. Reagents and reaction conditions: (a) HNO_3 fuming, −10 to −17 °C, 1 h, 87%. (b) H_2, 3 bar, Pd/C, CH_3OH, rt, 44 h, 38%. (c) $ClCO_2CH_3$, NEt_3, THF, reflux, 24 h, 62%. (d) HNO_3, fuming, HOAc, rt, 22 h, 16% (**12**), 33% (**13**).

Several methods for the cyclization of amino substituted carbamates to form cyclic ureas have been reported. Generally, simple heating, acids (e.g. glacial acetic acid, *p*-toluenesulfonic acid) [34], coupling agents (e.g. carbonyl diimidazole (CDI)) [35] and bases (e.g. K_2CO_3, triazabicyclodecen, NaH) [36,37] can be used for this cyclization. However, heating of the aminocarbamate **18** in methanol, THF or toluene, addition of HOAc, *p*-toluenesulfonic acid or CDI did not lead to the desired benzimidazolone **19**. However, an excess of the strong base NaH in boiling THF induced the cyclization of **18** and the benzimidazolone **19** was isolated in 77% yield.

In conclusion this seven-step procedure allows for the first time the synthesis of the linear tricyclic ring system **19** consisting of a benzene ring, which is annulated with a five-membered imidazolone ring and a seven-membered [7]annulene ring. The overall yield of **19** prepared via the optimized seven-step sequence starting with benzosuberone (**8**) was 28%.

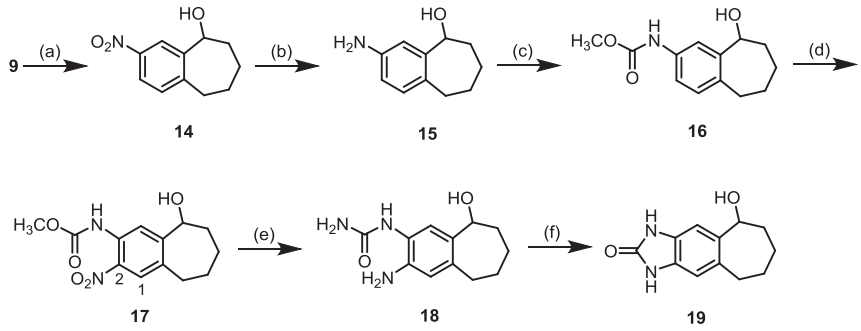
The tricyclic ketone **20** was envisaged as valuable building block for the introduction of amino substituents in α- and β-position (6- and 7-position) as well as at the ketone position itself (5-position). The ketone **20** was obtained in 75% yield upon oxidation of the secondary alcohol **19** with Dess-Martin-Periodinane. The α-bromination of ketone **20** was performed in DMSO using NBS as Br_2 source. The α-bromoketone **21** was obtained with high chemoselectivity in 75% yield. However, further reactions of the ketones **20** and **21** failed. In particular the synthesis of an α,β-unsaturated ketone by oxidation with IBX [37–40] or β-elimination of HBr, the reductive amination of **20** with primary or secondary amines and the substitution of Br in α-position of **21** were investigated. (Scheme 3)

The failure of these transformations is explained by the very low solubility of the ketones **20** and **21** in organic solvents. DMSO was the only solvent, which allowed the preparation of solutions with **20** and **21**. In addition to the low solubility, the carbonyl activity of the ketones **20** and **21** is reduced due to the N-atom in *p*-position of the benzene ring, which could explain the low reactivity during the reductive amination experiments. Moreover, the ketones **20** and **21** represent phenylogous imides, which are deprotonated upon treatment with base and thus are further deactivated for nucleophilic attack at the carbonyl moiety in 5-position.

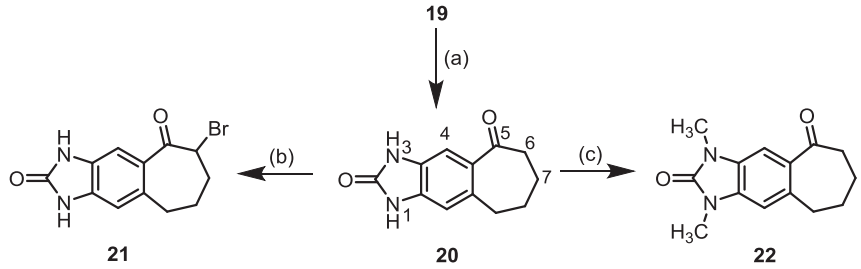
In order to avoid deprotonation and increase the solubility, the ketone **20** was methylated in a phase transfer-catalyzed transformation [41,42]. However, despite improved solubility of the dimethyl derivative **22**, the reactivity of **22** was not increased considerably.

In order to introduce amino moieties into the seven-membered part of the tricyclic system, the alcohol **19** was dehydrated with *p*-toluenesulfonic acid and the resulting alkene **23** was methylated in a phase transfer catalyzed reaction [41,42] to obtain the dimethyl derivative **24** in 71% yield. In contrast to the ketones **20** and **21** the solubility of the methylated alkene **24** was sufficient to convert it into the epoxide **25** upon treatment with *m*-chloroperbenzoic acid (MCPBA) in CH_2Cl_2. The epoxide **25** represents the central intermediate for the introduction of amino moieties in various positions. (see Scheme 4)

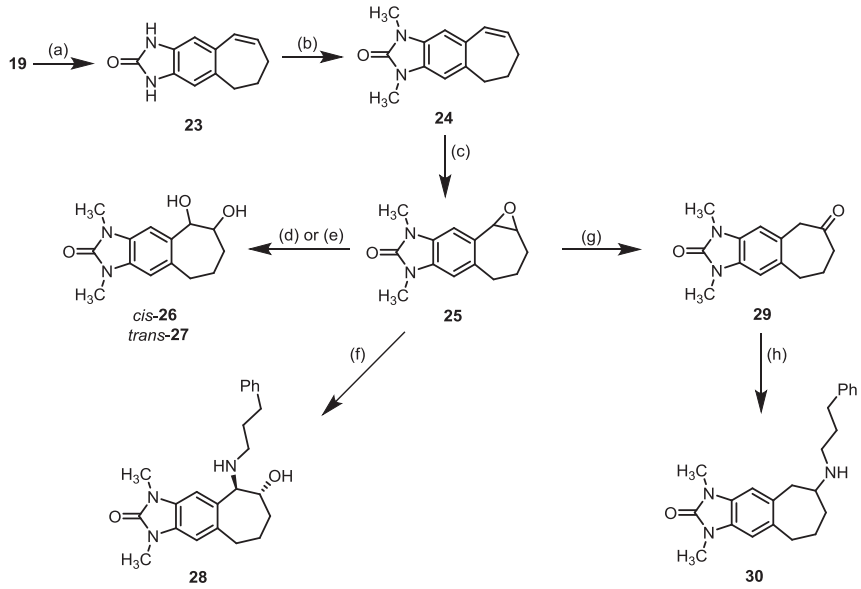
According to the structures of the lead compounds **1**-**6** the 3-phenylpropylamino moiety was selected as preferred substituent. Therefore the epoxide **25** was treated with phenylpropylamine under various reaction conditions. These transformations led to complex mixtures of products, which partly allowed the isolation of some products. The isolated products are summarized in Scheme 4. Treatment of **25** with 3-phenylpropylamine in the presence of NaH and $BF_3.OEt_2$ lead to an opening of the epoxide and the diols *cis*-**26** and *trans*-**27** could be isolated in 13% and 19% yield, respectively. The opening of **25** with the Lewis acid $BF_3.OEt_2$ seems to proceed via a S_N2-like mechanism. The reaction of **25** with CH_3MgBr in THF resulted in the isolation of ketone **29** in a 36% yield which was



Scheme 2. Second approach for the synthesis of annulated benzimidazolones by nitration of carbamate **16**. Reagents and reaction conditions: (a) $NaBH_4$, CH_3OH, rt, 0.5 h, 85%. (b) H_2, 4 bar, Pd/C, CH_3OH, rt, 0.5–1 h, 91%. (c) $ClCO_2CH_3$, NEt_3, THF, rt, 0.5 h, 92%. (d) HNO_3, fuming, HOAc, −10 °C, 0.5 h, 85%. (e) H_2, 4 bar, Pd/C, CH_3OH, rt, 0.5 h, 69%. (f) NaH, THF, reflux, 23 h, 77%.



Scheme 3. Oxidation of tricyclic alcohol **19**. Reagents and reaction conditions: (a) Dess-Martin-Periodinane (DMP), DMSO, rt, 2 h, 75%. (b) N-bromosuccinimide (NBS), DMSO, 40 °C, 24 h, 75%. (c) CH_3I, toluene, NaOH, H_2O, $Bu_4N^+I^-$, 30 °C, 20 h, 26%.



Scheme 4. Synthesis of tricyclic phenylpropylamines **28** and **30**. Reagents and reaction conditions: (a) *p*-TosOH, DMSO, 70 °C, 40 min, 70%. (b) CH_3I, toluene, NaOH, H_2O, $Bu_4N^+I^-$, 30 °C, 24 h, 71%. (c) MCPBA, CH_2Cl_2, Na_2CO_3, H_2O, rt, 2 h, 51%. (d) $Ph(CH_2)_3NH_2$, NaH, THF, rt, 10 d, 13% (*cis*-**26**). (e) $Ph(CH_2)_3NH_2$, $BF_3.OEt_2$, rt, 17 h, 19% (*trans*-**27**). (f) $Ph(CH_2)_3NH_2$, LiHMDS, THF, rt, 11 d, 27%. (g) $Ph(CH_2)_3NH_2$, CH_3MgBr, THF, 35 °C, 7.5 h, 36%. (h) $Ph(CH_2)_3NH_2$, $NaBH(OAc)_3$, CH_2Cl_2, rt, 45 min, 42%. The compounds in Scheme 4 represent racemic mixtures. In order to show the *trans*-configuration of compound **28** one enantiomer of the racemic mixture is displayed in Scheme 4.

converted by reductive amination into the amine **30** in 42% yield. The aminoalcohol **28** was obtained by treatment of epoxide **25** with 3-phenylpropylamine and LiHMDS in THF in 27% yield.

2.2. Receptor affinity

The affinity of the diastereomeric diols *cis*-**26** and *trans*-**27** and the amines **28** and **30** towards GluN2B containing NMDA receptors was investigated using the competitive receptor binding assay recently developed in our group [43]. Membrane fragments prepared by ultrasonic irradiation of L(tk-)-cells stably expressing recombinant human GluN1a and GluN2B subunits were employed as receptor material in this assay. The synthesis of functional NMDA receptors was induced by addition of dexamethasone to the growth

medium of these L(tk-)-cells. In order to protect the cells against cell death, ketamine was added to the medium, which blocks the NMDA receptor by interaction with the phencyclidine binding site within the channel pore. Tritium labeled [^{3}H]ifenprodil (**1**) served as radioligand. Although the selectivity of the radioligand is rather low, this assay is selective for the ifenprodil binding site of GluN2B containing NMDA receptors due to the high amount of receptors in this cell line.

In Table 1 the GluN2B affinity of the test compounds is summarized. Generally the GluN2B affinity of the *cis*- and *trans*-configured diols *cis*-**26** and *trans*-**27** is very low, since they are lacking a basic amino moiety. The aminoalcohol **28** shows also a very low GluN2B affinity, which is explained by the amino moiety in benzylic position resulting in a short distance between the basic amino moiety and the aromatic ring. Although the amine **30** fits nicely into the pharmacophore model developed by Tamiz et al. [27], it shows only moderate affinity (K_i = 697 nM) to GluN2B subunit containing NMDA receptors. It can be speculated that the methylated benzimidazolone is not an appropriate bioisostere of the phenol of **1**, **2a** and **4** and the methyl phenyl ether of **2b** and **3**. Moreover, it is possible that the tricyclic [7]annulenobenzimidazolone is too rigid to adapt to the complementary binding pocket of the GluN2B binding site.

The diols *cis*-**26** and *trans*-**27** and the amine **28** do not interact considerably with σ_1 and σ_2 receptors [44–46]. However, the phenylpropylamine **30** shows weak σ_1 affinity (K_i = 4 μM) and moderate σ_2 affinity (K_i = 549 nM). Thus the rigid tricyclic [7]annulenobenzimidazolone **30** shows >5-fold selectivity for the GluN2B receptor over the σ_1 receptor, but the same affinity towards the GluN2B receptor and σ_2 receptors.

3. Conclusion

Starting with the structures of potent GluN2B antagonists, the [7]annulenobenzimidazolone system **7** was designed. A seven-step sequence is described allowing the efficient synthesis of the linear tricyclic ring system **19** consisting of a benzene ring, which is annulated with a five-membered imidazolone ring and a seven-membered [7]annulene ring. The overall yield of **19** prepared via the optimized seven-step sequence starting with benzosuberone (**8**) was 28%. Five more steps including elimination of water, dimethylation to increase solubility, epoxidation followed by epoxide rearrangement and, finally, reductive amination led to the phenylpropylamine **30**. Although the amine **30** fits nicely into the postulated GluN2B pharmacophore, it shows only moderate GluN2B affinity (K_i = 697 nM). Additionally, **30** interacts with σ_2 receptors (K_i = 549 nM) with similar affinity, indicating poor selectivity. It is postulated that the benzimidazolone system is not a suitable bioisostere of the phenol or the corresponding methyl ether of potent GluN2B antagonists. Moreover, it is assumed that the tricyclic [7]annulenobenzimidazolone system is too rigid to induce a fit during approaching the ifenprodil binding site of the NMDA receptor. Nevertheless, the tricyclic amine **30** opens the possibility for several modifications at various ring positions. Therefore, **30** represents a promising lead compound for the development of novel types of GluN2B antagonists.

Table 1
Affinities of [7]annulenobenzimidazolones towards the ifenprodil binding site of GluN2B containing NMDA receptors, as well as towards σ_1 and σ_2 receptors.

Compd.	K_i ± SEM (nM)[a]		
	GluN2B[b]	σ_1[b]	σ_2[b]
cis-**26**	0%	11%	5%
trans-**27**	18%	0%	18%
28	0%	19%	32%
30	697 ± 18	4 μM	549 ± 84
Ifenprodil (**1**)	10 ± 0.7	125 ± 24	98.3 ± 34
Eliprodil	13 ± 2.5	–	–
Haloperidol	–	6.3 ± 1.6	78.1 ± 2.3
Di-*o*-tolylguanidine	–	89 ± 29	57.5 ± 18

[a] For low-affinity compounds only the inhibition of the radioligand binding at a test compound concentration of 1 μM is given in %.
[b] All K_i-values were recorded three times (n = 3).

4. Experimental

4.1. Experimental, chemistry

4.1.1. General

Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. Acetonitrile and dimethyl sulfoxide were dried over molecular sieves. CH_2Cl_2 was distilled from CaH_2, methanol was distilled from magnesium methanolate and tetrahydrofuran was distilled from sodium/benzophenone. Thin layer chromatography (TLC): Silica gel 60 F254 plates (Merck). Flash chromatography (FC): Silica gel 60 (40–63 μm, Macherey–Nagel); parentheses include: diameter of the column, length, fraction size, Rf value, eluent. Automatic flash column chromatography: Isolera™ One (Biotage®). Biotage® SNAP cartridge (10 g); stationary phase: silica gel 60 (40–63 μm, Macherey–Nagel); column volume (CV): 1CV = 15 mL; flow rate: 12 mL/min; max. fraction volume: 18 mL; detection at 254 nm (UV1) and 280 nm (UV2); baseline: on; start threshold: 5 mAU; solvents: A: cyclohexane; B: ethyl acetate; gradient elution: (A/B [%]): 2.0 CV: 20%, 10.0 CV: 20–100%, 6.0 CV: 100%. Melting point: Melting point apparatus SMP3 (Stuart Scientific), uncorrected. MS: microTOF-Q II (Bruker Daltronics); APCI, atmospheric pressure chemical ionization. IR: FT-IR MIRacle 10 (Shimadzu) equipped with ATR technique. Nuclear magnetic resonance (NMR) spectra were recorded on Agilent 600-MR (600 MHz for ^{1}H, 151 MHz for ^{13}C) or Agilent 400-MR spectrometer (400 MHz for ^{1}H, 101 MHz for ^{13}C); δ in ppm related to tetramethylsilane and measured referring to CDCl3 (δ = 7.26 ppm (^{1}H NMR) and δ = 77.16 ppm (^{13}C NMR)), CD_3OD (δ = 4.87 ppm (^{1}H NMR) and δ = 49.0 ppm (^{13}C NMR)) and CD_3OD (δ = 2.50 ppm (^{1}H NMR) and δ = 39.52 ppm (^{13}C NMR)); coupling constants are given with 0.5 Hz resolution. Analytical HPLC: Merck Hitachi Equipment; UV detector: L-7400; autosampler: L-7200; pump: L-7100; interface: D-7000; column: LiChrospher® 60 RP-select B (5 μm); LiChroCART® 250-4 mm cartridge; flow rate: 1.0 mL/min; injection volume: 5.0 μL; detection at λ = 210 nm; solvents: A: water with 0.05% (v/v) trifluoroacetic acid; B: acetonitrile with 0.05% (v/v) trifluoroacetic acid: gradient elution: (A %): 0–4 min: 90%, 4–29 min: 90 → 0%, 29–31 min: 0%, 31–31.5 min: 0 → 90%, 31.5–40 min: 90%. The purity of all compounds was determined by this method. The purity of all test compounds is higher than 95%.

4.1.2. 3-Nitro-6,7,8,9-tatrahydrobenzo[7]annulen-5-one (**9**)

Fuming nitric acid (29.5 mL, 703 mmol) was poured into a three necked flask. 1-Benzo-suberone (**8**) (9.3 mL, 62.42 mmol) was added dropwise under vigorous stirring and cooling to −10 to −17 °C by acetone and dry ice. After complete addition of 2 the mixture was stirred for 1 h at < −10 °C. The mixture was poured on ice and stirred till the ice was molten. The solid residue was filtered off in vacuo and washed several times with ice cold water. The crude product was purified by recrystallization from EtOH 96% to form a light yellow solid, mp 92–93 °C, yield 11.2 g (87%). $C_{11}H_{11}NO_3$ (205.2). TLC (petroleum ether: ethyl acetate = 8:2): Rf = 0.29. ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.82–1.90 (m, 2H, 7-H), 1.91–1.99 (m, 2H, 8-H), 2.83–2.87 (m, 2H, 6-H), 3.02–3.07 (m, 2H, 9-H), 7.40 (d, 3J = 8.4Hz, 1H, 1-H), 8.26 (dd, 3J = 8.3 Hz, 4J = 2.5 Hz,

1H, 2-H), 8.56 (d, 4J = 2.5 Hz, 1H, 4-H). ^{13}C NMR ($CDCl_3$): δ [ppm] = 20.8 (1C, C-7), 25.0 (1C, C-8), 32.7 (1C, C-9), 40.7 (1C, C-6), 124.1 (1C, C-4), 126.4 (1C, C-2), 131.2 (1C, C-1), 140.0 (1C, C-4a), 148.2 (1C, C-3), 152.9 (1C, C-9a), 203.6 (1C, C-5). Exact mass (APCI): *m/z* = 206.0829 (calcd. 206.0812 for $C_{11}H_{12}NO_3$ [MH]+). IR (neat): ṽ [cm-1] = 2951 (w, νC-H), 1670 (s, νC = O aryl), 1604 (m, νC═C arom.), 1519 (s, νN═O), 1342 (s, νN═O konj.). Purity (HPLC): tR = 17.43 min, purity 97.4%.

4.1.3. 3-Amino-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (**10**)

Nitroketone **9** (0.30 g, 1.46 mmol) was dissolved in abs. CH_3OH (15 mL) and Pd/C (30 mg, 10% m/m) was added. The mixture was stirred under H_2-atmosphere (3 bar) for 44 h. The crude product was filtered over Celite 450® to remove the catalyst and Celite 450® was washed with CH_3OH (3×). The solvent was removed in vacuo and the residue was purified by fc (Ø = 2.3 cm, h = 19.5 cm, cyclohexane:ethyl acetate = 2:8, V = 10 mL) to form an orange solid, mp 104–105 °C, yield 0.1 g, (38%). $C_{11}H_{13}NO$ (175.2). TLC (cyclohexane:ethyl acetate = 7:3): Rf = 0.21. ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.75–1.88 (m, 4H, 7-H, 8-H), 2.68–2.73 (m, 2H, 6-H), 2.79–2.85 (m, 2H, 9-H), 6.75 (dd, 3J = 8.0 Hz, 4J = 2.6 Hz, 1H, 2-H), 6.99 (d, 3J = 8.0 Hz, 1H, 1-H), 7.06 (d, 4J = 2.6 Hz, 1H, 4-H). Signals for the protons of the amino group are not seen in the spectrum. ^{13}C NMR ($CDCl_3$): δ [ppm] = 21.1 (1C, C-7), 25.6 (1C, C-8), 31.8 (1C, C-9), 41.0 (1C, C-6), 114.9 (1C, C-4), 119.2 (1C, C-2), 130.9 (1C, C-1), 132.0 (1C, C-9a), 139.5 (1C, C-4a), 144.8 (1C, C-3), 206.4 (1C, C-5). Exact mass (APCI): *m/z* = 176.1063 (calcd. 176.1070 for $C_{13}H_{14}NO$ [MH]+). IR (neat): ṽ [cm-1] = 3429 (m, νN-H), 3348 (m, νN-H), 2928 (m, νC-H), 1639 (s, νC═O aryl), 1625 (s, νC = C arom). Purity (HPLC): tR = 8.76 min, purity 97.1%.

4.1.4. Methyl N-(9-oxo-6,7,8,9-tetrahydro-5H-benzo[7]annulen-2-yl)carbamate (**11**)

Aminoketone **10** (1.5 g, 8.6 mmol) was dissolved in abs. THF (70 mL). Triethylamine (3.23 mL, 21.4 mmol) and methyl chloroformate (1.67 mL, 21.4 mmol) were added. The mixture was heated to reflux under N_2-atmosphere for 24 h. The mixture was cooled to ambient temperature and the solvent was removed in vacuo. The residue was dissolved in CH_2Cl_2 and extracted with water and 2M HCl (4×). The separated organic layer was dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The crude product was purified by flash column chromatography (Ø = 4.5 cm, h = 13.5 cm, cyclohexane:ethyl acetate = 7:3, V = 30 mL) to give a colorless solid, mp 98–99 °C, yield 1.24 g (62%). $C_{13}H_{15}NO_3$ (232.3). TLC (cyclohexane:ethyl acetate = 7:3): Rf = 0.23. ^{1}H NMR (CD_3OD): δ [ppm] = 1.74–1.90 (m, 4H, 7-H, 6-H), 2.69–2.75 (m, 2H, 8-H), 2.89–2.95 (m, 2H, 5-H), 3.73 (s, 3H, $COOCH_3$), 7.19 (d, 3J = 8.2 Hz, 1H, 4-H), 7.57 (dd, 3J = 8.2 Hz, 4J = 2.3 Hz, 1H, 3-H), 7.69 (d, 4J = 2.3 Hz, 1H, 1-H). Signal for the NH group is not seen in the spectrum. ^{13}C NMR (CD_3OD): δ [ppm] = 22.0 (1C, C-7), 26.4 (1C, C-6), 32.6 (1C, C-5), 41.6 (1C, C-8), 52.6 (1C, $COOCH_3$), 119.4 (1C, C-1), 123.7 (1C, C-3), 131.5 (1C, C-4), 137.5 (1C, C-4a), 139.2 (1C, C-2), 140.2 (1C, C-9a), 156.5 (1C, $COOCH_3$) 207.9 (1C, C-9). Exact mass (APCI): *m/z* = 234.1087 (calcd. 234.1026 for $C_{13}H_{16}NO_3$ [MH]+). IR (neat): ṽ [cm^{-1}] = 3321 (m, ν–NH–), 2936 (m, νC-H), 1724 (s, νC═O Ketone), 1654 (s, νC═O Carbamate), 1609 (m, ν-C═C). Purity (HPLC): tR = 17.03 min, purity 99.9%.

4.1.5. Methyl (1-nitro-9-oxo-6,7,8,9-tetrahydro-5H-benzo[7]annulen-2-yl)carbamate (**12**) and Methyl (3-nitro-9-oxo-6,7,8,9-tetrahydro-5H-benzo[7]annulen-2-yl)carbamate (**13**)

Method 1: Synthesis by nitration of carbamate **11**

Carbamate **11** (0.5 g, 2.1 mmol) was dissolved in HOAc (10 mL) and fuming HNO3 (1 mL, 24.2 mmol) was dropwise added under vigorous stirring and cooling to 0 °C (water and ice). After complete addition, the mixture was stirred at ambient temperature for 22 h. The mixture was poured into H_2O and the mixture was stirred for 0.5 h CH_2Cl_2 was added and the layers were separated. The separated organic layer was extracted with 2 M NaOH (3×), dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The products were separated by flash column chromatography (Ø = 2.3 cm, h = 18.5 cm, petroleum ether: ethyl acetate = 8:2, V = 10 mL). Two fractions were isolated containing compound **12** (Rf = 0.24) and compound **13** (Rf = 0.29).

12: Pale yellow oil, yield 10 mg (16%). $C_{13}H_{14}N_2O_5$ (278.3). TLC (petroleum ether: ethyl acetate = 8:2): Rf = 0.24. ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.79–1.91 (m, 2H, 7-H), 1.90–2.01 (m, 2H, 6-H), 2.73–2.79 (m, 2H, 8-H), 2.83–2.87 (m, 2H, 5-H), 3.81 (s, 3H, $COOCH_3$), 7.34–7.37 (m, 1H, 4-H), 8.25 (d, 3J = 8.6 Hz,1H, 3-H), 8.73 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ [ppm] = 24.5 (1C, C-6), 25.9 (1C, C–7), 32.5 (1C, C-8), 41.8 (1C, C-5), 53.1 (1C, $COOCH_3$), 122.5 (1C, C-3), 131.8 (1C, C-9a), 132.8 (1C, C-2), 134.8 (1C, C-4), 138.8 (1C, C-4a), 146.0 (1C, C-1), 153.8 (1C, NCOO), 205.6 (1C, C-9). Exact mass (APCI): *m/z* = 279.0871 (calcd. 279.0975 for $C_{13}H_{17}N_2O_5$ [MH]+). IR (neat): ṽ [cm^{-1}] = 3356 (w, ν–CONH–), 2954 (w, νC-H), 1751 (m, νC═O Ketone), 1701 (m, νC═O Carbamate), 1612 (w, ν-C═C), 1578 (w,. ν-C═C), 1505 (s, νN = O), 1344 (m, νN═O). Purity (HPLC): tR = 17.87 min, purity 93.4%.

13: Pale yellow solid, mp 166–167 °C, yield 25 mg (33%). $C_{13}H_{14}N_2O_5$ (278.3). TLC (petroleum ether: ethyl acetate = 8:2): Rf = 0.29. ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.79–1.95 (m, 4H, 6-H, 7-H), 2.71–2.79 (m, 2H, 8-H), 2.88–2.96 (m, 2H, 5-H), 3.83 (s, 3H, $COOCH_3$), 8.03 (s, 1H, 4-H), 8.73 (s, 1H, 1-H), 9.63 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ [ppm] = 21.3 (1C, C-6), 25.3 (1C, C-7), 31.8 (1C, C-5), 40.9 (1C, C-8), 53.1 (1C, $COOCH_3$), 121.2 (1C, C-1), 126.6 (1H, C-4), 133.8 (1C, C-2), 134.3 (1C, C-3), 137.2 (1C, C4a), 145.5 (1C, C-9a), 153.5 (1C, NCOO), 204.5 (1C, C-9). Exact mass (APCI): *m/z* = 279.0952 (calcd. 279.0975 for $C_{13}H_{15}N_2O_5$ [MH]+). IR (neat): ṽ [cm^{-1}] = 3283 (w, ν–CONH–), 2959 (w, νC-H), 1732 (m, νC═O Ketone), 1667 (m, νC═O Carbamate), 1612 (w, ν-C═C), 1516 (s, νN═O), 1358 (w, νN═O), 1230 (s, νC-O). Purity (HPLC): tR = 18.81 min, purity 95.7%.

Method 2: Synthesis of **13** by oxidation of nitroalcohol **17**

A solution of nitroalcohol **17** (0.20 g, 0.71 mmol) in abs. DMSO (3 mL) was added dropwise to a solution of Dess-Martin Periodinane (0.36 g, 0.86 mmol) in abs. DMSO (10 mL) under vigorous stirring. The mixture was stirred under N_2-atmosphere at ambient temperature for 2 h. Then, 1 M NaOH (20 mL), ethyl acetate (40 mL) and brine (25 mL) were added. The separated aqueous layer was extracted with ethyl acetate (6×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The crude product was purified by flash column chromatography (Ø = 4.0 cm, h = 19.5 cm, petroleum ether: ethyl acetate = 8:2, V = 30 mL) to give a paella yellow solid, mp 166–167 °C, yield 55.2 mg (28%). $C_{13}H_{14}N_2O_5$ (278.3). TLC (petroleum ether: ethyl acetate = 8:2): Rf = 0.29. Purity (HPLC): tR = 19.97 min, purity 96.6%.

4.1.6. 3-Nitro-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-ol (**14**)

Nitroketone **9** (12.5 g, 61 mmol) was dissolved in abs. CH_3OH (525 mL) and $NaBH_4$ (2.8 g, 73 mmol) were added portionwise under N_2-counter flow. The mixture was stirred under N_2-atmosphere at ambient temperature for 0.5 h. Water and 2 M HCl (110 mL) were added. Then, CH_2Cl_2 was added and the layers were separated. The separated aqueous layer was extracted with CH_2Cl_2 (12×). The combined organic layers were dried (Na_2SO_4) and the

solvent was removed in vacuo. The crude product was purified by recrystallization from EtOH 96% to form a fine colorless solid, mp 117–118 °C, yield 10.7 g (85%). $C_{11}H_{13}NO_3$ (207.2). TLC (cyclohexane:ethyl acetate = 7:3): Rf = 0.29. ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.33–1.46 (m, 1H, 8-H), 1.68–1.82 (m, 1H, 6-H), 1.80–1.93 (m, 2H, 7-H, 8-H), 1.96 (d, 3J = 4.1 Hz, 1H, OH), 2.01–2.11 (m, 2H, 6-H, 7-H), 2.74–2.83 (m, 1H, 9-H), 2.99 (dd, 2J = 14.2 Hz, 3J = 7.4 Hz, 1H, 9-H), 4.99 (dd, 3J = 9.0 Hz, 3J = 3.6 Hz, 1H, 5-H), 7.23 (d, 3J = 8.2 Hz, 1H, 1-H), 8.00 (dd, 3J = 8.3 Hz, 4J = 2.5 Hz, 1H, 2-H), 8.40 (d, 4J = 2.5 Hz, 1H, 4-H). ^{13}C NMR ($CDCl_3$): δ [ppm] = 26.9 (1C, C-8), 28.2 (1C, C-7), 35.8 (1C, C-9), 37.2 (1C, C-6), 72.9 (1C, C-5), 119.9 (1C, C-4), 122.1 (1C, C-2), 130.3 (C, C-1), 146.0 (1C, C-4a), 146.8 (1C, C-9a), 148.3 (1C, C-3). Exact mass (APCI): *m/z* = 208.0979 (calcd. 208.0968 for $C_{11}H_{14}NO_3$ [MH]+). IR (neat): ṽ [cm^{-1}] = 3499 (w, νO-H), 3345 (wbr, νO-H), 2928 (m, νC-H), 1520 (m, νN═O), 1330 (s, νN═O konj.). Purity (HPLC): tR = 17.29 min, purity 99.9%.

4.1.7. 3-Amino-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-ol (**15**)

Nitroalcohol **14** (21.5 g, 103.7 mmol) was dissolved in abs. CH_3OH (645 mL) and Pd/C (2.15 g, 10% m/m) was added. The mixture was divided in six parts (5 × 4.0 g of 19, 19.3 mmol, and 1 × 1.5 g of 7, 7.2 mmol). The separated mixtures were shaken under H_2 atmosphere (4 bar) for 0.5–1 h. The crude product was filtered over Celite 450® to remove the catalyst and Celite 450® was washed with CH_3OH (5×). The separated mixtures were combined and the solvent was removed in vacuo. The residue was purified by flash column chromatography (Ø = 8.0 cm, h = 11.5 cm, cyclohexane:ethyl acetate = 6:4, V = 100 mL) to give a light yellow solid, mp 165–166 °C, yield 17.0 g (91%). $C_{11}H_{15}NO$ (177.2). TLC (cyclohexane:ethyl acetate = 6:4): Rf = 0.15. ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.31–1.45 (m, 1H, 8-H), 1.57 (s br, 2H, NH2), 1.75 (tdd, 2J = 13.7 Hz, 3J = 6.4 Hz, 3J = 4.7 Hz, 3H, 6-H, 8-H), 1.91–2.07 (m, 2H, 7-H), 2.61 (ddd, 2J = 14.5 Hz, 3J = 10.8 Hz, 3J = 1.5 Hz, 1H, 9-H), 2.77 (dd, 2J = 14.7 Hz, 3J = 7.5 Hz, 1H, 9-H), 3.59 (s br, 1H, OH), 4.84 (dd, 3J = 8.3 Hz, 3J = 1.5 Hz, 1H, 5-H), 6.47 (dd, 3J = 7.8 Hz, 4J = 2.5 Hz, 1H, 2-H), 6.84 (d, 4J = 2.5 Hz, 1H, 4-H), 6.88 (d, 3J = 7.9 Hz, 1H, 1-H). ^{13}C NMR ($CDCl_3$): δ [ppm] = 28.2 (1C, C-8), 28.3 (1C, C-7), 35.1 (1C, C-9), 37.1 (1C, C-6), 74.0 (1C, C-5), 112.0 (1C, C-4), 113.2 (1C, C-2), 130.5 (1C, C-1), 130.9 (1C, C-9a), 144.7 (1C, C-4a), 145.5 (1C, C-3). Exact mass (APCI): *m/z* = 178.1233 (calcd. 178.1226 for C11H16NO [MH]+). IR (neat): ṽ [cm^{-1}] = 3383 (w, νN-H), 3267 (wbr, νO-H), 2928 (m, νC-H), 1612 (m, νC═C arom), 1049 (s, ΓC-O). Purity (HPLC): tR = 10.85 min, purity 98.6%.

4.1.8. Methyl N-(9-hydroxy-6,7,8,9-tetrahydro-5H-benzo[7] annulen-2-yl)carbamate (**16**)

Aminoalcohol **15** (2.8 g, 15.8 mmol) was dissolved in abs. THF (120 mL). Triethylamine (3.29 mL, 23.7 mmol) and methyl chloroformate (1.83 mL, 23.7 mmol) were added. The mixture was stirred under N_2 atmosphere at ambient temperature for 0.5 h. The solvent was removed in vacuo, the residue was dissolved in CH_2Cl_2 and the mixture was washed with water and 2 M HCl (6×). The separated organic layer was dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The crude product was purified by recrystallization from ethyl acetate to give a fawn solid, mp 109–110 °C, yield 2.63 g (92%). $C_{13}H_{17}NO_3$ (235.3).

TLC (cyclohexane:ethyl acetate = 7:3): Rf = 0.13. ^{1}H NMR (CD_3OD): δ [ppm] = 1.32–1.44 (m, 1H, 6-H), 1.60–1.71 (m, 1H, 8-H), 1.72–1.85 (m, 2H, 6-H, 7-H), 1.90–1.98 (m, 1H, 8-H), 1.99–2.07 (m, 1H, 7-H), 2.67 (ddd, 2J = 14.3 Hz, 3J = 10.8 Hz, 3J = 1.7 Hz, 1H, 5-H), 2.84 (dd, 2J = 14.2 Hz, 3J = 7.6 Hz, 1H, 5-H), 3.72 (s, 3H, $COOCH_3$), 4.81 (d, 3J = 9.8 Hz, 1H, 9-H), 6.98 (d, 3J = 8.0 Hz, 1H, 4-H), 7.22 (dd, 3J = 7.9 Hz, 4J = 2.4 Hz, 1H, 3-H), 7.42 (d, 4J = 2.3 Hz, 1H, 1-H). Signals for the OH and NH group are not seen in the spectrum. ^{13}C NMR (CD_3OD): δ [ppm] = 29.2 (1C, C-6), 30.7 (1C, C-7), 36.0 (1C, C-5), 38.1 (1C, C-8), 52.5 (1C, $COOCH_3$) 73.2 (1C, C-9), 117.0 (1C, C-1), 118.1 (1C, C-3), 130.7 (1C, C-4), 136.7 (1C, C-4a), 138.0 (1C, C-2), 146.8 (1C, C-9a), 156.8 (1C, $COOCH_3$). Exact mass (APCI): *m/z* = 218.1178 (calcd. 218.1176 for $C_{13}H_{16}NO_2$ [M − H2O + H]+). IR (neat): ṽ [cm^{-1}] = 3406 (w, ν–CONH–), 3275 (w, b, νO-H), 2931 (w, νC-H), 1694 (m, νC═O Carbamate), 1240 (s, ν O–H), 1069 (s, ν-C-O). Purity (HPLC): tR = 16.39 min, purity 97.2%.

4.1.9. Methyl (9-hydroxy-3-nitro-6,7,8,9-tetrahydro-5H-benzo[7] annulen-2-yl)carba-mate (**17**)

Carbamate **16** (10.0 g, 42.5 mmol) was dissolved in glacial acetic acid (100 mL). At −10 to 17 °C (acetone and dry ice) fuming nitric acid (19.8 mL, 479 mmol) was added dropwise under vigorous stirring. After complete addition, the mixture was stirred at < −10 °C for 0.5 h. The mixture was poured on ice and stirred until the ice was molten. The solid residue was filtered off in vacuo and washed several times with ice cold water. The crude product was purified by recrystallization from ethyl acetate to give a light yellow solid, mp 133–134 °C, yield 10.1 g (85%). $C_{13}H_{16}N_2O_5$ (280.3). TLC (petroleum ether: ethyl acetate = 8:2): Rf = 0.18. ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.32–1.45 (m, 1H, 6-H), 1.68–1.91 (m, 3H, 6-H, 7-H, 8-H), 1.99–2.09 (m, 2H, 7-H, 8-H),2.66–2.75 (m, 1H, 5-H), 2.85–2.94 (m, 1H, 5-H), 3.82 (s, 3H, COOCH3) 4.95 (d, 3J = 9.3 Hz, 1H, 9-H), 7.93 (s, 1H, 1-H), 8.67 (s, 1H, 4-H), 9.84 (1H, NH). The signal for the OH group is not seen in the spectrum. ^{13}C NMR (CD_3OD): δ [ppm] = 28.6 (1C, C-6), 29.3 (1C, C-7), 35.6 (1C, C-8), 38.1 (1C, C-5), 53.3 (1C, $COOCH_3$), 73.5 (1C, C-9), 119.2 (1C, C-4), 126.5 (1C, C-1), 133.6 (1C, C-2), 136.9 (1C, C-3), 137.3 (1C, C-4a), 155.3 (1C, C-9a), 155.5 (1C, NCOO). Exact mass (APCI): *m/z* = 281.1120 (calcd. 281.1132 for $C_{13}H_{17}N_2O_5$ [MH]+). IR (neat): ṽ [cm^{-1}] = 3502 (w, νOH), 3345 (w, ν–CONH–), 2932 (w, νC-H), 1721 (s, νC═O Carbamate), 1578 (m, ν-C═C), 1496 (s, νN═O), 1327 (s, νN═O), 1242 (s, ΓO-H), 1072 (m, ΓC-O). Purity (HPLC): tR = 18.40 min, purity 96.2%.

4.1.10. Methyl (3-amino-9-hydroxy-6,7,8,9- tetrahydro-5H-benzo [7]annulen-2-yl)carba-mate (**18**)

Nitrocarbamate **17** (2.2 g, 7.8 mmol) was dissolved in abs. CH_3OH (100 mL) and Pd/C (0.22 g, 10% m/m) was added. The mixture was shaken under H2-atmosphere (4 bar) for 0.5 h. The crude product was filtered over Celite 450® to remove the catalyst and Celite 450® was washed with CH_3OH (3×). The solvent was removed in vacuo and the residue was purified by flash column chromatography (Ø = 4.0 cm, h = 15.3 cm, cyclohexane:ethyl acetate = 2:8, V = 25 mL) to give an orange solid, mp 117–118 °C, yield 1.5 g (69%).

$C_{13}H_{18}N_2O_3$ (250.3). TLC (cyclohexane:ethyl acetate = 2:8): Rf = 0.25. ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.39–1.52 (m, 1H, 6-H), 1.63–1.80 (m, 3H,, 6-H, 7-H, 8-H), 1.87 (ddt, 2J = 10.6 Hz, 3J = 4.9 Hz, 3J = 2.3 Hz, 1H, 8-H), 1.98–2.09 (m, 1H, 7-H), 2.54–2.64 (m, 1H, 5-H), 2.83 (ddd, 2J = 13.8 Hz, 3J = 9.2 Hz, 3J = 1.7 Hz, 1H, 5-H), 3.72 (s, 3H, $COOCH_3$), 4.72 (d, 3J = 8.7 Hz, 1H, 9-H), 6.58 (s, 1H, 4-H), 7.13 (s, 1H, 1-H). The signals for the OH, NH_2 and NH groups are not seen in the spectrum. ^{13}C NMR (CDCl3): δ [ppm] = 28.7 (1C, C-7), 29.3 (1C, C-6), 36.4 (1C, C-5), 38.0 (1C, C-8), 52.8 (1C, $COOCH_3$), 74.4 (1C, C-9), 111.2 (1C, C-2), 119.7 (1C, C-4), 122.4 (1C, C-9a), 124.6 (1C, C-1), 136.3 (1C, C-4a), 143.3 (1C, C-3), 213.1 (1C, $COOCH_3$). Exact mass (APCI): *m/z* = 251.1392 (calcd. 251.1390 for $C_{13}H_{19}N_2O_3$ [MH]+). IR (neat): ṽ [cm^{-1}] = 3372 (w, νN-H), 3310 (w, b, ν-O-H-), 2924 (w, νC-H), 1728 (s, νC = O Carbamate), 1593 (m, ν-C═C), 1524 (s, ν-C═C), 1238 (s, νC-O), 1219 (s, ΓO-H), 1045 (s, ΓC-O). Purity (HPLC): tR = 5.34 min, 5.73 min, purity 94.8%.

4.1.11. 5-Hydroxy-3,5,6,7,8,9-hexahydro[7]annuleno[f]benz-imidazol-2(1H)-one (**19**)

Under N_2, sodium hydride (2.1 g, 60% dispersion in oil,

63.1 mmol) was suspended in abs. THF (350 mL). Aminoalcohol 18 (4.7 g, 18.9 mmol) was dissolved in abs. THF (249 mL) and the solution was slowly added dropwise to the sodium hydride suspension under vigorous stirring. The mixture was heated to reflux for 23 h. After cooling to ambient temperature, saturated ammonium chloride solution (100 mL), water (150 mL) and CH_2Cl_2 (350 mL) were added. The precipitate was collected and washed several times with CH_2Cl_2. The residue was dried in vacuo. Fawn solid, mp > 300 °C (decomposition), yield 3.2 g (77%). $C_{12}H_{14}N_2O_2$ (218.3). TLC (cyclohexane:ethyl acetate = 2:8): Rf = 0.37. ^{1}H NMR (D6-DMSO) δ [ppm] = 1.13–1.27 (m, 1H, 8-H), 1.37–1.52 (m, 1H, 6-H), 1.61–1.79 (m, 2H, 7-H, 8-H), 1.82–1.94 (m, 2H, 6-H, 7-H), 2.63 (dd, 2J = 13.7 Hz, 3J = 11.2 Hz, 1H, 9-H), 2.76 (dd, 2J = 13.6 Hz, 3J = 6.5 Hz, 1H, 9-H), 4.69 (dd, 3J = 9.5 Hz, 3J = 4.1 Hz, 1H, 5-H), 5.11 (d, 3J = 4.0 Hz, 1H, OH), 6.63 (s, 1H, 10-H), 7.05 (s, 1H, 4-H), 10.34 (s, 1H, NH), 10.37 (s, 1H, NH). ^{13}C NMR (D6-DMSO): δ [ppm] = 28.5 (1C, C-8), 28.7 (1C, C-7), 35.6 (1C, C-9), 38.5 (1C, C-6), 72.0 (1C, C-5), 106.0 (1C, C-4), 109.8 (1C, C-10), 110.0 (1C, C-10a), 128.1 (1C, C-3a), 133.0 (1C, C-4a), 139.2 (1C, C-9a), 156.3 (1C, C-2). Exact mass (APCI): *m/z* = 219.1116 (calcd. 219.1128 for $C_{12}H_{15}N_2O_2$ [MH]+). IR (neat): ṽ [cm^{-1}] = 3175 (w, b, νO-H), 2920 (m, νC-H), 1636 (s, νC = O Urea), 1485 (m, ΓO-H), 1018 (νC-O). Purity (HPLC): tR = 12.03 min, purity 96.5%.

4.1.12. 1,3,6,7,8,9-Hexahydro[7]annuleno[f]benzimidazole-2,5-dione (**20**)

A solution of alcohol **19** (0.4 g, 1.8 mmol) in abs. DMSO (6 mL) was added dropwise to a solution of Dess-Martin Periodinane (0.93 g, 2.2 mmol) in abs. DMSO (10 mL) under vigorous stirring. The mixture was stirred under N_2-atmosphere at ambient temperature for 2 h. Then, 1M NaOH (50 mL), ethyl acetate (100 mL) and brine (60 mL) were added. The separated aqueous layer was extracted with ethyl acetate (6×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The crude product was purified by flash column chromatography (Ø = 4.0 cm, h = 18.0 cm, cyclohexane:ethyl acetate = 2:8, V = 20 mL) to give an orange solid, mp > 345 °C (decomposition), yield 297 mg (75%). $C_{12}H_{12}N_2O_2$ (216.2). TLC (cyclohexane:ethyl acetate = 2:8): Rf = 0.19. ^{1}H NMR (D6-DMSO): δ [ppm] = 1.60 (quint, 3J = 6.3 Hz, 2H, 7-H), 1.71 (quint, 3J = 6.6 Hz, 2H, 8-H), 2.57–2.63 (m, 2H, 6-H), 2.83–2.90 (m, 2H, 9-H), 6.77 (s, 1H, 10-H), 7.16 (s, 1H, 4-H), 10.67 (s, 1H, NH), 10.86 (s, 1H, NH). ^{13}C NMR (D6-DMSO): δ [ppm] = 20.0 (1C, C-7), 24.9 (1C, C-8), 31.6 (1C, C-9), 40.2 (1C, C-6), 108.2 (1C, C-4), 109.3 (1C, C-10), 128.4 (1C, C-3a), 130.9 (1C, C-10a), 133.6 (1C, C-4a), 136.0 (1C, C-9a), 155.6 (1C, C-2), 203.6 (1C, C-5). Exact mass (APCI): *m/z* = 217.0979 (calcd. 217.0972 for $C_{12}H_{13}N_2O_2$ [MH]+). IR (neat): ṽ [cm^{-1}] = 2928 (m, νC-H), 1721 (s, νC = O Ketone), 1628 (s, νC = O Urea). Purity (HPLC): tR = 12.91 min, purity 96.5%.

4.1.13. 6-Bromo-1,3,6,7,8,9-haxahydro [7]annuleno[f] benzimidazole-2,5-dione (**21**)

Ketone **20** (0.05 g, 0.23 mmol) was dissolved in DMSO (10 mL) and N-bromosuccinimide (80 mg, 0.46 mmol) was added. The mixture was heated to 40 °C for 24 h. Then water was added. The separated aqueous layer was extracted witch CH_2Cl_2 (4×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The crude product was purified by flash column chromatography (Ø = 2.0 cm, h = 21.5 cm, cyclohexane:ethyl acetate = 2:8, V = 10 mL) to give a yellow solid, mp > 260 °C (decomposition), yield 51 mg (75%). $C_{12}H_{12}BrN_2O_2$ (295.1). TLC (cyclohexane:ethyl acetate = 2:8): Rf = 0.16. ^{1}H NMR (D6-DMSO): δ [ppm] = 1.62–1.79 (m, 1H, 8-H), 1.93–2.03 (1H, 8-H), 1.98–2.15 (1H, 7-H), 2.25–2.39 (m, 1H, 7-H), 2.87–3.04 (m, 2H, 9-H), 5.23 (dd, 3J = 8.9 Hz, 3J = 4.0 Hz, 1H, 6-H), 6.84 (s, 1H, 10-H), 7.12 (s, 1H, 4-H), 10.74 (s, 1H, NH), 10.95 (s, 1H, NH). ^{13}C NMR (D6-DMSO): δ [ppm] = 24.2 (1C, C-8), 32.08 (1C, C-7), 32.1 (1C, C-9), 55.7 (1C, C-6), 108.9 (1C, C-4), 109.3 (1C, C-10), 128.3 (1C, C-3a), 129.4 (1C, C-10a), 133.7 (1C, C-4a), 135.1 (1C, C-9a), 155.4 (1C, C-2), 197.4 (1C, C-5). Exact mass (APCI): *m/z* = 295.0073 (calcd. 295.0077 for $C_{12}H_{13}BrN_2O_2$ [MH]+). IR (neat): ṽ [cm^{-1}] = 3928 (w, νC-H), 1705 (m, νC = O Ketone), 1670(s, νC = O Urea), 714 (w, νC-Br alkyl). Purity (HPLC): tR = 15.89 min, purity 54.8%.

4.1.14. 1,3-Dimethyl-1,3,6,7,8,9- hexahydro[7]annuleno[f] benzimidazole-2,5-dione (**22**)

Method 2 by methylation of ketone **20**:

Ketone **20** (36 mg, 0.17 mmol) was suspended in toluene (3 mL) and 50% aqueous NaOH (1 mL). Tetrabutylammonium iodide (9.2 mg, 0.025 mmol) and methyl iodide (63 μL, 0.84 mmol) were added under vigorous stirring. The mixture was heated to 30 °C for 20 h. Then, water was added and the separated aqueous layer was extracted with CH_2Cl_2 (4×), dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The crude product was purified by automatic flash column chromatography (h = 5,5 cm) to form a colorless solid, mp 152–153 °C, yield 11 mg (26%). $C_{14}H_{16}N_2O_2$ (244.3). TLC (cyclohexane:ethyl acetate = 2:8): Rf = 0.33. $C_{14}H_{16}N_2O_2$ (244.3). TLC (cyclohexane:ethyl acetate = 2:8): Rf = 0.33. ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.74–1.86 (m, 2H, 7-H), 1.84–1.96 (m, 2H, 8-H), 2.73–2.79 (m, 2H, 6-H), 2.96–3.04 (m, 2H, 9-H), 3.43 (s, 6H, NCH_3), 6.77 (s, 1H, 4-H), 7.46 (s, 1H, 10-H). ^{13}C NMR ($CDCl_3$): δ [ppm] = 20.6 (1C, C-7), 25.5 (1C, C-8), 27.49 (1C, NCH_3), 27.52 (1C, NCH_3), 32.9 (1C, C-9), 40.9 (1C,C-6), 108.1 (1C, C-4), 108.2 (1C, C-10), 129.0 (1C, C-4a), 132.3 (1C, C-3a), 133.6 (1C, C-9a), 136.9 (1C, C-10a), 155.1 (1C,C-2), 205.1 (1C, C-5). Exact mass (APCI): *m/z* = 245.1298 (calcd. 245.1285 for $C_{14}H_{17}N_2O_2$ [MH]+). IR (neat): ṽ [cm^{-1}] = 2932(w, νC-H), 1701 (s, νC═O Ketone), 1659(m, νC═O Urea), 1508 (m, νC═C). Purity (HPLC): tR = 16.31 min, purity 90.6%.

4.1.15. 3,5,6,7-Tetrahydro[7]annuleno[f]benzimidazol-2(1H)-one (**23**)

A solution of alcohol 19 (0.2 g, 0.92 mmol) and *p*-toluenesulfonic acid (0.19 g, 1 mmol) in THF (28 mL) and DMSO (9 mL) was heated to 70 °C for 40 min. Then, water (23 mL) was added and the separated aqueous layer was extracted with CH_2Cl_2 (6×). The combined organic layer was dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The crude product was dried in vacuo without further purification to give a pale yellow solid, mp > 335 °C (decomposition), yield 0.13 g (70%).$C_{12}H_{12}N_2O$ (200.2). TLC (cyclohexane:ethyl acetate = 2:8): Rf = 0.17. ^{1}H NMR (D6-DMSO): δ [ppm] = 1.79–1.90 (m, 2H, 6-H), 2.28–2.37 (m, 2H, 7-H), 2.71–2.78 (m, 2H, 5-H), 5.73 (dt, 2J = 12.1 Hz, 3J = 4.1 Hz, 1H, 8-H), 6.36 (dt, 3J = 12.4 Hz, 3J = 1.9 Hz, 1H, 9-H), 6.68 (s, 1H, 10-H), 6.71 (s, 1H, 4-H), 10.42 (s, 1H, NH), 10.48 (s, 1H, NH). ^{13}C NMR (D6-DMSO): δ [ppm] = 27.2 (1C, C-6), 31.7 (1C, C-7), 35.4 (1C, C-5), 109.0 (1C, C-10), 110.5 (1C, C-4), 127.9 (1C, C-3a), 128.6 (2C, C-10a, C-9a), 129.5 (C-8), 130.0 (1C, C-9), 134.2 (1C, C-4a), 155.5 (1C, C-2). Exact mass (APCI): *m/z* = 201.1020 (calcd. 201.1022 for $C_{12}H_{13}N_2O$ [MH]+). IR (neat): ṽ [cm^{-1}] = 2929 (m, νC-H), 1712(s, νC═O Urea), 1639 (w, νC═C konj). Purity (HPLC): tR = 17.14 min, purity 96.4%.

4.1.16. 1,3-Dimethyl-3,5,6,7-tetrahydro[7]annuleno[f]benzimidazol-2(1H)-one (**24**)

Tetrabutylammonium iodide (0.44 g, 1.2 mmol) and methyl iodide (3.0 mL, 47.9 mmol) were added to a suspension of alkene 23 (1.2 g, 6.0 mmol) in toluene (70 mL) and 50% aqueous NaOH (23 mL). The mixture was stirred vigorously and heated to 30 °C for 24 h. Then, water was added and the separated aqueous layer was

extracted with CH_2Cl_2 (15×), dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The crude product was purified by flash column chromatography (Ø = 4.0 cm, h = 17.0 cm, cyclohexane:ethyl acetate = 2:8, V = 30 mL) to give a solid, mp 161–162 °C, yield 0.965 g (71%). $C_{14}H_{16}N_2O$ (228.3). TLC (cyclohexane:ethyl acetate = 2:8): Rf = 0.44. ^{1}H NMR (D6-DMSO): δ [ppm] = 1.83–1.91 (m, 2H, 6-H), 2.34 (tdd, 3 J = 6.6 Hz, 3 J = 4.5 Hz, 3 J = 2.0 Hz, 2H, 7-H), 2.78–2.85 (m, 2H, 5-H), 3.29 (s, 3H, NCH3), 3.28 (s, 3H, NCH_3), 5.79 (dt, 2J = 12.2 Hz, 3J = 4.5 Hz, 1H, 8-H), 6.43 (dt, 2J = 12.2 Hz, 3J = 2.1 Hz, 1H, 9-H), 6.91 (s, 1H, 10-H), 6.94 (s, 1H, 4-H). ^{13}C NMR (D6-DMSO): δ [ppm] = 26.88 (1C, NCH_3), 26.89 (1C,, NCH_3), 27.3 (1C, C-6), 31.7 (1C, C-7), 35.4 (1C, C-5), 108.2 (1C, C-10), 109.6 (1C, C-4), 127.8 (1C, C-10a), 128.4 (1C, C-9a), 129.1 (1C, C-3a), 129.8 (1C, C-8), 130.0 (1C, C-9), 134.7 (1C, C-4a), 154.0 (1C, C-2). Exact mass (APCI): *m*/*z* = 229.1369 (calcd. 229.1335 for $C_{14}H_{17}N_2O$ [MH]+). IR (neat): ṽ [cm^{-1}] = 2916(w, νC-H), 1697 (s, νC═O Urea), 1512 (m, νC═C). Purity (HPLC): tR = 21.24 min, purity 68.3%.

*4.1.17. (5RS, 6SR)- 1,3-dimethyl-5,6-epoxy-3,5,6,7,8,9-hexahydro[7]annuleno[f]imidazol-2(1H)-one (**25**)*

Alkene 24 (0.968 g, 4.2 mmol) was dissolved in CH_2Cl_2 (117 mL). 0.5 M aqueous sodium carbonate solution (40 mL) was added and the mixture was stirred vigorously. Then, *m*-chloroperbenzoic acid (1.46 g, 8.5 mmol) was added portionwise. The mixture was stirred vigorously for 2 h. The layers were separated and the organic layer was extracted with 1 M NaOH (10×) and water (10×). The organic layer was dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The crude product was recrystallized from ethyl acetate to give a yellow solid, mp 146–147 °C, yield 527 mg, (51%). $C_{14}H_{16}N_2O_2$ (244.3). TLC (cyclohexane:ethyl acetate = 2:8): Rf = 0.15. ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.23–1.37 (m, 1H, 7-H), 1.66 (ddt, 3J = 14.0 Hz, 2J = 11.7 Hz, 3J = 5.8 Hz, 3J = 4.2 Hz, 1H, 8-H), 1.90 (ddtd, 3J = 14.5 Hz, 2J = 11.4 Hz, 3J = 5.8 Hz, 3J = 3.3 Hz, 1H, 8-H), 2.17 (dq, 3J = 14.1 Hz, 3J = 4.4 Hz, 7-H), 2.72 (ddd, 2J = 14.2 Hz, 3J = 5.4 Hz, 3J = 3.2 Hz, 1H, 9-H), 3.04 (ddd, 2J = 14.2 Hz, 3J = 11.8 Hz, 3J = 5.9 Hz, 1H, 9-H), 3.37 (dt, 3J = 7.5 Hz, 3J = 4.4 Hz, 1H, 6-H), 3.39 (s, 3H, NCH_3), 3.42 (s, 3H, NCH_3), 4.10 (d, 3J = 4.4 Hz, 1H, 5-H), 6.68 (s, 1H, 10-H), 7.11 (s, 1H, 4-H). ^{13}CNMR ($CDCl_3$): δ [ppm] = 22.3 (1C, C-8), 27.3 (1C, NCH_3), 27.4 (1C, NCH_3), 27.4 (1C, C-7), 32.1 (1C, C-9), 55.0 (1C, C-6), 56.2 (1C, C-5), 108.0 (1C, C—10), 109.5 (1C, C-4), 127.7 (1C, C-3a), 129.0 (1C, C-10a), 130.1 (1C, C-9a), 132.1 (1C, C-4a), 154.9 (1C, C-2). Exact mass (APCI): *m*/*z* = 245.1286 (calcd. 245.1285 for $C_{14}H_{17}N_2O_2$ [MH]+). IR (neat): ṽ [cm^{-1}] = 2928 (w, νC-H), 1690 (s, νC = O Uea), 1512 (m, νC = C), 1265 (w, νC-O Epoxid).

*4.1.18. (5RS,6SR)-5,6-Dihydroxy-1,3-dimethyl-3,5,6,7,8,9-hexahydro[7]annuleno[f]benz-imidazol-2(1H)-one (cis-**26**)*

Under N_2, sodium hydride (4.7 mg, 0.20 mmol) was added to a solution of 3-phenylpropan-1-amine (28 μL, 0.20 mmol) in abs. THF (5 mL). The mixture was stirred at ambient temperature for 1.5 h. Epoxide 25 (40 mg, 0.16 mmol) was added and the mixture was stirred at ambient temperature for 10.5 d. Then, water and saturated NH4Cl solution (20 mL) were added and the separated aqueous layer was extracted with CH_2Cl_2 (4×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The crude product was purified by flash column chromatography (Ø = 2.0 cm, h = 15.6 cm, CH_3OH:CH_2Cl_2= 0.5:9.5 + 0.75% NH3 (25%), V = 5 mL) to give a colorless solid, mp 227–228 °C, yield 5.7 mg (13%). $C_{14}H_{18}N_2O_3$ (262.3). TLC (CH_3OH:CH_2Cl_2 = 0.5:9.5 + 0.75% NH3 (25%)): Rf = 0.09. ^{1}H NMR (D6-DMSO): δ [ppm] = 1.43 (q, 2J = 10.2 Hz, 1H, 8-H), 1.60–1.79 (m, 2H, 7-H, 8-H), 1.95–2.07 (m, 1H, 7-H), 2.59 (dd, 2J = 13.8 Hz, 3J = 8.6 Hz, 1H, 9-H), 2.94–3.05 (m, 1H, 9-H), 3.28 (s, 3H, NCH_3), 3.29 (s, 3H, NCH_3), 3.67–3.74 (m, 1H, 6-H), 4.34 (d, 3J = 4.8 Hz, 1H, 6-OH), 4.74 (d, 3J = 4.2 Hz, 1H, 5-H), 5.10 (d, 3J = 4.1 Hz, 1H, 5-OH), 6.84 (s, 1H, 10-H), 7.07 (s, 1H, 4-H). ^{13}C NMR (D6-DMSO): δ [ppm] = 24.6 (1C, C-8), 27.0 (2C, 2 × NCH_3), 34.4 (1C, C-9), 34.5 (1C, C7), 72.7 (1C, C-6), 77.6 (1C, C-5), 108.0 (1C, C-10), 108.6 (1C, C-4), 127.3 (1C, C-3a), 128.0 (1C, C-10a), 134.0 (1C, C-4a), 154.1 (1C, C-9a), 206.7 (1C, C-2). Exact mass (APCI): *m*/*z* = 263.1373 (calcd. 263.1390 for $C_{14}H_{17}N_2O_2$ [MH]+). IR (neat): ṽ [cm^{-1}] = 3345 (w, br, νO-H), 2924(w, νC-H), 1670(s, νC═O Urea), 1508 (m, νC = C), 1026 (m, ΓC-O). Purity (HPLC): tR = 12.63 min, purity 96.1%.

*4.1.19. (5RS,6RS)-5,6-Dihydroxy-1,3-dimethyl-3,5,6,7,8,9-hexahydro[7]annuleno[f]benz-imidazol-2(1H)-one (trans-**27**)*

Epoxide **25** (40 mg, 0.16 mmol) was dissolved in abs. CH_2Cl_2 (5 mL). 3-Phenylpropylamine (28 μL, 0.20 mmol) and $BF_3.OEt_2$ (catalytic amount) were added and the mixture was stirred at ambient temperature for 17 h. Then, aqueous saturated sodium hydrogen carbonate solution was added and the separated aqueous layer was extracted with CH_2Cl_2 (4×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The crude product was purified by flash column chromatography (Ø = 2.0 cm, h = 15.5 cm, CH_3OH:CH_2Cl_2 = 0.5:9.5 + 0.75% NH_3 (25%), V = 5 mL) to give a colorless solid, mp 214–215 °C, yield 8.3 mg (19%). $C_{14}H_{18}N_2O_3$ (262.3). TLC (CH_3OH:CH_2Cl_2 = 0.5:9.5 + 0.75% NH_3 (25%)): Rf = 0.19. ^{1}H NMR (D6-DMSO): δ [ppm] = 1.20–1.34 (m, 1H, 8-H), 1.63 (tdd, 2J = 12.9 Hz, 3J = 8.5 Hz, 3J = 3.4 Hz, 1H, 7-H), 1.78–1.90 (m, 1H, 8-H), 1.92–2.02 (m, 1H, 7-H), 2.62–2.71 (m, 1H, 9-H), 2.82 (dd, 2J = 14.5 Hz, 3J = 6.2 Hz, 1H, 9-H), 3.28 (s, 3H, NCH_3), 3.30 (s, 3H, NCH_3), 4.54 (dd, 3J = 8.2 Hz, 2J = 3.3 Hz, 1H, 5-H), 4.77 (d, 3J = 3.8 Hz, 1H, 6-OH), 5.26 (d, 2J = 3.4 Hz, 1H, 5-OH), 6.88 (s, 1H, 10-H), 7.24 (s, 1H, 4-H). The signal by the CH of the OH group is not seen in the spectrum. ^{13}C NMR (D6-DMSO): δ [ppm] = 24.6 (1C, C-8), 26.9 (1C, NCH3), 27.0 (1C, NCH3), 34.1 (1C, C-9), 36.1 (1C, C-7), 72.0 (1C, C-6), 75.0 (1C, C-5), 105.4 (1C, C-4), 108.2 (1C, C-10), 127.7 (1C, C-10a), 128.0 (1C, C-3a), 133.5 (1C, C-9a), 134.6 (1C, C-4a), 154.1 (C-2). Exact mass (APCI): *m*/*z* = 263.1378 (calcd. 263.1390 for $C_{14}H_{19}N_2O_3$ [MH]+). IR (neat): ṽ [cm^{-1}] = 3352 (w, br, νO-H), 2916(w, νC-H), 1701(m, νC = O Urea), 1508 (m, νC = C), 1026 (m, ΓC-O). Purity (HPLC): tR = 12.92 min, purity 98.4%.

*4.1.20. (5RS,6RS)-6-Hydroxy-1,3-dimethyl5-[(3-phenylpropyl)-amino]-3,5,6,7,8,9-hexahydro[7]annuleno[f]-benzimidazol-2(1H)-one (**28**)*

Under N_2, 1.0 M lithium bis(trimethylsilyl)amide (169 μL, 0.20 mmol) was added to a solution of 3-phenylpropan-1-amine (28 μL, 0.20 mmol) in abs. THF (5 mL). The mixture was stirred at ambient temperature for 0.5 h. Epoxide 25 (40 mg, 0.16 mmol) was added and the mixture was stirred at ambient temperature for 11.5 d. Then, water was added and the separated aqueous layer was extracted with CH_2Cl_2 (4×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The crude product was purified by flash column chromatography (Ø = 2.0 cm, h = 15.3 cm, CH_3OH:CH_2Cl_2 = 0.5:9.5 + 0.75% NH_3 (25%), V = 5 mL) to give a pale yellow solid, mp 65–66 °C, yield 16.9 mg (27%). $C_{23}H_{29}N_3O_2$ (379.5). TLC (CH_3OH:CH_2Cl_2 = 0.5:9.5 + 0.75% NH_3 (25%)): Rf = 0.21. ^{1}H NMR (CDCl3): δ [ppm] = 1.37–1.50 (m, 1H, 8-H), 1.66–1.78 (m, 1H, 7-H), 1.96 (dtd, 2J = 13.8 Hz, 3J = 7.2 Hz, 3J = 3.1 Hz, 3H, 8-H, $NHCH_2CH_2CH_2Ph$), 2.09–2.19 (m, 1H, 7-H), 2.57 (dt, 2J = 11.7 Hz, 3J = 7.1 Hz, 1H, 9-H), 2.72 (t, 3J = 7.7 Hz, 2H, $NHCH_2CH_2CH_2Ph$), 2.80–2.91 (m, 3H, 9-H, $NHCH_2CH_2CH_2Ph$), 3.39 (s, 3H, NCH_3), 3.41 (s, 3H, NCH_3), 3.87 (d, 3J = 8.5 Hz, 5-H), 6.75 (s, 1H, 10-H), 6.91 (s, 1H, 4-H), 7.15–7.20 (m, 3H, Ar–H), 7.24–7.30 (m, 2H, Ar–H). Signals for the protons of the 6-CH group and NH and OH groups are not seen in the spectrum. ^{13}C NMR ($CDCl_3$): δ [ppm] = 24.9 (1C, C-8),

27.4 (2C, 2 × NCH3), 31.5 (1C, $NHCH_2CH_2CH_2Ph$), 33.5 (1C, $NHCH_2CH_2CH_2Ph$), 34.8 ($NHCH_2CH_2CH_2Ph$), 37.2 (1C, C-7), 47.5 (1C, C-9), 66.4 (1C, C-5), 71.3 (1C, C-6), 104.6 (1C, C-4), 109.0 (1C, C-10), 126.1 (1C, C-Arp), 128.5 (2C, C-Aro), 128.55 (2C, C-Arm), 128.6 (1C, C-10a), 128.8 (1C, C-3a), 131.1 (1C, C-4a), 135.7 (1C, C-9a), 141.7 (1C, C-Arq), 155.0 (1C, C-2). Exact mass (APCI): m/z = 380.2306 (calcd. 380.2333 for $C_{14}H_{17}N_2O_2$ [MH]+). IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3390 (w, br, νO-H), 3337 (w, νN-H), 2924(w, νC-H), 1682(s, νC = O Urea), 1508 (m, νC = C), 1396 (m, ΓO-H), 1045 (w, ΓC-O), 741 (w, Γmonosubst. arom), 698 (w, Γmonosubst. arom). Purity (HPLC): tR = 16.09 min, purity 83.3%.

4.1.21. 1,3-Dimethyl-5,7,8,9-tetrahydro[7]annuleno[f] benzimidazole-2,6(1H,5H)-dione (**29**)

Under N_2, a solution of 3-phenylpropan-1-amine (28 μL, 0.20 mmol) in abs. THF (4 mL) was slowly added dropwise to a 1 M solution of methylmagnesium bromide (200 μL, 0.20 mmol). The mixture was heated to 35 °C for 1 h. The mixture was cooled to ambient temperature. A solution of epoxide 25 (40 mg, 0.16 mmol) in abs THF (3 mL) was slowly added and the reaction mixture was heated to 35 °C for 7.5 h. Then, water (5 mL), saturated aqueous NH_4Cl solution (20 mL) and CH_2Cl_2 (10 mL) were added. 2 M HCl (6 mL) was added and the separated aqueous layer was extracted with CH_2Cl_2 (2×). The separated aqueous layer was alkalized by addition of 10% aqueous NaOH (10 mL) and extracted with CH_2Cl_2 (2×). The combined organic layers were dried (Na2SO4), filtered and the solvent was removed in vacuo. The crude product was purified by flash column chromatography (Ø = 2.0 cm, h = 15 cm, CH_3OH:CH_2Cl_2 = 0.5:9.5 + 0.75% NH_3 (25%), V = 5 mL) to give a pale yellow solid, mp 162–163 °C, yield 14.5 mg (36%). $C_{14}H_{16}N_2O_2$ (244.3). TLC (CH_3OH:CH_2Cl_2 = 0.5:9.5 + 0.75% NH_3 (25%)): Rf = 0.33. ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.96–2.04 (m, 2H, 8-H), 2.56 (t, 3J = 6.9 Hz, 2H, 7-H), 2.97–3.01 (m, 2H, 9-H), 3.39 (s, 3H, NCH_3), 3.40 (s, 3H, NCH_3), 3.75 (s, 2H, 5-H), 6.78 (s, 2H, 4-H, 10-H). ^{13}C NMR ($CDCl_3$): δ [ppm] = 27.2 (2C, 2 × NCH_3), 27.4 (1C, C-8), 33.3 (1C, C-9), 43.9 (1C, C-7), 50.2 (1C, C-5), 108.4 (1C, C-10), 108.8 (1C, C-4), 126.6 (1C, C-3a), 129.0 (1C, C-10a), 129.3 (1C, C-9a), 133.9 (1C, C-4a), 154.8 (1C, C-2), 208.9 (1C, C-6). Exact mass (APCI): m/z = 245.1345 (calcd. 245.1285 for $C_{14}H_{17}N_2O_2$ [MH]+). IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2920(w, νC-H), 1686(m, νC = O Urea), 1508 (m, νC = C). Purity (HPLC): tR = 15.73 min, purity 78.2%.

4.1.22. 1,3-Dimethyl-6-[(3-phenylpropyl)amino]-3,5,6,7,8,9-hexahydro[7]annuleno[f]-benzimidazol-2(1H)-one (**30**)

3-Phenylpropan-1-amine (24 μL, 0.168 mmol) and NaBH(OAc)3 (35.6 mg, 0.168 mmol) were added to a solution of ketone 29 (20.5 mg, 0.084 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred at ambient temperature for 45 min. Saturated aqueous $NaHCO_3$ solution (10 mL) was added and the separated aqueous layer was extracted with CH_2Cl_2 (4×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The crude product was purified by flash column chromatography (Ø = 2.0 cm, h = 16 cm, CH_3OH:CH_2Cl_2 = 0.5:9.5 + 0.75% NH_3 (25%), V = 5 mL) to give a yellow oil, yield 12.9 mg (42%). $C_{23}H_{29}N_3O$ (363.5). TLC (CH_3OH:CH_2Cl_2 = 0.5:9.5 + 0.75% NH3 (25%)): Rf = 0.16. ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.43–1.57 (m, 1H, 8-H), 1.70–1.79 (m, 1H, 7-H), 1.79–1.90 (m, 3H, 8-H, NHCH2CH2CH2Ph), 1.95–2.05 (m, 1H, 7-H), 2.61–2.85 (m, 7H, 9-H, 6-H, NHCH2CH2CH2Ph), 2.93 (d, 3J = 7.8 Hz, 2H, 5-H), 3.38 (s, 6H, 2 × NCH3), 6.71 (s, 1H, 10-H), 6.74 (1H, 4-H), 7.13–7.21 (3H, Ar–H), 7.22–7.30 (2H, Ar–H). ^{13}C NMR ($CDCl_3$): δ [ppm] = 25.8 (1C, C-8), 27.29 (1C, NCH_3), 27.33 (1C, NCH3), 31.6 (1C, $NHCH_2CH_2CH_2Ph$), 33.8 (1C, $NHCH_2CH_2CH_2Ph$), 36.1 (1C, C-9), 38.1 (1C, C-7), 42.0 (1C, C-5), 46.5 (1C, $NHCH_2CH_2CH_2Ph$), 56.7 (1C, C-6), 108.2 (1C, C-10), 109.4 (1C, C-4), 126.0 (1C, Ar–C), 128.3 (2C, C-3a, C-10a), 128.45 (2C, Ar–C), 128.47 (2C, Ar–C), 131.5 (1C, C-4a), 136.7 (1C, C-9a), 142.0 (1C, Ar-Cq). Exact mass (APCI): m/z = 364.2370 (calcd. 364.2383 for $C_{23}H_{30}N_{3O}$ [MH]+). IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3503 (w, νN-H), 2920(w, νC-H), 1686(s, νC = O Urea), 1508 (m, νC = C), 1458 (m, ΓN-H), 1045 (w, ΓC-O), 741 (w, Γmonosubst. arom), 698 (w, Γmonosubst. arom). Purity (HPLC): tR = 17.73 min, purity 94.3%.

4.2. Receptor binding studies

4.2.1. Materials and general procedures

Centrifuge: High-speed cooling centrifuge model Sorvall RC-5C plus (Thermo Finnigan). Filter: Printed Filtermat Type B (Perkin–Elmer), presoaked in 0.5% aqueous polyethylenimine for 2 h at rt before use. The filtration was carried out with a MicroBeta FilterMate-96 Harvester (Perkin–Elmer). The scintillation analysis was performed using Meltilex (Type A) solid scintillator (Perkin–Elmer). The scintillation was measured using a MicroBeta Trilux scintillation analyzer (Perkin–Elmer). The overall counting efficiency was 20%.

4.2.2. Cell culture and preparation of membrane homogenates for the GluN2B assay [43]

In the assay mouse L(tk-)-cells stably transfected with the dexamethasone inducible eukaryotic expression vectors pMSG NR1a, pMSG NR2B in a 1:5 ratio were used. The transformed L(tk-)-cells were grown in Modified Earl's Medium (MEM) containing 10% of standardized FCS (Biochrom AG, Berlin, Germany). The expression of the NMDA receptor at the cell surface was induced after the cell density of the adherent growing cells had reached approximately 90% of confluency. For the induction, the original growth medium was replaced by growth medium containing 4 μM dexamethasone and 4 μM ketamine (final concentration). After 24 h the cells were harvested by scraping and pelleted (10 min, 5000 × g, Hettich Rotina 35R centrifuge, Tuttlingen, Germany).

For the binding assay, the cell pellet was resuspended in phosphate buffer saline (PBS, pH 7.4) buffer and the number of cells was determined using an improved Neubauer's counting chamber (VWR, Darmstadt, Germany). Subsequently, the cells were lysed by sonication (4 °C, 6 × 10 s cycles with breaks of 10 s, device: Soniprep 150, MSE, London, UK). The resulting cell fragments were centrifuged with a high performance cool centrifuge (20,000 × g, 4 °C, Sorvall RC-5 plus, Thermo Scientific). The supernatant was discarded and the pellet resuspended in a defined volume of PBS yielding cell fragments of approximately 500,000 cells/mL. The suspension of membrane homogenates was sonicated again (4 °C, 2 × 10 s cycles with a break of 10 min) and stored at −80 °C.

4.2.3. Performing of the GluN2B binding assay [43]

The competitive binding assay was performed with the radioligand [3H]ifenprodil (60 Ci/mmol; Perkin Elmer) using standard 96-well-multiplates (Diagonal, Münster, Germany). The thawed cell membrane preparation (about 20 μg protein) was incubated with 6 different concentrations of test compounds, 5 nM [3H]-ifenprodil, and TRIS/EDTA-buffer (5 mM/1 mM, pH 7.5) in a total volume of 200 μL for 120 min at 37 °C. The incubation was terminated by rapid filtration through the presoaked filtermats by using the cell harvester. After washing each well five times with 300 μL of water, the filtermats were dried at 95 °C. Subsequently, the solid scintillator was placed on the filtermat and melted at 95 °C. After 5 min, the solid scintillator was allowed to solidify at rt. The bound radioactivity trapped on the filters was counted in the scintillation analyzer. The non-specific binding was determined with 10 μM unlabeled ifenprodil. The Kd-value of ifenprodil is 10 nM [43].

4.2.4. Affinity towards σ1 and σ2 receptors

The affinity towards σ1 and σ2 receptors [44–46] was recorded as previously described.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.03.065.

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