Title: Coumarin and 3,4-dihydroquinolinone derivatives: synthesis, antidepressant activity, and molecular docking studies

Authors: Shi-Ben Wang, Hui Liu, Guang-Yong Li, Jun Li, Xiao-Jing Li, Kang Lei, Li-Chao Wei, Zhe-Shan Quan, Xue-Kun Wang, Ren-Min Liu

PII: S1734-1140(19)30303-2
DOI: https://doi.org/10.1016/j.pharep.2019.07.011
Reference: PHAREP 1089

To appear in:

Received date: 25 April 2019
Revised date: 8 July 2019
Accepted date: 30 July 2019

Please cite this article as: Wang S-Ben, Liu H, Li G-Yong, Li J, Li X-Jing, Lei K, Wei L-Chao, Quan Z-Shan, Wang X-Kun, Liu R-Min, Coumarin and 3,4-dihydroquinolinone derivatives: synthesis, antidepressant activity, and molecular docking studies, *Pharmacological Reports* (2019), https://doi.org/10.1016/j.pharep.2019.07.011

Coumarin and 3,4-dihydroquinolinone derivatives: synthesis, antidepressant activity, and molecular docking studies

Running head title

Antidepressant activity of coumarin and 3,4-dihydroquinolinone derivatives

Shi-Ben Wang[*1], Hui Liu[2], Guang-Yong Li[1], Jun Li[1], Xiao-Jing Li[1], Kang Lei[1], Li-Chao Wei[1], Zhe-Shan Quan[3], Xue-Kun Wang[*1], Ren-Min Liu[*1]

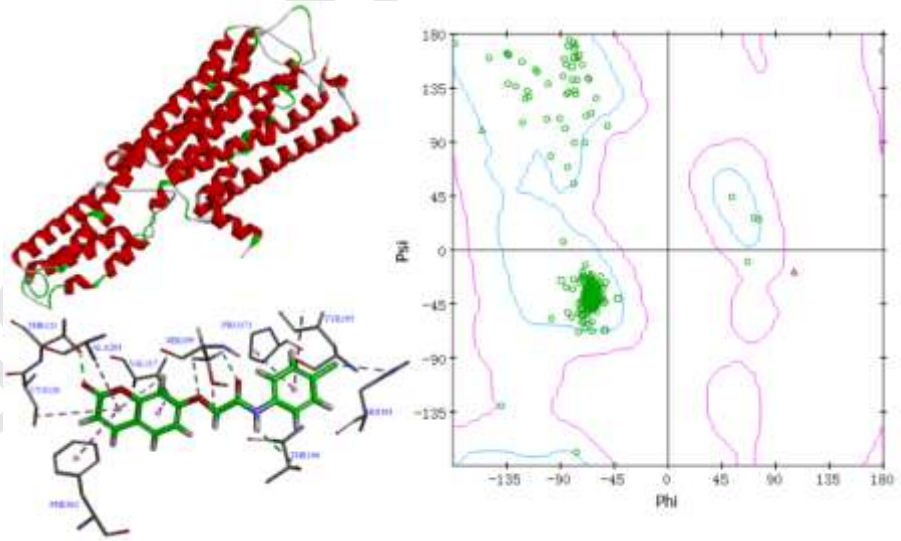
[1] *College of pharmacy, Liaocheng University, Liaocheng, Shandong 252059, China*

[2] *College of life sciences, Liaocheng University, Liaocheng, Shandong 252059, China*

[3] *College of pharmacy, Yanbian University, Yanji, Jilin 133002, China*

*Corresponding author: Shi-ben Wang, College of Pharmacy of Liaocheng University, 1 Hunan Road, Liaocheng 252059, Shandong, China. Email: wangshiben110@163.com.

Graphical abstract



Coumarin and 3,4-dihydroquinolinone derivatives: synthesis, antidepressant activity, and molecular docking studies.

Highlights

- Derivatives of coumarin and 3,4-dihydroquinolinone were designed and synthesized.
- Compound **7** showed the best antidepressant activity.
- Compound **7** exhibits good affinity for the 5-HT_{1A} receptor.

Abstract

Background: Coumarin and 3,4-dihydroquinolinone nuclei are two heterocyclic rings that are important and widely exploited for the development of bioactive molecules. Here, we designed and synthesized a series of 3,4-dihydroquinolinone and coumarin derivatives (Compounds **8**, **9**, **11**, **14**, **15**, **18**-**20**, **23**, **24** and **28** are new compounds) and studied their antidepressant activities.

Methods: Forced swimming test (FST) and tail suspension test (TST) were used to evaluate the antidepressant activity of the target compounds. The most active compound was used to evaluate the exploratory activity of the animals by the open-field test. 5-HT concentration was estimated to evaluate if the compound has an effect on the mouse brain, by using ELISA. A 5-HT_{1A} binding assay was also performed. The biological activities of the compounds were verified by molecular docking studies. The physicochemical and pharmacokinetic properties of the target compounds were predicted by Discovery Studio and ChemBioDraw Ultra.

Results: Of all the compounds tested, compound **7** showed the best antidepressant activity, which decreased the immobility time by 65.52s in FST. However, in the open-field test, compound **7** did not affect spontaneous activity. The results of 5-HT concentration estimation *in vivo* showed that compound **7** may have an effect on the mouse brain. Molecular docking results indicated that compound **7** showed significant interactions with residues at the 5-HT_{1A} receptor using homology modeling. The results show that compound **7** exhibits good affinity for the 5-HT_{1A} receptor.

Conclusion: Coumarin and 3,4-dihydroquinolinone derivatives synthesized in this study have a significant antidepressant activity. These findings can be useful in the design and synthesis of novel antidepressants.

Keywords: coumarin; 3,4-dihydroquinolinone; molecular docking studies; antidepressant; 5-HT

Introduction

Depression is one of the most commonly encountered neurological disorders [1], and is caused by many psychological, social, environmental, and genetic factors [2]. Current antidepressant drugs can only alleviate some symptoms of depression, and the occurrence of side effects is common. Therefore, the development of more effective and less toxic antidepressants has attracted significant attention in recent years. Coumarin (2*H*-chromen-2-one) (**Fig. 1**), one of the most useful heterocyclic nuclei, has gained prominence in medicinal chemistry owing to its diverse biological activities [3,4]. Several studies have demonstrated the antidepressant potential of coumarin derivatives [5,6].

<**Fig. 1** Insert here>

The 3,4-dihydroquinolinone nucleus is one of the most important and widely exploited heterocyclic ring for the development of bioactive molecules (**Fig. 1**). The literature contains many examples of 3,4-dihydroquinolinone derivatives that exhibit a range of biological properties, including analgesic [7], antibacterial [8], antimalarial [9], anticancer [10,11], anti-inflammatory [12], anticonvulsant [13,14], and antidepressant [15,16] activities.

In the field of depression research, molecules with carboxylic acid amide scaffolds display a broad scope of pharmacological and biological activities and have been widely used as frameworks in drug design. For example, many commercial amide antidepressants, such as Benzamide, Toloxatone, Isocarboxazid, and Agomelatin (**Fig. 2**) showed high activity and were successfully used to control a variety of depression symptoms.

<**Fig. 2**. Insert here>

Given these findings, we designed a formulation containing the combination of coumarin and 3,4-dihydroquinolinone framework with carboxamide units (**Fig. 3**) and examined their antidepressant activities in a mouse model. Forced swimming test (FST) was used to evaluate the antidepressant activity of the target compounds. Moreover, the most active compound was also tested using the tail suspension test (TST). From the three series compounds, three compounds **7**, **16**, and **24** with the best antidepressant activity in FST test were selected for binding assays. Binding affinity values (K_i) of the compounds at 5-HT_{1A} receptor were determined. The concentration of 5-HT in mouse brain was determined to explore the possible mechanism of action for compound **7**, which showed the highest activity. A homology model of 5-HT_{1A} receptor was constructed using Discovery Studio software, and

molecular docking study was performed by this model. We predicted the physicochemical and pharmacokinetic properties of these compounds, and the newly synthesized target compounds were characterized by infrared spectroscopy, high resolution mass spectrometry, and ^{1}H-nuclear/^{13}C-nuclear magnetic resonance techniques.

<**Fig. 3**. Insert here>

Material and methods

Chemistry

An X-4 binocular microscope melting point apparatus was used to determine melting points (Mp). High resolution mass spectra (HRMS) were measured by mass spectrometer (Bruker Daltonik, Germany). All NMR (^{1}H and ^{13}C NMR) results were measured by AV-300 spectrometer (solvent: $CDCl_3$ or DMSO-d_6; internal standard: tetramethylsilane). Most chemicals used for the synthesis were commercial products without further purification.

*Synthesis of compound **2***

Appropriate amine (10.0 mmol), triethylamine (12.0 mmol), and 30 ml CH_2Cl_2 were placed in a 100 ml round-bottomed flask, and the resulting mixture was stirred for 0.5 h at room temperature. A solution of $ClCH_2COCl$ (12.0 mmol) in 5 ml of CH_2Cl_2 was added drop wise to the mixture in an ice-bath. After the reaction was completed, the solvent was removing under reduced pressure to obtain a white solid.

*Synthesis of target compounds **4-11**, **13-20**, and **22-28***

7-Hydroxy-2*H*-chromen-2-one/4-hydroxy-2*H*-chromen-2-one/7-hydroxy-3,4-dihydroquinolin-2(1*H*)-one (10.0 mmol), K_2CO_3 (10.0 mmol), and 50 ml acetone were combined in a 100 ml round-bottomed flask, and the resulting mixture was stirred for 6-12 h at 80 ^{o}C. After the reaction was completed (TLC), the mixture was poured into water (50 ml), and then extracted with CH_2Cl_2. The combined organic extracts were washed with water, dried ($MgSO_4$), and concentrated to dryness in vacuum. The residue was purified by chromatography on silica gel, eluting with CH_2Cl_2, to extract target compounds **4**-**11**, **13**-**20**, and **22**-**28**. Half of the compounds thus obtained were novel compounds (Compounds **4**, **5** and **6** are reported by reference [17]; Compounds **7**, **13** and 1**6** are reported by reference [18]; Compounds **10** and **17** are reported by reference [19]; Compounds **22**, **25** and **27** are reported by reference [20]). The spectral data of the synthesized known compounds were consistent with the previously described papers [17-20]. The yield, melting point, and nuclear magnetic data of the new target compounds are shown below.

*2-[(2-Oxo-2H-chromen-7-yl)oxy]-N-phenylacetamide (**4**)*

The spectral data of this compound can be found in reference [17].

*N-benzyl-2-[(2-oxo-2H-chromen-7-yl)oxy]acetamide (**5**)*

The spectral data of this compound can be found in reference [17].

*2-[(2-Oxo-2H-chromen-7-yl)oxy]-N-phenethylacetamide (**6**)*

The spectral data of this compound can be found in reference [17].

*N-(4-chlorophenyl)-2-[(2-oxo-2H-chromen-7-yl)oxy]acetamide (**7**)*

The spectral data of this compound can be found in reference [18].

*2-[(2-Oxo-2H-chromen-7-yl)oxy]-N-[4-(trifluoromethyl)phenyl]acetamide (**8**)*

An white solid. Yield: 47 %. Mp: 237-238 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm) δ: 4.91 (s, 2H, CH_2), 6.32 (d, 1H, J = 8.00 Hz, COCH=), 7.06 (s, 1H, =CH), 7.06-8.02 (m, 7H, Ar-H), 10.54 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 67.69, 102.13, 113.18, 113.39, 120.00, 120.00, 123.43, 124.06, 126.13, 126.56, 126.60, 130.02, 142.39, 144.69, 155.64, 160.65, 161.33, 167.01. ESI-HRMS calcd for $C_{18}H_{13}F_3NO_4^+$ ([M + H]$^+$): 364.0791; found: 364.0798.

*2-[(2-Oxo-2H-chromen-7-yl)oxy]-N-(p-tolyl)acetamide (**9**)*

An white solid. Yield: 51 %. Mp: 231-232 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm) δ: 2.25 (s, 3H, CH_3), 4.83 (s, 2H, CH_2), 6.32 (d, 1H, J = 8.00 Hz, COCH=), 7.04 (s, 1H, =CH), 7.04-8.02 (m, 7H, Ar-H), 10.08 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 20.93, 67.78, 102.09, 113.21, 113.30, 113.34, 120.16, 120.16, 129.62, 129.62, 130.00, 133.21, 135.24, 144.72, 155.64, 160.67, 161.41, 166.03. ESI-HRMS calcd for $C_{18}H_{16}NO_4^+$ ([M + H]$^+$): 310.1074; found: 310.1082.

*N-(4-methoxyphenyl)-2-[(2-oxo-2H-chromen-7-yl)oxy]acetamide (**10**)*

The spectral data of this compound can be found in reference [19].

*N-cyclohexyl-2-[(2-oxo-2H-chromen-7-yl)oxy]acetamide (**11**)*

An white solid. Yield: 53 %. Mp: 239-240 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm) δ: 1.10-3.61 (m, 11H, cyclohexane-H), 4.59 (s, 2H, CH_2), 6.31 (d, 1H, J = 8.00 Hz, COCH=), 6.95 (s, 1H, =CH), 6.95-8.02 (m, 3H, Ar-H), 8.01 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 25.08, 25.08, 25.61, 32.71, 32.71, 47.98, 67.66, 102.06, 113.23, 113.27, 129.93, 144.73, 155.62, 160.68, 161.42, 166.25. ESI-HRMS calcd for $C_{17}H_{20}NO_4^+$ ([M + H]$^+$): 302.1387; found: 302.1396.

*3-[(2-Oxo-2H-chromen-4-yl)oxy]-N-phenylacetamide (**13**)*

The spectral data of this compound can be found in reference [18].

*N-benzyl-2-[(2-oxo-2H-chromen-4-yl)oxy]acetamide (**14**)*

An white solid. Yield: 60 %. Mp: 250-251 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm) δ: 4.39 (d, 2H, J = 8.00 Hz, CH_2), 4.88 (s, 2H, CH_2), 5.87 (s, 1H, COCH=), 7.25-8.07 (m, 9H, Ar-H), 8.79 (t, 1H, J = 6.00 Hz, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 42.44, 68.16, 91.91, 115.47, 116.84, 124.07,

124.60, 127.34, 127.67, 128.77, 127.67, 128.77, 133.38, 139.56, 153.21, 161.89, 164.85, 166.35. ESI-HRMS calcd for $C_{18}H_{16}NO_4^+$ ([M + H]$^+$): 310.1074; found: 310.1081.

*2-[(2-Oxo-2H-chromen-4-yl)oxy]-N-phenethylacetamide (**15**)*

An white solid. Yield: 48 %. Mp: 267-268 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm) δ: 2.79 (t, 2H, J = 8.00 Hz, CH_2), 3.41 (q, 2H, J = 6.80 Hz, CH_2), 4.77 (s, 2H, CH_2), 5.83 (s, 1H, COCH=), 7.21-8.00 (m, 9H, Ar-H), 8.31 (t, 1H, J = 6.00 Hz, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 35.49, 68.14, 91.82, 115.43, 116.84, 123.99, 124.58, 126.63, 128.82, 128.82, 129.12, 129.12, 133.37, 139.68, 153.20, 161.89, 164.76, 166.13. ESI-HRMS calcd for $C_{19}H_{18}NO_4^+$ ([M + H]$^+$): 324.1230; found: 324.1238.

*N-(4-chlorophenyl)-2-[(2-oxo-2H-chromen-4-yl)oxy]acetamide (**16**)*

The spectral data of this compound can be found in reference [18].

*N-(4-fluorophenyl)-2-[(2-oxo-2H-chromen-4-yl)oxy]acetamide (**17**)*

The spectral data of this compound can be found in reference [19].

*2-[(2-Oxo-2H-chromen-4-yl)oxy]-N-[4-(trifluoromethyl)phenyl]acetamide (**18**)*

An white solid. Yield: 63 %. Mp: 249-251 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm) δ: 5.09 (s, 2H, CH_2), 5.93 (s, 1H, COCH=), 7.40-7.97 (m, 8H, Ar-H), 10.65 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 68.03, 91.80, 115.48, 116.69, 116.94, 119.30, 119.99, 119.99, 123.71, 124.46, 124.76, 126.68, 132.51, 133.44, 142.31, 153.25, 161.93, 165.03, 165.68. ESI-HRMS calcd for $C_{18}H_{13}F_3NO_4^+$ ([M + H]$^+$): 364.0791; found: 364.0798.

*2-[(2-Oxo-2H-chromen-4-yl)oxy]-N-(p-tolyl)acetamide (**19**)*

An white solid. Yield: 62 %. Mp: 234-236 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm) δ: 2.25 (s, 3H, CH_3), 5.01 (s, 2H, CH_2), 5.89 (s, 1H, COCH=), 7.14-7.97 (m, 8H, Ar-H), 10.19 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 20.93, 68.18, 91.70, 115.52, 116.92, 120.17, 120.17, 123.77, 124.73, 129.69, 129.69, 133.35, 133.42, 136.15, 153.24, 161.95, 164.72, 165.11. ESI-HRMS calcd for $C_{18}H_{16}NO_4^+$ ([M + H]$^+$): 310.1074; found: 310.1077.

*N-cyclohexyl-2-[(2-oxo-2H-chromen-4-yl)oxy]acetamide (**20**)*

An grey solid. Yield: 67 %. Mp: 242-243 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm) δ: 1.24-1.77 (m, 11H, cyclohexane-H), 4.77 (s, 2H, CH_2), 5.78 (s, 1H, COCH=), 7.39-7.94 (m, 4H, Ar-H), 8.10 (d, 1H, J = 8.00 Hz, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 25.71, 25.71, 26.30, 33.35, 33.35, 48.82, 68.85, 92.29, 116.22, 117.58, 124.51, 125.37, 134.07, 153.91, 162.61, 165.77, 165.77. ESI-HRMS calcd for $C_{17}H_{20}NO_4^+$ ([M + H]$^+$): 302.1387; found: 302.1393.

*2-[(2-Oxo-1,2,3,4-tetrahydroquinolin-7-yl)oxy]-N-phenylacetamide (**22**)*

The spectral data of this compound can be found in reference [20].

*N-benzyl-2-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)oxy]acetamide (**23**)*

An white solid. Yield: 58 %. Mp: 234-235 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm) δ: 2.47 (t, 2H, J = 8.00 Hz, $COCH_2$), 2.85 (t, 2H, J = 8.00 Hz, CH_2), 4.39 (d, 2H, J = 4.00 Hz, CH_2), 4.53 (s, 2H, CH_2), 6.57-7.37 (m, 8H, Ar-H), 8.68 (t, 1H, J = 6.40 Hz, NH), 10.13 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 24.50, 31.18, 42.24, 67.52, 102.72, 108.01, 116.87, 127.21, 127.67, 127.67, 128.67, 128.67, 128.83, 139.71, 139.78, 157.42, 168.14, 170.78. ESI-HRMS calcd for $C_{18}H_{19}N_2O_3^+$ ([M + H]$^+$): 311.1390; found: 311.1398.

*N-(4-chlorophenyl)-2-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)oxy]acetamide (**24**)*

An white solid. Yield: 71%. Mp: 246-248 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm) δ: 2.41 (t, 2H, J = 6.00 Hz, $COCH_2$), 2.79 (t, 2H, J = 6.00 Hz, CH_2), 4.63 (s, 2H, CH_2), 6.52-7.69 (m, 7H, Ar-H), 10.07 (s, 1H, NH), 10.23 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 24.49, 31.14, 67.68, 102.57, 108.05, 116.88, 121.74, 121.74, 127.73, 128.89, 129.10, 129.10, 137.85, 139.74, 157.52, 167.18, 170.75. ESI-HRMS calcd for $C_{17}H_{16}ClN_2O_3^+$ ([M + H]$^+$): 331.0844; found: 331.0850.

*N-(4-fluorophenyl)-2-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)oxy]acetamide (**25**)*

The spectral data of this compound can be found in reference [20].

*2-[(2-Oxo-1,2,3,4-tetrahydroquinolin-7-yl)oxy]-N-(p-tolyl)acetamide (**26**)*

An white solid. Yield: 55 %. Mp: 253-254 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm) δ: 2.25 (s, 3H, CH_3), 2.41 (t, 2H, J = 6.00 Hz, $COCH_2$), 2.79 (t, 2H, J = 6.00 Hz, CH_2), 4.60 (s, 2H, CH_2), 6.52-7.53 (m, 7H, Ar-H), 9.99 (s, 1H, NH), 10.08 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 20.92, 24.49, 31.15, 67.70, 102.59, 108.05, 116.82, 120.20, 120.20, 128.87, 129.54, 129.54, 133.09, 136.34, 139.72, 157.58, 166.71, 170.78. ESI-HRMS calcd for $C_{18}H_{19}N_2O_3^+$ ([M + H]$^+$): 311.1390; found: 311.1396

*N-(4-methoxyphenyl)-2-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)oxy]acetamide (**27**)*

The spectral data of this compound can be found in reference [20].

*N-cyclohexyl-2-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)oxy]acetamide (**28**)*

An white solid. Yield: 73 %. Mp: 242-243 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm) δ: 1.09-3.60 (m, 11H, cyclohexane-H), 2.78 (t, 2H, J = 8.00 Hz, CH_2), 4.35 (s, 2H, CH_2), 6.46-7.06 (m, 3H, Ar-H), 7.84 (d, 1H, J = 4.00 Hz, NH), 10.06 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 24.48, 25.15, 25.15, 25.62, 31.16, 32.70, 32.70, 47.90, 67.56, 102.68, 108.00, 116.74, 128.80, 139.66, 157.58, 166.84, 170.78. ESI-HRMS calcd for $C_{17}H_{23}N_2O_3^+$ ([M + H]$^+$): 303.1703; found: 303.1710.

Pharmacology

All the target compounds were submitted for evaluation *in vivo*. All the target compounds were dissolved in dimethyl sulfoxide or 0.5% methylcellulose before the experiment, and Fluoxetine (racemic mixture) was used as the positive control. Kunming mice (body weight, 18–22 g) were used as experimental animals. Before the experiment, all the mice were allowed free access to food and

water. Two experimental tests, FST and TST, were used to evaluate the preliminary antidepressant activity of the target compounds. The 5-HT enzyme-linked immunosorbent assay (ELISA) kits were used to determine the concentrations of 5-HT in mice. The binding affinity for 5-HT_{1A} receptor was determined by competition binding studies with [^{3}H] 8-OH-DPAT using racemic 8-OH-DPAT as the reference compound [21].

Antidepressant activity

According to the method reported in the literature, the preliminary antidepressant activities of the target compounds were determined by the FST [22,23]. According to the preliminary screening results, compound 7 was selected for further evaluation of its antidepressant activity using the TST model [24,25]. Preliminary tests were performed with compound **7** to evaluate the exploratory activity of the animals by the open-field test [26]. 5-HT concentration was tested using ELISA to determine whether compound **7** has an effect on the mouse brain. 5-HT_{1A} binding assay was then carried out to determine the binding capacity of compound **7** to receptors, serotonin was used to define nonspecific binding [21].

Molecular docking studies and prediction of ADME properties

In this study, the FASTA sequence of 5-HT_{1A} receptor was selected from RCSB-PDB (web site: https://www.rcsb.org/), and structure crystals with high homology (PDB: 4IAR, 4IAQ, 5V54, and 6G79) were used to construct a homology model of 5-HT_{1A} receptor by Discovery Studio software. The structures of the ligands were sketched on ChemBioDraw Ultra 14.0. LibDock protocol was executed and the top LibDock Score was used for further analysis. The docking results were analyzed by Discovery Studio.

The structures of the ligands were sketched on ChemBioDraw Ultra 14.0 and their physicochemical properties were calculated by Discovery Studio or ChemBioDraw Ultra 14.0. The ADME properties of these compounds were predicted by Discovery Studio and ChemBioDraw Ultra 14.0, including molecular number of hydrogen donors (nHBD), acceptors (nHBA), weight (MW), number of rotatable bonds (RotB), polar surface area (tPSA), and cLogP. The absorption (ABS) level and blood-brain barrier (BBB) permeability level were also calculated using the Discovery Studio.

Statistical analysis

Data are expressed as mean ± standard deviation. One-Way analysis of variance (ANOVA) was performed using GraphPad Prism 5.0 statistical software. $0.01 < p < 0.05$ indicated statistically

significant differences. $p < 0.01$ indicated highly significant differences.

Results

Chemistry

For the preparation of all compounds described in this paper, 7-hydroxy-2*H*-chromen-2-one/4-hydroxy-2*H*-chromen-2-one/7-hydroxy-3,4-dihydroquinolin-2(1*H*)-one were used as the starting material (**Scheme 1**). A chemical reaction with $ClCOCH_2Cl$ and an appropriate amine, triethylamine was set at room temperature to obtain intermediate compound **2**. Treatment of compound **2** with 7-hydroxy-2*H*-chromen-2-one/4-hydroxy-2*H*-chromen-2-one/7-hydroxy-3,4-dihydroquinolin-2(1*H*)-one in acetone yielded target compounds **4-11**, **13-20**, and **22-28** in good amounts. The structures of the target compounds were characterized by spectral methods.

< **Scheme 1**. Insert here>

Pharmacology

FST

The synthesized compounds were then evaluated for the antidepressant activity. Most of the compounds showed antidepressant activity at 40 mg/kg body weight, and seven compounds **4**, **5**, **13**, **18**, **20**, **21**, and **27** showed significant antidepressant activity compared with the control group ($0.01 < p < 0.05$); four compounds **7**, **8**, **16**, and **24** showed highly significant antidepressant activity compared with the control group ($p < 0.01$).

<**Table 1**. Insert here>

The antidepressant activity tests were then performed with 10, 20, and 40 mg/kg body weight of the most active compound **7** and Fluoxetine (**Table 2**). At the dose of 40 mg/kg, both, compound **7** and Fluoxetine showed the best antidepressant activity, and reduced the immobility time of the mice to 61.28 s and 67.21s, respectively.

<**Table 2**. Insert here>

TST

After FST, TST was performed, and we noted that compound **7** showed a highly significant antidepressant activity compared to the control group ($p < 0.01$), and the immobility time of the mice was reduced to 64.31 s, which was superior to Fluoxetine which reduced the immobility time of the mice to 71.35 s) (**Table 3)**.

<**Table 3**. Insert here>

Open-field test

In the open-field test, there were no significant differences in effects seen upon treatment with compound **7** ($p > 0.05$, motor activity: crossing, rearing, and grooming) as compared with the control group. The results suggest that compound **7** did not affect the spontaneous locomotor activity in mice (**Fig. 4**).

<**Fig. 4**. Insert here>

Determination of 5-HT concentration

The estimation of 5-HT concentration by ELISA showed that the 5-HT content in the brain tissue of compound **7** and Fluoxetine group (40 mg/kg body weight) was significantly ($0.01 < p < 0.05$) higher than that of the control group (**Table 4**).

<**Table 4**. Insert here>

5-HT$_{1A}$ binding assay

Compounds **7**, **16**, and **24**, which showed the most potent antidepressant activity, were used for analyzing the binding with 5-HT$_{1A}$ receptor. Binding affinity analysis showed that compound **7** exhibits better affinity with the 5-HT$_{1A}$ receptor (K_i = 1.4 nM), serotonin has a 1.5 nM Ki for 5-HT$_{1A}$ receptor.

<**Table 5**. Insert here>

Docking study

Docking investigation showed that compound **7** interacts with amino acid residues at the 5-HT$_{1A}$ receptor active site, by chemical interactions, such as the hydrogen bond (Thr121, Thr196, and Ser 199) and π-π stacking (Phe362 and Tyr195) interactions (**Fig. 5**).

<**Fig. 5**. Insert here>

Prediction of ADME properties

We further observed that none of the compounds violated Lipinski's "rule of 5" (drug-like compounds have MW ≤ 500, nHBA ≤ 10, nHBD ≤ 5, clogP ≤ 5, and RotB ≤ 10), as shown in **Table 6.** Moreover, we observed that tPSA of all the synthesized compounds was < 140. In addition, most compounds showed favorable BBB permeability and good absorption rates.

<**Table 6**. Insert here>

Discussion

FST is an effective method for screening antidepressants. A variety of antidepressants have been

developed using this method. In this study, we used the FST model to perform a preliminary antidepressant activity test on all the synthesized target compounds, with Fluoxetine used as a positive control. The antidepressant activity data are shown in **Table 1**. Among all the compounds, compound **7** showed the best antidepressant activity, which was observed to be better than the positive control.

The structure-activity relationship was obtained based on the pharmacological results shown in **Table 1**. Overall, the antidepressant activity of coumarin derivatives was slightly better than that of quinolinone derivatives. Coumarin (-COO-) shows higher fat-solubility than 3,4-dihydroquinolinone (-CONH-), and hence is more likely to be absorbed and transported in the body, thereby exerting stronger biological activity. The activity of the compound derived from the 7^{th} position of the coumarin is not significantly different from the activity of the compound derived from the 4^{th} position. At the same time, we also noted a significant antidepressant activity of the compounds with Cl atom that showed superior activity as compared to the compounds substituted by other groups, such as F, CF_3, CH_3, and OCH_3. This indicates that Cl atoms have a certain influence on improving antidepressant activity. The antidepressant activity of compound **7** was observed to be better than that of other compounds, which could be due to the charge distribution of the compound after the introduction of Cl atom, and is more suitable for enhancing the electrical binding effect with the receptor. At the same time, the introduction of Cl atoms on the benzene ring can increase the fat-solubility, thereby allowing the compound to exert better biological activity.

To investigate whether the antidepressant activity of the compounds was dose-dependent, antidepressant activity of compound **7** and Fluoxetine were performed. Pharmacological results showed that the antidepressant activity of both compound **7** and fluoxetine increased with increasing dose.

The TST model is another effective way to screen antidepressants. **Table 3** shows that compound **7** showed a highly significant antidepressant activity compared with the control group ($p < 0.01$), which was superior to Fluoxetine.

The open-field test is a classical animal experimental model for assessing the autonomic effects of drugs and the general activities of animals. Since the shortening of animal immobility time in behavioral despair and depression animal models may be caused by the excited sympathetic nerves of the drug, the open-field experiment was performed to examine the central excitability of compound **7** [27,28]. In this study, we used an open-field test to determine whether compound **7** affected the spontaneous locomotor activity in mice. Compared with the control group, there was no significant

difference in animals treated with compound **7** ($p > 0.05$), which excluded the false positive results of the mice due to central activity excitability.

Studies show that monoamine neurotransmitter pathways primarily control the body's physiological activities, and changes in transmitters affect monoamine-based transmitter pathways result in a variety of clinical depressive symptoms. At present, most people recognize that monoamine neurotransmitters such as 5-HT may be involved in the neurobiochemical mechanisms of depression. The results of pathological autopsy of depression showed a decrease in 5-HT levels in the brainstem and frontal lobe, and a decrease in the total amount of 5-HT receptors in the hippocampus [29]. The effect of compound **7** on the content of 5-HT in brain tissue of mice was determined here by ELISA. The results showed that compound **7** and Fluoxetine significantly increased the 5-HT content in the brain.

The 5-HT_{1A} receptor plays a role in the pathogenesis of various mental and neurological diseases. Activation of postsynaptic 5-HT_{1A} receptors is important for the favorable response to antidepressants [30,31]. In 5-HT_{1A} binding assay, compound **7**, **16** and **24** exhibited higher affinities for the 5-HT_{1A} receptor, at or below the level of the native neurotransmitter, serotonin (Ki= 1.5 nM).

Molecular docking is an important means to study the possible mechanisms of action for biologically active compounds. In the present study, we docked the compound **7** to the constructed 5-HT_{1A} receptor by homology modeling. Molecular docking and results analysis were performed using Discovery Studio, and the docking results are shown in **Fig. 5**. According to the literature [32], amino acid residues at the active site of 5-HT_{1A} receptor homology model are Ala93, Ala263, Ala365, Ala383, Asn386, Asp116, Cys120, Thr379, Gln97, Gly382, Ile113, Ile124, Ile167, Phe112, Phe361, Phe362, Ser199, Thr196, Thr121, Thr200, and Val117. Docking results showed that compound **7** interacts with amino acid residues at the 5-HT_{1A} receptor active site by hydrogen bond and π-π stacking interactions.

The drug-like properties of designed compounds based on Lipinski's "rule of five" are an effective way of discovering new antidepressant drugs. Statistically, extremely few drugs violate the "rule of five," because compliance with this rule enables the drugs to be bioavailable. In addition, tPSA is closely related to the bioavailability of drugs; compounds with a tPSA > 140 tend to have a lower oral bioavailability [33]. In addition, BBB permeability is an important factor affecting drug activity in the CNS. We used Discovery Studio software to predict BBB permeability of the target compounds. Treatment of depression, Parkinson's disease, epilepsy, and brain tumors require the drugs to cross the

BBB. Due to the complexity of the BBB, it is particularly important to design compounds with suitable physicochemical properties to penetrate the BBB. According to previous reports [34,35], penetration of the BBB by drugs is closely related to the clogP, tPSA, and MW of the compounds. Pajouhesh and Lenz studied the physicochemical properties of the marketed CNS drugs, and highlighted that a successful CNS drug should have the following physical and chemical properties: clogP < 5, tPSA < 70, and MW < 450 [36]. In this study, the clogP, tPSA, and MW of the compounds we designed are consistent with the physicochemical properties of CNS drugs. The absorption of the synthesized compounds was predicted using Discovery Studio. As shown in **Table 6**, most compounds showed favorable BBB permeability and good absorption.

Conclusions

In the present study, a series of coumarin and 3,4-dihydroquinolinone derivatives were synthesized. Two experimental methods, FST and TST, were used to evaluate the antidepressant activity of the target compounds. The pharmacological results showed that most of the target compounds exhibited significant antidepressant activity in the FST model. Among these compounds, compound 7, *N*-(4-chlorophenyl)-2-[(2-oxo-2*H*-chromen-7-yl)oxy] acetamide, was noted to be the most potent compound. In the open-field test, compound **7** did not affect spontaneous activity. The results of *in vivo* 5-HT concentration estimation showed that compound **7** may have an effect on the mouse brain. In the 5-HT_{1A} binding assay, the results showed that compound **7** exhibits strong affinity for the 5-HT_{1A} receptor. Molecular docking results revealed that compound **7** showed significant interactions with residues at the 5-HT_{1A} receptor by homology modeling. The results of molecular properties prediction tests showed that all the compounds exhibited BBB permeability and favorable bioavailability via the oral route.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

The work was supported by Natural Science Foundation of Shandong (No. ZR2017BH037), Doctoral Foundation of Liaocheng University (No. 318051517), National Natural Science Foundation of China (No. 21675071, 81402512, and 81803360), A Projet of Shandong Province Higher Education Science and Technology Program (J13LK17).

References

[1] Grahek I, Shenhav A, Musslick S, Krebs RM, Koster EHW. Motivation and cognitive control in depression. Neurosci Biobehav Rev 2019; 102: 371-381.

[2] Bilbao A, Blanco E, Luque-Rojas MJ, Suárez J, Palomino A, Vida M, Araos P, Bermúdez-Silva FJ, Fernández-Espejo E, Spanagel R, Rodríguez de Fonseca F. Oleoylethanolamide dose-dependently attenuates cocaine-induced behaviours through a PPAR α receptor-independent mechanism. Addict Biol 2013; 18: 78-87.

[3] Pe´rez-Cruz K, Moncada-Basualto M, Morales-Valenzuela J, Barriga-Gonza´lez G, Navarrete-Encina P, Nu´n˜ez-Vergara L, Squella JA, Olea-Azar C. Synthesis and antioxidant study of new polyphenolic hybrid-coumarins. Arab J Chem 2018; 11: 525-537.

[4] Mangasuli SN, Hosamani KM, Satapute P, Joshi SD. Synthesis, molecular docking studies and biological evaluation of potent coumarin–carbonodithioate hybrids via microwave irradiation. Chemical Data Collections 2018; doi: 10.1016/j.cdc.2018.04.001.

[5] Sashidhara KV, Modukuri RK, Singh S, Bhaskara Rao K, Aruna Teja G, Gupta S, Shukla S. Design and synthesis of new series of coumarin–aminopyran derivatives possessing potential anti-depressant-like activity. Bioorg Med Chem Lett 2015; 25: 337-341.

[6] Sashidhara KV, Rao KB, Singh S, Modukuri RK, Aruna Teja G, Chandasana H, Shukla S, Bhatta RS. Synthesis and evaluation of new 3-phenylcoumarin derivatives as potential antidepressant agents. Bioorg Med Chem Lett 2014; 24: 4876-4880.

[7] Lan Y, Chen Y, Xu X, Qiu Y, Liu S, Liu X, Liu BF, Zhang G. Synthesis and biological evaluation of a novel sigma-1 receptor antagonist based on 3,4-dihydro-2(1H)-quinolinones caffold as a potential analgesic. Eur J Med Chem 2014; 79: 216-230.

[8] Jayagobi M, Raghunathan R, Sainath S, Raghunathan M. Synthesis and antibacterial property of pyrrolopyrano quinolinones and pyrroloquinolines. Eur J Med Chem 2011; 46: 2075-2082.

[9] Xiao Z, Waters NC, Woodard CL, Li Z, Li PK. Design and synthesis of Pfmrk inhibitors as potential antimalarial agents. Bioorg Med Chem Lett 2001; 11: 2875-2878.

[10] Kumar NP, Thatikond S, Tokala R, Kumari SS, Lakshmi UJ, Godugu C, Shankaraiah N, Kamal A. Sulfamic acid promoted one-pot synthesis of phenanthrene fused-dihydrodibenzo-quinolinones: Anticancer activity, tubulin polymerization inhibition and apoptosis inducing studies. Bioorg Med Chem Lett 2018; 26: 1996-2008.

[11] Joseph B, Darro F, Behard A, Lesur B, Collignon F, Decaestecker C, Frydman A, Guillaumet G,

Kiss R. 3-Aryl-2-quinolone derivatives: synthesis and characterization of in vitro and in vivo antitumor effects with emphasis on a new therapeutical target connected with cell migration. J Med Chem 2002; 45: 2543-2555.

[12] Shiro T, Fukaya T, Tobe M. The chemistry and biological activity of heterocycle-fused quinolinone derivatives: A review. Eur J Med Chem 2015; 97: 397-408.

[13] Quan ZS, Wang JM, Rho JR. Synthesis of 6-alkyloxyl-3,4-dihydro-2(1*H*)-quinoliones and their anticonvulsant activities. B Korean Chem Soc 2005; 26: 1757-1760.

[14] Kumar S, Bawa S, Gupta H. Biological Activities of Quinoline Derivatives. Mini Rev Med Chem 2009; 9: 1648-1654.

[15] Sun XY, He XJ, Pan CY, Liu YP, Zou YP. Synthesis and study of the antidepressant activity of novel 4,5-dihydro-7-alkoxy(phenoxy)-tetrazolo[1,5-*a*]quinoline derivatives. Med Chem Res 2012; 21: 3692-3698.

[16] Kumar S, Bawa S, Drabu S, Gupta H, Machwal L, Kumar R. Synthesis, antidepressant and antifungal evaluation of novel 2-chloro-8-methylquinoline amine derivatives. Eur J Med Chem 2011; 46: 670-675.

[17] Peperidou A, Bua S, Bozdag M, Hadjipavlou-Litina D, Supuran CT. Novel 6- and 7-Substituted Coumarins with Inhibitory Action against Lipoxygenase and Tumor-Associated Carbonic Anhydrase IX. Molecules 2018; 23: 153.

[18] Saeedi M, Goli F, Mahdavi M, Dehghan G, Faramarzi MA, Foroumadi A, Shafiee A. Synthesis and Biological Investigation of some Novel Sulfonamide and Amide Derivatives Containing Coumarin Moieties. Iran J Pharm Res 2014; 13: 881-892.

[19] Chang KM, Chen HH, Wang TC, Chen IL, Chen YT, Yang SC, Chen YL, Chang HH, Huang CH, Chang JY, Shih C, Kuo CC, Tzeng CC. Novel oxime-bearing coumarin derivatives act as potent Nrf2/ARE activators in vitro and in mouse model. Eur J Med Chem 2015; 106: 60-74.

[20] Chen IL, Chen JJ, Lin YC, Peng CT, Juang SH, Wang TC. Synthesis and antiproliferative activities of *N*-(naphthalen-2-yl)acetamide and N-(substitutedphenyl)acetamide bearing quinolin-2(1*H*)-one and 3,4-dihydroquinolin-2(1H)-one derivatives. Eur J Med Chem 2013; 59: 227-234.

[21] Gu ZS, Xiao Y, Zhang QW, Li JQ. Synthesis and antidepressant activity of a series of arylalkanol and aralkyl piperazine derivatives targeting SSRI/5-HT1A/5-HT7. Bioorg Med Chem Lett 2017;

27: 5420-5423.

[22] Porsolt RD. Behavioral despair in mice: A primary screening test for antidepressants. Arch Int Pharmacodyn Ther 1977, 229, 327-336.

[23] Zomkowski AD, Santos AR, Rodrigues AL. Evidence for the involvement of the opioid system in the agmatine antidepressant-like effect in the forced swimming test. Neurosci Lett 2005; 381: 279-283.

[24] Streu L, Chermat R, Thierry B, Simon P. The tail suspension test: A new method for screening antidepressants in mice. Psychopharmacol 1985; 85: 367-370.

[25] Sairanen M, Lucas G, Ernfors P, Castrén M, Castrén E. Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronalturnover, proliferation, and survival in the adult dentate gyrus. J Neurosci 2005; 25: 1089-1094.

[26] Elliott PJ, Chan J, Parker YM, Nemeroff CB. Behavioral effects of neurotensin in the open field: structure-activity studies. Brain Res 1986; 381: 259-265.

[27] Galdino PM, Nascimento MV Sampaio BL, Ferreira RN, Paula JR, Costa EA. Antidepressant-like effect of Lafoensia pacari A. St.-Hil. ethanolic extract and fractions in mice. J Ethnopharmacol 2009; 124: 581-585.

[28] Sakakibara H, Ishida K, Grundmann O, Nakajima J, Seo S, Butterweck V, Minami Y, Saito S, Kawai Y, Nakaya Y, Terao J. Antidepressant effect of extracts from Ginkgo biloba leaves in behavioral models. Biol Pharm Bull 2006; 29: 1767-1770.

[29] Meyer JH, McMain S, Kennedy SH, Korman L, Brown GM, DaSilva JN, Wilson AA, Blak T, Eynan-Harvey R, Goulding VS, Houle S, Links P. Dysfunctional attitudes and 5-HT_{2A} receptors during depression self-harm. Am J Psychiatry 2003; 160: 90-99.

[30] Ostrowska K, Grzeszczuk D, Głuch-Lutwin M, Gryboś A, Siwek A, Leśniak A, Sacharczuk M, Trzaskowski B. 5-HT_{1A} and 5-HT_{2A} receptors affinity, docking studies and pharmacological evaluation of a series of 8-acetyl-7-hydroxy-4-methylcoumarin derivatives. Bioorg Med Chem 2018; 26: 527-535.

[31] Blier P, Ward NM. Is there a role for 5-HT_{1A} agonists in the treatment of depression?. Biol Psychiatry 2003; 53: 93-103.

[32] Sherin DR, Geethu CK, Prabhakaran J, Mann JJ, Dileep JS, Manojkumar TK. Molecular docking, dynamics simulations and 3D-QSAR modeling of arylpiperazine derivatives of

3,5-dioxo-(2*H*,4*H*)-1,2,4-triazine as 5-HT_{1A}R agonists. Comput Biol Chem 2019; 78: 108-115.

[33] Allinger NL. Conformational analysis. 130. MM2. A hydrocarbon force field utilizing V1 and V2 torsional terms. J Am Chem Soc 1977; 99: 8127-8134.

[34] Clark DE. In silico prediction of blood-brain barrier permeation. Drug Discov Today 2003; 8: 927-933.

[35] Cecchelli R, Berezowski V, Lundquist S, Culot M, Renftel M, Dehouck MP, Fenart L. Modelling of the blood-brain barrier in drug discovery and development. Nature Rev Drug Discov 2007; 6: 650-661.

[36] Pajouhesh H, Lenz GR. Medicinal chemical properties of successful central nervous system drugs. NeuroRx 2005; 2: 541-553.

Captions:

Fig. 1. The chemical structure of coumarin and 3,4-dihydroquinolinone.

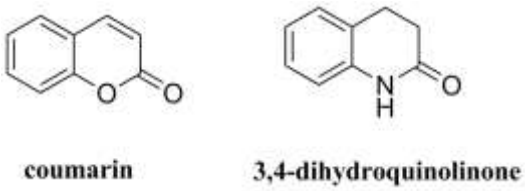


Fig. 2. Representative structures of carboxylic acid amide antidepressants.

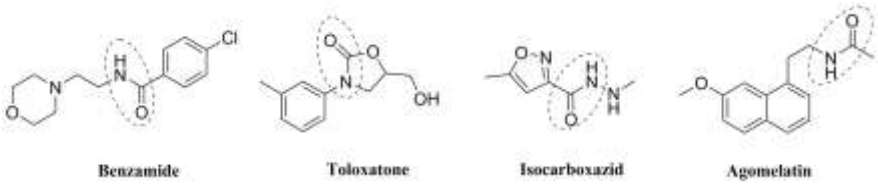


Fig. 3. Design strategy for target molecules.

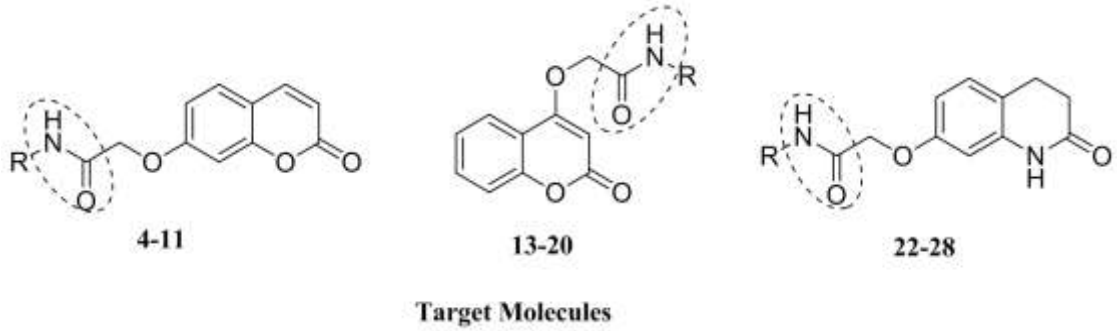


Fig. 4. Exploratory activity (counts) in the open-field test. The behavioral parameters were recorded for 3 min. Locomotion: number of line crossings; rearing: number of times seen standing on hind legs; grooming: number of modifications; **7** (40 mg/kg) was administered 60 min before the test. The values represent the mean ± SEM (*n* =8).

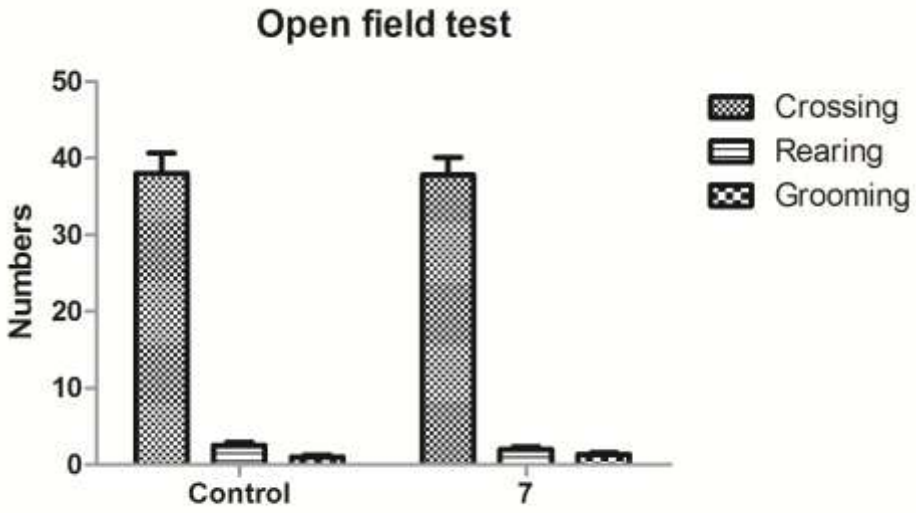
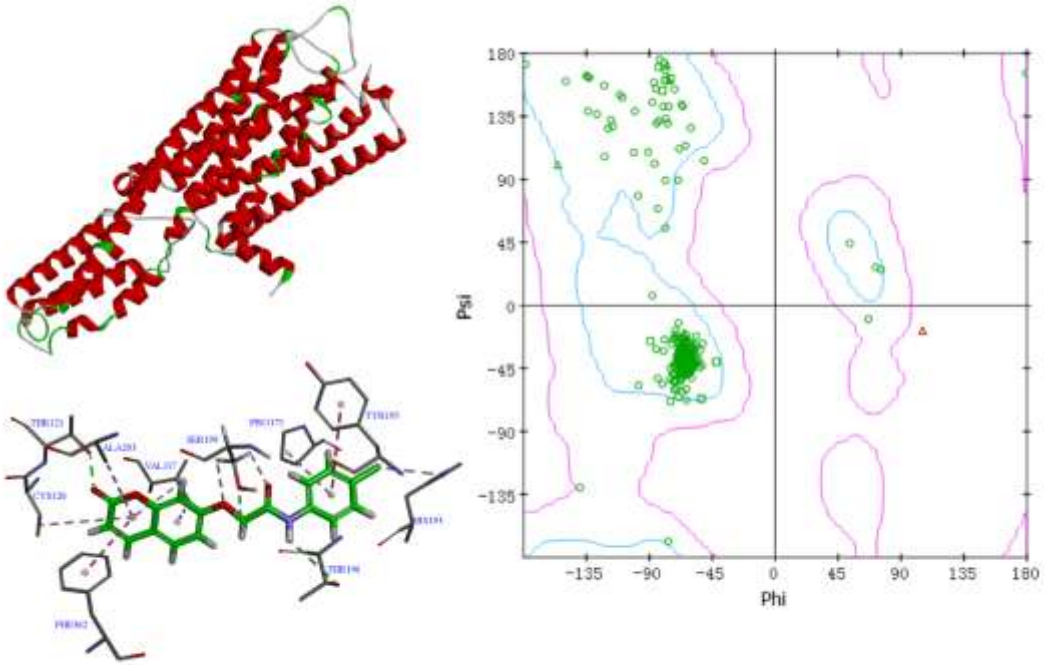


Fig. 5. (**a**) 3D model of 5-HT_{1A} receptor homology model; (**b**) Ramachandran plot; (**c**) Compound **7**

show interactions with residues at the 5-HT_{1A} receptor.



Scheme 1. Synthesis of the target compounds **4-11**, **13-20** and **22-28**. Reagents and conditions: (**a**) triethylamine, CH_2Cl_2, rt, 3-8h; (**b**) K_2CO_3, acetone, reflux, 6-12h.

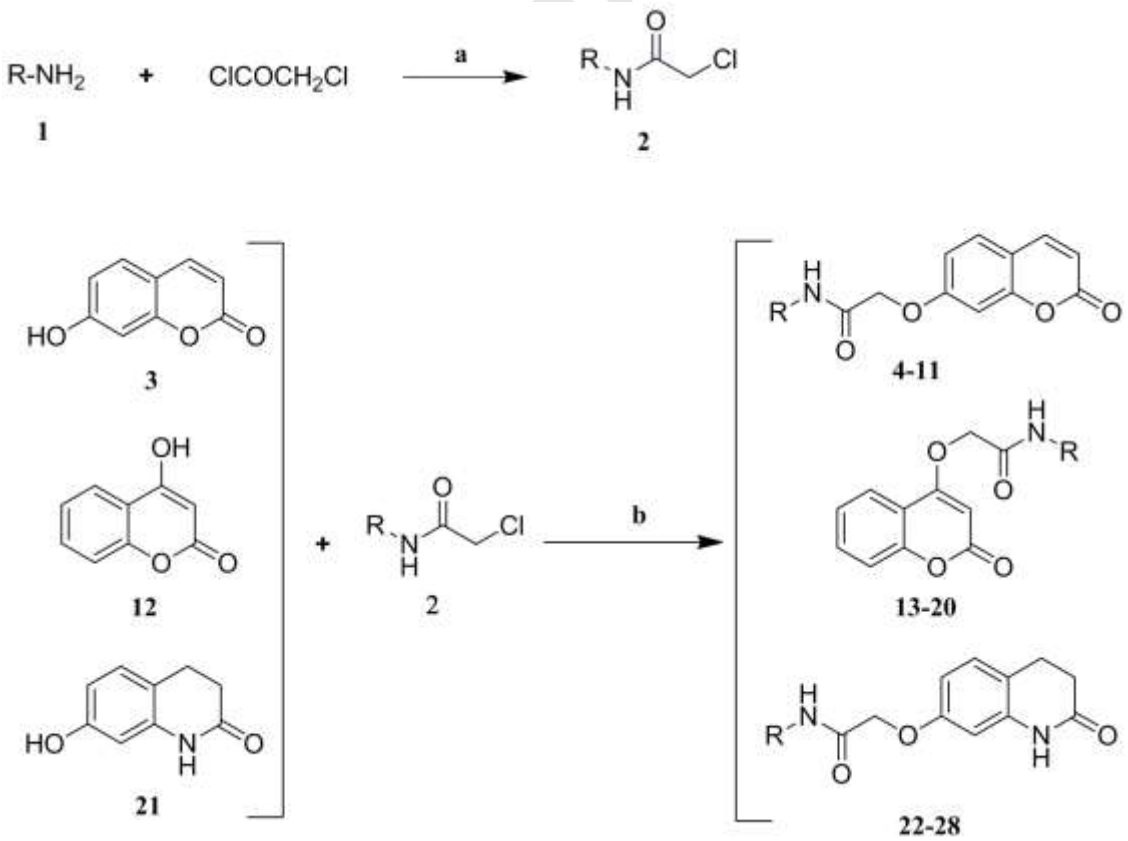


Table 1. Antidepressant activities of compounds **4-11**, **13-20** and **22-28** in FST.

Compds	R	Antidepressant activities[a]	
		Duration of immobility (s) (mean ± SEM)[b]	Change from control (%)
4	Ph	84.41 ± 18.81*	49.73
5	CH_2Ph	94.82 ± 15.38*	43.53
6	CH_2CH_2Ph	128.83 ± 9.87	23.27
7	(4-Cl) Ph	65.52 ± 9.70**	60.98
8	(4-CF_3) Ph	75.27 ± 17.29**	55.17
9	(4-CH_3) Ph	154.17 ± 14.01	8.18
10	(4-OCH_3) Ph	125.95 ± 20.38	24.99
11	Cyclohexane	100.82 ± 19.38	39.96
13	Ph	86.38 ± 22.21*	48.55
14	CH_2Ph	128.56 ± 22.88	23.44
15	CH_2CH_2Ph	109.72 ± 19.21	34.66
16	(4-Cl) Ph	71.91 ± 15.38**	57.17
17	(4-F) Ph	130.12 ± 12.01	22.51
18	(4-CF_3) Ph	89.21 ± 14.78*	46.87
19	(4-CH_3) Ph	151.69 ± 15.48	9.66
20	Cyclohexane	85.16 ± 15.51*	49.28
22	Ph	96.83 ± 18.96*	42.33
23	CH_2Ph	137.32 ± 21.09	18.22
24	(4-Cl) Ph	76.45 ± 17.87**	54.47
25	(4-F) Ph	134.92 ± 21.40	19.65
26	(4-CH_3) Ph	122.01 ± 21.79	27.34
27	(4-OCH_3) Ph	91.25 ± 17.88*	45.65
28	Cyclohexane	131.31 ± 11.07	21.80
Fluoxetine	-	68.15 ± 9.41**	59.41
Control	-	167.92 ± 22.09	-

[a] Target compounds and Fluoxetine were administered at 40 mg/kg. [b] Values represent the mean ± SEM (n = 8).

* significantly compared to control ($0.01 < p < 0.05$). ** very significantly compared to control ($p < 0.01$).

Table 2. Antidepressant activities of compounds **7** and Fluoxetine in FST at different doses.

Compds	Dose (mg/kg)	Antidepressant activities[a] Duration of immobility(s) (mean ± SEM)[b]	Change from control (%)
7	10	83.22 ± 17.21*	47.46
	20	68.32 ± 13.88**	56.87
	40	61.28 ± 11.79**	60.97
Fluoxetine	10	85.76 ± 16.81*	45.86
	20	72.54 ± 15.67**	54.29
	40	67.21 ± 13.34**	57.57
Control	-	158.41 ± 18.61	-

[a] Compound **7** and Fluoxetine were administered at 40, 20, 10 mg/kg, respectively. [b] Values represent the mean ± SEM (n = 8). * significantly compared to control ($0.01<p<0.05$). ** very significantly compared to control ($p<0.01$).

Table 3. Antidepressant activities of compounds **7** and Fluoxetine in the TST test.

Compds	Dose (mg/kg)	Antidepressant activities[a] Duration of immobility (s) (mean ± SEM)[b]	Change from control (%)
7	40	64.31 ± 15.72**	54.88
Fluoxetine	40	71.35 ± 14.48**	50.55
Control	-	144.31 ± 21.07	-

[a] Compound **7** and Fluoxetine were administered at 40 mg/kg. [b] Values represent the mean ± SEM (n = 8). * significantly compared to control ($0.01<p<0.05$). ** very significantly compared to control ($p<0.01$).

Table 4. Effect of compounds **7** and Fluoxetine on brain 5-HT level in mice.

Compds	Dose (mg/kg)	5-HT (ng/mg)[a]
7	40	1.314 ± 0.113*[b]
Fluoxetine	40	1.298 ± 0.095*
Control	-	1.023 ± 0.107

[a] Compounds **7** and Fluoxetine were analyzed (The concentration of 5-HT in the mice brain) 1 hour after oral administration. [b] Represent the mean ± SEM (n = 8). * significantly compared to control ($0.01<p<0.05$).

Table 5. 5-HT_{1A} receptor binding of selected compounds and reference serotonin.

Compds	5-HT_{1A} (K_i [nM] ± SEM)[a]
7	1.4 ± 0.1
16	2.5 ± 0.2
24	6.3 ± 0.4
Serotonin	1.5 ± 0.1

[a] K_i values were obtained from 8 concentrations of the compound, each in duplicate.

Table 6. Drug-likeness parameters of target compounds.

Comds	R	MW[a]	ClogP	nHBD	nHBA	tPSA	nRotB	BBB (level)	ABS (level)
4	Ph	295.08	2.36	1	4	64.63	4	2	0
5	CH_2Ph	309.10	2.38	1	4	64.63	5	2	0
6	CH_2CH_2Ph	323.11	2.60	1	4	64.63	6	2	0
7	(4-Cl) Ph	329.04	3.33	1	4	64.63	4	2	0
8	(4-CF_3) Ph	363.07	3.69	1	4	64.63	5	2	0
9	(4-CH_3) Ph	309.10	2.86	1	4	64.63	4	2	0
10	(4-OCH_3)Ph	325.09	2.43	1	5	73.86	5	3	0
11	Cyclohexane	301.13	2.53	1	4	64.63	4	2	0
13	Ph	295.08	2.50	1	4	64.63	4	2	0
14	CH_2Ph	309.10	2.52	1	4	64.63	5	2	0
15	CH_2CH_2Ph	323.11	2.74	1	4	64.63	6	2	0
16	(4-Cl) Ph	329.04	3.47	1	4	64.63	4	2	0
17	(4-F) Ph	313.07	2.90	1	4	64.63	4	2	0
18	(4-CF_3) Ph	363.07	3.83	1	4	64.63	5	2	0
19	(4-CH_3) Ph	309.10	2.99	1	4	64.63	4	2	0
20	Cyclohexane	301.13	2.67	1	4	64.63	4	2	0
22	Ph	296.11	1.98	2	3	67.43	4	3	0
23	CH_2Ph	310.13	2.01	2	3	67.43	5	3	0
24	(4-Cl) Ph	330.07	2.95	2	3	67.43	4	2	0
25	(4-F) Ph	314.10	2.38	2	3	67.43	4	3	0
26	(4-CH_3) Ph	310.13	2.48	2	3	67.43	4	2	0
27	(4-OCH_3)Ph	326.12	2.06	2	4	76.66	5	3	0
28	Cyclohexane	302.16	2.16	2	3	67.43	4	3	0

[a] MW, molecular weight; CLogP, Calculated lipophilicity; nHBD, number of hydrogen bond donors; nHBA, number of hydrogen bond acceptors; tPSA, topological polar surface area; nRotB, number of rotatable bonds; BBB-Level, 0 (very high penetration), 1 (high penetration), 2 (medium penetration), 3 (weak penetration); Absorption-Level, 0 (very well absorption), 1 (well absorption), 2 (poorly absorption), 3 (very poorly absorption).