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CCK-Antagonist PNB-081 (Isobutyl-5-Hydroxy-5-Phenyl-Pyrrol-2-One) as Adjunct to Opiates

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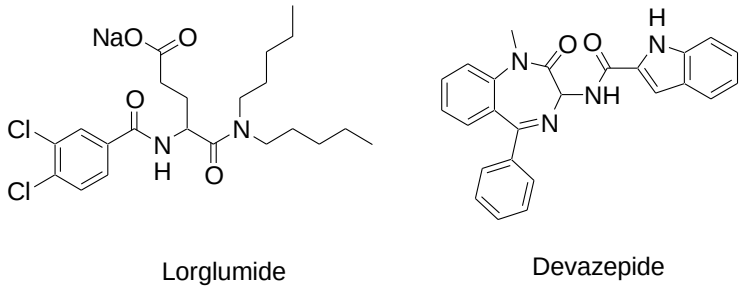
Abstract: *Arylated 5-hydroxy–pyrrol-2-ones were prepared in 2 synthetic steps from muco- chloric acid and were optimised as CCK selective ligands using radiolabelled binding assays. A potent CCK1 selective ligand was identified (PNB-081: CCK1=20nM) as part of systematic SAR optimisation. The antagonism was confirmed for the ligands by using isolated tissue preparations with CCK8S. The cholecystokinin antagonist PNB-081 potentiated the analgesic effect of morphine and reversed opiate tolerance in mice from doses >1 mg/kg by oral administration.*

Keywords: *Phenyl-pyrrolone, CCK antagonist, cholecystokinin, analgesic, opiate adjunct.*

1. INTRODUCTION

In terms of cholecystokinin-physiology[1], CCK_8 is the most common peptide hormone, which is extensively found throughout the gastrointestinal tract (GIT) and is also widely distributed through the nervous system[2]. Originally, cholecystokinin was discovered to cause contractions of the gallbladder[3]. It was then rediscovered as pancreozymin, triggering the release of pancreatic enzymes. Finally, it was confirmed that both peptides are identical[4]. Cholecystokinin acts as a neuromodulator as well as gut hormone. CCK-ligands, agonists and antagonists have been extensively investigated as potential drug molecules[5].

Cholecystokinin antagonists have been extensively investigated as potential drug targets[6]. They were studied as growth inhibitors in certain forms of cancer[7], as anxiolytics[8], in the treatment of schizophrenia[9] and satiety[10]. An agonist, the shortened CCK_4 was found to induce panic in patients[11] andthe CCK_2 receptor is known to mediate anxiety[12] and panic attacks[13]. Cholecystokinin does cause proliferation in colon- and pancreatic cancer cell lines and therefore, CCK-antagonists were studied as growth factor inhibitors in certain forms of cancer.



Lorglumide Devazepide

Fig 1. *CCK antagonists.*

Asperlicin was the first non-peptidal lead structure from nature[14] and analogues thereof were studied as CCK ligands[15]. Simplification of this lead structure by Merck led to Devazepide[16], a potent CCK_1 selective cholecystokinin antagonist (Figure 1), containing a 1,4-benzodiazepine template and an indole moiety. Proglumide[17]was the first glutamic acid based agent, marketed as Milid for the treatment of ulcer. Lorglumide, a derivative of proglumide[18], (Figure 1) is one of several CCK

receptor antagonists[19]and served as experimental standard.The indolyl amide of devazepide was replaced by a urea linkage and Merck's L-365,260 resulted in a CCK_2 selective antagonist[20].

All structural optimisations did only partly address the main underlying problem with respect to poor pharmacokinetic properties, such as a low water solubility and very low membrane penetration, as a result of a large polar surface area of the molecules and a relatively high molecular weight.

Again, having realized the poor pharmacokinetic properties these agents, a search for a completely novel, smaller template with a molecular weight <350, a log p about 3 and a polar surface area for membrane penetration of less that 100A, with no urea linkage was initiated.Aim of the drug discovery programme,initiated by PNB Vesper Life Sciences,was to systematically investigate and design the 2(5H)-furanone scaffold[21] into a hydroxy-pyrrolone scaffold with ligands for both CCK pathways.

Molecular pain targets have been reviewed recently[22] and the results are quite disappointing in terms of efficacy and FDA approval rate. Even, this review missed out on CCK antagonists[23] and most importantly on a very positive report, publicised only in form of an abstract[24]. In summary in this study, devazepide at 5 mg was found very efficient in pain management as adjunct to strong opiates in a phase 2 trial carried out at leading UK pain research centres.

Initial results for CCK antagonists of the pyrrolone scaffold were communicated in the area of cancer therapeutics[25]and GI inflammation[26].

Here, a full biological evaluation of PNB-081 is reported in detail with respect to opiates and pain management[27].

2. Materials and Methods

2.1. Synthesis

The chemicals were obtained from Aldrich (Gillingham, UK) and Lancaster (Lancaster, UK). Atmospheric pressure chemical ionisation mass spectroscopy (APCI), negative or positive mode, was carried out using a Hewlett-Packard 5989b quadrupole instrument (Vienna, Austria). Proton and Carbon NMR spectra were obtained on a Bruker AC 250 instrument (Follanden, Switzerland), operating at 250 MHz, calibrated with the solvent reference peak or TMS. IR spectra were plotted from KBr discs on a Mattson 300 FTIR Spectrometer. Melting points were recorded using a Stuart Scientific (Coventry, UK) apparatus and are uncorrected.

2.2. Synthesis Of 3,4-Dichloro-5-Phenyl-5H-Furan-2-One, Lactone A

Dry and powdered aluminium chloride (20g, 0.15 mol) was added slowly to a mixture of mucochloric acid (16.9g, 0.1 mol) and benzene / chlorobenzene (250 ml). The reaction mixture was stirred overnight. It was then poured into a mixture of 100g ice and 32 ml concentrated hydrochloric acid. The organic layer was separated by separating funnel and washed with 3 x 100 ml water. The combined organic layers were dried over magnesium sulphate and the solvent was removed under vacuum. The oily residue was crystallized in n-hexane.

Yield = 70%; mp: 78-79 ^{o}C;MS (APCI(+)): 195/197 (M+), 230/232 (M+1) m/z; ^{1}H NMR ($CDCl_3$) 250 MHz: δ = 7.22-7.51 (m, 5H), 5.81 (s, 1H); ^{13}C NMR ($CDCl_3$):165.3, 152.2, 139.8, 130.5, 129.3, 128.5, 127.4, 127.2, 121.2, 83.5; IR (KBr-disc) υ max: 3445, 3074, 3035, 2959, 2056, 1768, 1630, 1499, 1457 1294, 1224, 1028, 910, 772, 705 cm^{-1}.

2.3. General Method PNB-081

The relevant amine (2.3 times excess) was added to a solution of lactone **A**(0.7 mol) in MTB ether (10 ml) and it was stirred on ice for 30 minutes, allowing to warm up to RT over time. The resultant mixture was poured into 5 ml of water and was separated by a separating funnel. The organic mixture was washed with water three times. The organic layer was dried over magnesium sulphate and the solvent was removed under vacuum. All compounds gave an oily solid, which were passed through a short silicagelcolumn (80% ether, 20% petrol ether). The resulting fractions were dried from excess solvent under a stream of argon to yield crystals.

2.4. 4-Chloro-5-Hydroxy-1-Isobutyl-5-Phenyl-1,5-Dihydro-Pyrrol-2-One 7

Yield = 85 %; mp: 167-169 ^{o}C; MS (APCI(+)):266/268 (M+) m/z; ^{1}H NMR ($CDCl_3$) 250 MHz: 7.38-7.51 (m, 5H), 6.24 (s, 1H), 4.79 (bs, 1H), 3.23 & 2.18 (m, 2H), 1.71(m, 1H), 0.76 (m, 6H) ^{13}C NMR ($CDCl_3$) 168.5, 155.7, 137.1, 129.2, 128.7, 126.2, 121.7, 93.1, 47.6, 27.5, 20.4 ppm. IR (KBr-

disc)3237, 3114, 2965, 2926, 2881, 2374, 2343, 1675, 1614, 1460, 1416, 1299, 1251, 1202, 1150, 1072, 1027, 878, 758, 696 cm^{-1}.

2.5. Molecular Modeling

For target preparation the protein structures, pdb identifier 1HZN for the CCK_1 and 1L4T for the CCK_2 –gastrin receptor were downloaded from the protein data bank (www.rcs.org) and docking was performed using AutodockVina and Hex. After several docking trials for the CCK_1 / CCK_2 receptor the results were analysed and visualized using Chimera and Designer studio 4.5. After visual inspection the results were presented to rationalize drug ligand interactions with the each CCK receptor subtype.

2.6. Radioligand Cholecystokinin Binding Assay

CCK_2 and CCK_1 receptor binding assays were performed, by using guinea pig cerebral cortex or rat pancreas. Male guinea pig brain tissues were prepared according to the modified method described by Saita et al[28]. Pancreatic membranes were prepared as described by Charpentier et al[29]. Tissues were homogenized in ice cold sucrose (0.32 M, 25 ml) for 15 strokes at 500 rpm and centrifuged at 13000 rpm for 10 minutes. The supernatant was re-centrifuged at 13000 rpm for 20 minutes. The resulting pellet was re-dispersed to the required volume of buffer at 500 rpm and stored in aliquots at 70^0C.

Binding was achieved using radioligand^{125}I-Bolton-Hunter labeled CCK, NEN at 25 pM. The samples were incubated with membranes (0.1 mg/ml) in 20 mMHepes, 1mM EGTA, 5 mM $MgCl_2$, 150mMNaCl, at pH 6.5 for 2 hrs at RT and then centrifuged at 11000 rpm for 5 minutes. The membrane pellets were washed twice with water and the bound radioactivity was measured in a Packard Cobra Auto-gamma counter (B5005). Binding assays were carried out with L-363, 260 as control.

2.7. Isolated Tissue Preparations

Male Sprague Dawley rats, weighing 200-250g were used and all animal care and experimental protocols adhered to the relevant laws and guidelines of the institution. The animals were housed under standard conditions of temperature (25°C) with unrestricted access to food and water. The animals were sacrificed using cervical dislocation without anaesthesia. From the abdomen of the animals, the duodenum was carefully excised and washed with physiological solution. The mesentery of the tissue was removed and the lumen was gently flushed with Tyrode's solution to clear luminal contents. The prepared isolated tissue was rapidly incubated in Tyrode's solution maintained at 32^oC and gassed with 95% O_2 / 5% CO_2.Tyrode's solution was freshly prepared daily (g/l): NaCl, 8.0; KCl, 0.2; $CaCl_2$, 0.2; $MgSO_4$, 0.1; NaH_2PO_4,0.05; $NaHCO_3$,1.0; Glucose,1.0.The main equipment used was the Radnoti single unit tissue bath system with a chamber capacity of 35ml. Bath aeration with carbogen (O_2 95%, CO_2 5%) was maintained at a constant temperature (32°C). The force in grams was measured with an isometric transducer linked to a power lab data acquisition system.

2.8. General Procedure

From the isolated tissue preparation, strips of appropriate length were mounted vertically in organ bath containing Tyrode's solution, under a tension of 1g and allowed to equilibrate for 30 minutes.Agonists, such as CCK_{8S}were directly applied in the bath and antagonists were pre-incubated for 10min. Stock solutions of all test compounds including the standard were prepared in DMSO.

2.9. Cholecystokinin CCK8 Preparations

CCK_{8S} was dissolved in distilled water to prepare a stock solution of 500µM solution, from which cumulative additions of increasing concentrations (0.1 nM, 1 nM, 5 nM, 10 nM, 20 nM, 30 nM, and 40 nM) were tested to plot a dose response curve. Test molecules and lorglumide were added to the organ bath 10 minutes before exposure to the next CCK_{8S} serial concentrations.

2.10. Electrically Stimulated Muscle Contractions

The intramural nerves within the ileal strips were excited by rectangular pulses of 2 ms, 25 mA and a frequency of 0.2 Hz. Transmural stimulation was applied using two platinum electrodes, one placed in the lumen of the ileum and the other outside the tissue.

2.11.Animal Studies

Experiments were conducted in male standard IRC mice obtained from the animal house, Faculty of Medicine, KhonKaen University. Each experimental group consisted of 6 animals and the treatment procedures were approved by the ethical committee, Faculty of Medicine, KhonKaen University (BEA030699).Mice were intraperitoneal injected with either test compound dissolved in 5% DMSO at the volume not more than 0.2 ml/animal. At 30 min after treatment, animals were tested as described in the following sections.

2.12.Nociception Tests

The tail immersion test: The thermal response latency was measured by the tail immersion test. The animals were placed into individual restraining cages leaving the tail hanging freely. The tail was immersed into water at 50^{o}C. The response time, at which the animal reacted by withdrawing its tail from water, was recorded and the cut-off time was 10 sec in order to avoid tissue damage. The base line withdrawal thresholds (BT) were recorded prior to the first injection. Test thresholds (TT) were measured 60 min after the second injection. The test thresholds were expressed as a percentage of Maximal Possible Effect (% MPE) using the equation: % MPE = {(TT-BT) / (45-BT)} x 100

DMSO (5%), pyrrolone (in 5% DMSO) was intraperitoneally injected and morphine was administered subcutaneously.

2.13.PK Analysis

6-8 week male rats purchased from Harlan Research Laboratories, North America Registration Number : Syngene-IAEC-412-08-2013 aged 6 to 8 weeks old Identification : They were identified individually with tail marking using permanent marker Acclimatization : At least for one week under laboratory conditions, after veterinary examination. Only animals without any visible signs of illness were used for the study.

Time points for blood sampling (IV dose) were pre-dose, 0.083, 0.25, 0.5, 1, 2, 4,6, 8, & 24 hr post dose (10 time points). Time-points for blood collection (PO dose) were, pre-dose, 0.25, 0.5, 1, 2, 4, 6, 8, & 24 hr post dose (9 time points). At each time point, approximately 150µL of blood was collected through jugular vein in labeled tubes containing K2-EDTA as anticoagulant. The tubes were mixed gently and centrifuged at 2500 g for 10 minutes at 4°C. The plasma was separated into labeled polypropylene tubes (~75µl of plasma) and stored immediately at -80°C until analysis.

Analysis of samples by LC-MS/MS was done using API Sciex 4000 system operated with Nexera ™ UHPLC (Shimadzu) as front-end. Samples were separated on a Phenomenexkinetex C18 (50X2.1 mm, 5µ) using a gradient mode at a flow rate of 1 ml/min. The mobile phase consisted of 0.1% formic acid in MilliQ water (A) and 0.1% formic acid in acetonitrile (B). MS instrument was operated in positive mode. The multiple reactions monitoring transition of test molecule was 247.9/192.0 (Q1/Q3) with a declustering potential of 70V, entrance potential 10 V, and collision energy of 25 V. The curtain gas (5 V), ion-spray voltage (5500 V), temperature (500°C), nebulizer gas (GS1), and auxiliary gas (GS2) were set at 45 psi & 55 psi respectively, and the interface heater was on.

2.14.Statistical Methods

The data were expressed as mean $\pm$ SD and one-way analysis of variance (ANOVA) and supplementary Tukey test for pair wise comparison were tested to determine for any significant difference at $p< 0.05$.

3. RESULTS AND DISCUSSIONS

3.1.Chemistry

5-arylated dichloro-2(5H)-furanones were synthesised from mucochloric acid (Scheme 1), which is commercially available from furfural under oxidising conditions with hydrochloric acid. Theses intermediates were evaluated previously as anticancer agents[30].

Mucochloric acid was reacted with benzene as reagent and solvent at RT under the development of hydrogenchloride gas. Depending on the scale of the reaction cooling with ice was required. For benzene the powdered or most preferred granulated aluminium chloride served as the best catalyst and during work up with hydrochloric acid on ice the inorganic salts were easily removed from the organic phase. For the small scale synthesis aluminium chloride worked well as Lewis acid. However,

during scale up aluminium chloride was replaced by trifluoroborane in THF as the exothermic reaction become problematic on a kg scale.

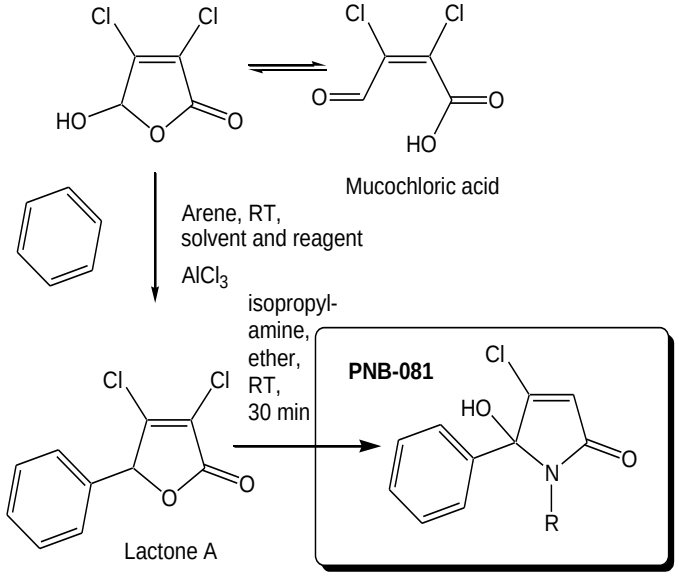
Subsequent reaction of the 5-arylated 3,4-dichloro-2(5H)-furanones(Stage 1 intermediate) in MTB etherwith alkyl-amines furnished N-alkylated hydroxyl-pyrrolones(Stage 2 products) in high yields under mild conditions. The general synthetic sequence is outlined in Scheme 1.

Overall, the desired N-alkylated unsubstituted 5-phenyl pyrrolones were obtained in only a 2 stage process as white crystalline material.

The molecule is not present in the ring opened keto form and fully occurred in the 5-membered ring form, as a hydroxy–pyrrolone.

The 5-arylated 2(*5H*)-furanones reacted selectively in the ester position and no reaction in the 4-position was observed here. Previously the IPSO substitution (4-position) was described for pseudo-esters[31], and here in scheme 1, a ring-opening ring-closure mechanism is proposed for the formation of hydroxy-pyrrolones.

Thus, the first step in the reaction sequence of the dichlorinated 2(*5H*)-furanone is the ring opening and amide formation from the corresponding lactone. Subsequently, the keto form of the acyclic amide was *in situ* converted into a lactame under the elimination of hydrogen chloride (middle, scheme 1).



a) Benzene, RT, 10 h, workup: hydrochloric acid

b) Isobutyl-lamine, excess, ether, RT, 30 min

Scheme 1. *Preparation of lactame PNB-081 from mucochloric acid.*

The analysis of lactame **PNB-081** by chiral HPLC showed a 50:50 racemic mixture of both enantiomers in solution in methanol.

3.2. SAR Optimisation

The first step was to screen for potent binding affinity and to identify a CCK_1or CCK_2-selective ligand for subsequent *in vitro* and *in vivo* evaluation.

Using radiolabelled iodinated cholecystokinin, inhibition of binding was determined for all test molecules and the IC_{50} are outlined in Table 1. Lorglumide served as CCK_1 standard and L-365,260 was used as CCK_2 standard.

The change from N-propyl into the N-butyl group resulted in a manifold increase of activity and the best substituent on the central nitrogen atom was found iso-butyl, as seen for derivative **7, PNB-081**. The introduction of a halogen atom into the para- position of the phenyl group resulted in an increase of binding affinity, possibly due to enhanced lipophilicity.

Table1. *CCK binding affinity expressed in IC_{50} in micromolarusing iodinated hot CCK_8 as radioligands with cortexand pancreatic membranes;N=3.*

Lactame	X=	R=	CCK_1 **[μM]**	CCK_2 **[μM]**
7	H	Isobutyl-	0.020±0.01	1.2±0.3
8	Cl	Isobutyl-	0.008±0.01	0.4±0.2
Lorglumide	-	-	0.17±0.01	>10
L-356,260	-	-	0.25±0.01	0.003±0.001

Overall, the introduction of alkyl groups, most preferred an isobutyl- group,provided a CCK_1 selective antagonist **PNB-081**, which was the selected development candidate. A fluorinated analogue had potent anticancer properties via the CCK_C receptor[32].

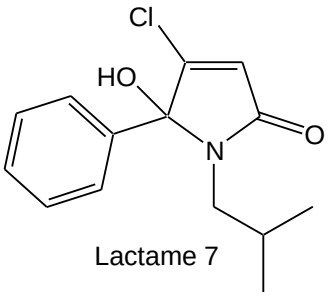


Fig 2. *Development candidate PNB-081 a CCK_1 antagonists*.

Lactame**8**(pA_2 = 8.1) has a nominally higher binding affinity compared with lactame**7** (Lactame**7**= **PNB-081**, pA2= 7.7)but they were found identical in vivo.

In addition PNB-081 has a higher melting point (higher chemical stability) and better physical properties (crystal properties). It can be produced during scale up with an increasing chemical yield and excellent (>99.7 %) purity, required by cGMP synthesis.

Therefore, PNB-081 chosen for preclinical development. Most importantly, *in vivo* it may be metabolized into its para hydroxy-analogue, in line with its pharmacological profile.

3.3. Molecular Modelling

Molecular modelling studies were performed for PNB-081 with the CCK_1 receptor (Figure 3b).The isobutyl group of the ligand interacted with a hydrophobic cave of the receptor, centred at Ala-14. The carbonyl group in the 2- position bond via hydrogen binding towards the CCK receptor with Arg-9 and the N- atom of the lactame interacted with Glu-17. The 5-hydroxy- group of the ligand displayed interactions with of Asn-6, while the phenyl group has no interaction with tryptophan or phenylalanine.

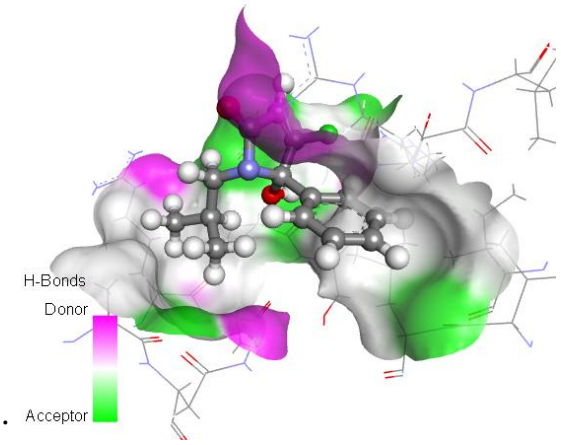


Fig 3a. *Molecular modelling of possible drug receptor interactions*

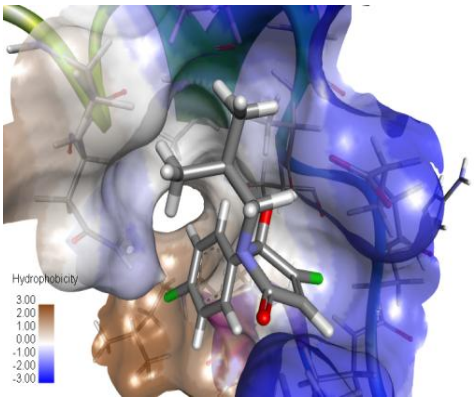


Fig 3b. *Drug receptor interactions of **PNB-081** with the CCK_1 receptor.*

Pi-alkyl interactions only may explain the small increase in binding affinity of the chlorinated analogue of PNB-081, based on interaction with Leu-29 and Ile-28. Most interestingly hydroxylation may also enhance CCK_1 affinity due to interactions with Arg 9.

3.4. Pharmacology

3.4.1. In Vitro Experiments Using Isolated Tissue Preparations

Initially CCK_4 was used, an agent, which trigged panic attacks in patients[33], but *in vitro* CCK_4 has a low solubility and low potency in the micro-molar range. The best full CCK agonist *in vitro* and in humans[34]is CCK_{8s}.

Cholecystokinin, CCK_{8s}, induced contractions of the guinea pig gall bladder[35] and this tissue based assay was adopted to the rat duodenum preparation.CCK-8s induced dose dependently contractions of the rat duodenum over a wide concentration range. These contractions were reduced dose dependently for PNB-081, which is outlined in fig. 4.

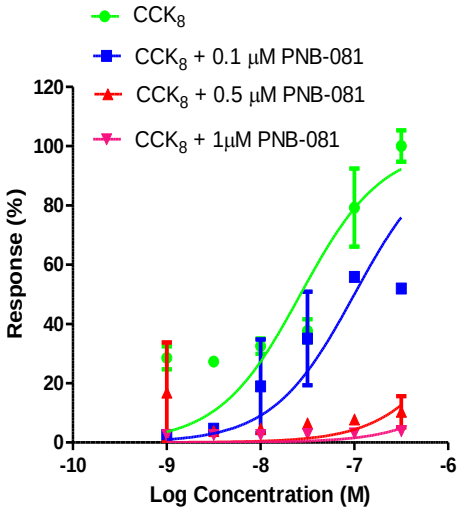


Fig 4. *Mean cumulative concentration–response curves for CCK-8s in the presence and absence of **PNB-081**.*

Increased concentrations of the antagonist, PNB-081 were added to the bath cumulatively and a shift of the curve to the right was observed. PNB-081 was acting as non-competitive antagonist[36].

The problem with the gall bladder based assay is the limit of the tissue and the rat duodenum represented an excellent alternative with a good expression of the CCK_1 receptor.

Opiate agonists, such as morphine and CCK antagonists, such as lorglumide and devazepide reduced electrically induced contractions on the GPI. From the radioligand binding assay the iso-butyl-pyrrolones were identified as the most potent ligands, and the classical isolated tissue preparation served as initial functional assay, confirming the antagonistic properties of these ligands.

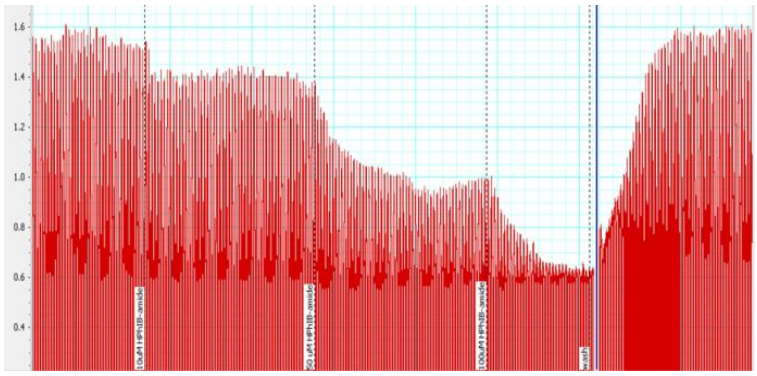


Fig 5. *The inhibitory effect of 0.1, 0.5 and 1.0 µM of pyrrolonePNB-081 on electrically stimulated rat duodenum tissue contractions.*

Using the isolated rat duodenum preparation, a stable amplitude was generated and a reduction of this amplitude was observed dose dependently for the isobutyl series, which is outlined in figure 5 for the pyrrolonePNB-081. This assay represents a fast and efficient way to screen for CCK antagonists using classical isolated tissue preparations.

In conclusion, the CCK antagonising properties of PNB-081 were clearly established using selective tissues and selective ligands. Under consideration of failed clinical trials for panic[37], positive pain results,our attention turned to a systematic *in vivo* evaluation of the agent as an adjunct to opiates.

3.4.2.In Vivo Evaluation

In order to evaluate the pain managing properties of PNB-081,the tail immersion assay were used in rodents.CCK antagonists potentiated the analgesia of opiates and usually (Lattmann, 2016) have no analgesic effect on their own.

3.4.3.Potentiation of Opiate Analgesia

It was now focussed on the potentiation of morphine analgesia for our selected cholecystokinin antagonist PNB-081.

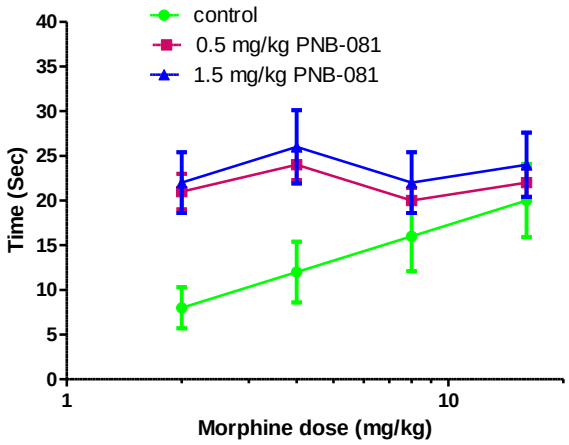


Fig 6. *Potentiation of morphine analgesia of a dose range from 2-16mg/kg in conjunction with 0.5 and 1.5 mg/kg* ***PNB-081****in the tail immersiontest.*

The analgesic effect of morphine served as baseline and a linear correlation of the response time in s against the log dose of morphine from 2 to 16 mg/kg was obtained in the tail immersion test in mice (Figure 6). An IP dose of 0.5 and 1.5 mg/kg of PNB-081, potentiated the low morphine dose by factor 3. The 2 mg/kg dose of morphine was shifted in presence of the CCK antagonist to an equivalent dose of 16 mg/kg morphine.

Previously 2 different chemical classes, the anilino- benzodiazepines (Lattmann, 2006) and pyrazols (Lattmann, 2005) showed pain potentiation in the same standard assays.

3.4.4.Opiate Tolerance

Opiates and endorphines, so small organic molecules as well as peptides act as opiate agonist and produce analgesia. This analgesic effect is reversed by cholecystokinin, not gastrin. The established

model of tolerance[38] is that, opiates induce the formation of cholecystokinin receptors and then cholecystokinin in form of CCK_{8s}, which is the main circulating form, neutralises opiate analgesia. A CCK antagonist does reverse this by blocking the newly formed CCK receptors.

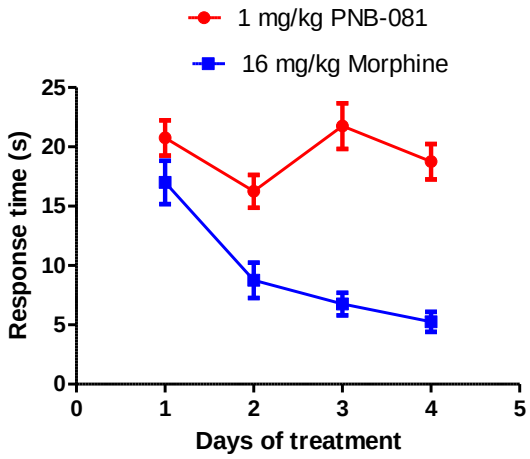


Fig 7. *Reversal of opiate tolerance: 16mg/kg of morphine as single agent and in conjunction with by PNB-081, 1mg/kg, in the tail immersiontest.*

Experimentally, in line with the proposed model, a16mg / kg dose of morphine, exhibited the anticipated analgesic effect and this analgesia faded out within days based on bydaily sc administration of morphine (Figure 7). In presence of 1 mg **PNB-081** administed intraperitonally, no tolerance was observed and a continuous analgesic effect was observed over the duration of 4 days.

This is clear confirmation, that opiates and cholecystokinin are supposed to have opposing effects. Prolonged exposure to opiates induced the expression of CCK receptors and these CCK receptors were blocked by the CCK antagonist PNB-081.

PNB-081 may be hydroxylated *in vivo* in mice gaining CCK_2 selectivity[39] . Thus, a dual acting CCK antagonist may be produced, alleviating best the anti-nociceptive effects of morphine.

3.4.5. Pharmakinetic Analysis

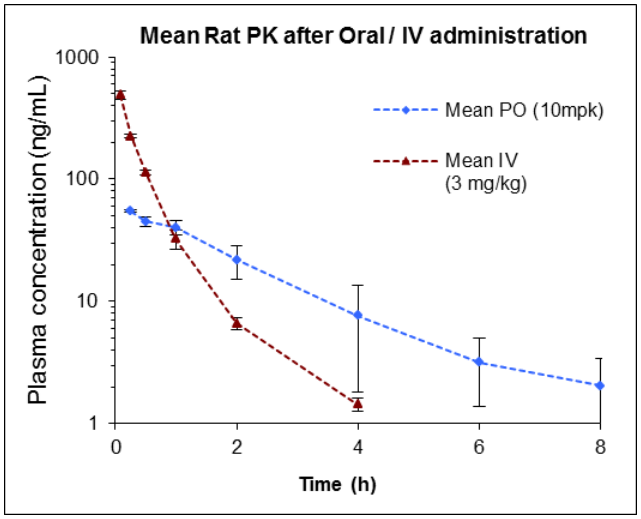


Fig 8. *Kinetics of PNB-081.*

For lactam PNB-081 an oral bioavailability was determined as 24% in rats for the 10mg/kg dose. The analysis of the plasma concentration in rats was analysed for PNB-081 (24%) and is outlined in figure 8.

The isobutyl group, as aliphatic side chain, is inert towards metabolism and the bioavailability for PNB-081 is therefore medium.

As part of the preclinical development, the pharmacokinetics of PNB-081were fully analysed. In summary PNB-081 showed a good half-life in dogs and rats. Protein binding was determined of 82.4% in human plasma and a very high membrane permeability was determined by using the Caco-2 monolayer assay. PNB-081 is orally available and entered preclinical toxicology.

4. Conclusion

The target molecule PNB-081 was synthesised in only 2 steps from one readily available starting materials and will potentially deliver affordable therapeutic agents for long term pain management.

A first in class analgesic CCK antagonist was developed under the consideration of membrane penetration, half-life and bioavailability.

Overall the cholecystokinin antagonist PNB-081 has shown the expected CNS profile with opiate potentiation and the reversal of opiate tolerance.

PNB-081 may help to alleviate the current opiate crisis in industrialised countries.

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No conflict of interest is declared.

The animal studies were performed in compliance with relevant laws and institutional guidelines.

References

[1] Maton P, Sutliff V, Jensen R, et al. Carbobenzoxy amino acids: Structural requirements for cholecystokinin receptor antagonist activity. *AmJ Physiol.* 1985;248:479.

[2] Herranz R. Cholecystokinin Antagonists: Pharmacological and Therapeutic Potential. *MedResRev*. 2003;23:559.

[3] Mawe GM. The role of cholecystokinin in ganglionic transmission in the guinea-pig gall-bladder. *JPhysiol*. 1990;439:89.

[4] Jorpes E, Mutt V. Cholecystokinin and Pancreozymin, one single Hormone? *Acta PhysiolScand.* 1966;66:196.

[5] McDonald IM. CCK_2 receptor antagonists. *ExpOpinTher Patents*. 2001;11:445.

[6] Bock MG, DiPardo RM,Mellin EC, et al.Second-generation benzodiazepine CCK-B antagonists. Development of subnanomolar analogues with selectivity and water solubility. *J Med Chem*. 1994;37: 722.

[7] Lattmann E, Arayarat P. From CNS-drugs to anti-neoplastic agents: Cholecystokinin (CCK) antagonists as modern anti-cancer agents.*KKU Science J.* 2003;31: 178.

[8] Dourish CT. Cholecystokinin and anxiety. *Trends Pharmacol Sci*.1990;11: 271.

[9] Rasmussen K, Czachura, JF, Stockton, ME, et al. Electrophysiological effects of diphenylpyrazolidinone cholecystokinin-B and cholecystokinin-A antagonists on midbrain dopamine neurons. *J PharmacolExpTher*. 1993;264:480.

[10] Dourish CT, Rycroft,W, Iversen, SD. Postponement of satiety by blockade of brain cholecystokinin-B receptors. *Science.* 1989;245:1509.

[11] Bradwejn J, Koszycki D, Meterissian G. Cholecystokinin tetrapeptide induces panic attacks in patients with panic disorder. *Can J Psychiaty.* 1990;35: 83.

[12] Hughes J, BodenP, Costallt B, et al. Development of a class of selective cholecystokinin type B receptor antagonists having potent anxiolytic activity. *Proceedings of the National Academy of Sciences USA*. 1990;87:6728.

[13] Trivedi K, Bharat J. Cholecystokinin receptor antagonists:Current status, *CurrMedChem.* 1994;1:313.

[14] Chang R, Lotti V, Monaghan R, et al. A potent nonpeptide cholecystokinin antagonist selective for peripheral tissues isolated from Aspergillus alliaceus. *Science*. 1985;230:177.

[15] Lattmann E, Billington DC, Poyner DR, et al. Synthesis and evaluation of Asperlicin analogues as non-peptidal Cholecystokinin-antagonists. *Drug DesDiscov.*2001;17:219.

[16] Evans BE, Rittle KE, Bock MG, et al. Methods for drug discovery: Development of potent, selective, orally effective cholecystokinin-A antagonists. *J Med Chem*. 1988;31:2235.

[17] Hahne W, Jensen R, Lemp G, et al. Proglumide and benzotript: Members of a different class of cholecystokinin receptor antagonists. *Proceedings of the National Academy of Sciences USA*. 1981;78:6304.

[18] Makovec F, Chistè R, Bani M, et al. New glutaramic acid derivatives with potent competitive and specific cholecystokinin-antagonistic activity. *Arzneimittelforschung.*1985;35:1048.

[19] Noble F, Wank SA, Crawley JN, et al. International Union of Pharmacology XXI. Structure, Distribution, and Functions of Cholecystokinin Receptors. *PharmacolRev.* 1999;51: 4.

[20] Woodruff GN, Hughes J. Cholecystokinin antagonists.*Annu Rev PharmacolToxicol*. 1991;31:469.

[21] Lattmann E, Sattayasai J, Schwalbe CH, et al. Analgesic Effects of 5-Alkyloxy-4-amino-2(5H)-furanones as Cholecystokinin-2 antagonists. *Arch Pharm (Weinheim).* 2016;349:456.

[22] YekkiralaAS, Roberson DP, Bean BP, et al. Breaking barriers to novel analgesic drug development. *Nat Rev Drug Discov.* 2017;16:545.

[23] DourishC, O'Neill M, Coughlan J, et al. The selective CCK-B receptor antagonist L-365,260 enhances morphine analgesia and prevents morphine tolerance in the rat. *Eur JPharmacol.* 1990;176:35.

[24] Simpson KH, Serpell M, McCubbins TD, et al. A multi-dose study: Management of neuropathic pain in patients using a CCK antagonist devazepide (DEVACADE®) as an adjunct to strong opioids. In World Congress of 10th IASP 2002; San Diego, CA, U.S.A; (abstract)

[25] LattmannE, RussellST, SchwalbeCH, et al. Cholecystokinin-1 receptor antagonists: 5-hydroxy-5-aryl-pyrrol-2-ones as anticancer agent. *Med ChemCommun.* 2016;7:1138.

[26] Lattmann E,Sattayasai J, Narayanan R, et al. Cholecystokinin-2/gastrin antagonists: 5-hydroxy-5-aryl-pyrrol-2-ones as anti-inflammatory analgesics for the treatment of inflammatory bowel disease. *Med ChemCommun.* 2017:8:680.

[27] BaberS, Dourish T, HillR.The role of CCK caerulein, and CCK antagonists in nociception. *Pain*. 1989;39:307.

[28] Saita Y, Yazawa H, Honma, Y, Nishida A, Miyata K, Honda K. Characterization of YM022: its CCKB/gastrin receptor binding profile and antagonism to CCK-8-induced Ca^{2+} mobilization. *Eur J Pharmacol.* 1994;269: 249-281.

[29] Charpentier B, Pelaprat D, Durieux C, Dor A, Reibaud M, Blanchard JC, Roques BP. Cyclic cholecystokinin analogues with high selectivity for central receptors. *Proc Natl Acad Sci*.U.S.A. 1988;85:1968-1973.

[30] Lattmann E, Ayuko WO, Kinchinaton D, et al. Synthesis and evaluation of 5-arylated 2(5H)-furanones and 2-arylated pyridazin-3(2H)-ones as anticancer agents. *J Pharm Pharm.* 2003;55:1259.

[31] Lattmann E, Sattayasai N, Schwalbe CS, et al. Novel anti-bacterials against MRSA: synthesis of focussedcombinatorial libraries of tri-substituted 2(5H)-furanones. *Curr Drug Discov Technol.* 2006;3:125.

[32] Ponnusamy S, Lattmann E, Lattmann P, et al. Novel, isoform-selective, cholecystokinin A receptor antagonist inhibits colon and pancreatic cancers in preclinical models through novel mechanism of action. *OncolRep.* 2016;35:2097.

[33] BradwejnJ, Koszycki D, Couetoux du Tertre A, et al. The panicogenic effects of cholecystokinin-tetrapeptide are antagonized by L-365,260, a central cholecystokinin receptor antagonist, in patients with panic disorder. *Arch Gen Psychiat.* 1994;51:486.

[34] D'Amato M, Stamford I, Bennett A. The effects of cholecystokinin octa-peptide on human isolated alimentary muscle. *BrJPharmacol*. 1990;100:126.

[35] Bishop L, GerskowitchV, Hull R, et al. Combined dose-ratio analysis of cholecystokinin receptor antagonists, devazepide, lorglumide and loxi-glumide in the guinea-pig gall bladder. *Br JPharmacol*. 1992;106:61.

[36] Kenakin T, Jenkinson S, Watson C. Determining the Potency and Molecular Mechanism of Action of Insurmountable Antagonists. *J PharmacolExpTher*.2006;319:710.

[37] Sramek JJ, Kramer MS, Reines SA, et al. Pilot study of a CCK_B antagonist in patients with panic disorder: Preliminary findings. *Anxiety.*1994;1:141.

[38] Wiesenfeld-Hallin Z, Xu XJ. The role of cholecystokinin in nociception, neuropathic pain and opiate tolerance. *RegulPept.* 1996;65:23.

[39] Zhou Y, Sun Y, Zhang Z, et al.Increased release of immunoreactive cholecystokinin octapeptide bymorphineandpotentiation of mu-opioid analgesia by CCKB receptor antagonist L-365,260 in rat spinal cord. *EurJPharmacol*.1993;234**:**147.

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