Cholecystokinin-2/gastrin antagonists: 5-Hydroxy-5-aryl-pyrrol-2-ones as anti-inflammatory analgesics for the treatment of inflammatory bowel disease†

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Arylated 5-hydroxy–pyrrol-2-ones were prepared in 2 synthetic steps from muco- chloric acid and optimised as CCK_2 selective ligands using radiolabelled binding assays. CCK antagonism was confirmed for the ligands in isolated tissue preparations. DSS (dextran sulfate sodium) induced inflammation was analysed for derivative 7 and PNB-001 with L-365,260 as standard. The IC_{50} of PNB-001 was determined as 10 nM. Subsequent in vivo evaluation confirmed anti-inflammatory activity with respect to IBD assays. The best molecule, PNB-001, showed analgesic activity in the formalin test and in the hotplate assay the analgesic effect of 1.5 mg/kg PNB-001 was equivalent to 40 mg/kg tramadol. The CCK_2 selective antagonist PNB-001 protected rats against indomethacin induced ulceration at similar doses. The GI protection activity was found more potent than the 10 mg/kg dose of prednisolone, which served as standard.

Introduction

Inflammatory bowel syndrome (IBS) is a complex symptom of unknown ethology, characterized by abdominal pain or discomfort associated with disturbed defecation and often bloating. Inflammatory bowel disease (IBD) encompasses at least two forms of intestinal inflammation being expressed as ulcerative colitis and Crohn's disease. Although many other inflammatory disorders affect the gastrointestinal tract, most can be distinguished by a specific underlying process from IBD. Stress may exacerbate IBS symptoms and the CCK_2 receptor is known to mediate anxiety[1] and panic attacks. Though several drugs such as anti-cholinergic-, anti-spasmodic- agents and tricyclic anti-depressants are of limited use for IBS and steroids for IBD, inflammatory bowel disease is still classified as unmet medical need and hundreds of studies are ongoing worldwide.

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Aim of the drug discovery programme was to target the underlying mechanism of the disease, thus targeting Cholecystokinin (CCK) pathways. CCK, a peptide hormone, which is extensively found in the gastrointestinal tract (GIT) is widely distributed through the nervous system[2]. Cholecystokinin acts as a neuromodulator / gut hormone and CCK-ligands, agonists as well as antagonists[3], have been extensively investigated as potential drug targets[4]. CCK-antagonists were studied as growth inhibitors in certain forms of cancer[5], as anxiolytics[6], in the treatment of schizophrenia[7], satiety[8] and as anti-panic agents[9]. An agonist, the shortened CCK tetrapeptide, was found to induce panic in patients[10].

Despite the progress of several CCK receptor antagonists[11] to different phases of clinical trials, only proglumide was marketed as Milid for the treatment of gastric ulcer.

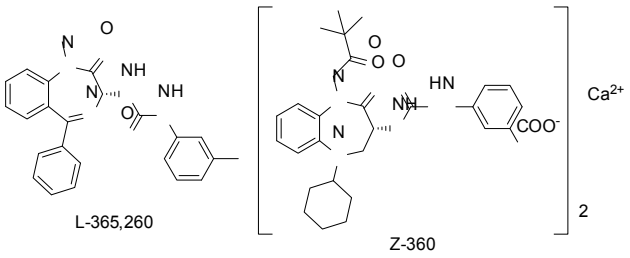


Figure 1. CCK_2 / gastrin antagonists, Merck standard and most recent addition.

Merck's standard CCK_2 antagonist[12] is outlined in Figure 1. Z-360 is the most recent derivative derived from this original lead structure, in which the N was moved to the 5-position. The N-1 was alkylated, the 5 phenyl group was replaced by a cyclohexyl group and the water solubility was enhanced by converting the Me group on the side chain into a carboxylic acid. All structural optimisations did not address the main underlying problem with respect to poor pharmacokinetic properties, such as a low solubility and very low membrane penetration resulting from the large polar surface area of the molecule.

PNB Vesper Life Science systematically investigated the hydroxyl-pyrrolone scaffold and the isobutyl derivative[13] served as lead structure on this programme towards novel anti-inflammatory

analgesics targeting the underlying mechanism of inflammatory ulceration.

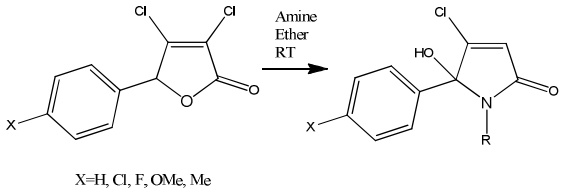
A full biological evaluation of lactame **14**, now PNB-001, will be reported here, in this publication from a CCK-5 pentagastrin antagonism to initial anti-inflammatory analgesic activity.

Results and discussions

Synthesis

Mucochloric acid was reacted in presence of a Lewis acid via S_E reactions into a series of arylated dichlorinated furanones. In addition to halogenated X=Cl, F 5-arylated 2(5H)-furanones containing further donor substituents were prepared. The chemical yields were generally lower and binding affinity was also decreased in the resulting final molecules. Arylated 2(5H)-furanones, containing nitro- groups or trifluoro-methyl groups could not be prepared.

Subsequent reaction of the 5-arylated 3,4-dichloro-2(5H)-furanones with amines, in particular with aryl alkyl amines, furnished 15 arylated N-alkylated hydroxyl-pyrrolones and the 2nd step of the synthetic sequence is outlined in Scheme 1.

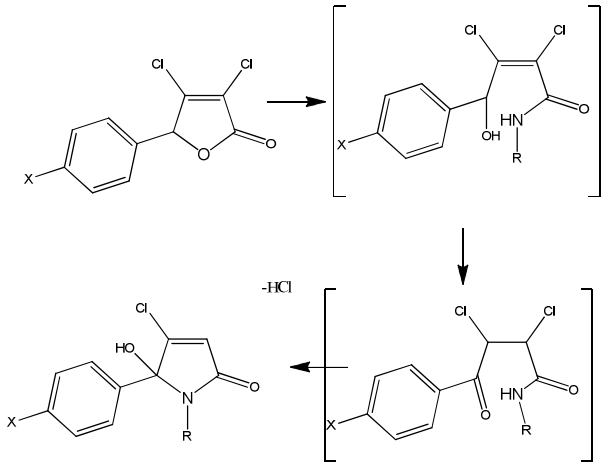


Scheme 1. Synthesis of lactames **1-15** from 3,4-dichloro- 5-arylated 2(*5H*) furanones. For the synthesis of building blocks from mucochloric acid see Lattmann et al, MedChemComm 2016.

The 5-arylated 2(*5H*)-furanones reacted selectively in the ester position and no reaction in the 4-position was observed. Previously the substitution in the 4-position was described for pseudo-esters[14], and here a ring -opening ring -closure mechanism for the formation of hydroxyl-pyrrolones is proposed in scheme 2.

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

Analysis by chiral HPLC showed a 50:50 racemic mixture of the novel template in solution in methanol.



Scheme 2. Chemical mechanism for the formation of 5-hydroxy-5-aryl-pyrrol-2-ones with amines.

SAR optimisation

Initially SAR were fully explored and optimised for the CCK_2/gastrin receptor, using a radiolabelled binding assay with iodinated CCK_8. The results, expressed in IC_{50}, are outlined in table 1.

Lorglumide served as CCK_1 standard and L-365,260 was used as CCK_2 standard. For a better comparison the initial starting point, the CCK_1 antagonist PNB-028, is also included in Table 1.

Cyclic substituents on the N-position, such as cyclopropyl-, cyclopentyl- and cyclohexyl provided activity in the micromolar range (Lactame **1-5**). Anilines were inactive and the introduction of the benzyl group furnished a dual ligand in nanomolar range (lactame **7**). Halogen atoms on the 5 phenyl group marginally enhanced the binding affinity and the best derivative was the fluorinated N-benzyl lactame **9**.

Donor substituents on the 5-phenyl group, such as para-methoxy and methyl groups resulted in ligands with manifold lower activity (lactame **10**, **11**). The introduction of a chiral amine, such as methyl benzyl amine provided diastereoisomers (**12**, **13**), which were separated by column chromatography, but both isomers occurred a lower affinity than the parent benzyl derivative **7**. Further optimisation on the N-benzyl group was performed, but the introduction of a para F- and methoxy- group, using p-fluorobenzyl amine and p-methoxy benzylamine, did not enhance the binding affinity for the CCK receptor.

The introduction of a spacer, a single CH_2 group, resulted in a phenyl-ethyl derivative **14**, which represented a highly CCK_2 selective ligand.

Lactame **14** is 450 times selective for the CCK_2 / gastrin receptor. Halogenation, the introduction of a para- chlorine atom on the phenyl–position, lactame **15**, did not enhance binding affinity any further; possibly due to drug receptor interaction of the phenyl group with a lipophilic cavity within the CCK receptor.

Table 1. CCK binding affinity using radioligands with cortex and pancreatic membranes. IC_{50} is presented in micromolar; N=3.

Lactame	X=	R=	CCK-A **[μM]**	CCK-B **[μM]**
Lorglumide	-	-	0.17±0.01	>10
L-356,260	-	-	0.25±0.01	0.003±0.001
PNB-028	F	Isobutyl-	0.012±0.001	0.75±0.21
1	H	Cyclopropyl-	7.5±0.3	>10
2	Cl	Cyclopropyl-	4.0±0.3	>10
3	H	Cyclopentyl-	0.36±0.30	0.84±0.40
4	Cl	Cyclopentyl-	2.50±0.20	>10
5	H	Cyclohexyl-	>10	>10
6	H	Ph-	>10	>10
7	H	Bz-	0.80±0.04	0.022±0.002
8	Cl	Bz-	0.51±0.04	0.020±0.004
9	F	Bz-	0.32±0.01	0.017±0.002
10	MeO	Bz-	0.21±0.02	4.5±0.4
11	Me	Bz-	1.5±0.04	4.1±0.3
12	H	Methylbenzyl-	0.60±0.04	0.21±0.01
13	H	Methylbenzyl-	0.42±0.03	0.21±0.15
14	H	Phenylethyl-	>10	0.022±0.002
15	Cl	Phenylethyl-	>10	0.030±0.001

Molecular modelling - Ligand docking

The docking of the phenylethyl pyrrolone **14** into the CCK_2 receptor is outlined in figure 2 for one final pose of minimal energy and some key drug receptor interactions are highlighted.

The 2-carbonyl group of the central pyrrolone template interacts via hydrogen binding with the N group of Trp-114. The phenyl group of the N-phenylethyl- side chain binds towards the aromatic indole system of Trp-114 and electron withdrawing groups should enhance these aromatic interactions. The lipophilic pocket in the CCK_2 receptor comprising of Ile-184 and Leu-133, allows principally a wide range of substituents, but only phenyl and not cyclo-hexyl can be realised synthetically. The 5-phenyl group of the pyrrolone template binds via Ile-184 and Leu-133, based on van der Waals interactions and not aromatic interactions. Therefore, the introduction of electron withdrawing groups on the 5- phenyl group, such as a chlorine atom, do not enhance binding affinity and optimisation on this side is overall with limited effect. Interestingly, the metabolism of the 5-phenyl derivative **14**, may result in a p-hydroxy-phenyl metabolite, which may interact additionally with His-122 via a strong hydrogen bond and the role of metabolites is currently under investigation.

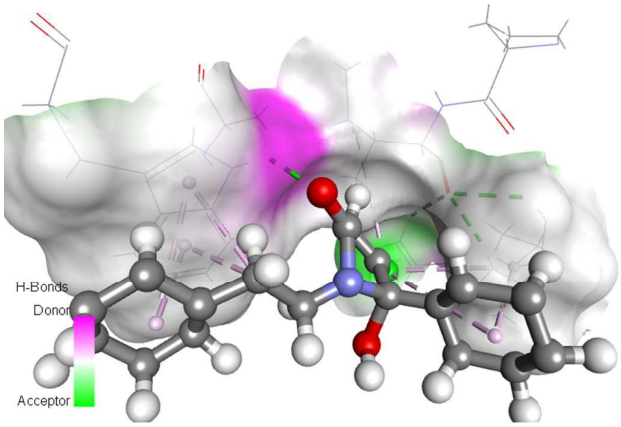


Figure 2. Docking of CCK antagonist **PNB-001** into the CCK_2 receptor.

The molecular structure of lactame **14**, (Table 1) which is now **PNB-001** was designed from an iso-propyl lead structure via the benzyl derivative **7** (Figure 3). PNB-001 contains the N-phenyl-ethyl substituent, which is required for high CCK_2 / gastrin selectivity[15].

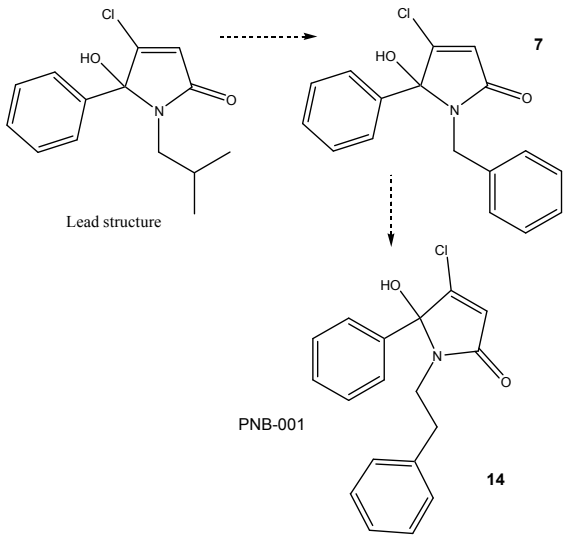


Figure 3. Lead design. From a CCK_1 antagonist to a CCK_2 selective molecule.

The mixed dual acting N- benzylated pyrrolone **7** (Table 1) is showing the expected pharmacological profile of a mixed CCK

antagonist[16] and its role in brain cancer is currently being elucidated.

The phenyl ethyl derivative PNB-001 completed preclinical development as anti-inflammatory analgesic and progressed into Phase 1 clinical trials for the treatment of IBD.

Isolated tissue preparations

CCK antagonism

The CCK_2 selective ligand PNB-001, occurred a potent binding affinity and the CCK-antagonism was studied[17] using pentagastrin, CCK-5 induced contractions of the rat duodenum.

Initially CCK_4 was used[18], but CCK_4 has a low solubility and low potency in the micro-molar range. The best CCK_2 selective agonist is CCK-5 and this was used to analyse the agonist or antagonist properties of the ligand.

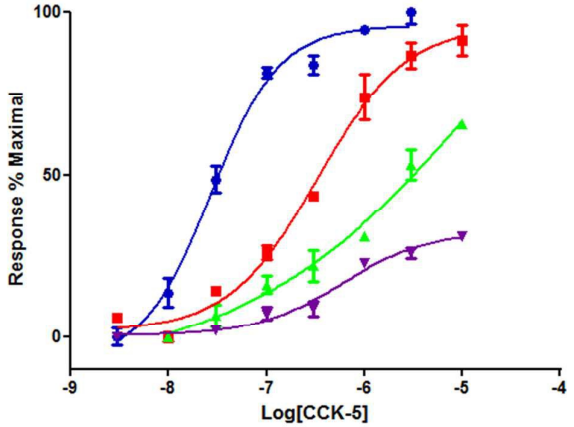


Figure 4. Responses to CCK-5 in the absence and presence of PNB-001; CCK-5, CCK-5 +10 nM PNB-001, CCK-5 +30 nM PNB-001, CCK-5 + 100 nM PNB-001; N = 2 for each data point. Values are ±SEM.

The concentration response curve of pentagastrin, CCK-5, was recorded and shifted to the right by a nanomolar concentration of PNB-001, thus confirming the antagonistic properties[19] of this ligand.

100 nM of PNB-001 with 30 min of incubation time fully blocked the CCK_4 and CCK_5 induced contractions. For the 5 min incubation cycle, 10 nM of PNB-001 shifted the CCK-5 concentration response curve to the right and from 30 nM onwards, the antagonist PNB-001 showed a reduced maximum response. At high nano molar concentrations PNB-001 acted as unsurmountable gastrin/cholecystokinin antagonist.

As the clinical trial outcome of selective CCK/gastrin antagonists was found questionable in the treatment of anxiety and depression[20], the aim of the programme was, to focus on selectively designing CCK_2 antagonists for the gastrointestinal system and to focus on inflammation thereof.

Inflammatory bowel disease, IBD, is classified an unmet medicinal need by the FDA and allows fast track approval. The indomethacin induced ulceration is a standard *in vivo* assay for IBD[21].

In vitro inflammation

In vitro, spontaneous contractions correlate with inflammation and anti-inflammatory steroids such as dexamethasone reduced spontaneous contractions of the duodenum[22].

DSS, dextran sulfonic acid sodium, is a standard agent to induce inflammation for IBD in vivo and in vitro the effect is slower and milder than for Trinitro-benzene sulfonic acid, TNBSA[23].

DSS was used here in vitro using isolated organ preparations and preparations of the duodenum worked best in this assay[24].

L-365,260 was applied as CCK_2 standard and the mixed CCK antagonist HPhBz, lactame **7**, together with the highly CCK_2 selective antagonist PNB-001 were all tested under the same conditions. The concentration response curves, showing how these agents inhibit contractions a very low concentrations, are outlined in Figure 5.

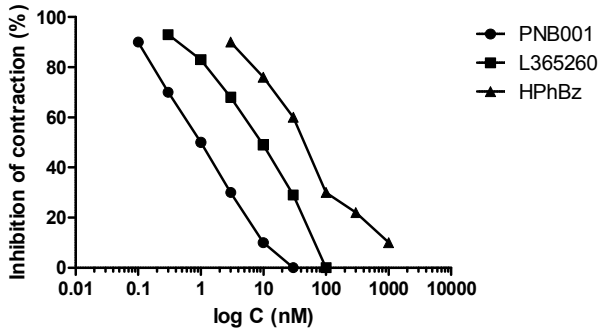


Figure 5.The inhibitory concentration-response curves of PNB-001, L-365,260 and HPhBz (lactame **7**) for DSS stimulated contractions.

An IC_{50} of 1 nM was determined for CCK_2 antagonist PNB-001. The Merck CCK_2 standard occurred a ten times lower IC_{50} and the benzylated derivative HPhBz, lactame **7**, had the lowest anti-inflammatory effect. Considering the chemical similarity between the lactame **7** and PNB-001, the addition of an extra CH_2 group resulted in a remarkable effect. The CCK_1 antagonism may oppose the anti-inflammatory effect, but the possible mechanistic pathway is outside the scope of this publication.

Based on the *in vitro* results with respect to anti-inflammatory activity, PNB-001 was selected for further *in vivo* assays. This programme was concerned with inflammation of the GI tract and the indomethacin induced ulceration is the best model for IBD, inflammatory bowel disease and ulcerative colitis, which is associated with the release of gastric acid. Here, the anti-inflammatory action of the molecule, supported by anti-gastric activity, was supposed to create a synergistic therapeutic effect.

In vivo evaluation

Inflammatory/ulceration protection

Gastrin triggers the release of gastric acid in the stomach and the content of the GI system moves from the duodenum, jejunum via ileum to the colon. The inflammation was assessed in this order by organ weight and inflammation scores. The measurement of weight as inflammation parameter is reliable and the induction of inflammation / ulceration resulted in an increased organ weight.

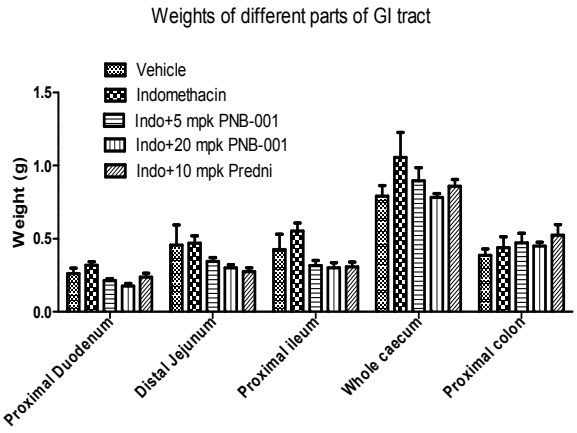


Figure 6. Indomethacin induced ulceration. Weights of the GI tract treated by PNB-001.

Indomethacin increased weight in all observed organ parts and this increase of weight was reduced dose dependently by PNB-001 for the duodenum, jejunum, and ileum. The effect of protection is observed for all parts of the GI system and PNB-001 performed better than the steroid standard prednisolone.

The second accessed parameter was scores of inflammation and the results are outlined in Figure 7.

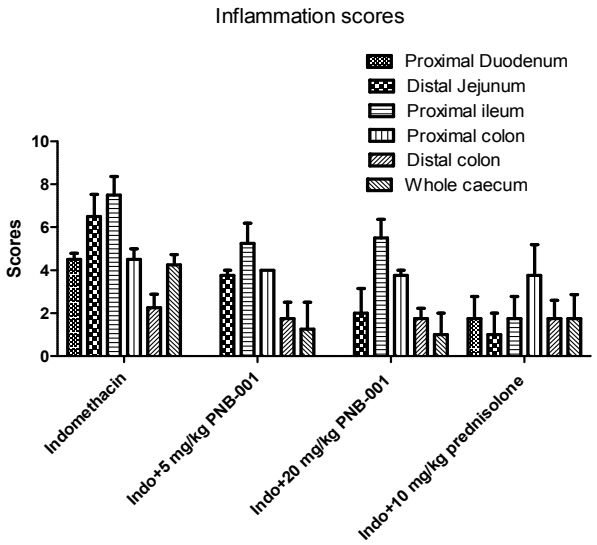


Figure 7. Inflammation score of the GI tract treated by PNB-001.

Treatment is presented for each individual organ and scores of inflammation were determined. Most interestingly the gaps in the treatment groups with zero inflammation are present in the duodenum for both the 5 and the 20 mg /kg dose of PNB-001 via oral administration. Indomethacin induced an inflammation score of 5 in the duodenum, which was fully protected by both doses of the CCK_B antagonist and reduced to 2 by prednisolone at 10 mg/kg.

In figure 8 selected photos of the gastrointestinal system with ulceration are depicted. In the duodenum the perforation is clearly visible and this is fully prevented by a low 5 mg /kg dose of PNB-001. A similar effect is seen for the jejunum on indomethacin induced ulcers and this damage of the GI system was prevented by PNB-001.

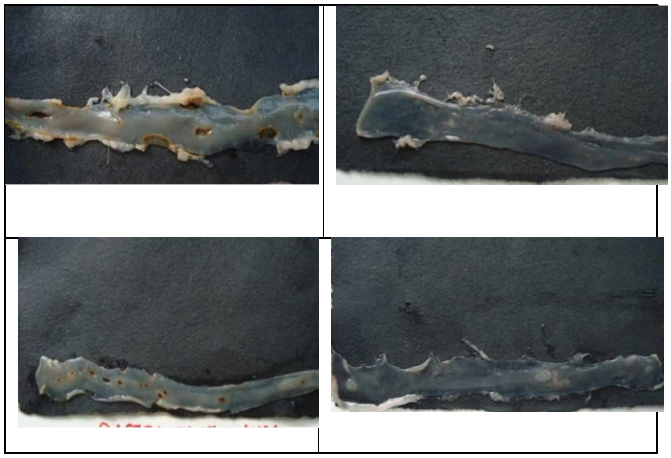


Figure 8. Top: Photos of duodenum; below jejunum. Selected parts of the GI system, indomethacin induced ulcers and prevented by CCK_2 / gastrin antagonist PNB-001.

Analgesic evaluation

CCK antagonists potentiate the analgesia of opiates and usually, except for proglumide, have no analgesic effect on their own [25]. For Z-360 an interesting weak analgesic effect in the formalin test was observed[26].

Thus, a first evaluation of the analgesic properties of PNB-001 was performed using the formalin test.

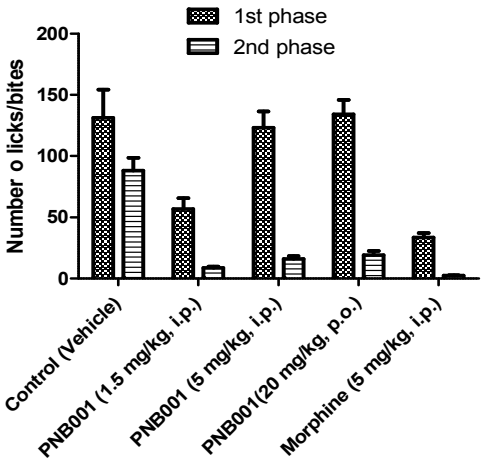


Figure 9. Analgesic anti-inflammatory activity of PNB-001 on formalin induced pain in rats.

Opiates, such as morphine, reduce the response in form of licks and bites to formalin in the first phase of the assay. Anti-inflammatory agents are active in this assay in the second phase.

PNB-001 showed only at the 1.5 mg/kg dose some opiate like effects (phase 1) and displayed at this dose also the best anti-inflammatory effect (phase 2). The 5 mg dose by IP administration is equivalent to the 20 mg/kg dose by oral administration, but had a lower analgesic effect than the 1.5mg/kg dose. However, the activity in the second phase of the formalin test, reconfirmed the anti-inflammatory activity found *in vitro* and *in vivo*.

In order to evaluate the pain managing properties of the new agent, the partial opiate agonist tramadol was included in a last study, in which the hot plate assay was used to evaluate analgesic activity.

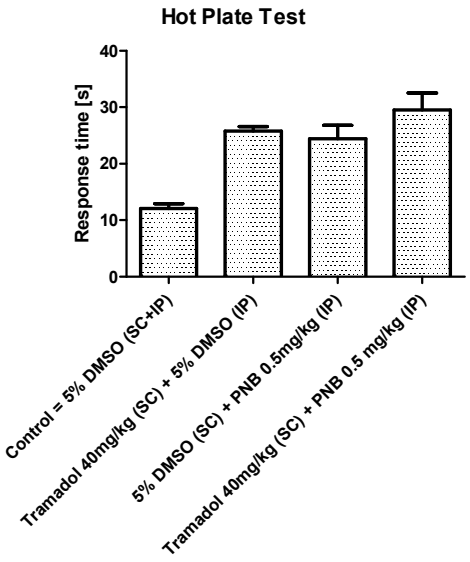


Figure 10. Hotplate test of PNB-001 in mice by IP – administration.

Bar 1 is the baseline with a response time about 12 s. The maximum analgesic effect in the hotplate is 26 s for 40 mg/kg tramadol, a more than 100% of change from the control.

The analgesic efficacy of 0.5 mg/kg PNB-001 by IP administration as a single agent was found equivalent to 40 mg/kg tramadol (SC administration). Interestingly, there is no significant potentiation of tramadol analgesia with PNB-001. The selective CCK_2 / gastrin antagonist, worked here in this assay as analgesic on its own. This analgesic activity may be found useful in the treatment of IBD/IBS pain, additionally.

Conclusions

This novel template, which originally occurred CCK_1 activity[27] was optimised as CCK_2/gastrin selective antagonist.

The target molecule was synthesised in only 2 steps from readily available starting materials and will potentially deliver affordable therapeutic agents.

By designing a potent CCK_2 /gastrin antagonist for inflammatory-diseases and not CNS disorders, a first in class CCK_2 antagonist with analgesic anti-inflammatory activity was developed under the consideration of membrane penetration, half-life and bioavailability.

By focussing on unmet medical needs such as inflammatory bowel disease, IND was granted in India and the FDA approval is expected for late 2019.

The mild analgesic effect of PNB-001 may offer an alternative drug treatment to Tramadol, which is closing the gap of pain management between NSAIDs and opiates.

Acknowledgements

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