

Full Paper

Analgesic Effects of 5-Alkyloxy-4-amino-2(5H)-furanones as Cholecystokinin-2 Antagonists

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4-Amino-2(5H)-furanones were synthesized in high yields over two synthetic steps from readily available mucochloric acid. These 5-alkyloxy-4-amino-2(5H)-furanones were screened in a [¹²⁵I]-CCK-8 radioligand receptor binding assay for CCK₂ affinity and novel active ligands in the nanomolar range were identified. SAR was optimized leading to the cyclohexyl derivative **25** with an IC₅₀ of 27 nM. Furanone **18** was obtained as a stable crystalline material with an IC₅₀ of 85 nM, but had a higher CCK₂ selectivity. It was subsequently tested in the isolated guinea pig ileum assay with sulfated CCK₈, and the CCK antagonizing properties of the ligand were confirmed. The CCK₂ selective antagonist **18** was found to potentiate analgesia in the tail flick assay in mice, for the strong opiate morphine, the partial opiate agonist tramadol and the tricyclic antidepressant desimipramine.

Keywords: 2(5H)-Furanones / Analgesia potentiation / CCK antagonists / Isolated tissue preparations / Radioligand binding

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Introduction

Cholecystokinin acts as a neuromodulator as well as gut hormone and CCK-ligands, agonists as well as antagonists [1], have been extensively investigated as potential drug molecules [2]. CCK-antagonists were studied as growth inhibitors in certain forms of cancer [3], as anxiolytics [4], in the treatment of schizophrenia [5], satiety [6], and as anti-panic agents [7]. The most simplified agonist, the shortened CCK tetrapeptide, was found to induce panic in patients and these effects were blocked by CCK antagonists [8]. In addition to anxiety [9] and depression [10], the clinically most relevant therapeutic area for CCK antagonists is pain management.

Asperlicin (Fig. 1) was the first non-peptidal CCK antagonist lead structure from nature and analogs thereof were studied as CCK ligands [11]. Simplification of the lead structure by Merck led to devazepide [12], a potent CCK₁ selective cholecystokinin antagonist, containing a 1,4-benzodiazepine template and an indole moiety. A phase II trial of devazepide, which was only published in form of a conference report [13] showed a significant enhancement of the effect of opiates in the treatment of chronic and severe pain and the CCK neuropeptide pathway was reviewed and a featured clinical application for pain [14].

The indolyl amide of devazepide was replaced by a urea linkage and this resulted in Merck's L-365,260, a CCK₂ selective antagonist. Further structure–activity relationship (SAR) optimization led to Zeria's Z-360, in which the *N*-alkyl side chain and the 5-position (cyclohexyl-) was optimized for potency (Fig. 1). Additionally, a *meta*-carboxylic acid on the aryl urea linkage was introduced to enhance water solubility of this most recent CCK₂ antagonist [15] (Fig. 1).

In our search for new CCK ligands, the 1,4-benzodiazepine template was varied by a combinatorial solid phase synthesis [16] and optimized in terms of CCK binding affinity [17].

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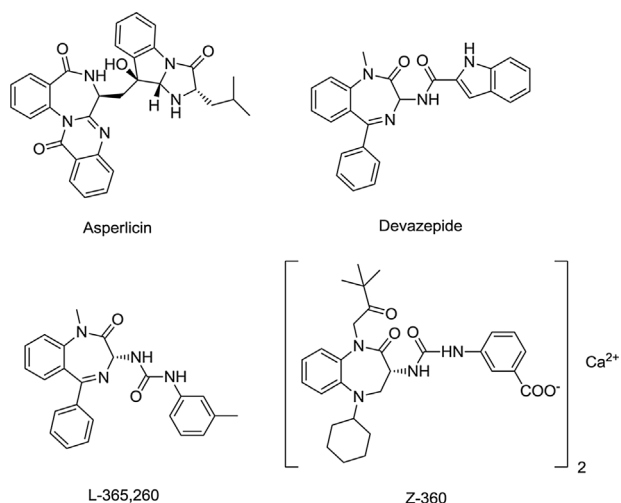


Figure 1. Drug design from asperlicin, an isolated natural product, via devazepide, a CCK₁ selective antagonist, to a CCK₂ selective urea-based antagonist, L-365,260. Drug optimization from L-365,260 toward Z-360, a CCK₂ antagonist with improved potency and water solubility.

The 1,4-benzodiazepine structure [18] was replaced by an achiral diphenyl pyrazolone template, giving novel CCK antagonists with an indole carboxylic acid [19] and a phenyl urea moiety [20] and they displayed excellent animal data on anxiety and depression [21].

Again, having realized the poor pharmacokinetic properties of 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 than 100 Å, with no urea linkage was initiated.

This resulted in the discovery and SAR-optimization of 4-amino-2(5*H*)-furanones, which are reported here in this publication. For the first time now, a totally novel, non-urea, and non-benzodiazepine template [22] was available as potent CCK₂ receptor antagonist. *In vivo* evaluation in mice using the tail flick test [23] was initiated with tramadol, morphine, and finally desimipramine and resulted in a relevant potentiation of analgesia.

Results and discussion

Chemistry

Alkoxy-2(5*H*)-furanones were generally prepared from mucochloric acid using previously developed methods [24]. The overall starting material of this short chemical sequence is furfural, which is converted into mucochloric acid on an industrial scale, mainly for the use of this intermediate in plant protection and pharmaceuticals.

Propargylalcohol, allylalcohol, benzylalcohol, and cyclohexyl methanol were reacted in the presence of toluene

under reflux using a Dean-Stark trap (Method A). For the isopropoxy-3,4-dichloro-2(5*H*)-furanone excess of the alcohol was applied (Method B) and the intermediate was obtained in large crystals once crystalline (Scheme 1).

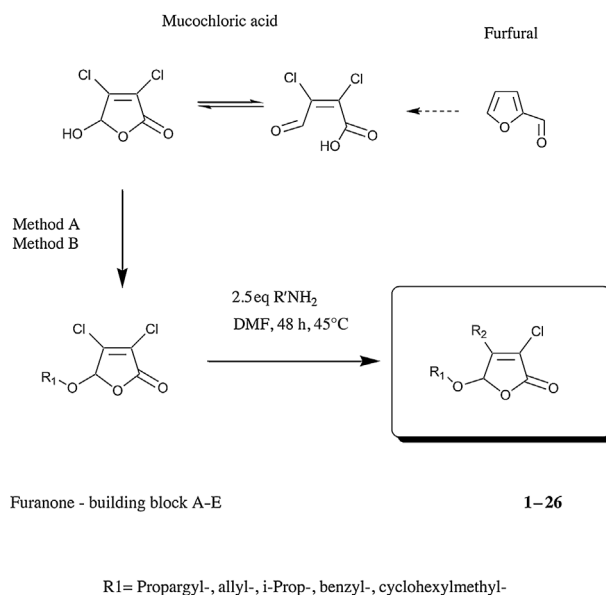
The 5-alkoxy (5-propargyl-, allyl-, isopropoxy-, benzyloxy, and cyclohexyl-methoxy-) 2(5*H*)-furanone intermediates were reacted at slightly elevated temperatures with an excess of the parent amine into the desired 4-amino-5-alkoxy-2(5*H*)-furanones **1–26**.

The target molecules were obtained in a low yield for the propargyl series and in good yields for the allyloxy, isopropoxy, and benzyloxy series. *Isopropoxy* derivatives were generally inactive, but crystalline solids, while the benzyloxy series provided active and crystalline target molecules. Chemical stability is linked with crystalline properties via the melting point, explaining the desire of the medicinal chemist for white crystalline materials.

SAR optimization

The propargyl series contained the original lead structure **1**, which was identified, when the combinatorial library of antibacterial 4-amino-furanones was screened in a CCK radioligand binding assay. The 4-isobutyl lead structure **1**, which was identified by chance, was obtained in a low yield and occurred as sticky brown oil and the optimization of SARs are outlined in Table 1.

The change of the butyl group in this series resulted generally in lower binding affinity and the benzyl derivative **2** was found of twofold lower and the *N*-methyl benzyl analog of a similar activity.



Scheme 1. See experimental section for furanone building blocks. Synthetic route for the formation of 5-alkoxy-4-amino-furan-2(5*H*)-ones **1–26**.

Table 1. SAR optimization with respect to receptor binding affinity for 4-amino-5-alkoxy-furan-2(5H)-ones 1–26.

Entry	R1	R2	CCK-B	CCK-A
1	Propargyl-	Isobutylamino-	220 ± 29	>10000
2	Propargyl-	Benzylamino-	470 ± 34	–
3	Propargyl-	<i>N</i> -Methyl-benzylamino-	280 ± 32	–
4	Propargyl-	Benzylpiperazinyl-	>10000	–
5	Propargyl-	3,5-Dimethylmorpholino-	>10000	–
6	Allyl-	Isobutylamino-	1239 ± 103	–
7	Allyl-	Sec butylamino-	>10000	–
8	Allyl-	<i>n</i> -Butylamino-	4312 ± 242	–
9	Isopropyl-	Isopropylamino-	1200	–
10	Isopropyl-	Cyclopropylamino-	4300	–
11	Isopropyl-	Cyclopentylamino-	5700	–
12	Isopropyl-	Cyclohexylamino-	2300	–
13	Isopropyl-	Phenylethylamino-	6700	–
14	Isopropyl-	Benzylamino-	1300	–
15	Isopropyl-	<i>N</i> -Methyl-benzylamino-	2700	–
16	Isopropyl-	Dimethylanilino-	2600 ± 120	–
17	Isopropyl-	Indolino-	5400	–
18	Benzyl-	Isobutylamino-	85 ± 11	2566 ± 343
19	Benzyl-	<i>n</i> -Butylamino-	548 ± 43	–
20	Benzyl-	Sec butylamino-	627 ± 43	–
21	Benzyl-	3-Methylpyrazolo-	162 ± 13	2344 ± 321
22	Benzyl-	Cyclopropylamino-	263 ± 18	>10000
23	Benzyl-	Cyclopentylamino-	180 ± 33	2598 ± 436
24	Benzyl-	Cyclohexylamino-	503 ± 48	–
25	Cyclohexylmethyl-	Isobutylamino-	27 ± 6	260 ± 21
26	Cyclohexylmethyl-	Methylamino-	>10000	–

IC₅₀ in nM. N = 3.

The introduction of piperazinyl-, morpholinyl-, and other heterocyclic groups was found to result in the inactive molecules **4** and **5**.

The isobutyl analog **6** containing an allyloxy-group, was five times less potent and the closely related *n*-butyl derivative **8** was found 20 times less potent in the receptor binding assay.

Subsequently, the 5-alkoxy C3 unit was varied and a series of isopropoxy furanones **9–17** was prepared and tested and an unspecific micromolar activity was determined.

The benzyl group is a classical bioisostere of the propargyl group, and the benzyl analog of the isobutyl furanone **18** was three times more potent than lead structure **1**. It was also obtained in a good chemical yield as a white, crystalline material.

For *n*-butyl and for the secondary butyl 5-benzyloxy-furanones **19** and **20**, the bioactivity was decreased.

The pyrazol derivative **21**, as well as the cyclopropyl furanone **22** and the cyclopentyl derivative **23** were found of a high nanomolar binding affinity, while the cyclohexyl analog **24** had a significantly lower activity.

For the 5-cyclohexyl methyl aminofuranones series, the methyl analog **26** showed no binding affinity up to 10 micromolar and the isobutyl derivative **25** was identified as the most potent derivative of the entire series. The replacement of the aromatic phenyl group by a cyclohexyl group

resulted in enhanced binding affinity for the privileged isobutyl amino structure **25**. A similar enhancement phenyl versus cyclohexyl was previously observed in Z-360, a recent CCK₂ antagonist [25].

Thus, overall four potent CCK ligands in the nanomolar range were identified. The furanone **18** showed an IC₅₀ of 85 nM and displayed 30 times selectivity toward the CCK₂ receptor. Furanones **21** and **23** displayed slightly lower binding affinities and are outlined in Fig. 2.

The cyclohexyl derivative **25** showed an enhanced IC₅₀ of about 27 nM, but was less selective, only 10 times for the CCK_{2R}. It was obtained as an oily solid, which makes purification under GMP conditions by recrystallization impossible.

The isobutyl-amino-benzyloxy-furanone **18** was recrystallized from methanol and the crystal structure was determined.

The crystal structure of the high yield – high purity – compound, furanone **18**, is outlined in Fig. 3, supporting excellent manufacturing properties due to appropriate physical chemical properties. In the crystal structure, the 5-benzyloxy moiety is orientated below the furanone plain and the 4-amino substituent is located above.

In order to rationalize drug ligand interactions of furanone **18** with the CCK₂/gastrin receptor, molecular modeling studies were performed.

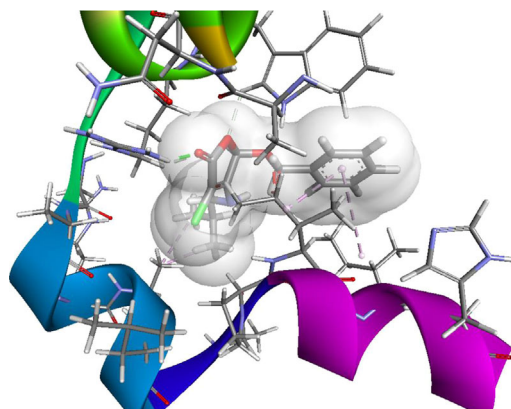


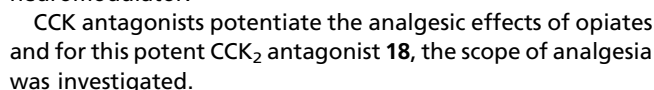
Figure 4. Docking of the furanone **18** into the active site of the CCK₂ receptor.

Functional assay – isolated guinea pig ileum preparation

In a radio-labeled binding assay with native brain and pancreatic membranes, potent and selective ligands were identified. The CCK ligands may act as antagonists, and this was investigated using isolated tissue preparations.

Sulfated CCK₈ resulted in dose-dependent contractions of the GPI, which are outlined in Fig. 5. From 1 nM onwards, a small response was obtained, which reached a plateau at about 100 nM concentrations. A total of 500 nM final bath concentration of the test molecule **18** shifted the curve to the right and 1 μM concentration of the furanone **18** resulted in nearly a complete inhibition of CCK₈-induced contractions, so that it is now confirmed, that the ligand furanone **18** acted as CCK antagonist.

The small alkyl amino-benzoyloxyfuranone molecule **18** showed a cLogP of about 2.6 compared with a cLogP of diazepam of 2.7. Therefore, a sufficient brain penetration should be anticipated, essential for CNS activity. A high percentage of preclinical candidates drop out due to a poor pharmacokinetic profile and this is in particular complex for a neuromodulator.



In vivo analgesic tests

The effects of CCK on the modulation of pain transmission and the opioid effects are well established [27, 28], and typically a low dose of morphine or a high dose of a weak opiate agonist will result in a good potentiation of analgesia [29] in the tail immersion test [30].

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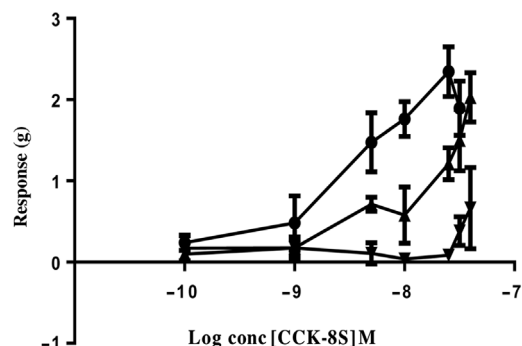


Figure 5. Isolated GPI. Cumulative log concentration response curves to CCK_{8S} only (●) and CCK_{8S} in the presence of **18**: 0.5 μM (▲), 1.0 μM (▼) on the guinea pig ileum, expressed as change of tension in gram. Each point represents the mean and the standard errors obtained from three different experiments.

The furanone **18** was intraperitoneally injected as a first injection to build up a plasma concentration of the CCK antagonist and then, tramadol/morphine/desimipramine was subcutaneously injected and subsequently, the analgesic effect was evaluated in the tail flick assay (Fig. 6).

In all treated groups, no effect on nociception [29] for doses of up to 5 mg/kg was observed for the furanone **18** in the tail immersion test [30] as a single agent. Administered in conjunction with tramadol/morphine and desimipramine at 0.05 mg/kg showed no potentiation of analgesia and a clinically relevant significant effect was observed at 0.5 mg/kg.

For tramadol (20 mg/kg) and concomitant administration of furanone **18** (0.5 mg), the analgesic effect in the tail flick test is equivalent to a 2 mg/kg dose of morphine.

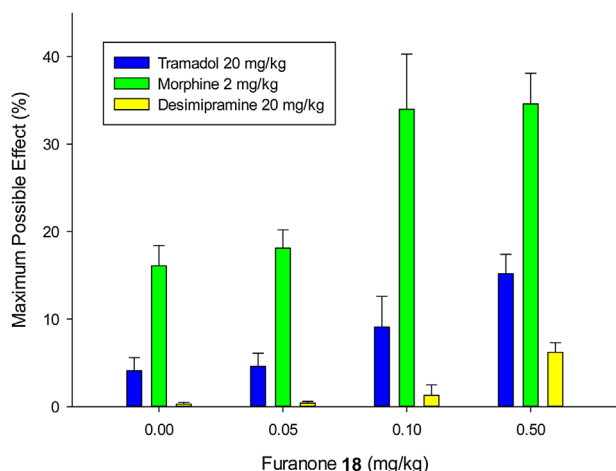


Figure 6. Maximum possible effect (MPE) of three doses of furanone **18** in the tail flick test in mice in % MPE for tramadol-, morphine-, and desimipramine potentiation.

For morphine, the potentiation of analgesia was also confirmed. Morphine analgesia is potentiated by a CCK antagonist and the 2 mg/kg morphine dose is, in the presence of furanone **18**, equivalent to 8 mg/kg morphine, which is therapeutically relevant to reduce side effects of opiates.

Most interestingly, for desimipramine at 0.5 mg/kg, a maximum possible effect of 6% was determined. Tricyclic antidepressants (TCAs), such as desimipramine, show clinically a useful analgesic effect in man and in mice, this effect is very small (0.3% MPE). Here, in the presence of 0.5 mg/kg CCK antagonist **18**, the 20 mg/kg desimipramine dose is equivalent to 20–40 mg tramadol. Thus, 0.5 mg/kg of furanone **18** potentiated the analgesic effect of a classical TCA by factor 20.

The effects of CCK on the modulation of pain transmission and the opioid effects are well established [27, 28] and we have shown here the useful adjunct therapy of these agents in pain management. Previously, it was shown that the CCK₂ antagonist CI-988 potentiated the analgesia of morphine and clomipramine [31] and therefore, it may be concluded that this represents a general therapeutic application of CCK₂ antagonists. Trimipramine is in use as second line treatment for persistent neuropathic pain, which still represents an unmet medical need. CCK antagonists also block the development of morphine tolerance [32, 33], another further possible therapeutic feature in pain management.

Conclusions

Chemically, the bis-substituted amino-furanones are not related to previously known CCK antagonizing small organic molecules and they do not contain the widely used urea linkage. Our amino-furanones represent a totally novel “new chemical entity” for a CCK antagonist, a CNS drug-like molecule and most preferred an adjunct in pain management.

The tail immersion test is a robust assay to evaluate analgesia of selected CCK ligands in combination with approved analgesics. A classical tissue preparation confirmed reliably the antagonism of the ligands. Overall, a good *in vitro/in vivo* correlation was found.

During scale up, the yield for the solid isobutyl derivative **18** was increased to >70% over two steps, and this molecule is available in >99.7% purity and was selected for preclinical development. The greater CCK₂ selectivity of **18**, compared with **25** may result in less toxicity due to less interference with the physiological role of cholecystokinin, mediated by CCK₁ receptors.

Experimental

General synthesis

The chemicals were obtained from Aldrich (Gillingham, UK) and Lancaster (Lancaster, UK). Atmospheric pressure chemical ionization 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 from a Stuart Scientific (Coventry, UK) melting point analyzer and are uncorrected.

The InChI codes of the synthesized compounds are provided in the Supporting Information.

Preparation of 3,4-dichloro-5-alkoxy-furan-2(5H)-one building blocks

Preparation of 3,4-dichloro-5-propargyloxy-furan-2(5H)-one A

Method A (toluene-Dean-Stark): To a solution of (10 g, 0.06 mol) mucochloric acid in toluene (50 mL), propargyl alcohol (2 eq., 6.5 g, 0.12 mol) and concentrated sulfuric acid (0.2 mL) were added. The resulting solution was left for 2 days to reflux using Dean and Stark apparatus. The brown oily tar-like crude product was analyzed by TLC and then quenched with saturated sodium bicarbonate solution (50 mL). After leaving the crude mixture to settle for 10 min, the product was extracted with ether (3 × 50 mL). The organic layers were combined and dried using magnesium sulfate. The solvent was removed in vacuum, resulting in a dark brown-colored oil. Yield = 61%; ¹H NMR (CDCl₃) 250 MHz: δ = 6.11 (s, 1H), 4.58 (m, 2H), 2.96 (t, *J* = 2.4 Hz, 1H); ¹³C NMR (CDCl₃) δ = 163.1, 147.5, 128.2, 98.6, 78.1, 77.7, 57.4 ppm; IR (KBr-disc) ν max: 3578, 3293, 2938, 2881, 2122, 1799, 1634, 1450, 1355, 1232, 1143, 1017, 903, 748, 687 cm⁻¹.

Preparation of 5-allyloxy-3,4-dichloro-5H-furan-2-one B

Method A: To a solution of (10 g, 0.06 mol) mucochloric acid in toluene (50 mL), allyl alcohol (2 eq., 7.1 g, 0.12 mol) and concentrated sulfuric acid (0.2 mL) were added. The resulting solution was left for 2 days according to Method A. The crude furanone, which was obtained as dark brown-colored oil, was distilled under vacuum to give an orange liquid. Yield = 69%; ¹H NMR (CDCl₃) 250 MHz: δ = 5.99 (s, 1H), 5.78 (m, 1H), 5.27 (m, 1H), 5.48 (m, 1H), 4.32 (m, 2H) ppm; ¹³C NMR (CDCl₃) δ = 163.3, 147.9, 131.9, 124.2, 119.5, 99.7, 71.1 ppm; IR (KBr-disc) ν max: 3413, 3092, 2935, 2870, 2365, 2339, 1796, 1642, 1334, 1236, 1157, 1020, 899, 778, 748 cm⁻¹.

Preparation of 3,4-dichloro-5-isopropoxy-5H-furan-2-one C

Method B (excess alcohol): To a solution of (10 g, 0.06 mol) mucochloric acid in excess isopropanol (60 mL), concentrated sulfuric acid (0.2 mL) was added. The resulting solution was left for 2 days to reflux. The brown oily crude product was analyzed by TLC and then quenched with saturated sodium bicarbonate solution (50 mL). After leaving the crude mixture to settle for 10 min, the product was extracted with ether (3 × 50 mL). The organic layers were combined and dried using magnesium sulfate. The solvent was removed in vacuum,

resulting in a light-dark brown-colored oil, which was distilled under vacuum giving a colorless liquid. Yield = 73%; ¹H NMR (CDCl₃) 250 MHz: δ = 5.90 (s, 1H), 4.25 (m, 1H), 1.29 + 1.42 (d, 6H) ppm; ¹³C NMR (CDCl₃) 250 MHz: δ = 163.7, 148.0, 123.9, 100.3, 75.1, 22.6, 22.0 ppm; IR (KBr-disc) ν max: 3392, 2975, 2927, 1797, 1645, 1460, 1380, 1326, 1234, 1160, 1118, 951, 892, 746 cm⁻¹.

Preparation of 5-benzyloxy-3,4-dichloro-5H-furan-2-one D

Method A: To a solution of (10 g, 0.06 mol) mucochloric acid in toluene (50 mL), benzyl alcohol (2 eq., 13.8 g, 0.12 mol) and concentrated sulfuric acid (0.2 mL) were added and reacted according to Method A. The crude furanone, which was obtained as a light brown-colored oil, was distilled under vacuum giving a yellow liquid. Yield = 77%; ¹H NMR (CDCl₃) 250 MHz: δ = 7.48 (m, 5H), 5.94 (s, 1H), 4.88 (m, 2H) ppm; ¹³C NMR (CDCl₃) 250 MHz: δ = 163.3, 147.7, 135.2, 128.9, 128.5, 126.2, 124.5, 99.7, 71.4 ppm; IR (KBr-disc) ν max: 3432, 3034, 2928, 2371, 2344, 1794, 1642, 1452, 1330, 1231, 1148, 1022, 909, 746, 697 cm⁻¹.

Preparation of 3,4-dichloro-5-cyclohexylmethoxy-5H-furan-2-one E

Method A: To a solution of (10 g, 0.06 mol) mucochloric acid in toluene (50 mL), cyclohexyl methanol (2 eq. 13.5 g, 0.12 mol) and concentrated sulfuric acid (0.2 mL) were added and the reaction mixture was reacted according to Method A. The crude furanone was distilled under vacuum to give an orange liquid. Yield = 57%; ¹H NMR (CDCl₃) 250 MHz: δ = 5.73 (s, 1H), 3.90 (m, 2H), 1.39–1.76 (m, 11H) ppm; ¹³C NMR (CDCl₃) 250 MHz: δ = 161.4, 147.6, 125.3, 101.2, 68.6, 40.3, 29.5, 26.7, 25.7 ppm; IR (KBr-disc) ν max: 3334, 2926, 2851, 2669, 1794, 1732, 1634, 1444, 1177, 1018, 729, 697 cm⁻¹.

Preparation of 3-chloro-4-substituted amino-5-alkyloxy-5H-furan-2-ones

General method: Building block (2 mmol A–E/0.41, 0.42, 0.42, 0.52, 0.53 g) was dissolved in DMF (2 mL) and placed into 30 mL glass vials. Amines (5 mmol) were added carefully dropwise to each vial. The vials were placed in a heating block and heated to 45°C for 48 h. The compound was extracted with ether (10 mL) and washed twice with water (10 mL). The organic layer was dried using anhydrous magnesium sulfate and removed in vacuum to give a dark brown oil, which was purified using column chromatography (solvent system: 50:50 ether/petrol ether) to give the desired product.

3-Chloro-4-isobutylamino-5-prop-2-ynyloxy-5H-furan-2-one 1

Yield = 12% brown oil; MW: 243.7; MS (APCI(+)): 244/246 (M+1) *m/z*; ¹H NMR (CDCl₃) 250 MHz: δ = 5.92 (s, 1H), 5.19 (bs, NH), 4.41 (s, 2H), 3.28 (m, 2H), 2.55 (m, 1H), 1.84 (m, 1H), 1.08 (m, 6H) ppm; ¹³C NMR (CDCl₃) δ = 166.2 (C=O), 155.3, 104.5, 94.5, 76.7, 78.1, 56.1, 51.1, 29.6, 19.7 ppm; IR (KBr-disc) ν max: 3306, 3103, 2967, 2937, 2881, 2370, 1748, 1646, 1542, 1458, 1434, 1335, 1126, 969, 747, 701 cm⁻¹.

4-(Benzylamino)-3-chloro-5-(prop-2-ynyloxy)furan-2(5H)-one 2

Yield = 36% brown oil; MW: 277.7; MS (APCI(+)): 278/280 (M+1) *m/z*; ¹H NMR (CDCl₃) 300 K δ: 7.36 (m, 5H); 5.98 (s, 1H), 5.20 (s, NH), 4.66 (s, 2H), 4.45 (m, 1H), 4.41 (m, 2H), 2.55 (m, 1H) ppm. ¹³C NMR (CDCl₃) δ = 168.1, 155.8, 135.6, 129.0, 128.6, 126.3, 108.3, 96.6, 78.2, 76.6, 54.9 ppm. IR (KBr-disc) ν max: 3380, 3283, 2358, 2338, 1752, 1646, 1455, 1326, 1123, 971, 695 cm⁻¹.

4-(Benzyl-methyl-amino)-3-chloro-5-prop-2-ynyloxy-5H-furan-2-one 3

Yield = 49% oily solid; MW: 291.7; MS (APCI(+)): 292/294 (M+1) *m/z*; ¹H NMR (CDCl₃) 250 MHz: δ = 7.16–7.41 (m, 5H), 5.96 (s, 1H), 4.70 (m, 2H), 4.40 (m, 2H), 3.04 (s, 3H), 2.48 (m, 1H) ppm; ¹³C NMR (CDCl₃) δ = 168.2, 155.6, 135.7, 129.0, 128.6, 127.3, 107.3, 94.6, 78.1, 76.9, 55.9, 38.1 ppm. IR (KBr-disc) ν max: 3441, 3296, 3037, 2930, 2374, 2343, 2128, 1766, 1643, 1460, 1419, 1353, 1270, 1229, 1116, 983, 747, 703 cm⁻¹.

4-(4-Benzyl-piperazin-1-yl)-3-chloro-5-prop-2-ynyloxy-5H-furan-2-one 4

Yield = 50%; mp: 120–123°C; MW: 346.8; MS (APCI(+)): 346/348 (M+1) *m/z*; ¹H NMR (CDCl₃) 250 MHz: δ = 7.18–7.38 (m, 5H), 5.87 (s, 1H), 4.36 (s, 2H), 3.76 (m, 4H), 3.51 (s, 2H), 2.42–2.39 (m, 5H) ppm. ¹³C NMR (CDCl₃) 250 MHz: δ = 168.1, 154.0, 137.2, 129.2, 128.4, 127.5, 103.0, 94.3, 76.9, 76.8, 62.7, 55.7, 52.7, 47.5 ppm. IR (KBr-disc) ν max: 3253, 2935, 2815, 2126, 1758, 1446, 1347, 1277, 1111, 985, 849, 740, 693 cm⁻¹.

3-Chloro-4-(2,6-dimethyl-morpholin-4-yl)-5-prop-2-ynyloxy-5H-furan-2-one 5

Yield = 47% oily solid; MW: 285.7; MS (APCI(+)): 286/288 (M+1) *m/z*; ¹H NMR (CDCl₃) 250 MHz: δ = 5.94 (s, 1H), 4.36 (s, 2H), 3.89–4.25 (m, 2H), 3.58–3.85 (m, 2H), 2.51–2.98 (m, 3H), 1.46 (m, 6H) ppm. ¹³C NMR (CDCl₃) δ = 170.4, 153.7, 104.2, 94.3, 77.8, 77.6, 66.0, 55.8, 52.6, 18.5, 18.4 ppm. IR (KBr-disc) ν max: 3484, 3255, 2981, 2928, 2883, 2366, 2108, 1743, 1639, 1267, 1083, 981, 751, 697 cm⁻¹.

5-Allyloxy-3-chloro-4-isobutylamino-5H-furan-2-one 6

Yield = 62% oily solid; MW: 245.7; MS (APCI(+)): 188/190 (M+), 246/248 (M+1) *m/z*; ¹H NMR (CDCl₃) 250 MHz: δ = 5.89 (m, 1H), 5.73 (s, 1H), 5.34 (m, 2H), 4.79 (bs, NH), 4.27 (m, 2H), 3.22 (bs, 2H), 1.83 (m, 1H), 1.16–0.98 (m, 6H) ppm; ¹³C NMR (CDCl₃) δ = 172.1, 156.7, 132.2, 119.6, 108.0, 95.9, 69.3, 51.0, 29.6, 19.7 ppm; IR (KBr-disc) ν max: 3296, 3088, 2978, 2978, 2939, 2881, 1753, 1649, 1546, 1467, 1338, 1150, 972, 709 cm⁻¹.

5-Allyloxy-4-sec-butylamino-3-chloro-5H-furan-2-one 7

Yield = 49% oily solid; MW: 245.7; MS (APCI(+)): 190/192 (M+), 246/248 (M+1) *m/z*; ¹H NMR (CDCl₃) 250 MHz: δ = 5.89 (m, 1H), 5.74 (s, 1H), 5.28 (m, 2H), 4.27 (m, 2H), 1.68 (m, 2H), 1.82 (m, 3H), 0.99 (t, *J* = 4.6 Hz, 3H) ppm; ¹³C NMR (CDCl₃) δ = 168.3, 153.5, 132.5, 119.0, 106.3, 96.5, 69.6, 49.6, 30.5,

21.4, 10.3 ppm. IR (KBr-disc) ν max: 3296, 3088, 2978, 2978, 2939, 2881, 1753, 1649, 1546, 1467, 1338, 1150, 972, 709 cm⁻¹.

5-Allyloxy-4-butylamino-3-chloro-5H-furan-2-one 8

Yield = 57% oily solid; MW: 245.7 (APCI(+)): 188/190 (M+), 246/248 (M+1) *m/z*; ¹H NMR (CDCl₃) 250 MHz: δ = 5.90 (m, 1H), 5.76 (s, CH), 5.32 (m, 2H), 4.29 (m, 2H), 3.43 (bs, 2H), 1.63 (m, 2H), 1.38 (m, 2H), 0.98 (t, *J* = 8.2 Hz, 3H) ppm. ¹³C NMR (CDCl₃) δ = 165.9, 151.3, 132.4, 119.4, 105.6, 96.2, 69.6, 43.6, 32.7, 19.7, 13.7 ppm. IR (KBr-disc) ν max: 3332, 3095, 2974, 2941, 2892, 1754, 1655, 1540, 1457, 1333, 1227, 1138, 1087, 969, 748, 700 cm⁻¹.

5-Benzyloxy-3-chloro-4-isobutylamino-5H-furan-2-one 18

Yield = 76%; mp: 105–107°C; MW: 295.8; MS (APCI(+)): 188/190 (M+), 296/298 (M+1) *m/z*; ¹H NMR (CDCl₃) 250 MHz: δ = 7.01–7.49 (m, 5H), 5.79 (s, CH), 5.10 (bs, NH), 4.71 (bs, 2H), 3.22 (m, 2H), 1.79 (m, 1H), 0.97 (m, 6H) ppm; ¹³C NMR (CDCl₃) δ = 170.4, 170.4, 135.4, 128.8, 106.3, 95.6, 70.4, 51.0, 29.5, 19.7 ppm. IR (KBr-disc) ν max: 3460, 3252, 2977, 2937, 2877, 2368, 2341, 1741, 1631, 1433, 1353, 1329, 1255, 1128, 1028, 961, 750, 703 cm⁻¹.

C₁₅H₁₈ClNO₃, *M_r* = 295.75, *T* = 293(2) K, tabular, 0.20 × 0.15 × 0.05 mm, colorless, MoK_α radiation: λ = 0.71073 Å, monoclinic, *P*₂₁/*c*, *a* = 11.420(5) Å, *b* = 10.736(2) Å, *c* = 12.798(5) Å, β = 102.83(4)°, *V* = 1529.9(9) Å³, *Z* = 4, *D_x* = 1.284 Mg/m⁻³, *D_m* not measured, *R* [*F*² > 2σ(*F*²)] = 0.0791, *wR*(*F*²) = 0.1845, 3207 reflections, 187 parameters.

Selected geometric parameters are listed in Table 2.

5-Benzyloxy-4-butylamino-3-chloro-5H-furan-2-one 19

Yield = 68%; mp: 109–112°C; MW: 295.8; MS (APCI(+)): 188/190 (M+), 296/298 (M+1) *m/z*; ¹H NMR (CDCl₃) 250 MHz: δ = 7.39 (m, 5H), 5.74 (s, 1H), 4.64 (m, 3H), 3.31 (bs, 2H), 1.569 (m, 2H), 1.39 (m, 2H), 0.89 (t, *J* = 6.6 Hz, 3H) ppm. ¹³C NMR (CDCl₃) δ = 171.4, 153.4, 135.5, 128.8, 128.1, 124.5, 107.5, 95.5, 70.3, 43.7, 29.5, 19.7, 13.7 ppm; IR (KBr-disc) ν max: 3419, 2067, 3028, 2928, 2371, 2338, 1794, 1642, 1502, 1456, 1327, 1234, 1144, 1022, 972, 902, 750, 707 cm⁻¹.

5-Benzyloxy-4-sec-butylamino-3-chloro-5H-furan-2-one 20

Yield = 64%; mp: 93–96°C; MW: 295.8; MS (APCI(+)): 188/190 (M+), 296/298 (M+1) *m/z*; ¹H NMR (CDCl₃) 250 MHz: δ = 7.30 (m, 5H), 5.73 (s, 1H), 5.11 (bs, NH), 4.68 (m, 2H), 4.40 (bs, NH), 3.49 (bs, 1H), 1.46 (m, 2H), 1.16 (m, 3H), 0.84 (m, 3H) ppm; ¹³C NMR (CDCl₃) δ = 169.3, 155.5, 135.6, 128.9, 128.7, 124.3, 105.9, 95.7, 70.2, 51.1, 30.5, 21.6, 21.6 ppm; IR (KBr-disc) ν max: 3272, 3085, 2977, 2929, 2880, 2368, 1732, 1632, 1560, 1454, 1348, 1231, 1123, 966, 749, 700 cm⁻¹.

5-Benzyloxy-3-chloro-4-(3-methyl-pyrazol-1-yl)-5H-furan-2-one 21

Yield = 76%; mp: 89–91°C; MW: 304.7; MS (APCI(+)): 305/307 (M+1) *m/z*; ¹H NMR (CDCl₃) 250 MHz: δ = 8.25 (m, 1H), 7.31

Table 2. Selected geometric parameters (Å, °).

Cl(7)–C(2)	1.707(7)	O(12)–C(4)	1.386(7)
O(5)–C(1)	1.365(8)	C(3)–N(8)	1.331(7)
O(5)–C(4)	1.432(7)	C(2)–C(3)	1.354(9)
O(6)–C(1)	1.202(7)		
C(4)–O(12)–C(13)	113.7(5)	O(6)–C(1)–O(5)	120.3(5)
O(12)–C(13)–C(14)	108.2(6)	C(1)–C(2)–Cl(7)	122.9(5)
N(8)–C(3)–C(4)	124.5(6)	C(3)–C(2)–Cl(7)	127.0(5)
O(12)–C(4)–O(5)	111.5(5)	C(3)–N(8)–C(9)	124.5(6)

(m, 5H), 6.49 (s, 1H) 6.40 (m, 1H), 4.94 (m, 2H), 2.41 (s, 3H) ppm. ¹³C NMR (CDCl₃) δ = 165.7, 153.7, 147.3, 135.5, 130.9, 128.7, 128.6, 128.55, 111.3, 102.7, 99.1, 72.6, 13.7 ppm; IR (KBr-disc) ν max: 3440, 3176, 2950, 2941, 2885, 2362, 2336, 1790, 1674, 1551, 1443, 1324, 1264, 1122, 1016, 983, 735, 695 cm^{−1}.

5-Benzyloxy-3-chloro-4-cyclopropylamino-5H-furan-2-one 22

Yield = 81%; mp: 104–107°C; MW: 279.7; MS (APCI(+)): 278/280 (M+) *m/z*; ¹H NMR (CDCl₃) 250 MHz: δ = 7.80 (m, 5H), 5.95 (s, 1H), 4.84 (m, 3H), 2.99 (m, 1H), 0.79 (m, 7H) ppm; ¹³C NMR (CDCl₃) δ = 161.4, 153.4, 133.6, 128.8, 128.7, 126.4, 106.1, 95.7, 70.8, 25.5, 18.3 ppm; IR (KBr-disc) ν max: 3453, 3265, 3071, 2935, 2864, 2366, 1788, 1742, 1636, 1442, 1348, 1245, 983, 753, 701 cm^{−1}.

5-Benzyloxy-3-chloro-4-cyclopentylamino-5H-furan-2-one 23

Yield = 78%; mp: 101–103°C; MW: 307.8; MS (APCI(+)): 284/286 (M+), 306/308 (M+1) *m/z*; ¹H NMR (CDCl₃) 250 MHz: δ = 7.49 (m, 5H), 5.78 (s, 1H), 4.78 (m, 2H), 4.01 (bs, NH), 1.33–2.15 (m, 8H) ppm; ¹³C NMR (CDCl₃) δ = 165.2, 151.3, 135.5, 128.8, 128.2, 124.6, 104.2, 95.6, 70.2, 55.3, 34.6, 23.7 ppm; IR (KBr-disc) ν max: 3459, 3306, 3070, 2961, 2859, 2367, 2342, 1735, 1626, 1547, 1352, 1231, 1144, 1007, 959, 758, 707 cm^{−1}.

5-Benzyloxy-3-chloro-4-cyclohexylamino-5H-furan-2-one 24

Yield = 49% oily solid; MW: 321.8; MS (APCI(+)): 322/324 (M+) *m/z*; ¹H NMR (CDCl₃) 250 MHz: δ = 7.30 (m, 5H), 5.78 (s, 1H), 4.96 (bs, NH), 4.76 (m, 2H), 1.04–2.18 (m, 10H) ppm; ¹³C NMR (CDCl₃) δ = 167.5, 155.5, 135.5, 128.7, 128.4, 124.2, 104.5, 95.6, 67.0, 52.7, 34.5, 25.5, 25.1 ppm; IR (KBr-disc) ν max: 3293, 3067, 2941, 2861, 2375, 2355, 1754, 1648, 1550, 1446, 1337, 1231, 1134, 1091, 955, 746, 700 cm^{−1}.

3-Chloro-5-cyclohexylmethoxy-4-isobutylamino-5H-furan-2-one 25

Yield = 59%; mp: 119–122°C; MW: 301.8; MS (APCI(+)): 206/208 (M+), 302/304 (M+1) *m/z*; ¹H NMR (CDCl₃) 250 MHz: δ = 5.75 (s, 1H), 4.89 (bs, NH), 3.53 (m, 1H), 3.49 (m, 1H), 3.37 (bs, 2H), 2.76 (bs, 1H), 1.53–1.79 (m, 5H), 0.81–1.42 (m, 12H) ppm; ¹³C NMR (CDCl₃) δ = 165.5, 153.6, 104.2, 97.0, 74.0,

51.1, 37.7, 29.8, 29.7, 27.0, 25.7, 19.74, 19.71 ppm; IR (KBr-disc) ν max: 3285, 2971, 2933, 2863, 2370, 1745, 1678, 1630, 1470, 1333, 1256, 1147, 1025, 948, 750, 718 cm^{−1}.

3-Chloro-5-cyclohexylmethoxy-4-methylamino-5H-furan-2-one 26

Yield = 55%; mp: 117–120°C; MW: 259.7; MS (APCI(+)): 164/166 (M+1), 260/262 (M+) *m/z*; ¹H NMR (CDCl₃) 250 MHz: δ = 5.72 (s, 1H), 4.89 (bs, NH), 3.49 (m, 1H), 3.37 (m, 1H), 3.23 (m, 3H), 2.76 (bs, 1H), 1.72 (m, 5H), 0.77–1.39 (m, 6H) ppm; ¹³C NMR (CDCl₃) δ = 164.1, 151.3, 102.6, 97.0, 74.2, 37.7, 30.6, 29.7, 27.0, 25.7 ppm. IR (KBr-disc) ν max: 3267, 2925, 2848, 2370, 2338, 1742, 1684, 1632, 1451, 1329, 1255, 1159, 1016, 949, 755, 716 cm^{−1}.

Pharmacology

Cholecystokinin binding assay, [¹²⁵I]-CCK-8 receptor binding assay

CCK₁ and CCK₂ receptor binding assays were performed by using guinea pig cerebral cortex (CCK₂) or rat pancreas (CCK₁). Male guinea pig brain tissues were prepared according to the modified method described by Saita et al. [34]. Pancreatic membranes were prepared as described by Charpentier et al. [35].

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 min. The supernatant was re-centrifuged at 13000 rpm for 20 min. The resulting pellet was re-dispersed to the required volume of buffer at 500 rpm and stored in aliquots at 70°C.

Binding was achieved using radioligand ¹²⁵I-Bolton-Hunter-labeled CCK, NEN at 25 pM. The samples were incubated with membranes (0.1 mg/mL) in 20 mM Hepes, 1 mM EGTA, 5 mM MgCl₂, 150 mM NaCl, at pH 6.5 for 2 h at RT and then centrifuged at 11000 rpm for 5 min. The membrane pellets were washed twice with water and the bound radioactivity was measured in a Packard Cobra counter.

Isolated tissue preparation

Adult male guinea pigs, weighing 200–250 g, were used and from the abdomen of the animals, the ileum was carefully excised at a site 15 cm away from the ileocaecal junction and

washed with physiological solution. The mesentery of the ileum was removed and the ileal 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 37°C and gassed with 95% O₂/5% CO₂.

Tyrode's solution of the following quantity was freshly prepared daily (g/L): NaCl, 8.0; KCl, 0.2; CaCl₂, 0.2; MgSO₄, 0.1; NaH₂PO₄, 0.05; NaHCO₃, 1.0; glucose, 1.0. Stock solutions of test compounds were prepared weekly, diluted to required concentration as required, and stored at a temperature of 4°C.

From the isolated tissue preparation, ileal strips of appropriate length were mounted vertically in organ bath containing Tyrode's solution, under a tension of 1 g and allowed to equilibrate for 30 min. One end of the strip was attached to the hook at the bottom of the organ bath and the other end connected by a thread to the external isometric force transducer. During equilibration, tension was continuously adjusted to 1 g when required and the Tyrode's solution in the organ bath was changed every 20 min. All spontaneous contractions of longitudinal muscles were recorded with the aid of an isometric transducer linked to a power lab chart computer unit.

Effects on CCK-8 stimulated isolated guinea pig ileum

To study the effect of furanone **18** on strips prepared from guinea pig ileum, CCK_{8S} was dissolved in distilled water to prepare a stock solution of 500 μM solution, from which cumulative additions of increasing concentrations (0.1, 1, 5, 10, 20, 30, and 40 nM) were tested to plot a dose–response curve. Test compounds and Lorglumide/L365260 as standard were added to the organ bath 10 min before exposure to the next CCK_{8S} serial concentrations.

Animal studies

Experiments were conducted in male standard IRC mice obtained from the animal house, Faculty of Medicine, Khon Kaen University. Each experimental group consisted of six animals and the treatment procedures were approved by the ethical committee, Faculty of Medicine, Khon Kaen University (BEA030699).

Mice were intraperitoneally 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.

Nociception test

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 preset at 50°C. The response time, at which the animal reacted by withdrawing its tail from water, was recorded and the cut-off time was 10 s in order to avoid damaging the animal's tissue. 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 TT

were expressed as a percentage of Maximal Possible Effect (% MPE) using the equation:

$$\% \text{ MPE} = \{ (TT - BT) / (45 - BT) \} \times 100$$

DMSO (5%), furanone (in 5% DMSO) was intraperitoneally injected as the first injection. Twenty minutes after the first, the second injection was done by subcutaneously injecting with tramadol at either 10, 20, or 40 mg/kg body weight.

Molecular modeling

For target preparation, the protein structures, pdb identifier 1HZN for the CCK₁ and 1L4T for the CCK₂-gastrin receptor were downloaded from the protein data bank (www.rcs.org) and docking was performed using Autodock Vina and Hex. After several docking trials for the CCK₂ receptor, the results were analyzed and visualized using Chimera and Designer Studio 4.5. After visual inspection and scores, results were presented to understand drug–ligand interaction with the CCK₂ receptor.

Statistical methods

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

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