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Quinoline derivatives: candidate drugs for a Class B G-protein coupled receptor, the Calcitonin gene-related peptide receptor, a cause of migraines

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Abstract

Class B G-protein coupled receptors are involved in a wide variety of diseases and are a major focus in drug design. Migraines are a common problem, and one of their major causative agents is class B G-protein coupled receptor, Calcitonin gene-related peptide (CGRP) receptor, a target for competitive drug discovery. The calcitonin receptor-like receptor generates complexes with a receptor activity-modifying protein, which determines the type of receptor protein formed. The CGRP receptor comprises a complex formed from the calcitonin receptor-like receptor and receptor activity-modifying protein 1. In this study, an *in silico* docking approach was used to target calcitonin receptor-like receptor in the bound form with receptor activity-modifying protein 1 (CGRP receptor), as well as in the unbound form. In both cases, the resulting inhibitors bound to the same cavity of the calcitonin receptor-like receptor. The twelve evaluated compounds were competitive inhibitors and showed efficient inhibitory activity against the CGRP receptor and Calcitonin receptor-like receptor. The two studied quinoline derivatives demonstrated potentially ideal inhibitory activity in terms of binding interactions and low range nano-molar inhibition constants. These compounds could prove helpful in designing drugs for the effective treatment of migraines. We propose that quinoline derivatives possess inhibitory activity by disturbing CGRP

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Conflict of Interest

The authors have no conflict of interests.

binding in the trigeminovascular system and may be considered for further preclinical appraisal for the treatment of migraines.

Keywords

Calcitonin gene-related peptide; Calcitonin receptor-like receptor; Class B GPCR; Docking; G-protein coupled receptor; Migraine

Introduction

Guanine nucleotide-binding proteins (G-proteins) are heterotrimeric in nature and belong to the largest family of G-protein coupled receptors (GPCRs), which are eukaryotic transmembrane-type signal transduction receptors that communicate across cellular membranes. GPCR receptors are encoded by more than 1,000 genes in the human genome [1–4] and are activated through a diverse array of ligands, including hormones, peptides, amino acids, ions and transducer signals from an expansive domain of effectors [5].

Calcitonin gene-related peptide (CGRP) is a 37-amino-acid neuropeptide that is chiefly involved in the trigeminovascular system and primarily expressed within the nervous system [6]. CGRP is implicated in the pathogenesis of migraines [6] and is associated with multiple functions within the central nervous system (CNS) and its periphery [7]. Recent research has linked CGRP to migraine development due to its vasodilatory effect and localization at trigeminal nerve endings that innervate cerebral blood vessels [8, 9]. Migraines are prevalent, complex neurological disorders that manifest themselves as attacks of severe, throbbing headaches that are occasionally accompanied by sensory sensitivity to light, sound and head movements [9, 10]. In accord with this link, cranial CGRP levels are elevated in patients with migraines [11]. During spontaneous migraine attacks, the potent vasodilator CGRP is released. Accompanied by pain, this leads to increased CGRP blood levels from the external jugular vein, and its concentration is correlated with the headache intensity. Thus, studies have suggested that CGRP antagonists may prove efficacious for the treatment of acute migraines [12].

The CGRP receptor, a class B GPCR, has two key protein components, the calcitonin receptor-like receptor (CLR) and the receptor activity-modifying protein 1 (RAMP1), which form a stable, hetero-oligomeric complex [13]. Together, these constituents' extracellular domains form part of the CGRP peptide-binding site [14].

Moreover, CLR shares characteristic features with the Class B GPCR family, particularly an N-terminal extracellular domain (ECD): a transmembrane domain consisting of seven α -helices of approximately 120 residues [15, 16]. Through the use of X-ray crystallography and nuclear magnetic resonance spectroscopy, ECDs have been shown to have a common α - β - α folding topology that is strengthened by three conserved disulfide pairs [17]. Furthermore, a set of class B GPCR binding proteins are the RAMP proteins, which maintain a three-helix bundled structure containing a cleavable signal peptide, an N-terminal extracellular domain and an intracellular C-terminal domain [18]. This structure is stabilized by three disulfide bonds and has a single transmembrane segment [18]. Studies

have suggested that three known RAMP proteins, RAMP1, RAMP2 and RAMP3, and their complexes with the CLR confer specificity for different signaling peptides [19]. RAMP1 has roles in receptor trafficking, interactions and ligand binding and is critical for the determination of receptor phenotype and species-selectivity [6, 20]. Moreover, this binding assists in delineating the functionality of the CLR-RAMP1 complex [20].

In the current study, we investigated the interactions of class C-GPCR allosteric modulators against a class B-GPCR, CLR, both in complex with RAMP1 (CGRP receptor) and independently. *In silico* docking of twelve compounds (Figure 1), which are mainly the derivatives of pyrimidine, thiophene and quinoline (methanone), were performed using AutoDock4.2 [21] with the CGRP receptor and CLR. This was undertaken in order to elucidate the binding conformations of the selected compounds with the goal to find more potent inhibitors that could be used in designing drugs for the treatment of migraines.

All of the selected compounds have previously been evaluated in several different disorders, such as in depression, psychosis, acute and chronic pain, cerebral ischemia, neurodegenerative disorders, to reduce inflammation and epilepsy. These compounds include 7-hydroxyiminocyclopropan[b]chromen-1 α -carboxylic acid ethyl ester (CPCCOEt). The compound (3aS, 6aS)-hexahydro-5-methylene-6a-(2-naphthalenylmethyl)-1H-cyclopenta[c]furan-1-one that belongs to the cyclopentane family and is a hexahydro-5-methylene derivative, is mainly used as a prodrug [22]. These compounds are potentially useful in the treatment of autoimmune diseases [23]. 9-(dimethylamino)-3-(hexahydro-1H-azepin-1-yl)pyrido[3,2-d:4,5]thieno [3,2-d]pyrimidin-4(3H)-one and 4-(cycloheptylamino)-N-[[[(2R)-tetrahydro-2-furanyl]methyl]-thieno[2,3-d] pyrimidine-6-methanamine are pyridine, thiophene and pyrimidine derivatives. Thieno-pyrimidine derivatives are structural analogs of purines, which are known to have a wide range of pharmacological activities, including antibacterial, antifungal, analgesic, antipyretic, anti-inflammatory, antihistaminic, and anticancer activities, and also have been assessed as radioprotective drugs [24]. Numerous thieno[2,3-d]pyrimidines have been appraised in cerebral ischemia, malaria, tuberculosis, and Alzheimer's and Parkinson's diseases [25]. Two of the docked compounds, (3,4-dihydro-2H-pyrano[2,3-b]quinoline-7-yl)-(cis-4-methoxycyclohexyl)-methanone and 1-(3,4-dihydro-2H-pyrano[2,3-b]quinoline-7-yl)-2-phenyl-1-ethanone, respectively, are quinoline derivatives that are considered to be therapeutic candidates for transmissible spongiform encephalopathies (TSEs) [26] or prion diseases; a group of fatal, human neurodegenerative disorders that include Creutzfeldt-Jakob disease and Gerstmann-Straussler-Scheinker disease (GSS). Compound 1-ethyl-2-methyl-6-oxo-4-(1,2,4,5-tetrahydro-benzo[d]azepin-3-yl)-1,6-dihydro-pyrimidine-5-carbonitrile is a tetrahydro-benzo[D]azepin derivative that, likewise, has potential medicinal advantages and has similar therapeutic properties to 4-[1-(2-fluoropyridin-3-yl)-5-methyl-1H-1,2,3-triazol-4-yl]-N-isopropyl-N-methyl-3,6-dihydropyridine-1(2H)-carboxamide. Compound 6-amino-N-cyclohexyl-N,3-dimethylthiazolo[3,2-a] benzimidazole-2-carboxamide hydrochloride has previously been investigated as a drug candidate for psychiatric disorders, including schizophrenia, depression and anxiety, and is known to regulate glutamatergic transmissions [27].

In the current study, an *in silico* approach was employed to appraise these compounds for their potential inhibitory effects on a key migraine-causing protein.

Methodology

Receptor structure extraction and ligand selection

The X-ray diffracted crystal structure of stable CGRP receptor was available at Research Collaboratory for Structural Bioinformatics (RSCB) with accession number PDB ID 3N7S [19]. It encompassed eight helices and small beta sheets, and possessed a structure weight of 50180.91 A.U. (asymmetric unit) with a resolution of approximately 2.10 Å. From the protein data bank, 3N7S [19] is the crystal structure of a class B GPCR; CGRP was extracted along with information regarding the antagonist binding residues. CGRP interacts with CLR through critical hydrogen-bonds (H-bonds) with residues Asp94, Thr122, Ile41, Met42, Trp72, Phe92, Arg119, Trp121 and Tyr124 as well as via hydrophobic interactions. Furthermore, Asp71 of CGRP also showed hydrogen bonding with RAMP1 as well as salt bridge formation of Asp72 assisted by hydrophobic interactions with Trp74, Phe83 and Trp84.

To study the molecular interaction of the targeted receptor, twelve compounds showing negative allosteric modulation of the mGlu1 receptor (class C GPCR) were selected for the docking studies. These compounds include 7-hydroxyiminocyclopropan[*b*]chromen-1 α -carboxylic acid ethyl ester (CID 6324610; CPCCOEt; Compound 1; Figure 1a), (3*a*S,6*a*S)-hexahydro-5-methylene-6*a*-(2-naphthalenylmethyl)-1*H*-cyclopenta[*c*]furan-1-one (BAY36-7620; Compound 2; Figure 1b), 9-(dimethylamino)-3-(hexahydro-1*H*-azepin-1-yl)pyrido[3,2-*b*]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (A841720; Compound 3; Figure 1c), (3,4-dihydro-2*H*-pyrano[2,3-*b*]quinoline-7-yl)-(cis-4-methoxycyclohexyl)-methanone (JNJ16259685; Compound 4; Figure 1d), 6-amino-*N*-cyclohexyl-*N*,3-dimethylthiazolo[3,2-*a*]benzimidazole-2-carboxamide hydrochloride (YM-298198; Compound 5; Figure 1e), 1-ethyl-2-methyl-6-oxo-4-(1,2,4,5-tetrahydro-benzo[*d*]azepin-3-yl)-1,6-dihydro-pyrimidine-5-carbonitrile (EM-TBPC; CID 9904703; Compound 6; Figure 1f), 3-cyclohexyl-5-fluoro-6-methyl-7-(2-morpholin-4-ylethoxy)-4*H*-chromen-4-one (CFMMC; Compound 7; Figure 1g), 4-[1-(2-fluoropyridin-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl]-*N*-isopropyl-*N*-methyl-3,6-dihydropyridine-1(2*H*)-carboxamide (FTIDC; CID 11245287; Compound 8; Figure 1h), 4-(3,3-dimethylbutan-2-yl) 2-propyl 3,5-dimethyl-1*H*-pyrrole-2,4-dicarboxylate (Compound 9; Figure 1i), 2-[(4-indan-2-ylamino)-5,6,7,8-tetrahydroquinazolin-2-ylsulphonyl]-ethanol (Compound 10; Figure 1j), 4-(cycloheptylamino)-*N*-[[*(2R)*-tetrahydro-2-furanyl]methyl]-thieno[2,3-*d*]pyrimidine-6-methanamine (YM-230888; Compound 11; Figure 1k) and 1-(3,4-dihydro-2*H*-pyrano[2,3-*b*]quinoline-7-yl)-2-phenyl-1-ethanone (CID 10470232; R214127; Compound 12; Figure 1l). The molecular structures of these compounds were drawn using Chemoffice [28], and geometric optimization and energy minimization were performed using HyperChem [29].

Docking studies

AutoDock4.2 [21] was used to perform the docking and to elucidate the binding conformations of the selected compounds. Polar hydrogen atoms were added to the receptor

protein and ligands; all bonds were allowed to rotate and were flexible during docking. Using AutoGrid, grid maps were generated by marking the grids around the entire receptor. Each grid was centered at the structure of the corresponding receptor. The grid dimensions were $80 * 80 * 80 \text{ \AA}^3$ with a spacing of $0.41 \text{ \AA}$. The Lamarckian genetic algorithm was used for flexible ligand docking with 100 runs, a population size of 150, $2.5 * 10^6$ evaluations, a maximum number of $27 * 10^3$ iterations, an elitism value of 1, a mutation rate of 0.02 and a crossover rate of 0.80. The binding conformations were then analyzed on the basis of the energy values and the interacting residues.

Results

Docking with CGRP type 1 receptor

To analyze the experimental data, cluster analysis was performed using a root means square with a tolerance of $1.0 \text{ \AA}$. The clusters were ranked by the lowest energy representative of each cluster. When compared against the CGRP type 1 receptor, docking of all compounds yielded very low energy values and inhibition constants (kIs; Table 1). The compounds were stable as indicated by the approximately 0 Kcal/mol internal energy of nearly all twelve compounds. The docking interactions of the compounds with the receptor protein resulted in low docking energy values based on the observed stable internal state and low intermolecular energy values. This result is explained by the low binding energy and suitable torsional energy during the docking procedure, which resulted in stable protein-ligand interactions. Overall, the inhibitory capability of the compounds against CGRP from the docking procedure was mainly based on kI. However, the kIs for all twelve compounds are positioned in a favorable range; quinoline derivative 4 possessing a single digit nanomolar value for kI and compound 12 showed a very low kI value as well, indicative of a highly effective inhibitory activity for the receptor. Figure 2 collectively shows the binding of all compounds with the protein and CGRP. All potential inhibitory compounds appraised docked in the cavity that formed the active site of the receptor protein.

The interactions of the known active site residues with the investigated compounds were observed. Favorable numbers of residues were involved in docking with the compounds (Table 2). The most active residues of CGRP involved with these compounds were CLR Trp72, Arg119, Trp112, Thr122, Tyr124 and RAMP1 Trp84. The RAMP1 residues; Trp74 and Phe83 as well as the CLR residues; Ile41 and Phe92 also interacted with key compounds.

The CLR residue Thr122 appeared most actively involved in binding with all compounds, exhibiting strong H-bonds with favorable distance (Table 2). The best H-bonds from CLR residue Thr122 were formed with compounds 1 and 5 with H-bond distances of $2.00 \text{ \AA}$ and $2.07 \text{ \AA}$, respectively. CLR Trp121, Arg119 and RAMP1 Trp74 also formed H-bonds with some of the compounds. Figure 3 depicts the individual docking orientations of all compounds with the CGRP protein at the atomic-level, indicating the residues interacting with the compounds. Thus, of all the investigated compounds, compounds 4 and 12 appeared to be the most competent inhibitors of CGRP in terms of binding interactions, energy values and kI.

Docking with Calcitonin receptor-like receptor

Compound docking with CLR also resulted in low energy values (Table 3); however, the energy values and KI values for the compounds against CLR were higher when compared to those against CGRP. Similar to CGRP, compound stability and low binding and intermolecular energy values led to stable interactions and low docking energy values. Additionally, similar to the situation with CGRP, compounds 4 and 12 possessed the smallest binding energies, docking energies and KI values compared to those with CLR alone.

Figure 4 illustrates all 12 compounds collectively bound within the same binding pocket of CLR; thereby demonstrating the similarity in binding in terms of the orientation and positioning of the compounds within the protein cavity.

Table 4 defines the residues of CLR involved in binding with the evaluated compounds. As with CLR in CGRP, the most frequently interacting residues when CLR was individually docked to the compounds were Trp72, Arg119, Trp121, Thr122 and Tyr124. The residues Ile41, Phe92 and Asp94 also appeared to interact with key compounds. Similar to CGRP, Thr122 appeared to be the residue that most frequently formed H-bonds with the appraised compounds. Arg119 and Tyr124 similarly formed H-bonds with select compounds. Of all of the agents evaluated, compounds 1 and 5 showed the strongest H-bond interactions during docking. Figure 5 depicts the individual binding pattern or orientations of the protein CLR with all compounds, showing details at the atomic-level as well as the distance of the H-bonds. Similarly, compounds 1 and 4 were also found to be most efficient in terms of binding interactions and binding and docking energy.

Discussion

GPCRs comprise the largest protein superfamily within mammalian genomes. Sharing a common seven-transmembrane topology, they mediate a diverse host of cellular responses to a broad range of extracellular signals to maintain homeostasis and optimize function across health, aging and disease. The diversity of the extracellular ligands that bind and activate GPCRs is reflected in the structural diversity of some 1000 human GPCRs. These, on the basis of their amino acid sequences, can be broadly divided into five primary families and multiple subfamilies. Signal transduction subsequent to GPCR activation is fundamental across almost all physiological processes, particularly neurological ones, thereby making the GPCR superfamily a critical target for therapeutic intervention [30]. Indeed, GPCRs represent the largest class of targets for modern drugs [7]. Recent breakthroughs in GPCR crystallography have been critical in supporting a framework for biochemical, biophysical, and computational studies to define GPCR functions and dynamics.

Earlier studies have shown that allosteric modulators of GPCRs bind to receptors, thus altering the binding of the orthosteric ligands [31, 32]. The mathematical modeling of allosterism has been characterized by a cooperativity factor (α) and the KIs, which represent the strength of the interaction between the allosteric and orthosteric sites [2]. A previously reported novel compound N-[(3R,6S)-6-(2,3-difluorophenyl)-2-oxo-1-(2,2,2-trifluoroethyl)azepan-3-yl]-4- (2-oxo-2,3-dihydro-1H-imidazo[4,5-b]pyridin-1-

yl)piperidine-1-carboxamide (MK-0974), which is a potent, orally bioavailable CGRP antagonist for the treatment of migraines [33], is structurally related to compound 5, 6-amino-N-cyclohexyl-N,3-dimethylthiazolo[3,2-a] benzimidazole-2-carboxamide hydrochloride, in line with its predicted favored binding within the current study.

The receptor for CGRP has been a target for the development of novel small molecule antagonists for the treatment of migraines [14]. During the drug discovery process, optimizing the effects of substances on the target receptor is normally directed towards obtaining sufficiently high potency and efficacy [34]. In this study, two derivatives of quinoline, compound 4 (1-(3,4-dihydro-2H-pyrano[2,3-b]quinoline-7-yl)-2-phenyl-1-ethanone) and compound 12 (3,4-dihydro-2H-pyrano[2,3-b]quinoline-7-yl)-(cis-4-methoxycyclohexyl)-methanone) showed *K_i*s in the low nano-molar range, indicative of their highly effective inhibitory activities for the receptor, and these agents are hence of further interest to evaluate as potential drugs.

The key to harnessing the potential therapeutic benefits of GPCRs is to effectively identify highly selective pharmaceutical ligands for these receptors (endogenous or otherwise) with the dual aim of using them as tools to elucidate their functions as well as evaluating their clinical potential [35]. *In silico* docking procedures were performed on the unbound form of CLR and the bound form in complex with RAMP1. In both cases, all inhibitors were bound in the conserved cavity of the calcitonin receptor-like receptor. This area hence warrants further consideration in future analyses.

Our results of the docking study also indicate optimized conditions in which the docking of all compounds yielded very low and favorable energy values and *K_i*s (Table 1). The internal energy of approximately 0 Kcal/mol for nearly all twelve compounds suggests that the compounds are stable. Based on the observed stable internal state and the low intermolecular energy values, the docking interactions (complexes) of the compounds with the receptor protein resulted in low docking energy values (Table 3). Therefore, quinoline derivatives a) (3,4-dihydro-2H-pyrano[2,3-b]quinoline-7-yl)-(cis-4-methoxycyclohexyl)-methanone (compound 4) and b) 1-(3,4-dihydro-2H-pyrano[2,3-b]quinoline-7-yl)-2-phenyl-1-ethanone (compound 12) appeared the most effective inhibitors of CGRP in terms of binding interactions, energy values and *K_i*. On this basis, compounds 4 and 12, which are quinoline derivatives, may be considered as drug candidates - particularly for migraines - that warrant evaluation in preclinical models.

Perspectives

The results reported in this study suggest that the molecular docking of Calcitonin receptor-like receptor- and CGRP type 1 receptor-homology models (with 3D structures that approximate reality) sufficiently support the following applications: a) the design of site-directed mutagenesis experiments intended to experimentally probe interactions, which can then be incorporated into the models in an iterative manner; b) predictions of the conformation and orientation of the ligands, which can be applied to the construction of docking-based three-dimensional quantitative structure–activity relationship (3D-QSAR) models; and c) the design of fuzzy receptor-based pharmacophores to be applied in virtual screening experiments.

Abbreviations

CLR	Calcitonin receptor-like receptor
CGRP	Calcitonin gene-related peptide
RAMP1	Receptor activity-modifying protein 1
GPCR	G protein coupled receptor
K_I	inhibition constant

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Figure 1.
Compounds (a-l) used for docking evaluation against receptor proteins: a-l Compounds 1–12.

Figure 2.

The binding of all compounds occurred at the same position within the active binding cavity of CGRP. **a** all compounds are shown as surface mesh, CLR is shown in green ribbon and surface, RAMP1 is shown in blue ribbon and surface; **b** all compounds are shown as surface mesh, all residues are shown as sticks and labeled; H-atoms are colored white, O-atoms are colored red, N-atoms are colored dark blue, CLR C-atoms are colored green, and RAMP1 C-atoms are colored light blue.

Figure 3.

Docking interactions of CGRP residues with compounds 1-12 (a-l). (O-atoms are colored red, N-atoms blue, S-atoms gold, F-atoms light blue, CLR C-atoms green, RAMP1 C-atoms cyan, and compound C-atoms pink; H-bonds are shown in green dotted lines labeled with distance in Å).

Figure 4.

Binding of evaluated inhibitors within the active-site cavity of CLR. **a** compounds are shown in surface mesh, CLR protein are shown in green surface and ribbon; **b** compounds are shown in surface mesh, all residues are shown as sticks and labeled, H-atoms are colored white, O-atoms are colored red, N-atoms are colored dark blue, and C-atoms are colored green.

Figure 5.

Docking interactions of CLR residues with compounds 1–12 (a–l). (O-atoms are colored red, N-atoms blue, S-atoms gold, F-atoms light blue, CLR C-atoms green, RAMP1 C-atoms cyan, and compound C-atoms pink; H-bonds are shown in green dotted lines labeled with distance in Å).

Table 1

Energy values and kIs of compounds after docking with CGRP.

Compounds	Binding Energy (Kcal/mol)	Internal Energy (Kcal/mol)	Intermolecular Energy (Kcal/mol)	Docking Energy (Kcal/mol)	kI (nM)	Torsional energy (Kcal/mol)
1	-7.54	-0.24	-8.73	-8.97	2990	1.19
2	-10.54	-0.14	-11.14	-11.28	18.77	0.6
3	-9.37	0.4	-9.97	-9.57	135.71	0.6
4	-11.04	-0.41	-11.93	-12.34	8.08	0.89
5	-10.49	0.03	-11.39	-11.36	20.43	0.89
6	-9.69	-0.41	-10.59	-11	78.31	0.89
7	-8.95	-0.32	-10.15	-10.47	273.12	1.19
8	-9.85	-0.66	-10.74	-11.4	60.24	0.89
9	-8.38	-1.07	-10.76	-11.83	722.16	2.39
10	-9.52	-0.48	-10.42	-10.9	104.54	0.89
11	-9.92	-0.95	-11.71	-12.66	53.4	1.79
12	-10.87	-0.61	-11.76	-12.37	10.8	0.89

Active site residues of CGRP involved in docking interactions with the compounds. HP represents a hydrophobic interaction, the bond distances are represented in Å, and H-bond atoms are indicated as protein residue atom – ligand atom.

Table 2

Compounds	Binding residues											
	CLR						RAMP1					
	I41	W72	R119	W121	T122	Y124	W74	F83	W84			
1	-	HP	HP	HP	OH-O 2.00	HP	-	HP	HP			
2	-	HP	HP	HP	NH-O 2.72	HP	-	HP	HP			
3	-	HP	HP	HP	OH-O 2.71	HP	-	-	HP			
					NH-O 3.10							
4	-	HP	HP	HP	OH-O 3.09	HP	-	HP	HP			
5	HP	HP	HP	HP	O-HN 2.07	HP	HP	-	HP			
6	-	HP	HP	NH-O 3.15	OH-O 2.48	HP	HP	-	HP			
					NH-O 3.12							
7	HP	HP	HP	HP	NH-O 2.96	-	NH-F 2.81	-	HP			
8	-	HP	HP	HP	OH-F 2.96	HP	-	-	HP			
					NH-F 2.98							
9	-	HP	NH-O 3.02	HP	OH-O 2.94	HP	-	-	HP			
					NH-O 3.01							
10	-	HP	HP	HP	OH-O 2.69	HP	-	-	HP			
					NH-S 3.09							
11	-	HP	HP	HP	NH-S 2.91	HP	HP	HP	HP			
12	-	HP	HP	HP	NH-O 3.11	HP	-	HP	HP			
					OH-O 3.15							

Table 3

Energy values and kIs of compounds after docking with CLR.

Compounds	Binding Energy (Kcal/mol)	Internal Energy (Kcal/mol)	Intermolecular Energy (Kcal/mol)	Docking Energy (Kcal/mol)	kI (μM)	Torsional energy (Kcal/mol)
1	-6.51	-0.23	-7.71	-7.94	16.83	1.19
2	-7.54	-0.46	-8.13	-8.59	2.99	0.6
3	-6.76	0.39	-7.36	-6.97	11.04	0.6
4	-8.08	-0.42	-8.97	-9.39	1.2	0.89
5	-7.53	-0.37	-8.42	-8.79	3.04	0.89
6	-6.3	-0.40	-7.20	-7.60	24.07	0.89
7	-6.29	-0.32	-7.48	-7.80	24.51	1.19
8	-7.25	-0.49	-8.15	-8.64	4.83	0.89
9	-5.28	-0.82	-7.67	-8.49	134.98	2.39
10	-6.88	-0.56	-7.78	-8.34	9.04	0.89
11	-6.33	-0.59	-8.12	-8.71	22.94	1.79
12	-7.64	-0.46	-8.54	-9.00	2.51	0.89

Active site residues of CLR involved in docking interactions with the 12 evaluated compounds. HP represents a hydrophobic interaction, the bond distances are represented in Å, and H-bond atoms are indicated as protein residue atom – ligands.

Table 4

Compounds	Binding Residues							
	W72	F92	D94	R119	W121	T122		Y124
1	HP	-	-	NH-O	HP	NH-N	1.93	HP
						CO-H	2.04	
						NH-O	2.12	
2	HP	-	-	HP	HP	NH-O	2.16	HP
						NH-O	2.27	
3	HP	-	-	HP	HP	NH-N	2.21	NH-S 2.47
							OH-O	3.05
4	HP	HP	HP	NH-O	HP	NH-O	2.06	HP
5	HP	-	-	HP	HP	NH-O	1.92	NH-S 2.45
6	HP	-	-	HP	HP	NH-O	2.00	HP
						OH-O	3.07	
7	HP	HP	HP	HP	HP	NH-O	2.19	HP
						OH-O	2.74	
8	HP	-	-	HP	HP	NH-N	2.08	HP
9	HP	-	-	HP	HP	NH-O	2.17	HP
						OH-O	2.68	
10	HP	-	-	HP	HP	NH-S	2.14	2.14
						OH-O	2.75	
11	HP	HP	HP	HP	HP	NH-O	1.86	HP
12	HP	HP	HP	HP	HP	NH-O	1.97	HP