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Synthesis and investigations of double-pharmacophore ligands for treatment of chronic and neuropathic pain

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Abstract

Acids **9 a–f** as possible bivalent ligands designed as a structural combination of opioid μ-agonist (Fentanyl) and NSAID (Indomethacin) activities and produced compounds which were tested as analgesics. The obtained series of compounds exhibits low affinity and activity both at opioid receptors and as cyclooxygenase (COX) inhibitors. One explanation of the weak opioid activity could be stereochemical peculiarities of these bivalent compounds which differ significantly from the fentanyl skeleton. The absence of significant COX inhibitory properties could be explained by the required substitution of an acyl fragment in the indomethacin structure for 4-piperidyl.

1. Introduction

Since 1990, at least 50 new chemical entities for the treatment of pain have reached clinical development [1], but no one was marketed. Management of chronic pain is often highly individualized and combination therapy on a trial and error basis is not uncommon. During the last few years combined use of non-steroidal anti-inflammatory drugs (NSAIDs) and opioids has been indicated for achieving better analgesia with reduced side effects [2–15].

The synergistic effect in peripheral tissues is thought to result from actions on a common transduction pathway between NSAIDs and opioids [5,16–18], although other mechanisms have been proposed [19]. Multicomponent drugs where two or more agents are co-formulated in a single dosage form are increasingly popular in drug treatment. An alternative strategy is to develop a single chemical entity that is able to modulate multiple targets simultaneously [20–22].

There is an increasing readiness to challenge the current paradigm and to consider developing agents that modulate multiple targets simultaneously with the aim of enhancing efficacy or improving safety relative to drugs that address only a single target [22–29].

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For these reasons, we hypothesize that bivalent ligands designed as a combination of two different pharmacophores with μ opioid agonist and NSAID activity could show antinociceptive efficacy in chronic neuropathic pain states. For this paper, Fentanyl molecule was chosen as a structural framework for the creation of possible bivalent ligands for pain relief.

Fentanyl, an opioid with an analgesic potency of about 80 times greater than that of morphine, was introduced into medical practice in the 1960s [30–33].

NSAIDs (COX inhibitors) which are used widely for the treatment of pain and inflammation states have been reviewed [34–37].

In this paper, we will focus on indolyl-acetic acids in our construction of possible bifunctional opioid agonist/COX inhibitor ligands. The development of indolyl / indene-acetic acids derivatives have involved structural modifications of structures of the most popular drugs of this class of compounds, mainly Indomethacine [38,39]., Many structural changes have been made on indomethacin molecule. Compounds such as Sulindac [40] and Etodolac [41] are already marketed as pharmaceuticals, and others like L-748780 and, L-761066 [42,43] are still under investigation (Fig. 1).

2. Results and discussion

2. 1. Chemistry

The main goal of this investigation was to develop a single chemical entity combining pharmacophore elements of two powerful analgesics acting by different mechanisms into one molecule. By analogy with approaches to the creation of peptide drugs, where the combination of two active peptides gives excellent results, we hypothesized that combination of the essential parts of two small molecules - the known opioid Fentanyl with the known COX inhibitor Indomethacin might lead to new pharmacological properties (Fig. 2).

N-(Piperidin-4-yl)-N-phenylhydrazines **5** have been designed as starting materials for creation of indolylpiperidine derivatives (Scheme 1). They were synthesized starting from piperidine-4-ones **1a–c** which were condensed with aniline or substituted anilines to give the desired imines (Schiff bases) **2a–f**, then hydrogenated with sodium borohydride to obtain 4-anilinopiperidines **3 a–f**. These 4-anilinopiperidines were nitrosylated to produce N-nitroso-compounds **4 a–f**, then hydrogenated to the desired 4-piperidylphenylhydrazines **5 a–f**. 4-Piperidylphenylhydrazines **5 a–f** were condensed with levulinic acid or its esters and obtained hydrazones **6 a–f** and **7 a–f** underwent Fisher type reactions which led to indole derivatives – a series of indomethacine analogs **8 a–f** and **9 a–f**. Different catalysts have been evaluated for this reaction [44,45] and the optimal one was found to be the simplest – HCl in ethanol for both cases **6→9** and **7→8**. Basic hydrolysis of **8 a–f** led to the desired **9 a–f**. The sequence **5→7→8→9** was found optimal for its ease of separation and purification of products. These heterocyclization reactions present unlimited possibilities for creation of a wide diversity of noncondensed indolopiperidines.

2.2 Molecular Modeling

Docking of small molecules was performed using FlexiDock within the Sybyl 7.2 suite of programs [46]. Ligand molecules were constructed using Sybyl 7.2/Builder module. The piperidine nitrogen was protonated while the acid group was used as a carboxylate. The X-ray crystal structure of cyclooxygenase-2 with indomethacin (PDB code: 4cox) [47] was used for docking studies. Initially, the ligand molecule is placed within the active site of the protein and this complex was minimized using 1000 steps of minimization. This minimized complex was used as an input for FlexiDock calculations. FlexiDock calculations involved selecting

rotatable bonds for the ligand and then exploring orientations of the ligand within the protein active site. FlexiDock results were obtained as various orientations of the ligand within the active site. These different orientations were analyzed on the basis of FlexiDock score as well as interactions between ligand and the enzyme active site.

A homology model for the μ opiate receptor was built using Sybyl / Biopolymer module. The X-ray crystal structure of bovine rhodopsin (PDB code: 1F88) was used as a template protein [48]. Amino acid residues were replaced by the corresponding μ opioid receptor residues and the model structure was refined and used for Flexidock calculations. Flexidock scores for series **9a – f** were obtained as described above and the data is presented in the Table 1.

Since more negative the FlexiDock scores result in better binding, the compounds show better binding at both COX2 and the μ opiate receptor. Compounds **9a–f** show COX-2 inhibitor activity practically equal to indomethacin and opioid activity 2–3 times higher than that of Fentanyl. Thus, docking experiments showed marginal results; but unfortunately, the biological activity data is not consistent with the results from molecular modeling.

2.3 Pharmacology

Table 2 shows the functional characterization of compounds **9a–f** at δ- and μ-opioid receptors, using guinea pig isolated ileum/longitudinal muscle myenteric plexus (GPI/LMMP) as a source of μ opioid receptors and mouse vas deferens (MVD) as a source of δ opioid receptors.

The data indicate that the ligands have a very weak range of bioactivities at both δ and μ opioid receptors, practically independent on their respective structures. The weak or lack of opioid activity (agonist or antagonist) is likely due to low affinity of these compounds at opioid receptors, which was confirmed by radioligand competition assays. Compounds **9c** and **9f** (direct Fentanyl analogues) competed against the binding of the opioid receptor antagonist [^{3}H]diprenorphine in rat brain membranes with inhibition constants in the micromolar range (data not shown).

We tested the effect of **9a–f**, at a concentration of 50 nM, on COX-1 or COX-2 mediated prostaglandin production by an enzymatic immunoassay (EIA). We chose 50 nM as the test concentration based on the rationale that a lead compound should have an IC_{50} value at COX of equal or less than 100 nM, or within an order of magnitude of its affinity (commonly in the nanomolar range) for opioid receptors. The parent compound of **9a–f**, indomethacin, inhibits both COX-1 and COX-2, and is more potent at COX-1. The IC_{50} values for indomethacin in inhibiting prostaglandin production ranged between 40–80 nM for COX-1 and 600–1000 nM for COX-2 [49], whereas COX-2 selective compounds such as celecoxib showed IC_{50} values in the low nanomolar range for COX-2 in the same assay [49]. However, compounds **9a–f** at 50 nM did not inhibit prostaglandin production by COX-1 or COX-2.

Compounds **9a–f** were also evaluated in rodent models of acute and chronic pain. The compounds, each given as a single dose of 10 μg into the lumbar spinal cord of naïve Sprague-Dawley rats by intrathecal injection, did not prolong the latency to a noxious thermal stimulus to the hind paw, indicating a lack of antinociceptive activity at least at the dose given (data not shown). In a rat model of neuropathic pain, the same dose of these compounds given intrathecally also had no effect on the abnormal pain state associated with the nerve injury in the injured rats, or on the sensory thresholds of control rats that were given a sham surgery (data not shown). The lack of antinociceptive activity of these compounds is in line with the apparent low affinity and lack of biological activity at opioid receptors indicated by the *in vitro* assays.

3. Discussions

A possible explanation of these results could be related to the stereochemical peculiarities of these compounds which could differ from the fentanyl structure. For a comparison of structures of Fentanyl and the 2-methyl-1-(1-substituted-piperidin-4-yl)-1H-indol-3-yl]-acetic acids, an X-Ray analysis of the **8c** (Fig. 3) and superposition of its structure with the X-Ray structure of fentanyl has been examined (Fig. 4–Fig. 6). The Fentanyl data is taken from the Cambridge Structural Database [50,51].

An exact overlay of the phenyl and piperidine rings with the linker is prevented by differences in their torsion angles. Overlay 1 (Fig. 4) is a least-squares average overlay of these two rings. Overlay 2 (Fig. 5) has only the phenyl rings overlapping and Overlay 3 (Fig. 4) overlaps only the piperidine rings. In all three diagrams compound **8c** is in red.

Because it is well documented that the biological activity of Fentanyl and its derivatives is greatly influenced by their stereochemistry[50] the differences in the structure between compounds **9a–f** and Fentanyl could account for the loss of affinity and efficacy at μ opioid receptor. For optimal interaction with the μ opioid receptor it is necessary to have both a *trans* configuration for amide group with the orientation of phenyl ring practically perpendicular to the amide function a 4-N-propionylamide substituent with the ϕ angle (5-4-11-7) in the range of 0 – 30° and an extended conformation of the phenethyl group (Fig 7).

On the other hand, the compounds' lack of COX inhibitory activity at 50 nM suggests that their IC_{50} values may be significantly larger than 50 nM, or that the substitution of a necessary acyl fragment in the Indomethacin structure for 4-piperidyl has caused a right shift of the dose effect at COX.

4. Conclusions

The overall conclusion is that this series of novel hybrid fentanyl/indomethacin compounds exhibits low affinity and activity at the opioid receptors and COX. Identification of small molecule bi-ligand drugs with a high binding affinity is a very difficult task. Bi-ligand drug candidates are very dependent on the linker between the two active pharmacophores, its nature, length and flexibility, places of attachments, etc. These factors, which are not very decisive for peptide pairs, become critical for small molecules, which are very sensitive to the minor changes in structure, where arrangement of every atom is crucial. Our attempt to combine opioid and NSAID pharmacophores in one small molecule, which succeeded as a chemical approach, gave unsuccessful pharmacological results. The fentanyl/indomethacin hybrid molecule obtained lost both opioid and COX inhibition properties, which demonstrates that implementation of approaches in peptide science are not necessarily applicable to small molecules.

5. Experimental

5.1. Chemistry

The compounds were characterized by ^{1}H and ^{13}C NMR. Nuclear magnetic resonance spectra were recorded with on Bruker DRX-500 and DRX-600 spectrometers using tetramethylsilane as internal standard. IR spectra were recorded on Nicolet Avatar 360 FT-IR instrument in mineral oil. The compounds were analyzed by electrospray mass spectrometry on a Thermoelectron (Finnigan) LCQ classic ion trap mass spectrometer. Standard ESI conditions were applied and the masses of protonated molecules $[MH]^{+}$ were measured. Direct infusion (10 uL/min) was applied to ca. 50 uM solutions of the samples in MeOH:H_2O 1:1 2% AcOH.

High resolution mass spectra were obtained on the 9.4 T Bruker FT-ICR-MS spectrometer in MeOH:ACN 1:1. The purity of compounds was determined by TLC on silica gel plates (Analtech 02521), solvent system MeOH:$CHCl_3$ 1:4. Melting points are uncorrected.

5.1.1 General method for the synthesis of the N-(1-Substituted-piperidin-4-yl) anilines 3a–f—A solution of 0.1 mol of a 1-substituted-piperidin-4-one, 0.125 mol of the appropriate phenylamine and 2–3 drops of acetic acid in 200 mL of toluene was heated on stirring under reflux (2–3-hours) with a Dean-Stark trap till the full separation of water. The toluene and excess of phenylamine were removed *in vacuo*. The residue was dissolved in 200 mL of ether and filtered through a layer of neutral alumina. The remaining pretty pure compound was used for hydrogenation without further purification. In cases with 1-methyl-piperidin-4-ylidene derivatives distillation of the products is recommended 132°C (2 mm Hg) for **2a**; 138°C (4 mm Hg) for **2d**.

The 1-substituted-piperidin-4-ylidene derivatives **2a–f** (0.1 mol) were dissolved in 150 mL of methanol and 3.8 g (0.1 mol) of $NaBH_4$ was added gradually, following stirring during 30 minutes. Obtained solution was stirred for additional 4–5 hours an left over night. The residue was dissolved into ether, dried over $MgSO_4$, filtered and the solvent was removed in vacuum. Remaining solid was crystallized. Recrystallized from hexanes or from diethyl ether. Total yields are varying between 60–76%.

5.1.2. (1-Methyl-piperidin-4-yl)-phenyl-amine (3a)—Crystalline solid (60.7%), mp 81–83 °C, (bp 140–141 °C (4mm Hg). IR: ν (cm^{-1}) = 3390 (N–H); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.14 (m, 2H), 6.66 (m, 1H), 6.57 (m, 2H), 3.47 (d, 1 H, *J* = 7.79 Hz), 3.27 (m, 1 H), 2.81 (d, 2H, *J* = 11.00 Hz), 2.29 (s, 3 H), 2.13 (t, 2H, *J* = 11.46 Hz), 2.05 (m, 2H), 1.49 (2 H, m). EI-MS: *m/z* 191; HRMS calcd for $C_{12}H_{19}N_2$: 191.1543; found (ESI, $[M+H]^+$): 191.1539.

5.1.3. (1-Benznyl-piperidin-4-yl)-phenyl-amine (3b)—Crystalline solid (72,3%), mp 84–86 °C. IR: ν (cm^{-1}) = 3392 (N–H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.34 (m, 4 H), 7.27 (m, 1H), 7.17 (m, 2H), 6.69 (m, 1H), 6.60 (m, 2H), 3.55 (s, 2H), 3.52 (d, 1H, *J* = 7.72 Hz), 3.31 (m 1H), 2.87 (d, 2 H, *J* = 11.71 Hz), 2.17 (m, 2H), 2.05 (m, 2H), 1.50 (m 2H). EI-MS: *m/z* 267. HRMS calcd for $C_{18}H_{32}N_2$: 267.1856; found (ESI, $[M+H]^+$): 267.1859.

5.1.4. [1-(2-phenyl)-ethyl-piperidin-4-yl]-phenyl-amine (3c)—Crystalline solid (75.1%), mp 96–98 °C. IR: ν (cm^{-1}) = 3390 (N–H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.28 (m, 2H), 7.20 (m, 3H), 7.16 (m, 2H), 6.68 (m, 1H), 6.59 (m, 2H), 3.52 (d, 1H, *J* = 7.85 Hz), 3.32 (m, 1H), 2.98 (d, 2 H, *J* = 11.40 Hz), 2.83 (m, 2 H), 2.63 (m, 2H), 2.23 (t, 2H, *J* = 10.96 Hz), 2.09 (d, 2 H, *J* = 11.86 Hz), 1.53 (m, 2H). EI-MS: *m/z* 281. HRMS calcd for C19H24N2: 281.2012; found (ESI, $[M+H]^+$): 281.2007.

5.1.5. 4-Methoxyphenyl-(1-Methyl-piperidin-4-yl)-amine (3d)—Crystalline solid (65.0%), mp 46–47 °C, (bp 174–175 °C (4mm Hg). IR: ν (cm^{-1}) = 3391 (N–H). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.72 (m, 2H), 6.54 (m, 2H), 3.70 (s, 3H), 3.17 (m, 2H), 2.77 (d, 2 H, *J* = 11.19 Hz), 2.26 (s, 3H), 2.07 (t, 2H, *J* = 11.20 Hz), 2.00 (d, 2 H, *J* = 12.60 Hz), 1.43 (m, 2H). EI-MS: *m/z* 221. HRMS calcd for $C_{13}H_{20}N_2O$: 221.1648; found (ESI, $[M+H]^+$): 221.1655.

5.1.6. 4-Methoxyphenyl-(1-Benznyl-piperidin-4-yl)-amine (3e)—Crystalline solid (76,7%), mp 64–66 °C. IR: ν (cm^{-1}) = 3393 (N–H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.32 (m, 4H), 7.25 (m, 1H), 6.75 (m, 2H), 6.56 (m, 2H), 3.72 (s, 3H), 3.53 (s, 2H), 3.20 (m, 1H), 2.85 (d, 2H, *J* = 11.53 Hz), 2.14 (m, 2H), 2.01 (d, 2H, *J* = 12.21 Hz), 1.47 (m, 2H). EI-MS: *m/z* 297. HRMS calcd for $C_{19}H_{25}N_2O$: 297.1961 found (ESI, $[M+H]^+$): 297.1966.

5.1.7. 4-Methoxyphenyl-[1-(2-phenyl)-ethyl-piperidin-4-yl]-amine (3f)—Crystalline solid (74,8 %), mp 93–95 °C. IR: ν (cm^{-1}) = 3390 (N–H). ^1H NMR (600 MHz, $CDCl_3$) δ 7.27 (m, 2H), 7.19 (m, 3H), 6.76 (m, 2H), 6.57 (m, 2H), 3.73 (s, 3H), 3.24 (m, 1H), 3.00 (m, 2H), 2.84 (m, 2H), 2.65 (m, 2H), 2.24 (m, 2H), 2.08 (m, 2H), 1.53 (m, 2H). EI-MS: *m/z* 311. HRMS calcd for $C_{20}H_{27}N_2O$: 311.2118 found (ESI, $[M+H]^+$): 311.2126.

5.1.8. General method for the synthesis of N-(1-Substituted-piperidin-4-yl)-nitrosoanilines (4 a–f)—To a stirred cooled (ice) mixture of 0,1 mole of N-(1-Substituted-piperidin-4-yl)aniline 3(a–f) dissolved in 100 mL of ethanol and of 200 g of ice, was added (reaction was carried out under an argon atmosphere) dropvise, during 0.5 hour, 0.5 mol (~50mL of HCl conc.) diluted with 150 mL of ice cold water for N-Methyl derivatives **4a,d** and 0,75 mol (~75mL of HCl conc.) diluted with 225 mL of ice cold water for derivatives **4b,c,e,f**

After 15 min. to an ice cold solution of 0.2 mol of $NaNO_2$ in 40 mL of water was added dropvise, at −8°–5 ° C. Then the obtained mixture was stirred for an additional 4 hours at the same temperature. 200 mL of dichloromethane was added to the mixture and at the temperature below 0° C the mixture was made basic with a cold solution of 0.5 mol (for N-Methyl derivatives **4a,d**) and 0,75 mol (for derivatives **4b,c,e,f**) of K_2CO_3 in 125 (187.5) mL of water. Dichloromethane layer was separated, and the water layer was extracted two times with 50 mL portions of dichloromethane and the combained DCM dried over $MgSO_4$. The solvent was removed *in vacuo*. The residue was dissolved in 200 mL of ether and filtered through a layer of neutral alumina. Remaining after evaporation of ether pure (TLC) nitroso-compounds (sometime crystalline) were immediately used for hydrogenation without further purification. Total yields were vary between 80–95%. (Attempts to distill obtained compounds were unsuccessful due to very quick disintegration processes close to explosion which started during heating). Nevertheless Mass- Spectras registered immediately after preparation showed 100% presence of $[MH]^+$ for all cases.

5.1.9. N-(1-methyl-piperidin-4-yl)-nitrosoaniline (4a)—Crystalline residue (84.0%). IR: ν (cm^{-1}) = 1592 (N=O). EI-MS: *m/z* 220 $[M+H]^+$. MS/MS fragmentation base peak *m/z* 180 $[M+H-NO]^+$.

5.1.10. N-(1-benzyl-piperidin-4-yl)-nitrosoaniline (4b)—Crystalline residue (89.5%). IR: ν (cm^{-1}) = 1591 (N=O). EI-MS: *m/z* 296 $[M+H]^+$. MS/MS fragmentation base peak *m/z* 266 $[M+H-NO]^+$.

5.1.11. N-[1-(2-phenyl)-ethyl-piperidin-4-yl]-nitrosoaniline (4c)—Crystalline residue (95.0%). EI-MS: *m/z* 310 $[M+H]^+$. IR: ν (cm^{-1}) = 1590 (N=O). MS/MS fragmentation base peak *m/z* 280 $[M+H-NO]^+$.

5.1.12. 4-Methoxyphenyl-(1-methyl-piperidin-4-yl)-N-nitrosoamine (4d)—Light yellow liquid (77.5%). IR: ν (cm^{-1}) = 1590 (N=O). EI-MS: *m/z* 250 $[M+H]^+$. MS/MS fragmentation base peak *m/z* 220 $[M+H-NO]^+$.

5.1.13. 4-Methoxyphenyl-(1-benzyl-piperidin-4-yl)-N-nitrosoamine(4e)—Light yellow liquid (77.8%). IR: ν (cm^{-1}) = 1593 (N=O). EI-MS: *m/z* 326 $[M+H]^+$. MS/MS fragmentation base peak *m/z* 296 $[M+H-NO]^+$.

5.1.14. 4-Methoxyphenyl-[1-(2-phenyl)-ethyl-piperidin-4-yl]- N-nitrosamine (4f) —Crystalline residue (96%). IR: ν (cm^{-1}) = 1591 (N=O). EI-MS: *m/z* 340 $[M+H]^+$. MS/MS fragmentation base peak *m/z* 310 $[M+H-NO]^+$.

5.1.15. General method for the synthesis of (1-substituted-piperidin-4-yl)-N-phenyl-hydrazines (5a–f)—A N-(1-Substituted-piperidin-4-yl)-nitrosoaniline **4a–f** (0,1 mol) was dissolved in the mixture of 150 mL of dry THF and 50 mL of dry ether was dropped cautionously during one hour to a stirring and gently boiling (reaction was carried out under an argon atmosphere) mixture of 0.125 mol of $LiAlH_4$ in 450 mL of dry ether. Stirring and gentle boiling was continued for 3 hours and the mixture was left under Argon at room temperature overnight. Then the mixture which was cooled to −5°– 0° C and X very slowly, cautionously was added 3.8 mL of water, then after 10–15 min. 3.8 mL of 15% NaOH, and then after additional 10–15 min. 11,4 mL of water. The mixture was stirred at −5°– 0° C for additional 1–2 hours untill full disappearance of characteristic gray color which gave clear pale yellow solution with separated aluminum salts. This was filtered through Buchner funnel and the solution dried over $MgSO_4$. The solution was filtered through a layer of neutral alumina and the solvents were removed *in vacuo*. The remaining pure compound is possible to distill for cases **5a** and **5d** or crystallize from ether, hexanes or ethanol for cases **5b,c,e,f**. Total yields vary between 58–92%.

5.1.16. N-(1-methyl-piperidin-4-yl)- N-phenyl-hydrazine (5a)—Crystalline solid (71.5%), mp 58–60 °C. IR: ν (cm^{-1}) = 3352 (Hydrazine N-H). ^1H NMR (600 MHz, $CDCl_3$) δ 7.25 (m, 2H), 7.01 (m, 2H), 6.78 (m, 1H), 3.58 (tt, 1 H, *J* = 11.43, 3.90 Hz), 3.31 (bs, 2H), 2.96 (m, 2 H), 2.31 (s, 3H), 2.07 (dt, 2H, *J* = 2.00, 11.99 Hz), 1.95 (dq, 2H, *J* = 3.63, 12.20 Hz), 1.69 (m, 2H). EI-MS: *m/z* 206. HRMS calcd for $C_{12}H_{20}N_3$: 206.1652 found (ESI, $[M+H]^+$): 206.1660.

5.1.17. N-(1-benzyl-piperidin-4-yl)- N-phenyl-hydrazine (5b)—Crystalline solid (77.4%), mp 103–105 °C. IR: ν (cm^{-1}) = 3350 (Hydrazine N-H). ^1H NMR (600 MHz, $CDCl_3$) δ 7.36 (m, 4H), 7.27 (m, 3H), 7.01 (d, 2H, *J* = 8.16 Hz), 6.79 (t, 1H, *J* = 7.24 Hz), 3.62 (m, 3H), 3.35 (bs, 2H), 3.08 (d, 2H, *J* = 11.16 Hz), 2.20 (m, 2H), 2.05 (m, 2H), 1.71 (m, 2H). EI-MS: *m/z* 282. HRMS calcd for $C_{18}H_{24}N_3$: 282.1965 found (ESI, $[M+H]^+$): 282.1972.

5.1.18. N-[1-(2-phenyl)-ethyl-piperidin-4-yl]- N-phenyl-hydrazine (5c)—Crystalline solid (92.8%), mp 72–74 °C. IR: ν (cm^{-1}) = 3354 (Hydrazine N-H). ^1H NMR (600 MHz, $CDCl_3$) δ 7.30 (m, 4H), 7.24 (m 3H), 7.05 (m, 2H), 6.81 (m, 1H), 3.65 (tt, 1H, *J* = 11.48, 3.88 Hz), 3.35 (s, 2H), 3.17 (d, 2H, *J* = 11.67 Hz), 2.86 (m, 2H), 2.67 (m, 2H), 2.19 (t, 2H, *J* = 11.51 Hz), 2.02 (dq, 2 H, *J* = 3.68, 12.25 Hz), 1.77 (m, 2H). EI-MS: *m/z* 296. HRMS calcd for $C_{19}H_{26}N_3$: 296.2121 found (ESI, $[M+H]^+$): 296.2128.

5.1.19. 4-Methoxy-phenyl-(1-Methyl-piperidin-4-yl)-hydrazine (5d)—Light yellow liquid (64.5%). IR: ν (cm^{-1}) = 3351 (Hydrazine N-H). ^1H NMR (500 MHz, $CDCl_3$) δ 6.90 (m, 2H), 6.74 (m, 2H), 3.66 (s, 3H), 3.64 (m, 2H), 3.36 (bs, 2H), 3.26 (tt, 1H, *J* = 3.92, 11.38 Hz), 2.84 (m, 2 H), 1.93 (dt, 2 H, *J* = 2.49, 11.86 Hz), 1.78 (m, 2H), 1.59 (m, 2H). EI-MS: *m/z* 236. HRMS calcd for $C_{13}H_{22}N_3O$: 236.1757 found (ESI, $[M+H]^+$): 236.1748.

5.1.20. 4-Methoxy-phenyl-(1-Benzyl-piperidin-4-yl)- hydrazine (5e)—Crystalline solid (60.0%), mp 68–70 °C. IR: ν (cm^{-1}) = 3353 (Hydrazine N-H). 1H NMR (600 MHz, $CDCl_3$) δ 7.31 (m, 4H), 7.24 (m, 1H), 6.97 (m, 2H), 6.81 (m, 2H), 3.74 (s, 3H), 3.55 (s, 2H), 3.37 (tt, 1 H, *J* = 11.25, 3.79 Hz), 3.30 (s, 1H), 2.99 (d, 2 H, *J* = 11.70 Hz), 2.09 (m, 2H), 1.87 (m, 2H), 1.68 (d, 2 H, *J* = 11.02 Hz). EI-MS: *m/z* 312. HRMS calcd for $C_{19}H_{26}N_3O$: 312.2070 found (ESI, $[M+H]^+$): 312.2063.

5.1.21. 4-Methoxy-phenyl-[1-(2-phenyl)-ethyl-piperidin-4-yl]- hydrazine (5f)—Crystalline solid (81.5%), mp 95–96 °C. IR: ν (cm^{-1}) = 3350 (Hydrazine N-H). ^1H NMR (600 MHz, $CDCl_3$) δ 7.27 (m, 2H), 7.19 (m, 3H), 6.99 (m, 2H), 6.83 (m, 2H), 3.75 (s, 3H), 3.39 (tt,

1H, *J* = 11.05, 3.79 Hz), 3.30 (s, 2H), 3.13 (m, 2H), 2.85 (m, 2H), 2.64 (s, 2H), 2.17 (s, 2H), 1.91 (m, 2H), 1.76 (m, 2H). EI-MS: *m/z* 326. HRMS calcd for $C_{20}H_{28}N_3O$: 326.2227 found (ESI, $[M+H]^+$): 326.2222.

5.1.22. General method for the synthesis of N-[(1-substitutedl-piperidin-4-yl)-phenyl-hydrazono]-pentanoic acids (6a–f)—To a solution of 0,005 mol of any of the (1-substituted-piperidin-4-yl)-N-phenyl-hydrazines **5a–f** in 10 mL of ethanol was added drop by drop 0.0055 mol of levulinic acid in 5 mL of ethanol. The mixture was left overnight at room temperature. The crystals were filtered and dried in air. Sometimes it was necessary to separate product by flush chromatography on silica ($CHCl_3$/MeOH; 4/1). Yields were quantitative.

5.1.23. N-[(1-methyl-piperidin-4-yl)-phenyl-hydrazono]-pentanoic acid (6a)—Crystalline solid (65.4%), mp 124–126 °C. IR: ν (cm^{-1}) = 1610 (C=N), 1715 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.75 (bs, 1H), 7.21 (t, 2 H, *J* = 7.86 Hz), 6.89 (t, 1H, *J* = 7.26 Hz), 6.83 (d, 2H, *J* = 7.96 Hz), 3.51 (m, 1H,), 3.30 (d, 2H, *J* = 10.27 Hz), 2.74 (t, 2H, *J* = 6.93 Hz), 2.68 (m, 2H), 2.55 (s, 3H), 2.37 (m, 4H), 1.75 (d, 2H, *J* = 11.56 Hz), 1.66 (s, 3H). EI-MS: *m/z* 304. HRMS calcd for $C_{17}H_{26}N_3O_2$: 304.2020 found (ESI, $[M+H]^+$): 304.2022.

5.1.24. N-[(1-benzyl-piperidin-4-yl)-phenyl-hydrazono]-pentanoic acid (6b)—Crystalline solid (72.6%), mp 108–110 °C. IR: ν (cm^{-1}) = 1612 (C=N), 1718 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.63 (bs, 1H), 7.31 (m, 5H), 7.16 (t, 2H, *J* = 7.76 Hz), 6.84 (t, 1H, *J* = 7.29 Hz), 6.78 (d, 2H, *J* = 8.09 Hz), 3.90 (s 2H,), 3.41 (m, 1H), 3.23 (d, 2H, *J* = 6.88 Hz), 2.74 (t, 2H, *J* = 7.21 Hz), 2.67 (t, 2H, *J* = 6.96 Hz), 2.24 (m, 4H), 1.68 (d, 2H, *J* = 8.96 Hz), 1.64 (s, 3H). EI-MS: *m/z* 380. HRMS calcd for $C_{23}H_{30}N_3O$: 380.2333 found (ESI, $[M+H]^+$): 380.2340.

5.1.25. 4-[(1-phenethyl-piperidin-4-yl)-hydrazono]-pentanoic acid (6c)—Crystalline solid (91.1%), mp 212–214 °C. IR: ν (cm^{-1}) = 1612 (C=N), 1715 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.85 (bs, 1H), 7.27 (m, 2H), 7.19 (m, 5H), 6.86 (m, 1H), 6.82 (m, 2H), 3.49 (m, 1H), 3.38 (d 2H, *J* = 5.93 Hz), 2.96 (m, 2H), 2.89 (m, 2H), 2.77 (t, 2H, *J* = 7.23 Hz), 2.69 (t, 2 H, *J* = 7.23 Hz), 2.32 (m, 4H), 1.75 (m, 2H), 1.66 (s, 3H). EI-MS: *m/z* 394. HRMS calcd for $C_{24}H_{32}N_3O_2$: 394.2489 found (ESI, $[M+H]^+$): 394.2492.

5.1.26. 4-[(4-Methoxy-phenyl)-(1-methyl-piperidin-4-yl)-hydrazono]-pentanoic acid (6d)—Crystalline solid (67.4%), mp 182–184 °C. IR: ν (cm^{-1}) = 1615 (C=N), 1717 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.75 (bs 1H), 6.90 (m, 2H), 6.74 (m, 2H), 3.76 (s, 3H), 3.51 (m, 1H), 3.30 (d, 2H, *J* = 10.25 Hz), 2.74 (t, 2H, *J* = 6.93 Hz), 2.68 (m, 2H), 2.55 (s, 3H), 2.37 (m, 4H), 1.75 (d, 2H, *J* = 11.58 Hz), 1.66 (s, 3H). EI-MS: m/z 334. HRMS calcd for $C_{18}H_{28}N_3O_3$: 334.2125 found (ESI, $[M+H]^+$): 334.2131.

5.1.27. 4-[(1-benzyl-piperidin-4-yl)-(4-methoxy-phenyl) hydrazono]-pentanoic acid (6e)—Light yellow liquid (73.8%). IR: ν (cm^{-1}) = 1614 (C=N), 1714 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.07 (bs, 1H), 7.31 (m, 2H), 7.16 (m, 3H), 6.83 (m, 2H), 6.73 (m, 2H), 3.90 (s, 2H), 3.68 (s, 3H), 3.34 (m, 2H), 3.25 (m, 1H), 2.71 (t, 2H, *J* = 7.15 Hz), 2.63 (t, 2 H, *J* = 7.15 Hz), 2.27 (m, 2H), 1.74 (m, 2H), 1.61 (s, 3H). EI-MS: *m/z* 410. HRMS calcd for $C_{24}H_{32}N_3O_3$: 410.2438 found (ESI, $[M+H]^+$): 410.2443.

5.1.28. 4-[(4-Methoxy-phenyl)-1-(2-phenethyl-piperidin-4-yl)-hydrazono]-pentanoic acid (6f)—Crystalline solid (89.3%), mp 177–179 °C. IR: ν (cm^{-1}) = 1610 (C=N), 1715 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.57 (bs, 1H), 7.26 (m, 2H), 7.18 (m, 3H), 6.83 (m, 2H), 6.73 (m, 2H), 3.71 (s, 3H), 3.34 (m, 2H), 3.25 (m 1H), 2.94 (m, 2H), 2.87 (m, 2H),

2.71 (t, 2H, *J* = 7.15 Hz), 2.63 (t, 2H, *J* = 7.15 Hz), 2.27 (m, 2H), 1.74 (m, 2H), 1.61 (s, 3H). EI-MS: *m/z* 424. HRMS calcd for $C_{25}H_{34}N_3O_3$: 424.2595 found (ESI, $[M+H]^+$): 424.2602.

5.1.29. General method for the synthesis of N-[(1-substitutedl-piperidin-4-yl)-phenyl-hydrazono]-pentanoic acids ethyl ethers (7a–f)—A solution of 0.01 mol of any of (1-substituted-piperidin-4-yl)-N-phenyl-hydrazines **5a–f**, 0.011 mol of levulinic acid ethyl ether and 2–3 drops of acetic acid in 25–30 mL of toluene was heated under reflux for 1.5–2 hours with a Dean-Stark trap till the full separation of water. The toluene and excess of levulinic acid ethyl ether were removed *in vacuo*. Yields are close to quantitative. For analytical purposes product was purified by flash chromatography on silica ($CHCl_3$/MeOH; 4/1).

5.1.30. N-[(1-methyl-piperidin-4-yl)-phenyl-hydrazono]-pentanoic acid ethyl ether (6a)—Light yellow liquid (81.0%). IR: ν (cm^{-1}) = 1595 (C=N), 1740 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.15 (m, 2H), 6.85 (m, 1H), 6.79 (d, 2 H, *J* = 8.11 Hz), 4.09 (m, 2H), 3.30 (tt, 1H, *J* = 11.03, 3.86 Hz), 2.84 (d, 1H, *J* = 11.59 Hz), 2.69 (t, 1H, *J* = 6.58 Hz), 2.65 (t, 2H, *J* = 6.81 Hz), 2.57 (t, 2 H, *J* = 6.86 Hz), 2.52 (t, 1H, *J* = 6.58 Hz), 2.23 (m, 3H), 2.14 (s, 1H), 1.93 (t, 2H, *J* = 11.67 Hz), 1.84 (dq, 2H, *J* = 3.51, 12.30 Hz), 1.62 (m, 5H), 1.19 (m, 4H). EI-MS: *m/z* 332. HRMS calcd for $C_{19}H_{30}N_3O_2$: 332.2333 found (ESI, $[M+H]^+$): 332.2327.

5.1.31. N-[(1-benzyl-piperidin-4-yl)-phenyl-hydrazono]-pentanoic acid ethyl ether(6b)—Light yellow liquid (87.1%). IR: ν (cm^{-1}) = 1598 (C=N), 1742 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.63 (bs, 1H), 7.31 (m, 5H), 7.16 (t, 2H, *J* = 7.76 Hz), 6.84 (t, 1H, *J* = 7.29 Hz), 6.78 (d, 2H, *J* = 8.09 Hz), 4.15 (q, 2H, *J* = 7.17 Hz), 3.90 (s, 2H), 3.41 (m, 1H), 3.23 (d, 2H, *J* = 6.88 Hz), 2.74 (t, 2H, *J* = 7.21 Hz), 2.67 (t, 2H, *J* = 6.96 Hz), 2.24 (m, 4H), 1.68 (d, 2H, *J* = 8.96 Hz), 1.64 (s, 3H), 1.24 (t, 3 H, *J* = 7.14 Hz). EI-MS: *m/z* 408. EI-MS: *m/z* 332. HRMS calcd for $C_{25}H_{34}N_3O_2$: 408.2646 found (ESI, $[M+H]^+$): 408.2641

5.1.32. 4-[(1-phenethyl-piperidin-4-yl)-hydrazono]-pentanoic acid ethyl ether (6c)—Light yellow liquid (95.2%). IR: ν (cm^{-1}) = 1598 (C=N), 1740 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.85 (bs, 1H), 7.27 (m, 2H), 7.19 (m, 5H), 6.86 (m, 1H), 6.82 (m, 2H), 4.10 (q, 2H, *J* = 7.15 Hz), 3.49 (m, 1H), 3.38 (d, 2H, *J* = 5.93 Hz), 2.96 (m, 2H), 2.89 (m, 2H), 2.77 (t, 2H *J* = 7.23 Hz), 2.69 (t, 2H, *J* = 7.23 Hz), 2.32 (m, 4H), 1.75 (m, 2H), 1.66 (s, 3H), 1.22 (t, 3H, *J* = 7.12 Hz). EI-MS: *m/z* 422. HRMS calcd for $C_{26}H_{36}N_3O_2$: 422.2802 found (ESI, $[M+H]^+$): 422.2797.

5.1.33. 4-[(4-Methoxy-phenyl)-(1-methyl-piperidin-4-yl)-hydrazono]-pentanoic acid ethyl ether (6d)—Light yellow liquid (78.8%). IR: ν (cm^{-1}) = 1596 (C=N), 1741 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.82 (m, 2H), 6.72 (m, 2H), 4.08 (q, 2H, *J* = 7.15 Hz), 3.69 (s, 3H), 3.05 (tt, 1H, *J* = 10.74, 3.87 Hz), 2.81 (m, 2H), 2.59 (t, 2H, *J* = 6.91 Hz), 2.49 (t, 2H, *J* = 6.80 Hz), 2.20 (s, 3H,), 1.91 (m, 2H), 1.72 (m, 2H), 1.63 (d, 2H, *J* = 10.78 Hz), 1.56 (s, 3H), 1.20 (t, 3H, *J* = 7.12 Hz). EI-MS: *m/z* 362. HRMS calcd for $C_{20}H_{32}N_3O_3$: 362.2438 found (ESI, $[M+H]^+$): 362.2430.

5.1.34. 4-[(1-benzyl-piperidin-4-yl)-(4-methoxy-phenyl) hydrazono]-pentanoic acid ethyl ether (6e)—Light yellow liquid (82.5%). IR: ν (cm^{-1}) = 1598 (C=N), 1742 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.29 (m, 4H), 7.22 (m, 1H), 6.86 (m, 2H), 6.76 (m, 2H), 4.16 (q, 2H, *J* = 7.17 Hz), 3.73 (s, 3H,), 3.47 (s, 2H), 3.12 (tt, 1H, *J* = 11.06, 3.84 Hz), 2.88 (d, 2H, *J* = 11.72 Hz), 2.64 (t, 2H, *J* = 6.70 Hz), 2.56 (t, 2H, *J* = 6.75 Hz), 1.94 (t, 2H, *J* = 11.31 Hz), 1.75 (dq, 2H, *J* = 3.71, 12.09 Hz), 1.65 (d, 2H, *J* = 11.44 Hz), 1.61 (s, 3H), 1.26 (t, 3H, *J* = 7.14 Hz). EI-MS: *m/z* 438. HRMS calcd for $C_{26}H_{36}N_3O_3$: 438.2751 found (ESI, $[M+H]^+$): 438.2745.

5.1.35. 4-[(4-Methoxy-phenyl)-1-(2-phenethyl-piperidin-4-yl)-hydrazono]-pentanoic acid ethyl ether (6f)—Light yellow liquid (96.1%). IR: ν (cm^{-1}) = 1595 (C=N), 1740 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.22 (m, 2H), 7.13 (m, 3H), 6.84 (m, 2H), 6.74 (m, 2H), 4.11 (q, 2H, *J* = 7.14 Hz), 3.70 (s, 3H), 3.10 (tt, 1H, *J* = 10.84, 4.01 Hz), 2.97 (m, 2H), 2.76 (m, 2H), 2.61 (t, 2H, *J* = 6.81 Hz), 2.52 (m, 4H), 1.98 (m, 2H), 1.75 (m, 2H), 1.68 (m, 2H), 1.59 (s, 3H), 1.23 (t, 3H, *J* = 7.13 Hz). EI-MS: *m/z* 452. HRMS calcd for $C_{27}H_{38}N_3O_3$: 452.2908 found (ESI, $[M+H]^+$): 452.2989.

5.1.36. General method for the synthesis of [2-methyl-1-(1-methyl-piperidin-4-yl)-1H-indol-3-yl]-acetic acid ethyl esters (8 a–f)—N-[(1-substitutedl-piperidin-4-yl)-phenyl-hydrazono]-pentanoic acids ethyl ethers **7a–f** (0,01 mol) was dissolved in 20 mL of 10% ethanol solution of HCl and the solution was heated under reflux for 2.5 hours and then left at room temperature overnight. The solvent was removed in vacuum, and remaining residue was washed 2–3 times with boiling ether. Ether was added to remaining part and on cooling it was neutralized with NH_4OH solution. Ether layer was separated and the water layer extracted with ether one more time. Ether layer was dried over $MgSO_4$, filtered through a layer of neutral alumina. The solvent was removed *in vacuo*. Remaining pure product can be used without further purification. Yields are between 71–87%. For analytical purposes the product was purified by flash chromatography on silica ($CHCl_3$/MeOH; 4/1)for **8a** and **8d**, (hexane/ethyl acetate; 1/1) for **8 b,c.e.f.**

5.1.37. [2-methyl-1-(1-methyl-piperidin-4-yl)-1H-indol-3-yl]-acetic acid ethyl ester (8a)—Light yellow liquid (71.5%). IR: ν (cm^{-1}) = 1746 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.60 (d, 1H, *J* = 7.66 Hz), 7.55 (d, 1H, *J* = 7.77 Hz), 7.11 (t, 1H, *J* = 7.58 Hz), 7.07 (t, 1H, *J* = 7.36 Hz), 4.21 (m, 1H), 4.12 (q, 2H, *J* = 7.10 Hz), 3.69 (s, 2H,), 3.12 (d, 2H, *J* = 11.68 Hz), 2.75 (d, 2 H,*J* = 11.82 Hz), 2.44 (s, 3H,), 2.42 (s, 3H), 2.25 (t, 2H, *J* = 11.66 Hz), 1.84 (d, 2H, *J* = 12.60 Hz), 1.24 (t, 3H, *J* = 7.12 Hz). EI-MS: *m/z* 315. EI-MS: *m/z* 452. HRMS calcd for $C_{19}H_{27}N_2O_2$: 315.2067 found (ESI, $[M+H]^+$): 315.2059

5.1.38. [2-methyl-1-(1-benzyl-piperidin-4-yl)-1H-indol-3-yl]-acetic acid ethyl ester (8b)—Light yellow liquid (79.3%). IR: ν (cm^{-1}) = 1747 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 (m, 1H), 7.56 (m, 1H), 7.40 (m, 2H), 7.37 (m, 2H), 7.29 (m, 1H), 7.13 (m, 1H), 7.08 (m, 1H), 4.18 (m, 1H), 4.12 (q, 2H, *J* = 7.26 Hz), 3.90 (s, 2H), 3.69 (s, 2H), 3.62 (s, 2H), 3.10 (d, 2H, *J* = 11.16 Hz), 2.43 (s, 3H), 1.81 (m, 2H), 1.24 (t, 3H, *J* = 7.07 Hz). EI-MS: *m/z* 391. HRMS calcd for $C_{25}H_{31}N_2O_2$: 391.2380 found (ESI, $[M+H]^+$): 391.2385.

5.1.39. [2-methyl-1-(1-phenethyl-piperidin-4-yl)-1H-indol-3-yl]-acetic acid ethyl ester (8c)—Crystalline solid (85.6%). mp 93–94 °C. IR: ν (cm^{-1}) = 1744(C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 (d, 1H, *J* = 8.65 Hz), 7.31 (m, 2H), 7.22 (m, 3H), 7.02 (d, 1H, *J* = 2.51 Hz), 6.77 (dd, 1H, *J* = 8.65, 2.51 Hz), 4.11 (q, 2H, *J* = 7.16 Hz), 3.85 (s, 3H), 3.65 (s, 2H), 3.20 (d, 2H, *J* = 10.77 Hz), 2.87 (m, 2H), 2.68 (m, 2H), 2.62 (m, 2H), 2.41 (s, 3H), 2.21 (t, 2H, *J* = 11.23 Hz), 1.83 (m, 2H), 1.24 (t, 3H, *J* = 7.16 Hz). EI-MS: *m/z* 405. EI-MS: *m/z* 391. HRMS calcd for $C_{26}H_{33}N_2O_2$: 405.2537 found (ESI, $[M+H]^+$): 405.2543.

5.1.40. [5-methoxy- 2-methyl-1-(1-methyl-piperidin-4-yl)-1H-indol-3-yl]-acetic acid ethyl ester (8d)—Light yellow liquid (75,6%). IR: ν (cm^{-1}) = 1745 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (m, 1H), 6.99 (m, 1H), 6.72 (m, 1H), 4.09 (m+q 3H), 3.81 (s, 3H), 3.61 (s, 2H), 3.01 (d, 2H, *J* = 11.69 Hz), 2.59 (m, 2H,), 2.37 (s, 3H), 2.33 (s, 3H), 2.11 (m, 2H), 1.77 (m, 2H), 1.21 (m, 3H). EI-MS: *m/z* 345. HRMS calcd for $C_{20}H_{29}N_2O_3$: 345.2173 found (ESI, $[M+H]^+$): 345.2166.

5.1.41. [5-methoxy- 2-methyl-1-(1-benzyl-piperidin-4-yl)-1H-indol-3-yl]-acetic acid ethyl ester (8e)—Light yellow liquid (81.9%). IR: ν (cm^{-1}) = 1747 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.48 (m, 1H), 7.37 (m, 4H), 7.28 (m, 1H), 7.04 (m, 1H), 6.79 (m, 1H), 4.13 (m+q, 3H), 3.86 (s, 3H), 3.66 (s, 2H), 3.60 (s, 2H), 3.07 (m, 2H), 2.60 (m, 2H), 2.41 (s, 3H), 2.15 (m, 2H), 1.79 (m, 2H), 1.25 (t, 3H, *J* = 6.98 Hz). EI-MS: *m/z* 421. HRMS calcd for $C_{26}H_{33}N_2O_3$: 421.2486 found (ESI, $[M+H]^+$): 421.2493.

5.1.42. [5-methoxy- 2-methyl-1-(1-phenethyl-piperidin-4-yl)-1H-indol-3-yl]-acetic acid ethyl ester (8f)—Light yellow liquid (87.4%). IR: ν (cm^{-1}) = 1745 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 (d, 1H, *J* = 8.65 Hz), 7.31 (m, 2H), 7.22 (m, 3H), 7.02 (d, 1H, *J* = 2.51 Hz), 6.77 (dd, 1H, *J* = 8.65, 2.51 Hz), 4.11 (q, 2 H, *J* = 7.16 Hz), 3.85 (s, 3H), 3.65 (s, 2H), 3.20 (d, 2H, *J* = 10.77 Hz), 2.87 (m, 2H), 2.68 (m, 2H), 2.62 (m, 2H), 2.41 (s, 3H), 2.21 (t, 2H, *J* = 11.23 Hz), 1.83 (m, 2H), 1.24 (t, 3H, *J* = 7.16 Hz). EI-MS: *m/z* 435. HRMS calcd for $C_{25}H_{31}N_2O_3$: 407.2329 found (ESI, $[M+H]^+$): 407.2323.

5.1.43. General method for the synthesis of [2-methyl-1-(1-methyl-piperidin-4-yl)-1H-indol-3-yl]-acetic acids (9a–f)—a. 0,005 mol of one of the N-[(1-substitutedl-piperidin-4-yl)-phenyl-hydrazono]-pentanoic acids ethyl ethers **7a–f** dissolved in 8 mL of ethanol was added with cooling (ice) to a stirred solution of 0.00625 mol KOH in 4 mL of ethanol. The solution was left at room temperature for 48 hours. Ethanol was removed in vacuo. Remaining residue was washed several times with boiling ether. A new portion of ether was added and on cooling (ice) 0.00625 mol (0,375 mL) of acetic acid in 1 mL of water was added. Ether layer was separated and remaining part was extracted with $CHCl_3$ and dried ($MgSO_4$). The solvent was removed *in vacuo.* The product does not need further purification. Yields are between 55–84%.

b. For cases **9a,d** the work up during acidification of potassium salts differs. To the remaining residue after ethanol evaporation was added 10 mL acetonitrile, and neutralization with acetic acid (0.00625 mol) is done without water. After stirring the mixture for 30 minutes and filtering the potassium acetate, acetonitrile is evaporated under vacuo giving as a residue of the desired acids **9a,d**.

5.1.44. [2-methyl-1-(1-methyl-piperidin-4-yl)-1H-indol-3-yl]-acetic acid (9a)—Crystalline solid (55.2%), mp 83–85 °C. IR: ν (cm^{-1}) = 1718 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.93 (bs, 1H), 7.53 (d, 1H, *J* = 7.28 Hz), 7.44 (d, 1H, *J* = 7.70 Hz), 6.97 (m, 2H), 4.08 (m, 1H), 3.60 (s, 2H), 3.11 (d, 2H, *J* = 11.16 Hz), 2.60 (m, 3H), 2.27 (s, 3H), 2.21 (t, 2H, *J* = 11.17 Hz), 2.02 (s, 1H), 1.96 (s, 1H), 1.91 (s, 1H), 1.57 (d, 2H, *J* = 11.53 Hz), 2.38 (3 H, s). ^{13}C NMR (125 MHz, MeOD) δ 178.23, 176.56, 133.79, 129.47, 120.78, 118.95, 118.58, 111.05, 107.27, 54.16, 50.68, 42.78, 32.32, 27.65, 21.41, 10.46. EI-MS: *m/z* 287. HRMS calcd for $C_{17}H_{23}N_2O_2$: 287.1754 found (ESI, $[M+H^+]$): 287.1761.

5.1.45. [2-methyl-1-(1-benzyl-piperidin-4-yl)-1H-indol-3-yl]-acetic acid (9b)—Crystalline solid (83.0%), mp 78–80 °C. IR: ν (cm^{-1}) = 1720 (C=O). ^{1} H NMR (600 MHz, $CDCl_3$) δ 11.23 (bs, 1H), 7.39 (m, 7H), 6.99 (m, 1H), 6.91 (m, 1H), 4.02 (m, 1H), 3.89 (s, 2H), 3.61 (s, 2H), 3.17 (d, 2H, *J* = 10.99 Hz), 2.62 (m, 2H), 2.33 (m, 2H), 2.06 (s, 3H), 1.38 (d, 2H, *J* = 11.78 Hz). ^{13}C NMR (125 MHz, MeOD) δ 177.04, 175.42, 133.87, 131.21, 129.63, 129.25, 129.13, 120.67, 118.92, 118.39, 111.00, 106.55, 60.60, 52.09, 51.29, 31.59, 27.53, 20.75, 10.43. EI-MS: *m/z* 363. HRMS calcd for $C_{23}H_{27}N_2O_2$: 363.2067 found (ESI, $[M+H]^+$): 363.2061.

5.1.46. [2-methyl-1-(1-phenethyl-piperidin-4-yl)-1H-indol-3-yl]-acetic acid (9c)—Crystalline solid (80.%), mp 83–85 °C. IR: ν (cm^{-1}) = 1717 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.77 (bs, 1H), 8.97 (bs, 1H), 7.52 (m, 1H), 7.28 (m, 2H), 7.20 (m, 4H), 7.00 (m,

1H), 6.95 (m, 1H), 4.08 (m, 1H), 3.62 (s, 2H), 3.30 (d, 2H, *J* = 10.78 Hz), 2.95 (m, 2H,), 2.91 (m, 2H), 2.62 (d, 2H, *J* = 9.26 Hz), 2.38 (m, 2H), 2.05 (s, 3H), 1.39 (m, 2H). ^{13}C NMR (125 MHz, MeOD) δ 178.02, 137.55, 133.79, 129.45, 128.88, 128.84, 127.02, 120.80, 118.94, 118.59, 58.18, 52.47, 51.32, 32.38, 31.00, 27.82, 21.30, 10.59. EI-MS: *m/z* 377. HRMS calcd for $C_{24}H_{29}N_2O_2$: 377.2224 found (ESI, $[M+H]^+$): 377.2219.

5.1.47. [5-methoxy- 2-methyl-1-(1-methyl-piperidin-4-yl)-1H-indol-3-yl]-acetic acid (9d)—Crystalline solid (59.2%), mp 58–60 °C. IR : ν(cm^{-1}) = 1721 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.45 (bs, 1H), 7.43 (m, 1H), 6.99 (m, 1H), 6.72 (m, 1H), 4.09 (m, 1H), 3.81 (s, 3H), 3.61 (s, 2H), 3.01 (d, 2H, *J* = 11.69 Hz), 2.59 (m, 2H), 2.37 (s, 3H), 2.33 (s, 3H), 2.11 (m, 2H), 1.77 (m, 2H). ^{13} CNMR (125 MHz, MeOD) δ 178.32, 154.03, 134.55, 128.62, 112.25, 110.66, 107.11, 101.30, 55.04, 54.18, 50.30, 43.05, 32.24, 28.31, 10.48. EI-MS: *m/z* 317. HRMS calcd for $C_{18}H_{25}N_2O_3$: 317.1860 found (ESI, $[M+H]^+$): 317.1856.

5.1.48. [5-methoxy- 2-methyl-1-(1-benzyl-piperidin-4-yl)-1H-indol-3-yl]-acetic acid (9e)—Crystalline solid (82%), mp 135–137°C. IR: ν (cm^{-1}) = 1718 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.88 (bs, 1H), 7.48 (m, 1H), 7.37 (m, 4H), 7.28 (m, 1H), 7.04 (m, 1H), 6.79 (m, 1H), 4.13 (m, 1H), 3.86 (s, 3H), 3.66 (s, 2H), 3.60 (s, 2H), 3.07 (m, 2H), 2.60 (m, 2H), 2.41 (s, 3H), 2.15 (m, 2H), 1.79 (m, 2H). ^{13}C NMR (125 MHz, MeOD) δ 179.14, 153.83, 134.37, 133.46, 130.64, 130.16, 129.99, 128.82, 128.76, 111.79, 109.98, 107.11, 101.08, 61.46, 55.32, 52.60, 52.28, 32.97, 28.69, 10.52. EI-MS: *m/z* 393. HRMS calcd for $C_{24}H_{29}N_2O_3$: 393.2173 found (ESI, $[M+H]^+$): 393.2167.

5.1.49. [5-methoxy- 2-methyl-1-(1-phenethyl-piperidin-4-yl)-1H-indol-3-yl]-acetic acid (9f)—Crystalline solid (84.3%), mp 98–100 °C. IR: ν (cm^{-1}) = 1720 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.26 (bs, 1H), 7.42 (s, 1H), 7.29 (m, 2H), 7.21 (m, 4H), 6.99 (d, 1H *J* = 2.36 Hz), 6.71 (dd, 1H, *J* = 8.84, 2.36 Hz), 4.08 (m, 1H), 3.71 (s, 3H), 3.59 (s, 2H), 3.33 (d, 2H, *J* = 10.47 Hz), 3.00 (m 2H), 2.93 (m, 2H), 2.68 (m, 1H), 2.43 (s, 2H), 2.19 (s, 2H,), 2.06 (s, 3H), 1.46 (d, 2H, *J* = 11.32 Hz). ^{13}C NMR (125 MHz, MeOD) δ 178.48, 153.93, 137.67, 134.45, 130.04, 128.84, 126.97, 111.83, 110.21, 107.24, 101.17, 58.28, 55.29, 52.46, 51.42, 32.80, 31.07, 28.08, 10.61. EI-MS: *m/z* 407. HRMS calcd for $C_{25}H_{31}N_2O_3$: 407.2329 found (ESI, $[M+H]^+$): 407.2333.

5.2. Biological assays

5.2.1. Tissue bioassays

5.2.1.1. Guinea Pig Isolated Ileum/Longitudinal Muscle with Myenteric Plexus: Male Hartley guinea pigs under CO_2 anesthesia were sacrificed by decapitation and a nonterminal portion of the ileum removed. The longitudinal muscle with myenteric plexus (LMMP) was carefully separated from the circular muscle and cut into strips as described previously [52]. These tissues were tied to gold chains with suture silk and mounted between platinum wire electrodes in 20 mL organ baths at a tension of 1 g and bathed in oxygenated (95:5 O_2:CO_2) Kreb's bicarbonate buffer at 37C. They were stimulated electrically (0.1 Hz, 0.4 msec duration) at supramaximal voltage. Following an equilibration period, compounds were added cumulatively to the bath in volumes of 14–60 μl until maximum inhibition was reached. A dose-response curve of PL-017 was constructed to determine tissue integrity before analog testing.

5.2.1.2. Mouse Isolated Vas Deferens Preparation: Male ICR mice under CO_2 anesthesia were sacrificed by cervical dislocation and the vasa deferentia removed. Tissues were tied to gold chains with suture silk and mounted between platinum wire electrodes in 20 ml organ baths at a tension of 0.5 g and bathed in oxygenated (95:5 O_2:CO_2) magnesium free Kreb's buffer at 37C. They were stimulated electrically (0.1 Hz, single pulses, 2.0 msec duration) at

supramaximal voltage as previously described [53]. Following an equilibration period, compounds were added to the bath cumulatively in volumes of 14–60 μL until maximum inhibition was reached. A dose-response curve of DPDPE was constructed to determine tissue integrity before analog testing.

5.2.1.3. Agonist and Antagonist testings: Compounds were tested as agonists by adding cumulatively to the bath until a full dose-response curve was constructed or to a concentration of 1 μM. Compounds were tested as antagonists by adding to the bath 2 minutes before beginning the cumulative agonist dose-response curves of the delta (DPDPE) or μ (PL-017) opioid agonists.

5.2.1.4. Analysis: Percentage inhibition was calculated using the average tissue contraction height for 1 min preceding the addition of the agonist divided by the contraction height 3 min after exposure to the dose of agonist. IC_{50} values represent the mean of not less than 4 tissues. IC_{50} and E_{max} estimates were determined by computerized non-linear least-squares analysis (FlashCalc).

5.2.2. Radioligand binding analysis—Crude membranes were prepared from whole rat brains. The protein concentration of the membrane preparations was determined by the Lowry method and the membranes were stored at −80°C until use. Membranes were resuspended in assay buffer (50 mM Tris,pH 7.4, containing 50 μg/mL bacitracin, 30 μM bestatin, 10 μM captopril, 100 μM PMSF, 1 mg/mL BSA). Ten concentrations of a test compound were each incubated with 50 μg of membranes and 500 pM [^{3}H]diprenorphine (55 Ci/mmol). Naloxone at 10 μM was used to define non-specific binding of the radioligand in all assays. All samples were carried out in duplicates. The samples were incubated in a shaking water bath at 25°C for 3 hr, followed by rapid filtration through Whatman GF/B filter paper (Gaithersburg, MD) pre-soaked in 1% polyethyleneimine, washed 4 times each with 2 mL of cold saline, and the radioactivity determined by liquid scintillation counting (Beckman LS5000 TD).

5.2.3. Enzyme immunoassay—A COX inhibitor screening assay kit (Cayman Chemical, catalog#560101) was used to evaluate the effect of compounds **9a–f** on COX-1 or COX-2 mediated conversion of arachidonic acid to prostaglandin H_2; the latter was quantified by an enzyme immunoassay. The assay was carried out according to the manufacturer's recommendations. Briefly, compounds (50 nM final concentration) were pre-incubated with COX-1 or COX-2 for 5 min at 37C prior to the addition of arachidonic acid, and the reaction allowed to proceed for 2 min at 37C. Reaction was terminated with 1 M HCl and the amount of prostaglandin produced was determined by ELISA. The IC_{50} value of the reference compound indomethacin measured by this kit is around 200 nM.

5.2.4. Behavioral assays—Male Sprague-Dawley rats (220–275g) were used for all experiments. All surgical and testing procedures were approved by IACUC and have been previously described [54], including intrathecal catheterization for spinal cord drug delivery, peripheral nerve injury by unilateral ligation of the L5 and L6 spinal nerves of the left sciatic nerve, or sham operation as control, and behavioral testing for the subjects' response time to an infra read heat source, or their response to mechanical stimuli using a series of von Frey filaments. All drug testing was carried out 7 days after spinal nerve or sham surgery. Each test group consisted of 5–6 rats.

5.3. Crystal Structure for compound 8c

A colorless prism-like specimen of **8c** $C_{27}H_{34}N_2O_3$, approximate dimensions 0.06 mm × 0.11 mm × 0.15 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured at 100(2) K on a Bruker SMART APEXII system equipped with a graphite

monochromator and a Cu Kα fine-focus sealed tube (λ= 1.54178 Å) operated at 1.2 kW power (40kV, 30 mA). The detector was placed at a distance of 3.972 cm. from the crystal.

A total of 3889 frames were collected with a scan width of 0.5° in ω or φ and an exposure time of 10.0 sec/frame. The total data collection time was 15.34 hours. The frames were integrated with the Bruker SAINT software package using a narrow-frame integration algorithm. The integration of the data using a monoclinic unit cell yielded a total of 15841 reflections to a maximum θ angle of 68.17° (0.83 Å resolution), of which 4080 were independent (average redundancy 3.88, completeness = 97.3%, R_{int} = 5.55%, R_{sig} = 4.25%) and 3123 (76.54%) were greater than $2\sigma(F^2)$. The final cell constants of a = 9.9661(3) Å, b = 14.0923(5) Å, c = 16.4655 (5) Å, β = 96.182(2)°, volume = 2299.05(13) $Å^3$, are based upon the refinement of the XYZ-centroids of 4640 reflections above 20 σ(I) with 8.279° < 2θ < 135.275°. Analysis of the data showed negligible decay during data collection. Data were corrected for absorption effects using the multi-scan technique (SADABS). The ratio of minimum to maximum apparent transmission was 0.939. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.9078 and 0.9629.

The structure was solved and refined using the Bruker SHELXTL (Version 2008.2) Software Package, using the space group P2(1)/c, with Z = 4 for the formula unit, $C_{27}H_{34}N_2O_3$. The final anisotropic full-matrix least-squares refinement on F^2 with 327 variables converged at R1 = 3.88%, for the observed data and wR2 = 9.71% for all data. The goodness-of-fit was 1.009. The largest peak on the final difference electron density synthesis was 0.231 $e^-/Å^3$ and the largest hole was −0.184 $e^-/Å^3$ with an RMS deviation of 0.040 $e^-/Å^3$. On the basis of the final model, the calculated density was 1.255 g/cm^3 and F(000), 936 e^-.

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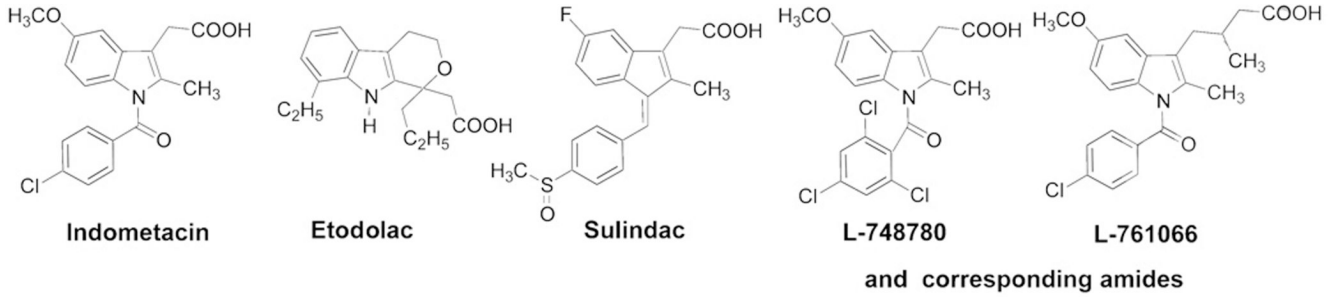


Fig. 1.
Indolyl / indene-acetic acids derivatives.

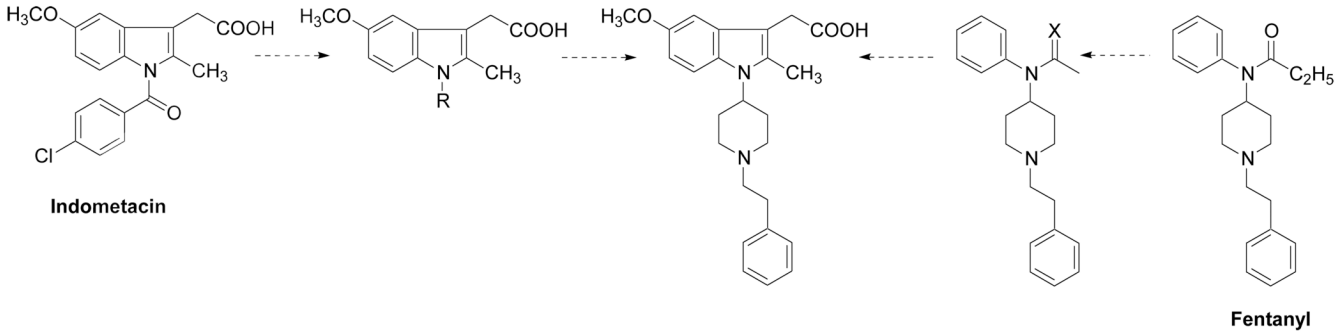


Fig. 2.
Combination of the essential parts of the known opioid fentanyl with the known COX inhibitor indomethacin.

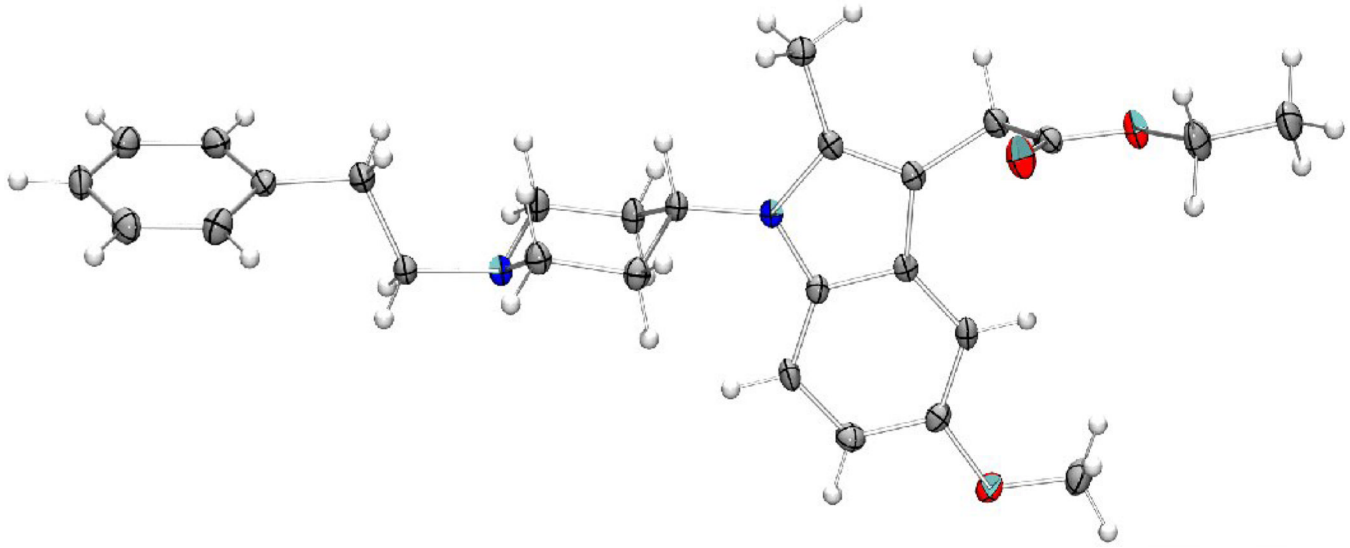


Fig. 3.
The molecular structure of **8c** with anisotropic displacement ellipsoids at the 50% probability level.

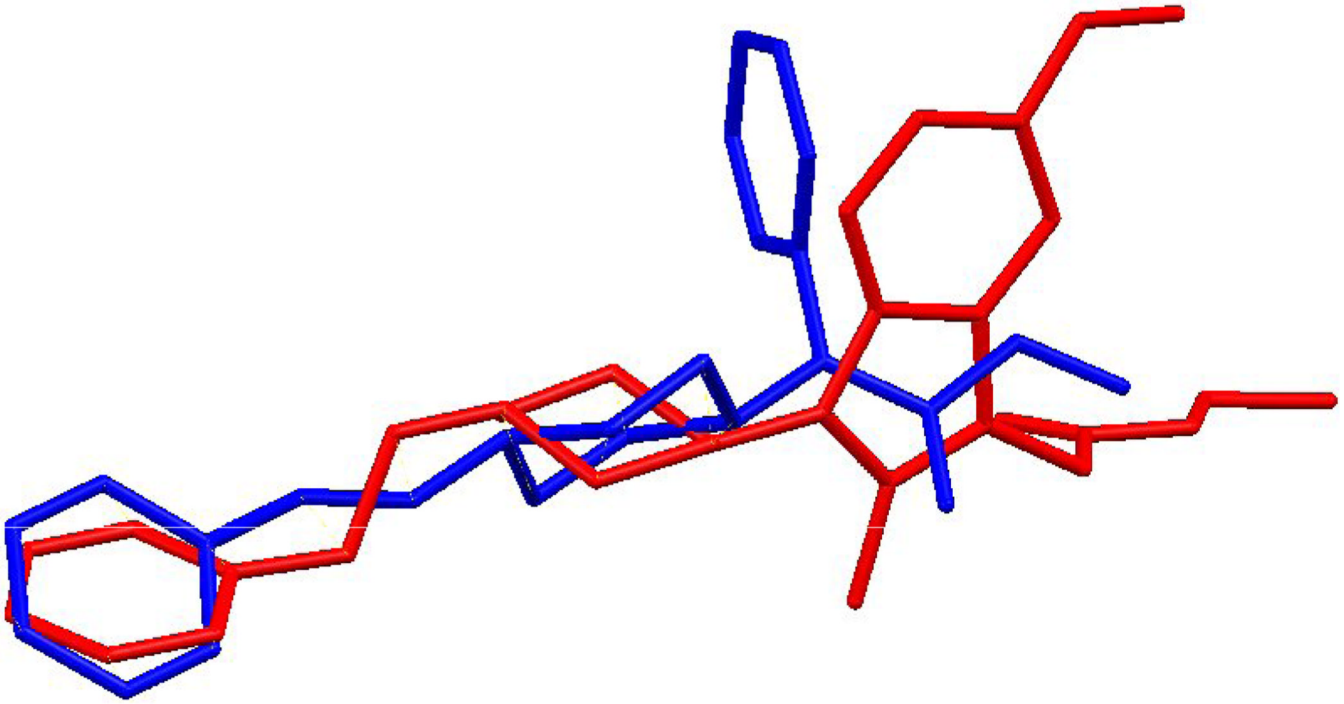


Fig. 4.
Least-squares average overlay of piperidine rings of Fentanyl (blue) and **8c** (red).

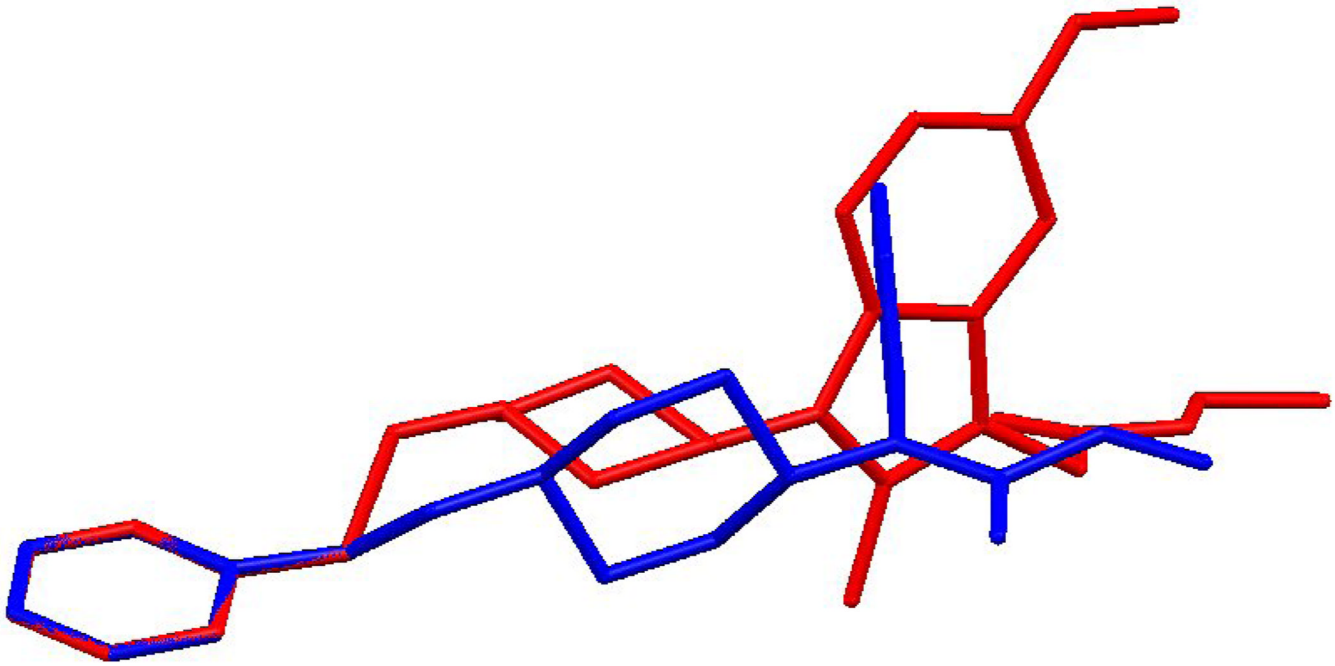


Fig. 5.
Overlapping the 1-(2-Phenethyl) phenyl rings of fentanyl (blue) and **8c**(red).

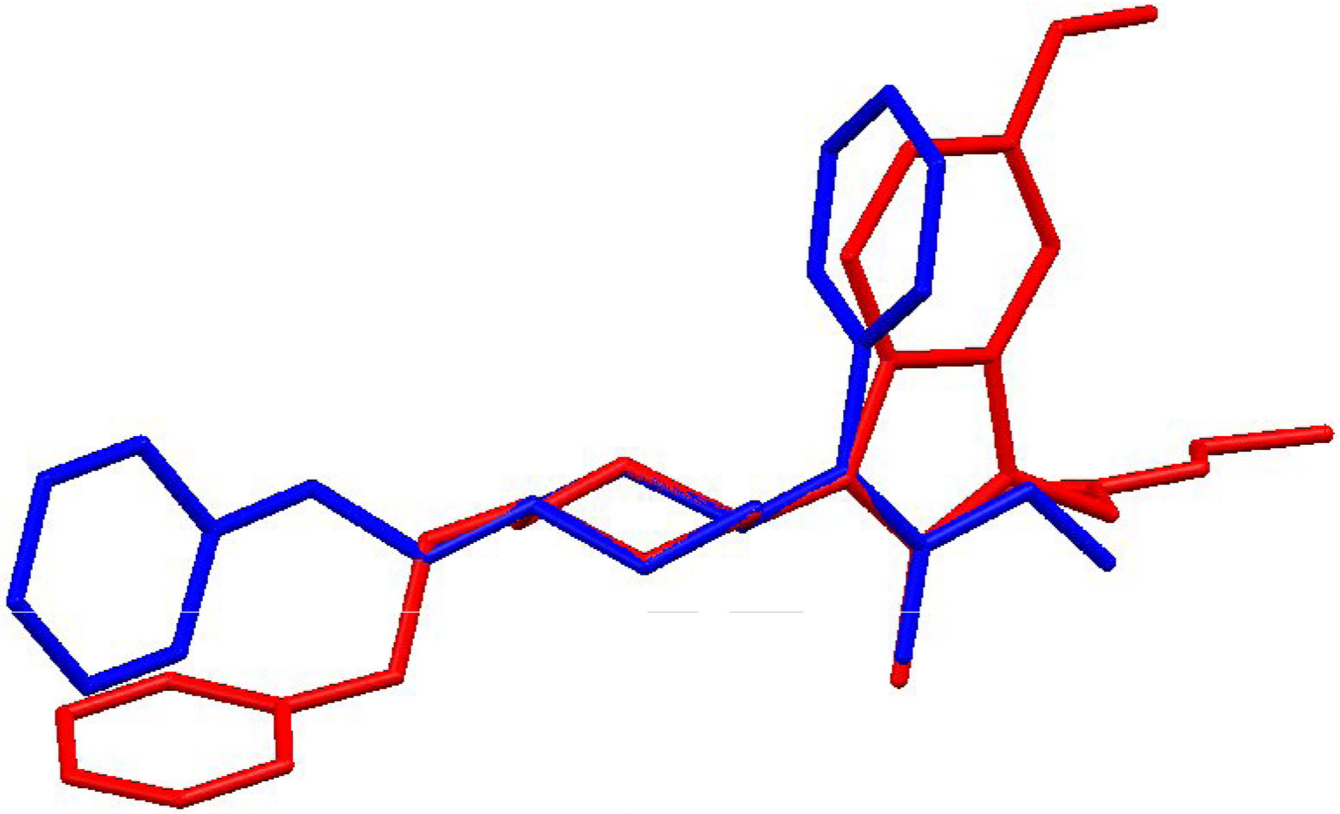


Fig. 6.
Overlapping the 4-(N-Phenyl) phenyl rings of fentanyl (blue) and **8c** (red).

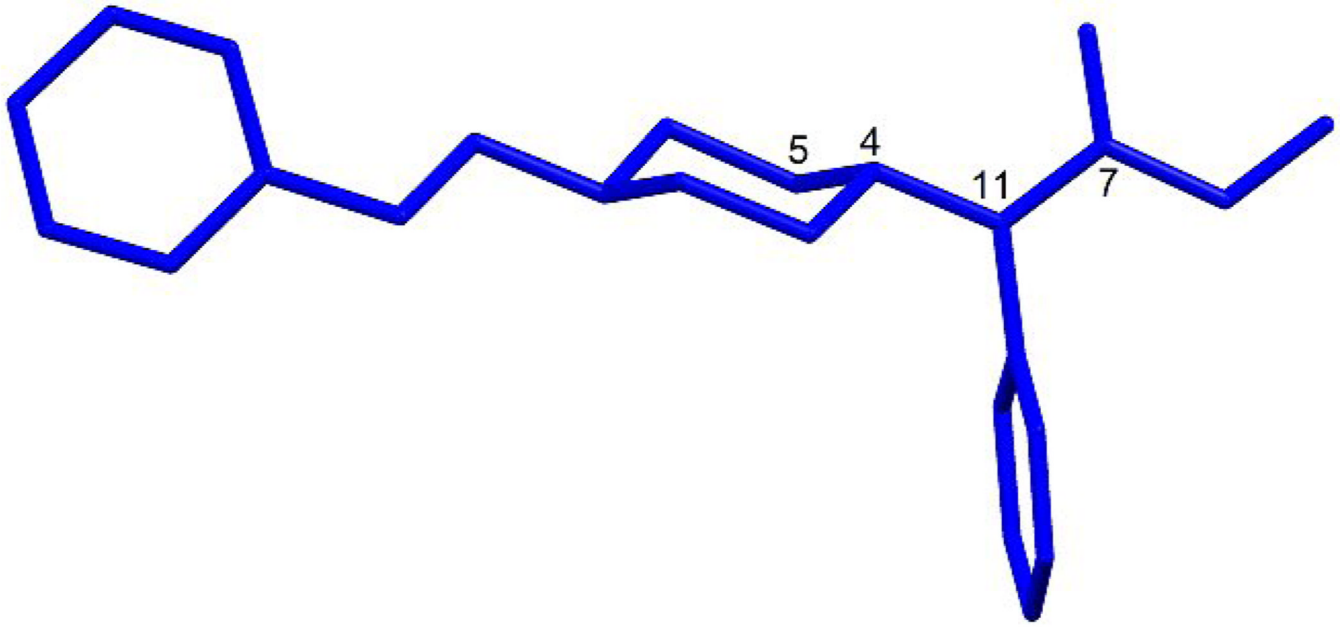
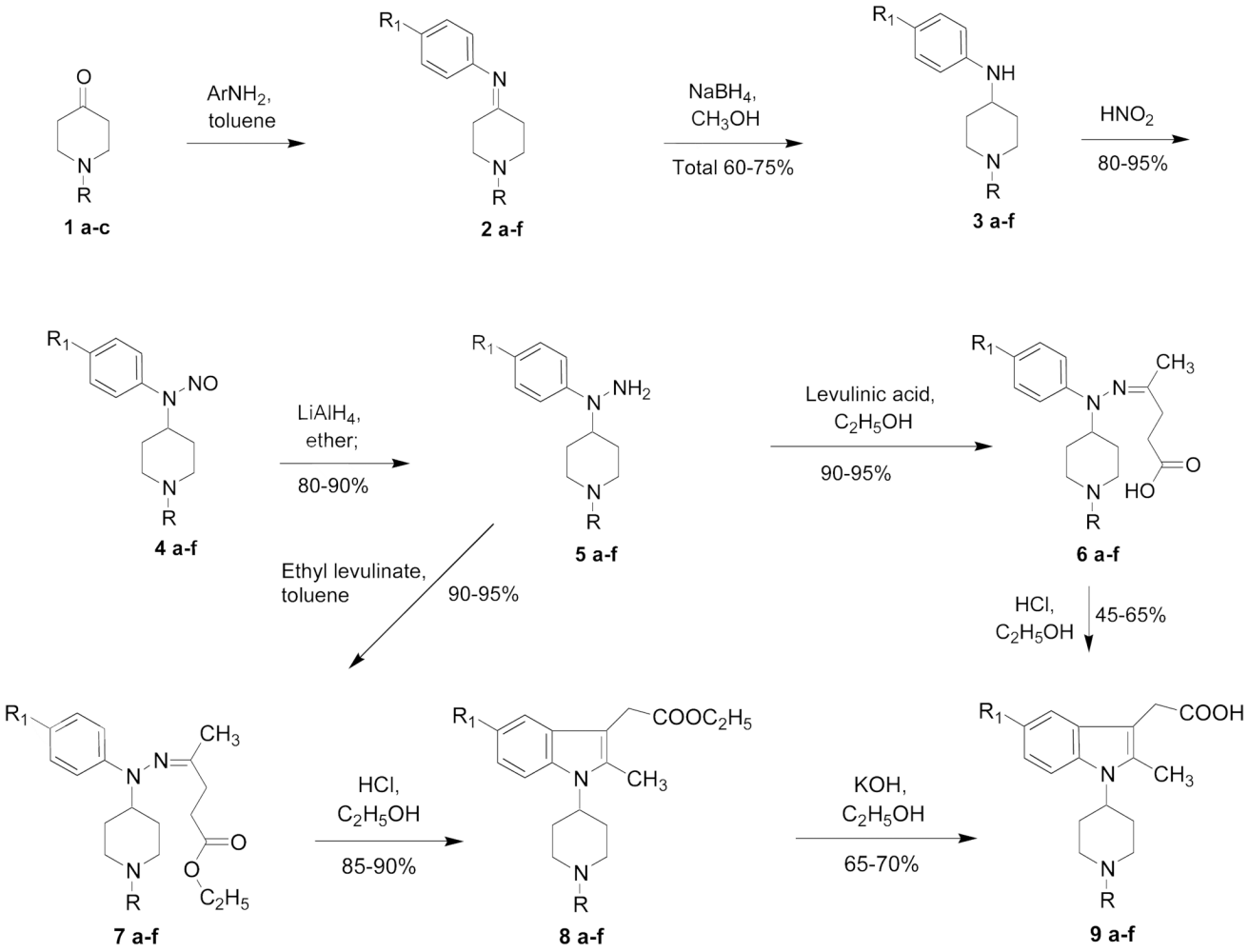


Fig 7.
The orientation of the phenyl rings in fentanyl.



a-series -R = CH_3-; R_1 = H-;
b-series -R = $PhCH_2$-; R_1 = H-;
c-series -R = $PhCH_2CH_2$-; R_1 = H-;
d-series -R = CH_3-; R_1 = CH_3O-;
e-series -$PhCH_2$-; R_1 = CH_3O-;
f-series -$PhCH_2CH_2$-; R_1 = CH_3O-;

Scheme 1.
Synthesis of [2-methyl-1-(1-methyl-piperidin-4-yl)-1H-indol-3-yl]-acetic acids

Table 1

Flexidock Score for molecules 9a–f with COX-2 and μ opiate receptor

Compound	FlexiDock scores with COX-2	Compound	FlexiDock scores with opiate μ-receptor
Indomethacin	− 101.7	Fentanyl	− 25.0
Compound **9a**	− 95.3	Compound **9a**	− 79.0
Compound **9b**	− 116.1	Compound **9b**	− 57.0
Compound **9c**	− 110.9	Compound **9c**	− 36.0
Compound **9d**	− 100.0	Compound **9d**	− 67.0
Compound **9e**	− 117.3	Compound **9e**	− 68.0
Compound **9f**	− 113.4	Compound **9f**	− 77.0

Table 2

Results in the MVD and GPI/LMMP

Assays	MVD	GPI/LMMP	DPDPE Antagonism at 1 μM in the MVD	PL-017 Antagonism at 1 μM in the GPI/LMMP
Compounds	**Agonist activity (% inhibition of contraction at 1 μM) or IC_{50} (nM) ± S.E.M.**			
9a	17.9 % at 1 μM	0.7 % at 1 μM	none at 1 μM	none at 1 μM
9b	1266 +/− 355	5164 +/− 2043	--	--
9c	19.5 % at 1 μM	3.1 % at 1 μM	none at 1 μM	none at 1 μM
9d	2.8 % at 1 μM	0 % at 1 μM	none at 1 μM	none at 1 μM
9e	8.3 % at 1 μM	3 % at 1 μM	none at 1 μM	none at 1 μM
9f	0 % at 1 μM	6 % at 1 μM	none at 1 μM	none at 1 μM

Footnotes: DPDPE ([D-Pen^2, D-Pen ^{5}]enkephalin) was used to inhibit MVD contraction by selective activation of the delta opioid receptors in this tissue. Compounds **9a–f** (at 1 μM) did not reverse the effect of DPDPE. PL-017 was used to inhibit GPI contraction by selective activation of the μ opioid receptors in this tissue. Compounds **9a–f** (at 1 μM) did not reverse the effect of PL-017.