Design, Synthesis and Anti-itch Activity Evaluation of Aromatic Amino Acid Derivatives as Gastrin-Releasing Peptide Receptor Antagonists

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Abstract: Eight aromatic amino acid derivatives (**9a-9h**) as gastrin-releasing peptide receptor (GRPR) antagonists were designed and synthesized. For the design, the tertiary structure of GRPR was predicted by blast searching in the premise of 1u19 protein as the template. Eight target compounds were docked into the binding pocket to investigate their possible binding interactions. Their anti-itch activities were tested by intrathecal injection using Kunming mice as experimental animals and chloroquine phosphate as a modeling medium. Compounds **9e** and **9f** significantly inhibited scratching behaviors. The anti-itch activities of these compounds decreased with the following sequence: **9e** > **9f** = **9d** > **9b** > **9g** > **9a** > **9h** > **9c** predicted by computer-aided drug design (CADD) and **9e** > **9f** > **9b** > **9h** > **9g** > **9d** > **9a** > **9c** evaluated by preliminary test. They were broadly in line with activity order pretested by CADD. It showed that the predicted tertiary structure of GRPR could be used for antipruritic drug design.

Key Words: Anti-itch activity, Aromatic amino acid derivatives, Computer-aided drug design, Gastrin-releasing peptide receptor, 2-(1*H*-imidazol-1-yl) ethanol, Molecular modeling, Pruritus, Tertiary structure.

1. INTRODUCTION

Itch is an unpleasant sensation that causes the desire or reflex to scratch. It usually caused by skin disease, biliary disease, thyroid disease, kidney disease, cancer, AIDS and so on [1-3]. Chronic itch is difficult to treat [4]. However, gastrin-releasing peptide receptor, situated in the central nervous system, is the first gene directly related to the itch which confirmed by Yan-Gang Sun and Zhou-Feng Chen in 2007 [5, 6]. So GRPR may be a central target for therapeutic treatment of chronic and intractable pruritus [5].

Gastrin-releasing peptide receptor belongs to the family of the bombesin-like peptides (BLP) [7]. The human GRPR has 384 amino acids. Hydropathy analysis of the predicted GRPR structure revealed seven regions of hydrophobic amino acids consistent with a seven-transmembrane structure typical for G protein-coupled receptors [8]. However, the tertiary structure of GRPR had not been reported.

In humans, GRPR is highly expressed in the pancreas and is also expressed in the stomach, adrenal cortex and brain [9]. GRPR activation has been proposed to be important in the mediation of a number of human disorders including disorders of lung development, various pulmonary diseases, CNS disorders, and the growth/differentiation of human cancers [10].

There have been a large number of different compounds reported to function as GRPR antagonists. They can be divided into six general classes. These six classes include substituted substance P analogs (class 1), [D-Phe12] bombesin analogs (class 2), modified position 13-14 bombesin or position 26-27 GRP analogs (class 3), desMet14 or GRP27 analogs (class 4), peptoids (class 5), and finally the nonpeptide analogs, kuwanon G and kuwanon H (class 6) [10-12]. Hereinto, PD 176252 is a peptoid antagonist that has nanomolar affinity for GRPR (Ki = 1 nM). Subsequent studies demonstrated that PD 176252 inhibited the growth of lung cancer cells, potentiated the growth inhibitory effects of histone deacetylase inhibitors; inhibited GRP/Bnstimulated signaling in lung cancer cells (Ca^{2+} and tyrosine phosphorylation of $p125^{FAK}$) and the stimulation of increases in c-fos mRNA and growth, and in rats had an anxiolytic effect *in vivo* [13]. All classes are peptides or peptoid antagonists, except for class 6, which are flavone derivatives, isolated from extracts of the mulberry tree Morus bombycis [14].

So far, few small molecule compounds with anti-itch activity as GRPR antagonists have been reported. The GRPR antagonists are mainly peptides, and they are not suitable as molecular design reference for the high molecular weights and complex structures. The non-peptides, kuwanon G and kuwanon H (Fig. **1**), have too many rotatable bonds and are difficult to synthesize by chemical methods. So they also don't fit as molecular design reference. The peptoids, especially PD 176252 with high affinity, can be used as reference. To discover some innovative drugs for anti-itch, the tertiary structure of GRPR was predicted with a homologous protein, then with the reported GRPR antagonists, especially PD 176252, eight compounds (Fig. **2**) were designed, synthesized and preliminarily tested the anti-itch activity.

2. RESULTS AND DISCUSSION

2.1. Chemistry

Compounds **9a-9h** were composed of three fragments, see Fig. (**2**). They were synthesized of L-phenylalanine or L-tryptophan (fragment 2) as the basic skeleton by esterifica-

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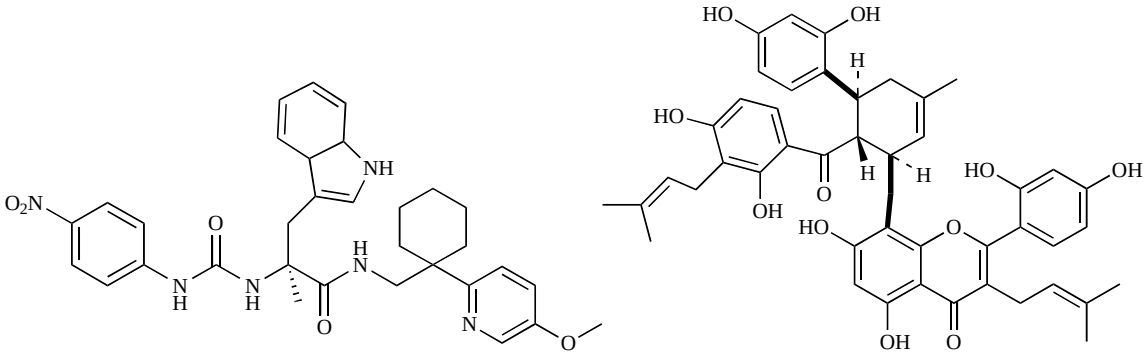
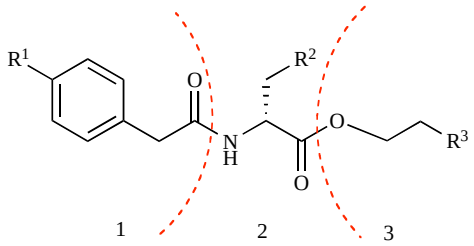


Fig. (1). The Structures of PD 176252 and Kuwanon H.



Comp. No	R^1	R^2	R^3
9a	NO_2	phenyl	1-imidazole
9b	F	phenyl	1-imidazole
9c	CH_3	phenyl	1-imidazole
9d	NO_2	3-indole	1-imidazole
9e	F	3-indole	1-imidazole
9f	CH_3	3-indole	1-imidazole
9g	F	3-indole	4-morpholine
9h	F	3-indole	4-(1-methylpiperazine)

Fig. (2). The Structures of Compounds **9a-9h**.

tion with the methanol, amidation with alphatoluic acid derivatives (fragment 1), then deprotection and esterification with ethanol derivatives (fragment 3).

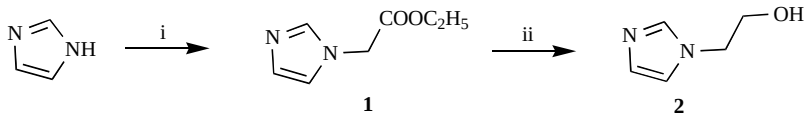
In Scheme **1**, ethyl 2-(1*H*-imidazol-1-yl) acetate (**1**) was prepared in 79.0% yield by refluxing imidazole in THF with ethyl 2-chloroacetate for 10 h [15, 16]. $NaBH_4$ was slowly added to a solution of **1** in MeOH at 0 °C. Then heating to 50 °C, 2-(1*H*-imidazol-1-yl) ethanol (**2**) was obtained for 8 h in a good yield [17]. While 2-morpholinoethanol and 2-(4-methylpiperazin-1-yl) ethanol were synthesized with the total yields of 76.6% and 72.2% applying the same preparation protocols followed in Scheme **1**. The only difference was the use of morpholine and methylpiperazine instead of imidazole used in Scheme **1**.

As shown in Scheme **2**, compounds **9a-9f** were synthesized using L-Phenylalanine or L-Tryptophan as starting material. Firstly, $SOCl_2$ was added to MeOH at 0 °C for 1 h. After the addition of **3** and refluxing for 5 h, **4** was obtained [18]. Secondly, **7** was carried out by the dropwise addition of the new prepared acetyl chloride derivative **6** to a solution of **4**. Then, the hydrolysis of **7** was conducted in a mixed solvent of MeOH and water in a (4:1, *V/V*) ratio and in the presence of Na_2CO_3 [19]. Finally, compounds **9a-9f** were prepared by the reaction of DCC condensation. Compounds **9g** and **9h** were synthesized applying the same preparation protocols followed in Scheme **2**. The only difference was the use of 2-morpholinoethanol and 2-(4-methylpiperazin-1-yl) ethanol instead of 2-(1*H*-imidazol- 1-yl) ethanol.

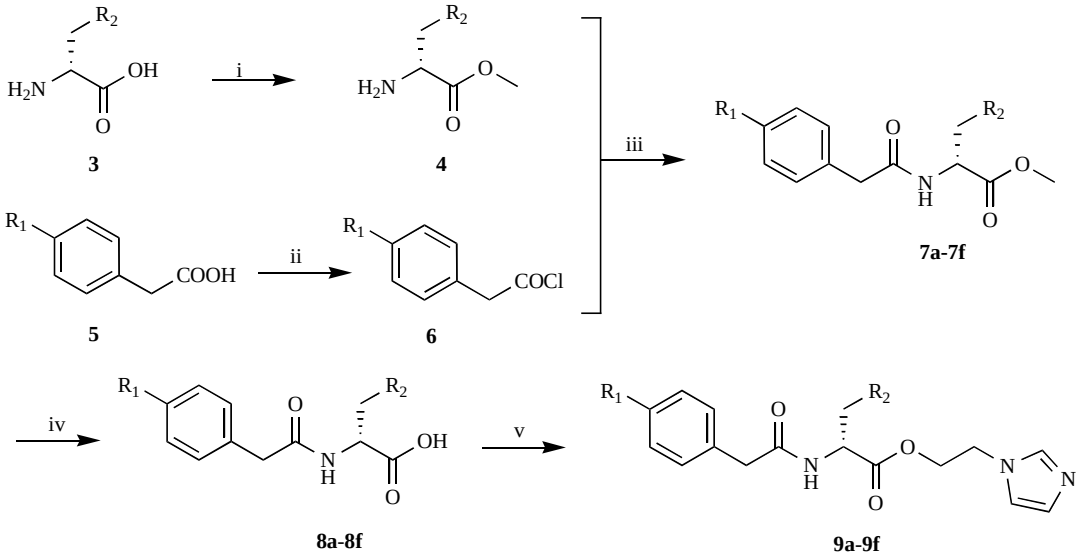
The structures of intermediates and target compounds were confirmed by FT-IR and ^1H NMR.

2.2. Molecular Modeling

In order to get a better insight into the binding affinity, molecular modeling studies were performed using Molecular Operating Environment (MOE). 2008. 10 software. Since no crystal structure of GRPR is yet available, the tertiary structure of GRPR was predicted using the crystal structure given in PDB code 1u19 (Fig. **3**).



Scheme 1. Reagents and Conditions: (i) Ethyl 2-chloroacetate, NEt_3, THF, Reflux, 10 h, 79%; (ii) $NaBH_4$, MeOH, 0 °C to 50 °C, 8 h, 90%.



Scheme 2. Reagents and Conditions: (i) $SOCl_2$, MeOH, 0 °C to Reflux, 5 h; (ii) $(COCl)_2$, NEt_3, Cat. DMF, DCM, N_2, 0 °C to rt, 2 h; (iii) NEt_3, DCM, N_2, 0 °C to rt, 5 h; (iv) Na_2CO_3, MeOH/H_2O (4:1), rt, 6 h; (v) 2-(1H-imidazol-1-yl)ethanol, DCC, cat. DMAP, THF, rt, 15 h.

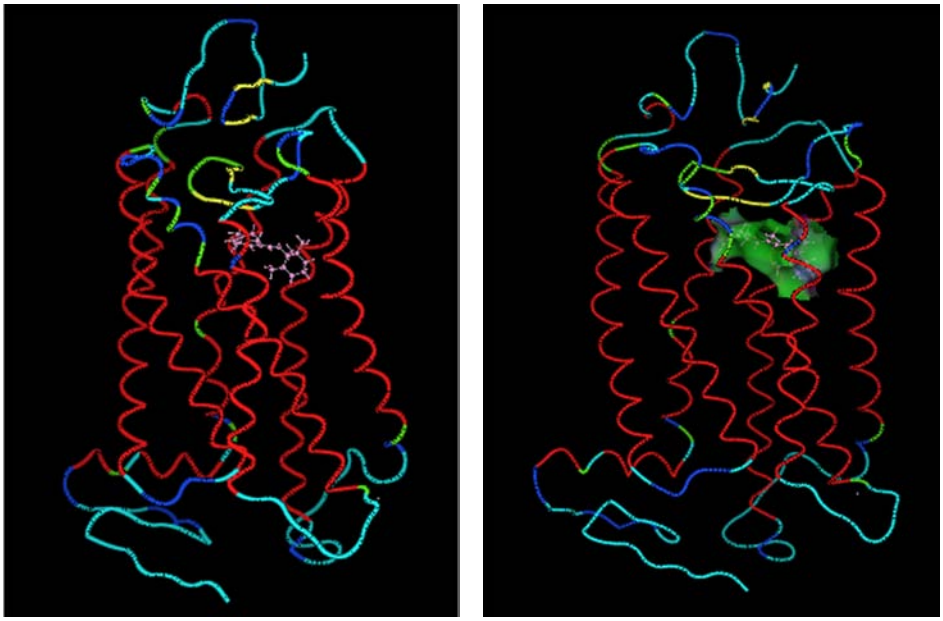


Fig. (3). The Tertiary Structure of GRPR.

As well as GRPR, 1u19 protein belongs to the family of G protein coupled receptor. By blast searching, the two proteins have a high degree of similarity in the structure and the identities and positives are 22% and 42%, respectively. So 1u19 protein was chosen as the template of homology modeling.

Compounds **9a**-**9h** were docked over the ligand atoms in the binding site. They occupy nearly the same space in the binding pocket, and are overlaid well over the skeleton of the ligand.

In compound **9a** (Fig. **4**), the benzene ring of L-phenylalanine establishes an arene-cation interaction with Lys-188. The left C=O forms a hydrogen bonding interaction with the backbone NH of Lys-265 and the right C=O forms a hydrogen bonding interaction with Val-187 through H_2O. The 4-nitro-benzene shows an arene-cation interaction with His-186. The binding interactions of compounds **9b** and **9c** (Table **1**) are the same with **9a**. Compounds **9d** and **9f** both have one more interaction with His-207 than **9a**. Compound **9e** as well as **9a**, interacts with Lys-265, Val-187, and His-

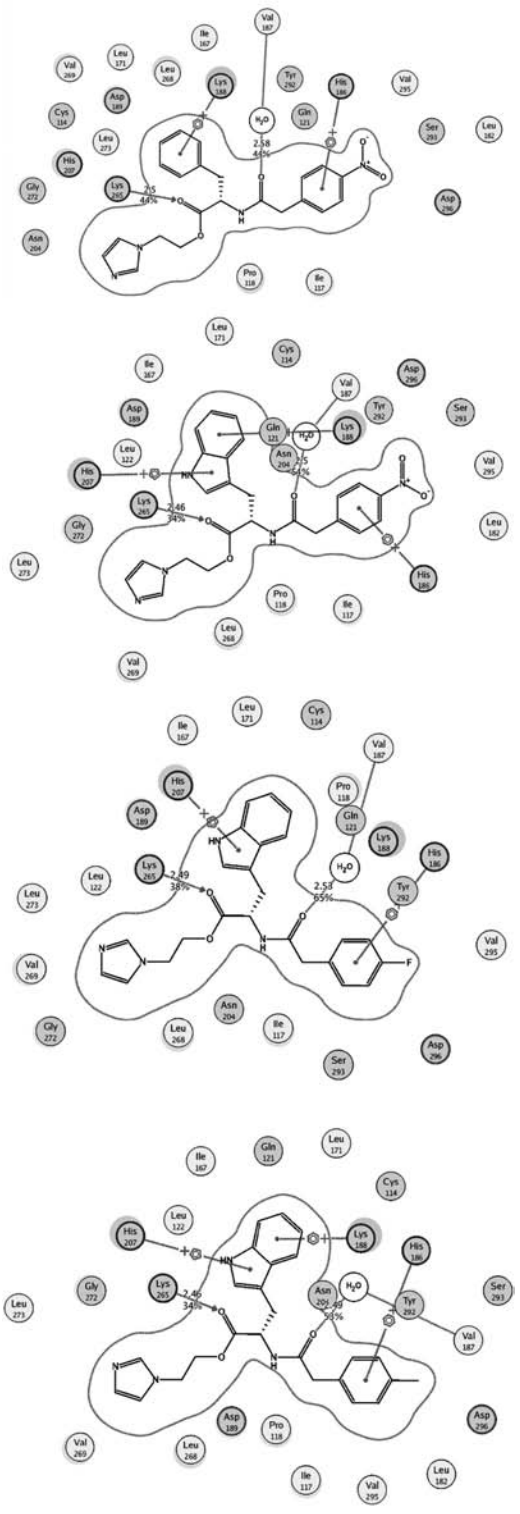


Fig. (4). 2D-presentation for the Binding Interactions of Compound **9a**, **9d**, **9e** and **9f** with Receptor.

186 respectively. The difference of the two compounds is that the pyrrole ring of L-tryptophan establishes the arene-cation interaction with His-207 rather than Lys-188. The binding interactions of **9g** and **9h** are the same with **9e**.

Eight compounds mainly form interactions with His-186, Val-187, Lys-188 and Lys-265. In addition, the pyrrole ring of L-tryptophan shows an interaction with His-207, while the benzene ring of L-phenylalanine does not.

2.3. Anti-itch Activity

Compounds **9a-9h** were tested the anti-itch activity. In the experiments, half female and half male Kunming mice were used as experimental animals and chloroquine phosphate was used as a modeling medium [5, 20]. The treatment animals were given intrathecal injection of the solution of target compounds (10 nmol/2.5 μL). While the control animals were given the solution not containing compounds. After 10 min, the chloroquine phosphate solution was applied to the nape adopting subcutaneous injection [21].

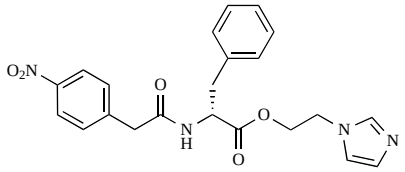
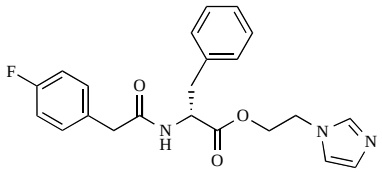
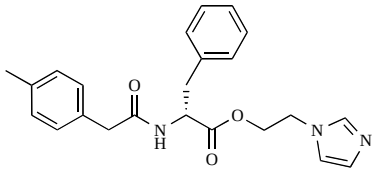
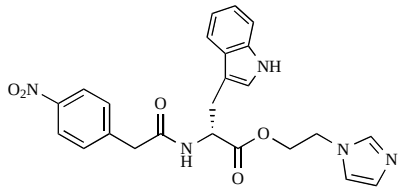
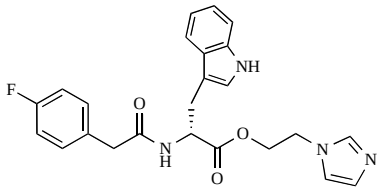
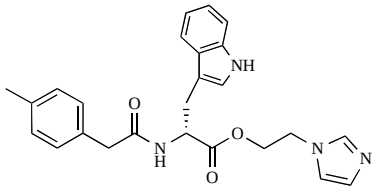
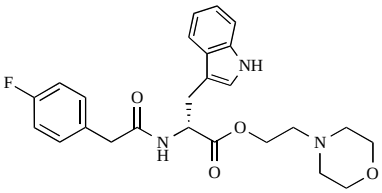
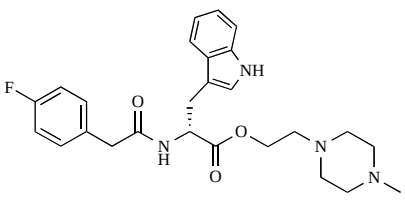
It was found that various concentrations (200 μg/100 μL, 400 μg/100 μL, 600 μg/100 μL, 645 μg/100 μL, 800 μg/100 μL) of chloroquine phosphate made scratching behaviors significantly different. When the concentrations $\leq$ 600 μg/100 μL, scratching frequency was faster and faster as the concentration increased. After 15 min of the injection of 600 μg/100 μL, scratching behaviors appeared, and lasted for 1 h. While the injection of 645 μg/100 μL, scratching behaviors appeared after 2 min, and lasted for 0.5 h. When injecting 800 μg/100 μL, backwardness emerged after 2 min, and scratching with a high frequency returned to normal after 25 min. So 645 μg/100 μL was chosen as the dose of itch modeling and 0.5 h was chosen as the time of counting the number of scratching behaviors.

The result of anti-itch activity of the target compounds was shown in Fig. (**5**). Compounds **9e** and **9f** had significant activities and compounds **9b**, **9d**, **9g** and **9h** had obvious activities. The concrete anti-itch activity decreased with the following sequence: **9e** > **9f** > **9b** > **9h** > **9g** > **9d** > **9a** > **9c**. By preliminary test, the optimum structure of fragment 1 was 2-(4-fluorophenyl) acetic acid, fragment 2 was L-tryptophan and fragment 3 was 2-(1H-imidazol-1-yl) ethanol. It was speculated that polycyclic aromatic structure of amino acid (fragment 2) may increase the activities. For it was near the active site of amino acid in space, and could enhance the electrostatic force with the active site.

Compared with the two results by CADD and preliminary test, the activity of compound **9d** was worse than **9f**, while they were the same by CADD. These two compounds were similar in structure and the only difference between them lied in the replacement of the nitro group of **9d** with a methyl group in **9f**. This change of group R^1 was associated with a large difference in activity, which suggested a possible critical binding role for this group in the binding site of the molecular target [22]. The activity of **9h** was better than **9g**, while they were on the contrary by CADD. It is to be presumed that the 4-(1-methylpiperazine)-yl (group R^3) has two nitrogen atoms for converting into salt while 4-morpholine-yl has one, and the water-solubility of **9h** may be greater than **9g** *in vivo*. Further study should be emphasized on this aspect so as to comprehend the differences clearly.

With the binding interactions of the eight compounds by CADD, the anti-itch activity of them decreased with the following sequence: **9e** > **9f** = **9d** > **9b** > **9g** > **9a** > **9h** > **9c**.

Table 1. The Binding Interactions of Compounds 9a-9h by CADD

Comp. No	Structure	Binding Interactions
9a		Lys-188, Lys-265, Val-187, His-186
9b		Lys-188, Lys-265, Val-187, His-186
9c		Lys-188, Lys-265, Val-187, His-186
9d		Lys-188, Lys-265, Val-187, His-186, His-207
9e		Lys-265, Val-187, His-186, His-207
9f		Lys-188, Lys-265, Val-187, His-186, His-207
9g		Lys-265, Val-187, His-186, His-207
9h		Lys-265, Val-187, His-186, His-207

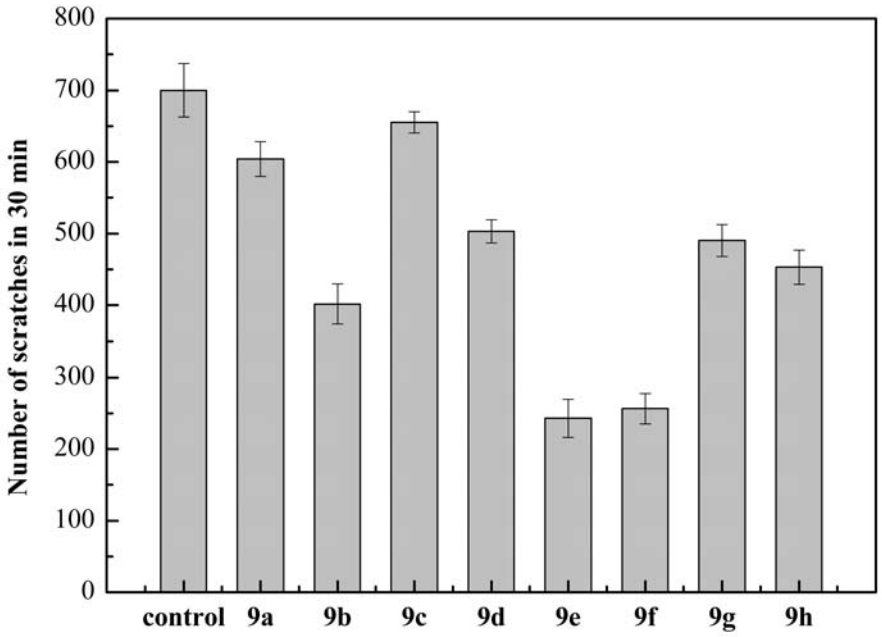


Fig. (5). The Anti-itch Activity Results of Compounds **9a-9h**. The First line was the Number of Scratching Behaviors of Control Animals and the others were the Numbers of Scratching Behaviors of Treatment Animals. All the Treatments Resulted in a Significant Decrease in Scratching Numbers Compared to the Control ($p < 0.05$).

3. CONCLUSION

Eight compounds were designed and synthesized. Compounds **9e** and **9f**, which had prominence activities, were screened out as GRPR activity antagonists. The optimum structure of fragment 1 was 2-(4-fluorophenyl) acetic acid, fragment 2 was L-tryptophan and fragment 3 was 2-(1H-imidazol-1-yl) ethanol. The anti-itch activity of them decreased with the following sequence: **9e** > **9f** = **9d** > **9b** > **9g** > **9a** > **9h** > **9c** by CADD and **9e** > **9f** > **9b** > **9h** > **9g** > **9d** > **9a** > **9c** by preliminary test. On the whole, they were broadly in line with activity order pretested by CADD. It showed that the predicted GRPR tertiary structure was of a certain reference value and it can be used for antipruritic drug design.

4. EXPERIMENTAL SECTION

4.1. General

The ^{1}H NMR spectra were recorded on a Bruker 300 MHz spectrometer. The chemical shifts were expressed in ppm using residual $CDCl_3$ and $(CD_3)_2SO$ as reference. The FT-IR spectra were recorded in solid-state KBr dispersion on a Perkins-Elmer FT-IR spectrophotometer. The melting points were taken on a Buchi apparatus. All reactions were monitored by TLC with HuanghaiGF 254 silica gel-coated plates. Commercially obtained reagents were used without further purification. Compounds **1**, **2** and **4** were prepared according to the literature methods as described [15-18].

4.2. Synthesis

4.2.1. General Procedure for the Synthesis of Compounds 7a-7h

The mixture of alphatoluic acid derivatives (**5**) (18 mmol), DCM (30 mL), TEA (2.6 mL, 18 mmol) and DMF (0.1 mL) was stirred at 0 °C under the protection of N_2 gas. $(COCl)_2$ (1.84 mL, 21.6 mmol) in DCM (10 mL) was slowly added to the mixture. After the dropwise addition, the mixture was stirred at room temperature for 2 h. Solvent and excess $(COCl)_2$ was removed under vacuum. The brown solid was obtained, which was directly used in the next step.

The suspension of new prepared acetyl chloride derivative **6** in DCM (15 mL) was added to the mixture of **4** (18 mmol), TEA (5.2 mL, 36 mmol) and DCM (20 mL) at 0 °C under the protection of N_2 gas. After the dropwise addition, the mixture was stirred at room temperature for 5 h. Compounds **7a-7h** were obtained by column chromatography (silica gel, 200-300 mesh, ethyl acetate/petroleum ether, 1:2, *V/V*).

4.2.1.1. (R)-methyl-2-(2-(4-nitrophenyl)acetamido)-3-phenylpropanoate (7a)

The title compound was separated by column chromatography. Yield (5.62 g, 91.2%); yellow solid; ^{1}H NMR ($CDCl_3$) δ: 6.94-8.17 (m, 9H), 4.88 (m, 1H), 3.72 (s, 3H), 3.66 (s, 2H), 3.11 (d, 2H); IR ν/cm^{-1}: 3284, 2925, 1743, 1665, 1542, 1345, 1280, 1172, 825, 701.

4.2.1.2. (R)-methyl-2-(2-(4-fluorophenyl)acetamido)-3-phenylpropanoate (7b)

The title compound was separated by column chromatography. Yield (5.23 g, 92.3%); cream white solid; ^{1}H NMR ($CDCl_3$) δ: 6.89-7.26 (m, 9H), 4.85 (m, 1H), 3.71 (s, 3H), 3.50 (s, 2H), 3.06 (dd, 2H); IR ν/cm^{-1}: 3285, 2953, 1755, 1669, 1546, 1512, 1364, 1222, 705.

4.2.1.3. (R)-methyl-3-phenyl-2-(2-p-tolylacetamido) propanoate (7c)

The title compound was separated by column chromatography. Yield (4.89 g, 87.3%); white solid; ^{1}H NMR ($CDCl_3$) δ: 6.87-7.24 (m, 9H), 4.84 (m, 1H), 3.69 (s, 3H), 3.48 (s,

2H), 3.02 (d, 2H), 2.34 (s, 3H); IR ν/cm^{-1}: 3319, 2925, 1757, 1646, 1533, 1438, 1373, 1275, 1171, 782, 699.

4.2.1.4. (R)-methyl-3-(1H-indol-3-yl)-2-(2-(4-nitrophenyl)acetamido)propanoate (7d)

The title compound was separated by column chromatography. Yield (6.03 g, 87.8%); brown solid; ^{1}H NMR ($CDCl_3$) δ: 7.03-8.04 (m, 9H), 4.91 (m, 1H), 3.72 (s, 3H), 3.54 (s, 2H), 3.29 (d, 2H); IR ν/cm^{-1}: 3381, 2925, 1734, 1655, 1520, 1438, 1346, 1106, 741.

4.2.1.5. (R)-methyl-2-(2-(4-fluorophenyl)acetamido)-3-(1H-indol-3-yl)propanoate (7e)

The title compound was separated by column chromatography. Yield (5.82 g, 91.2%); cream white solid; ^{1}H NMR ($CDCl_3$) δ: 8.07 (s, 1H), 6.74-7.44 (m, 9H), 4.90 (m, 1H), 3.68 (s, 3H), 3.45 (s, 2H), 3.25 (d, 2H); IR ν/cm^{-1}: 3407, 3291, 2923, 1733, 1662, 1547, 1513, 1451, 1361, 1220, 830, 746.

Compounds **7g** and **7h** are the same as **7e**.

4.2.1.6. (R)-methyl-3-(1H-indol-3-yl)-2-(2-p-tolylacetamido)propanoate (7f)

The title compound was separated by column chromatography. Yield (5.74 g, 91.1%); white solid; ^{1}H NMR ($CDCl_3$) δ: 8.02 (s, 1H), 6.72-7.42 (m, 9H), 4.90 (m, 1H), 3.66 (s, 3H), 3.48 (s, 2H), 3.24 (d, 2H), 2.32 (s, 3H); IR ν/cm^{-1}: 3331, 2924, 2855, 1751, 1652, 1517, 1461, 1377, 1198, 753, 659.

4.2.2. General Procedure for the Synthesis of Compounds 8a-8h

Compound **7** (5 mmol) was added to a solution of MeOH (32 mL), H_2O (8 mL) and Na_2CO_3 (0.79 g, 7.5 mmol) [19]. The mixture was stirred at room temperature for 6 h. MeOH was removed under vacuum, and H_2O (20 mL) was added to the residue. pH was adjusted to 5 to 6 using 3 N HCl. The mixture was extracted with ethyl acetate (50 mL × 3). The organic layer was separated, dried over anhydrous Na_2SO_4 and then evaporated under vacuum.

4.2.2.1. (R)-2-(2-(4-nitrophenyl)acetamido)-3-phenylpropanoic acid (8a)

The title compound was evaporated under vacuum. Yield (1.53 g, 93.2%); yellow solid; mp 165~168 °C; ^{1}H NMR ($(CD_3)_2SO$-d_6) δ: 12.73 (s, 1H), 7.19-8.53 (m, 9H), 4.45 (m, 1H), 3.57 (s, 2H), 3.01 (d, 2H); IR ν/cm^{-1}: 3319, 2894, 1711, 1621, 1520, 1347, 1246, 1050, 831, 700.

4.2.2.2. (R)-2-(2-(4-fluorophenyl)acetamido)-3-phenylpropanoic acid (8b)

The title compound was evaporated under vacuum. Yield (1.41 g, 94.0%); cream white solid; mp 152~154 °C; ^{1}H NMR ($CDCl_3$) δ: 6.94-7.26 (m, 9H), 4.81 (m, 1H), 3.51 (s, 2H), 3.08 (d, 2H); IR ν/cm^{-1}: 3295, 2928, 1709, 1613, 1559, 1355, 1251, 1225, 1158, 825, 700.

4.2.2.3. (R)-3-phenyl-2-(2-p-tolylacetamido)propanoic acid (8c)

The title compound was evaporated under vacuum. Yield (1.34 g, 90.4%); white solid; mp 124~125 °C; ^{1}H NMR ($CDCl_3$) δ: 6.91-7.25 (m, 9H), 4.80 (m, 1H), 3.49 (s, 2H), 3.06 (d, 2H), 2.35 (s, 3H); IR ν/cm^{-1}: 3358, 2924, 1770, 1743, 1611, 1534, 1396, 1209, 1170, 784, 700.

4.2.2.4. (R)-3-(1H-indol-3-yl)-2-(2-(4-nitrophenyl)acetamido)propanoic acid (8d)

The title compound was evaporated under vacuum. Yield (1.68 g, 91.4%); brown solid; mp 211~214 °C; ^{1}H NMR ($(CD_3)_2SO$-d_6) δ: 10.84 (s, 1H), 6.94-8.50 (m, 9H), 4.51 (m, 1H), 3.59 (s, 2H), 3.15 (d, 2H); IR ν/cm^{-1}: 3402, 3305, 2926, 1711, 1618, 1515, 1348, 1243, 747.

4.2.2.5. (R)-2-(2-(4-fluorophenyl)acetamido)-3-(1H-indol-3-yl)propanoic acid (8e)

The title compound was evaporated under vacuum. Yield (1.62 g, 95.2%); cream white solid; mp 196~197 °C; ^{1}H NMR ($(CD_3)_2SO$-d_6) δ: 10.83 (s, 1H), 8.32 (s, 1H), 6.94-7.52 (m, 9H), 4.47 (m, 1H), 3.42 (s, 2H), 3.14 (dd, 2H); IR ν/cm^{-1}: 3408, 3309, 2925, 1709, 1615, 1508, 1460, 1218, 1170, 793, 746.

Compounds **8g** and **8h** are the same as **8e**.

4.2.2.6. (R)-3-(1H-indol-3-yl)-2-(2-p-tolylacetamido)propanoic acid (8f)

The title compound was evaporated under vacuum. Yield (1.63 g, 96.9%); gray oily substance; ^{1}H NMR ($(CD_3)_2SO$-d_6) δ: 10.83 (s, 1H), 8.23 (s, 1H), 6.94-7.53 (m, 9H), 4.48 (m, 1H), 3.38 (s, 2H), 3.06 (d, 2H), 2.25 (s, 3H); IR ν/cm^{-1}: 3403, 2924, 1728, 1624, 1515, 1344, 1232, 1100, 743.

4.2.3. General Procedure for the Synthesis of Compounds 9a-9f

A mixture of compound **8** (4 mmol), THF (10 mL), DCC (0.99 g, 4.8 mmol) and DMAP (10 mg) was stirred at 0 °C for 1 h. Compound **2** in THF (5 mL) was added to the solution. The mixture was stirred at room temperature for 15 h. Target compound was obtained by column chromatography (silica gel, 200-300 mesh, DCM/MeOH, 70:1, *V/V*).

4.2.3.1.(R)-2-(1H-imidazol-1-yl)ethyl-2-(2-(4-nitrophenyl)acetamido)-3-phenylpropanoate (9a)

The title compound was separated by column chromatography. Yield (1.52 g, 90.1%); brown oily substance; ^{1}H NMR ($CDCl_3$) δ: 6.92-8.15 (m, 12H), 4.78 (m, 1H), 4.36 (t, 2H), 4.15 (t, 2H), 3.61 (s, 2H), 3.01 (d, 2H); IR ν/cm^{-1}: 3206, 2941, 1746, 1641, 1532, 1350, 1283, 1178, 820, 698.

4.2.3.2.(R)-2-(1H-imidazol-1-yl)ethyl-2-(2-(4-fluorophenyl)acetamido)-3-phenylpropanoate (9b)

The title compound was separated by column chromatography. Yield (1.43 g, 90.5%); yellow oily substance; ^{1}H NMR ($CDCl_3$) δ: 8.02 (s, 1H), 6.72-7.42 (m, 9H), 4.90 (m, 1H), 3.66 (s, 3H), 3.48 (s, 2H), 3.24 (d, 2H), 2.32 (s, 3H); IR ν/cm^{-1}: 3201, 2932, 1747, 1658, 1543, 1509, 1355, 1222, 1159, 825, 702.

4.2.3.3.(R)-2-(1H-imidazol-1-yl)ethyl-3-phenyl-2-(2-p-tolylacetamido)propanoate (9c)

The title compound was separated by column chromatography. Yield (1.41 g, 90.0%); white oily substance; ^{1}H NMR ($CDCl_3$) δ: 6.81-7.35 (m, 12H), 4.75 (m, 1H), 4.21 (t, 2H),

4.08 (t, 2H), 3.49 (s, 2H), 2.95 (d, 2H), 2.34 (s, 3H); IR ν/cm^{-1}: 3206, 2941, 1746, 1641, 1532, 1350, 1283, 1178, 820, 698.

4.2.3.4.(R)-2-(1H-imidazol-1-yl)ethyl-3-(1H-indol-3-yl)-2-(2-(4-nitrophenyl)acetamido)propanoate (9d)

The title compound was separated by column chromatography. Yield (1.72 g, 93.2%); brown oily substance; ^{1}H NMR ($CDCl_3$) δ: 8.78 (s, 1H), 6.65-8.02 (m, 12H), 4.81 (m, 1H), 4.26 (t, 2H), 3.96 (t, 2H), 3.55 (s, 2H), 3.17 (dd, 2H); IR ν/cm^{-1}: 2924, 1744, 1662, 1518, 1346, 1184, 822, 745.

4.2.3.5.(R)-2-(1H-imidazol-1-yl)ethyl-2-(2-(4-fluorophenyl)acetamido)-3-(1H-indol-3-yl)propanoate (9e)

The title compound was separated by column chromatography. Yield (1.63 g, 93.8%); yellow oily substance; ^{1}H NMR ($CDCl_3$) δ: 8.67 (s, 1H), 6.58-7.38 (m, 12H), 4.81 (m, 1H), 4.22 (t, 2H), 3.89 (t, 2H), 3.46 (s, 2H), 3.17 (dd, 2H); IR ν/cm^{-1}: 3274, 1744, 1657, 1509, 1356, 1223, 825, 745.

4.2.3.6.(R)-2-(1H-imidazol-1-yl)ethyl-3-(1H-indol-3-yl)-2-(2-p-tolylacetamido)propanoate (9f)

The title compound was separated by column chromatography. Yield (1.62 g, 94.1%); white oily substance; ^{1}H NMR ($CDCl_3$) δ: 8.36 (s, 1H), 6.56-7.38 (m, 12H), 4.81 (m, 1H), 4.20 (t, 2H), 3.86 (t, 2H), 3.48 (s, 2H), 3.15 (dd, 2H), 2.32 (s, 3H); IR ν/cm^{-1}: 3265, 2924, 1744, 1656, 1514, 1438, 1355, 1233, 1187, 745.

4.2.3.7.(R)-2-morpholinoethyl-2-(2-(4-fluorophenyl)acetamido)-3-(1H-indol-3-yl)propanoate (9g)

The title compound was separated by column chromatography. Yield (1.59 g, 87.6%); yellow oily substance; ^{1}H NMR ($CDCl_3$) δ: 8.13 (s, 1H), 6.82-7.46 (m, 9H), 4.90 (m, 1H), 4.20 (t, 2H), 3.65 (t, 4H), 3.48 (s, 2H), 3.26 (d, 2H), 2.53 (t, 2H), 2.38 (t, 4H); IR ν/cm^{-1}: 3293, 2926, 1740, 1657, 1509, 1457, 1354, 1222, 1115, 744.

4.2.3.8.(R)-2-(4-methylpiperazin-1-yl)ethyl-2-(2-(4-fluorophenyl)acetamido)-3-(1H-indol-3- yl)propanoate (9h)

The title compound was separated by column chromatography. Yield (1.68 g, 90.0%); yellow oily substance; ^{1}H NMR ($CDCl_3$) δ: 8.22 (s, 1H), 6.85-7.45 (m, 9H), 4.89 (m, 1H), 4.16 (t, 2H), 3.65 (t, 4H), 3.44 (s, 2H), 3.26 (d, 2H), 2.53 (t, 2H), 2.42 (s, 8H), 2.27 (s, 3H); IR ν/cm^{-1}: 3278, 2925, 1742, 1658, 1511, 1450, 1353, 1226, 1125, 742.

4.3. Biological Evaluation

4.3.1. Animals

In all biological experiments, Kunming mice, three of either sex, 6 to 12 weeks of age, and 18 to 22 g of weight, were used as experimental animals. The animals were housed for a week before this experiment in an air-conditioned room with controlled temperature (22 ± 2 °C) and illumination time (08: 00 to 20: 00). Food and water were provided ad libitum [20, 21].

4.3.2. Solution Preparation

4.3.2.1. The Preparation of Chloroquine Phosphate Solution

With saline as a solvent, different concentrations (200 μg/100 μL, 400 μg/100 μL, 600 μg/100 μL, 645 μg/100 μL, 800 μg/100 μL) of chloroquine phosphate solutions were prepared, filtered by 0.22 μm water film, and loaded 1 mL in each sample device in the sterile console.

4.3.2.2. The Preparation of Eight Target Compounds Solutions

With saline as a solvent, and 2 mL Tween 80 and 2.5 mL glycerol as solubilizers, 10 nmol/2.5 μL eight target compounds solutions were prepared, filtered by 0.22 μm water film, and loaded 1 mL in each sample device in the sterile console.

4.3.3. Statistical Analysis

Data were expressed as mean ± standard deviation and they were analyzed by Student's t-test. Values of $p < 0.05$ were considered significant.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGMENTS

This work was supported by Key Program of Natural Science Research by Education Department of Anhui Province (2011AJZR0919) and Condition Construction Cost of Hefei University of Technology (407-037022).

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Received: July 18, 2011 Revised: April 25, 2012 Accepted: April 26, 2012