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8-Hydroxyquinolin-2(1*H*)-one analogues as potential β_2-agonists: Design, synthesis and activity study

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ABSTRACT

β_2-Agonists that bind to plasmalemmal β_2-adrenoceptors causing cAMP accumulation are widely used as bronchodilators in chronic respiratory diseases. Here, we designed and synthesized a group of 8-hydroxyquinolin-2(1*H*)-one analogues and studied their β_2-agonistic activities with a cellular cAMP assay. Compounds **B05** and **C08** were identified as potent (EC_{50} < 20 pM) and selective β_2-agonists among the compounds tested. They behaved as partial β_2-agonists in non-overexpressed HEK293 cells, and possessed rapid smooth muscle relaxant actions and long duration of action in isolated guinea pig tracheal strip preparations. In summary, **B05** and **C08** are β_2-agonists with potential applicability in chronic respiratory diseases.

1. Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are common chronic diseases of the pulmonary system [1,2]. According to the Global Burden of Disease Study 2017, 544.9 million people worldwide had a chronic respiratory disease in 2017 [3], which account for 495,000 deaths due to asthma and 3.2 million deaths due to COPD [4]. The Global Initiative for Asthma (GINA) defined asthma as a heterogeneous disease of the airway characterized by typical symptoms such as wheeze, shortness of breath, chest tightness and cough. COPD is characterized by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases. Both genetic and environmental factors contribute to the development of asthma and COPD [5–7]. Smoking, second-hand smokes, household air pollution, ambient particulate matters, ozone, and occupational particulates are considered as the six external causes for COPD. Smoking and occupational exposures are also environmental risk factors for asthma [8].

β_2-Agonists, mAChR antagonists, PDE4 inhibitors and ICS (inhaled corticosteroids) constitute the major drug classes for pharmaceutical management of asthma and COPD [9,10]. β_2-Agonists are still the cornerstone for the treatment of chronic respiratory diseases [11]. Inhaled long-acting β_2-agonists have achieved a vast development in the past two decades, resulting in the marketing of drugs such as salmeterol [12,13], formoterol [14], indacaterol [15] and olodaterol [16,17], the latter two also known as the ultra-long-acting β_2-agonists. In addition, a number of small molecules are under clinical development, such as abediterol [18], bedoradrine [19], and PF-610355 [20](Fig. 1).

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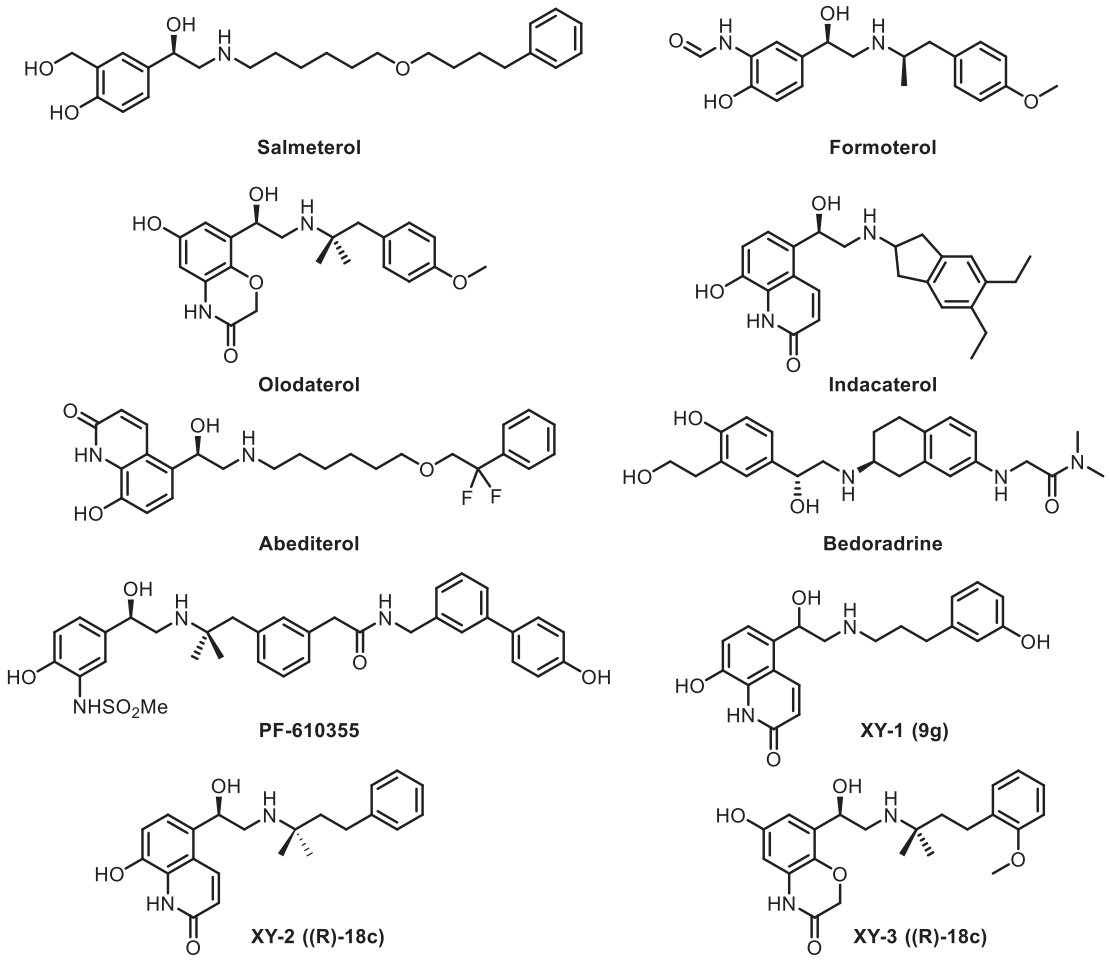


Fig. 1. The structure of salmeterol, formoterol, indacaterol, olodaterol, abediterol, bedoradrine, PF-610355, XY-1 (9g), XY-2 ((R)-18c), and XY-3 ((R)-18c).

We have previous synthesized compounds based on indacaterol and olodaterol, and studied their biological activities as β_2-agonists [21,22]. Representative compounds XY-1, XY-2, and XY-3 (Fig. 1), referring respectively to compounds 9g [21], (*R*)-18c [21] and (*R*)-18c [22] in the original articles, had EC_{50} values ranging from 21–36 pM. Their β_2/β_1-selectivity (β_2 pEC_{50} - β_1 pEC_{50}) varied widely from 1.77 to 5.68 with compound XY-2 being the most selective. A high β_2/β_1-selectivity is an important attribute for β_2-agonists used as bronchodilators for reduced cardiac side effects [23]. Here, we report our renewed efforts in the discovery of highly potent and selective β_2-agonists. In the present study, the 5-(2-amino-1-hydroxyethyl)-8-hydroxyquinolin-2(1*H*)-one scaffold of indacaterol was combined with the tail group of formoterol or olodaterol to produce compounds of series **A**, **B** and **C** with a varied number of methyl groups on the α-carbon of the amino group and different substituents on the tail benzene moiety (Fig. 2). The biological activities of these target compounds were studied. Structure-activity relationship analyses were then performed on these compounds to see how methyl substitution on the α-carbon affect β_2-agonistic activity.

2. Results and discussion

2.1. Chemistry

The synthetic pathways for the primary amines starting through commercially available different substituted benzaldehydes are provided in Scheme 1, Scheme 2, and Scheme 3. Compounds **4**–**01** to **4**–**08** were obtained by Wittig reaction and reduction reaction from benzaldehyde. Derivatives **8**–**01** to **8**–**14** were achieved through aldol condensation, reduction, and Leuckart reaction. Under the same condition to prepare compound **11** through Ritter reaction and deacetylation, intermediate **11** was finally converted into compounds **13**–**01** to **13**–**11** by treatment with MeMgBr.

The key intermediate **14** was prepared as described in our previous study [21]. The synthetic pathway of the target compounds was depicted in Scheme 4. Different amines reacted with intermediate **14** in the presence of DMSO, MeOH and $NaBH_4$ and the subsequent Bn was removed under the condition of H_2/Pd/C, yielding compounds **A01** to **A08**, **B01** to **B14**, and **C01** to **C13**. Compounds (***R,R*)-B04**, (***R*)-C07**, and (***R*)-C08** were synthesized as

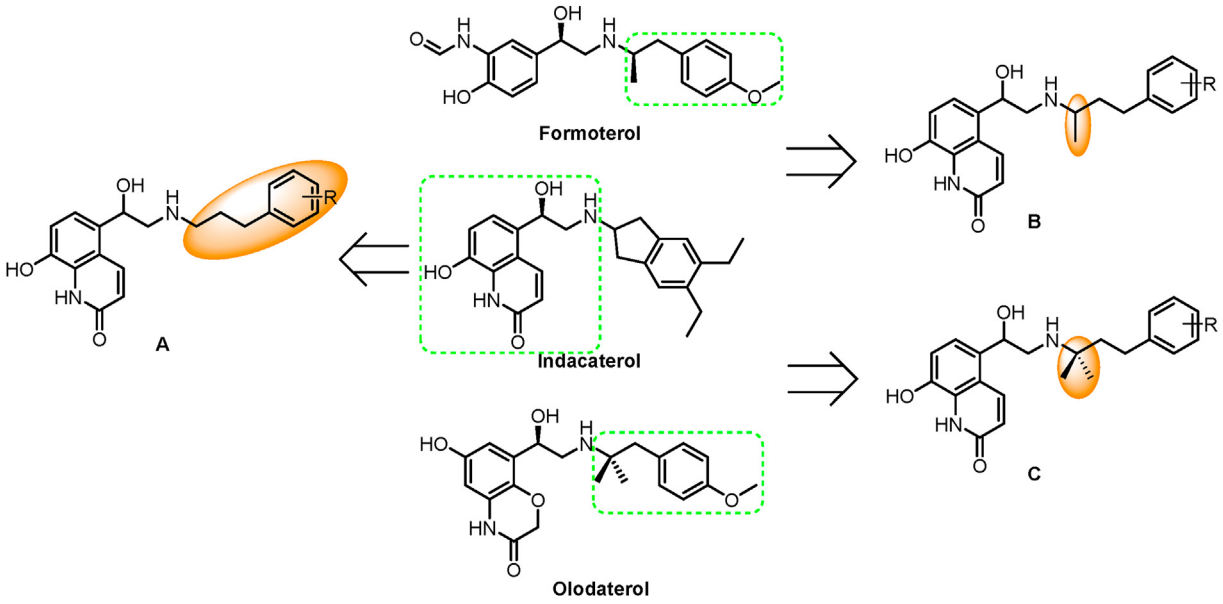
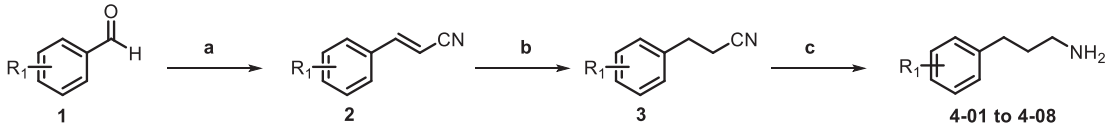
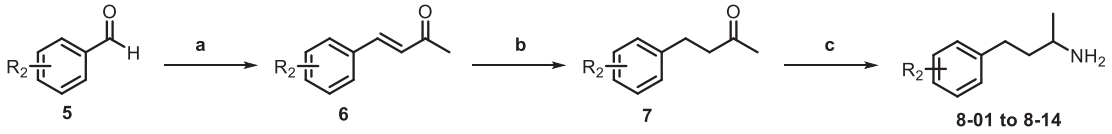


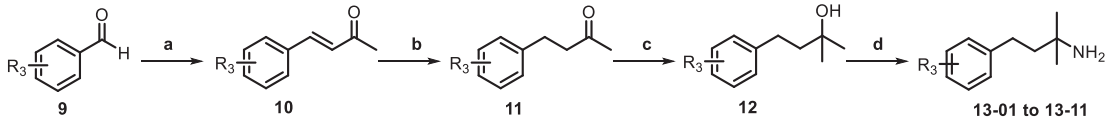
Fig. 2. Structure-aided and design strategy for the target compounds.



Scheme 1. Reagents and conditions: (a) 2-diethoxyphosphorylacetonitrile, NaH, dry oxolane, 30 min, 90–95%; (b) 10% Pd/C, H_2, MeOH, 1 h, 95–98%; (c) BH_3, dry oxolane, 80 °C, 2 h, 85–95%.



Scheme 2. Reagents and conditions: (a) 10% NaOH, acetone, 70–80%; (b) 10% Pd/C, H_2, EtOH, 1 h, 95–98%; (c) 10% Pd/C, $HCOONH_4$, MeOH, 70 °C, 53–62%.



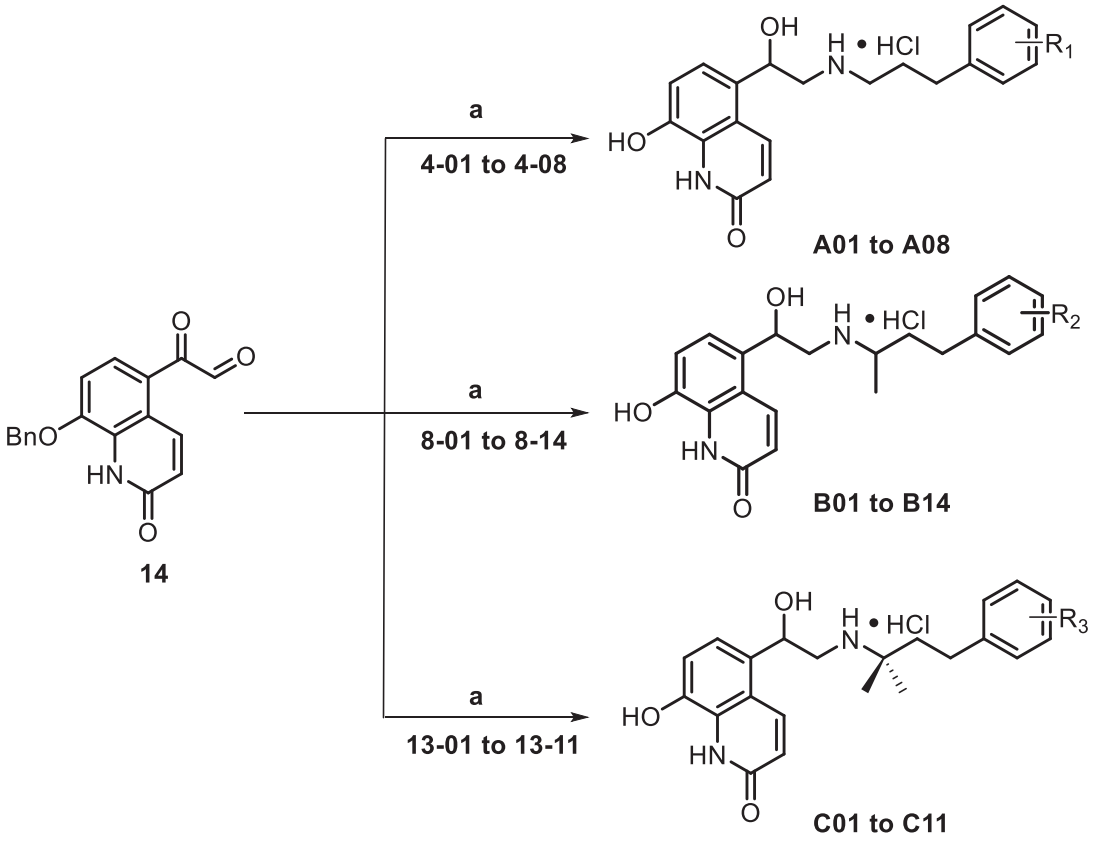
Scheme 3. Reagents and conditions: (a) 10% NaOH, acetone, 70–80%; (b) 10% Pd/C, H_2, EtOH, 1 h, 95–98%; (c) MeMgBr, dry oxolane, 0 °C, 40 min, 80–90%; (d) (i) CH_3CN, concentrated H_2SO_4, 0 °C, 5 h, 70–80%; (ii) KOH, ethylene glycol, 180 °C, 20 h, 80–95%.

described in Scheme 5. The key intermediate **15** was prepared from asymmetric induction with the use of a chiral catalyst (*R*)-2-methyl-CBS-oxazaborolidine ((*R*)-CBS) [24]. We obtained **(*R*,*R*)-B04** by semipreparative HPLC with a stereochemical purity value of 99.3%. The ee values of **(*R*)-C07** and **(*R*)-C08** were determined by HPLC and found to be 99.32% and 99.15%, respectively. (refer to the HPLC spectra in Supporting Information).

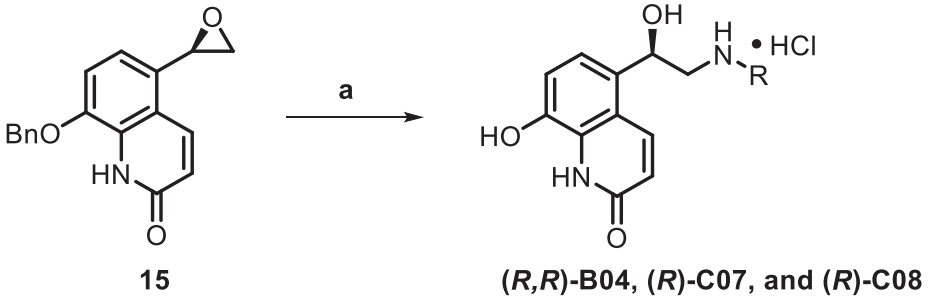
2.2. β_2-Agonistic activity and structure-activity relationship analysis

Adrenoceptors (adrenergic receptors, ARs) belong to the superfamily of the G protein-coupled receptors (GPCRs). ARs are broadly classified into the α and the β subtypes. β-ARs can be further classified into the β_1-, β_2-and β_3-AR [25]. In airway smooth muscles, β_2-ARs are abundantly expressed, whereas β_1-ARs are the predominant subtype expressed in the heart [26]. Activation of the β_2-AR by an agonist results in the accumulation of cAMP which leads to smooth muscle relaxation in airways [27]. We evaluated the biological activities of the titled compounds in human embryonic kidney 293 (HEK-293) cells overexpressing the human β_1-AR or β_2-AR. The intrinsic activities of a subset of compounds were also studied in non-overexpressed HEK293 cells with endogenous human β_2-ARs.

Compounds **A01**-**A08** in Table 1 have no side groups on the aminopropylbenzene backbone of the tail group. Compounds **A01**-



Scheme 4. Reagents and conditions: (a) (i) sodium borohydride, MeOH, DMSO, 1 h, 40–55%; (ii) 5% Pd/C, MeOH, H_2, 1 h, 85–99%; (iii) ethyl acetate-HCl, 2 h.

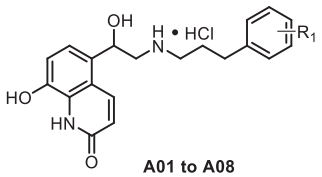


Scheme 5. Preparation of (*R*,*R*)-B04, (*R*)-C07, and (*R*)-C08. (a) (i) RNH_2, ^{i}PrOH, 85 °C, 12 h, 45%; (ii) 10% Pd/C, H_2, MeOH, 1 h, 90–95%.

A03 contain a terminal methyl-substituted benzene ring at various positions. The *meta*-substituted compound **A02** exhibited a higher activity as compared with compounds **A01** and **A03**, with similar subtype selectivity. Compound **A02** showed the same level of β_2-agonistic activity and lower selectivity compared with its analogue, compound XY-1. Fluorine is a potential bioisoster for substituent groups such as hydrogen, carbonyl, sulfonyl and cyanide on a drug molecule. We attempted to introduce fluorine-containing groups into the terminal benzene ring of the 5-(2-amino-1-hydroxyethyl)-8-hydroxyquinolin-2(1*H*)-one derivatives. Compounds **A04**-**A07** had comparable levels of β_2-agonistic activity, with **A07** having the highest potency. Compound **A04** and **A06** were roughly equipotent to compound XY-2 and displayed a reduced β_2/β_1-selectivity compared with compounds XY-1 and XY-2. Compound **A07** had a potency exceeding those of the reference compounds isoprenaline, salmeterol and olodaterol, and an excellent selectivity. It showed the same levels of β_2-agonistic activity and selectivity compared with compound XY-1. The 3,4,5-trimethoxy-substituted compound **A08** had an activity comparable to that of olodaterol and a selectivity about 50-fold higher than those of salmeterol and olodaterol. It was only slightly less potent compared with compound XY-1 but its selectivity was much higher, approaching that of XY-2.

A previous study has suggested that an 8-hydroxyquinolin-2(*1H*)-one analogue with an α-methyl aminoindane tail had higher binding affinity towards the β_2-AR compared with the parent compound indacaterol [28]. Conversely, a demethylated derivative of (*R*,*R*)-fenoterol had a lower affinity towards the β_2-AR compared with (*R*,*R*)-fenoterol [29]. We investigated whether introduction of a methyl group on the carbon atom next to the amino group as in formoterol [30] and fenoterol would increase activity. Compounds in Table 2 were synthesized and assayed. All single substituted analogues exhibited higher activities as compared with the

Table 1
Chemical Structures of Compounds A01 to A08 and Their Activities in Stimulating cAMP Accumulation in HEK293 Cells Expressing β_2-or β_1-Adrenoceptors.

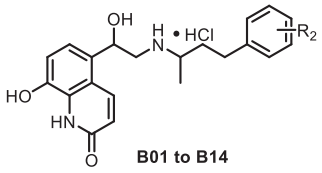


A01 to A08

Compound	R_1	β_1 pEC_{50}[a]	β_2 pEC_{50}[a]	β_2 - β_1[b]
A01	*o*-CH_3	7.77	10.24 ± 0.09	2.46
A02	*m*-CH_3	7.84	10.42	2.57
A03	*p*-CH_3	7.52	10.02 ± 0.20	2.49
A04	*m*-F	8.35	10.61 ± 0.08	2.25
A05	*p*-F	7.79	10.33 ± 0.09	2.53
A06	3,5-diF	8.37	10.67	2.30
A07	*m*-CF_3	7.11	10.55	3.44
A08	3,4,5-triOCH_3	5.00	10.08	5.08
XY-1		6.66 ± 0.10[c]	10.44 ± 0.02[c]	3.66[c]
XY-2		5.00 ± 0.00[c]	10.68 ± 0.28[c]	5.68[c]
Isoprenaline		8.90	9.67	0.77
Salmeterol		6.43	9.87	3.44
Olodaterol		6.91	10.06	3.15

[a] Data represent mean ± SEM obtained from at least four independent experiments.
[b] Selectivity is defined as pEC_{50} at the β_2-adrenoceptor - pEC_{50} at the β_1-adrenoceptor.
[c] Adopted from Ref. 21.

Table 2
Chemical Structures of Compounds B01 to B14 and Their Activities in Stimulating cAMP Accumulation in HEK293 Cells Expressing β_2-or β_1-Adrenoceptors.



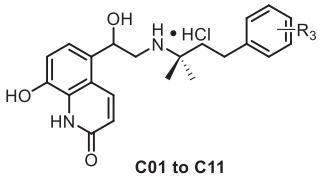
B01 to B14

Compound	R_2	β_1 pEC_{50}[a]	β_2 pEC_{50}[a]	β_2 - β_1[b]
B01	*o*-Cl	8.34 ± 0.03	10.47 ± 0.12	2.13
B02	*m*-Cl	8.35 ± 0.16	10.14 ± 0.25	1.79
B03	*p*-Cl	8.20 ± 0.15	10.48 ± 0.05	2.28
B04	*o*-OCH_3	8.81 ± 0.07	10.83 ± 0.15	2.02
B05	*m*-OCH_3	8.59 ± 0.27	10.67 ± 0.14	2.08
B06	*p*-OCH_3	7.35 ± 0.11	10.74 ± 0.22	3.39
B07	*m*-F	8.51 ± 0.06	10.63 ± 0.26	2.12
B08	*p*-F	7.98 ± 0.01	10.55 ± 0.19	2.57
B09	*o*-CH_3	8.17 ± 0.19	10.76 ± 0.39	2.59
B10	*p*-CH_3	7.98 ± 0.12	10.42 ± 0.37	2.44
B11	*m*-CH_3	8.39 ± 0.03	10.89 ± 0.05	2.50
B12	*p*-OH	8.35 ± 0.02	10.97 ± 0.03	2.62
B13	*m*-OH	8.63 ± 0.39	10.87 ± 0.01	2.24
B14	3,4,5-triOCH_3	7.45 ± 0.07	10.35 ± 0.10	2.90
(*R*,*R*)-B04	*o*-OCH_3	8.73	10.54	1.81
Isoprenaline		8.90	9.67	0.77
Salmeterol		6.43	9.87	3.44
Olodaterol		6.91	10.06	3.15

[a] Data represent mean ± SEM obtained from at least four independent experiments.
[b] Selectivity is defined as pEC_{50} at the β_2-adrenoceptor - pEC_{50} at the β_1-adrenoceptor.

reference compounds isoprenaline, salmeterol and olodaterol. We incorporated a chlorine group into the benzene ring obtaining

Table 3
Chemical Structures of Compounds C01 to C11 and Their Activities in Stimulating cAMP Accumulation in HEK293 Cells Expressing β_2-or β_1-Adrenoceptors.



C01 to C11

Compound	R_3	β_1 pEC_{50}[a]	β_2 pEC_{50}[a]	β_2 - β_1[b]
C01	*p*-OCH_3	8.11 ± 0.35	10.51 ± 0.35	2.40
C02	*m*-OCH_3	8.12 ± 0.24	10.58 ± 0.02	2.46
C03	*o*-OCH_3	8.69 ± 0.11	10.65 ± 0.17	1.96
C04	*p*-CH_3	7.61 ± 0.13	10.78 ± 0.22	3.17
C05	*m*-CH_3	8.58 ± 0.05	10.71 ± 0.08	2.13
C06	*o*-CH_3	8.67 ± 0.18	10.75 ± 0.24	2.08
C07	*p*-Cl	8.51 ± 0.07	10.90 ± 0.04	2.39
C08	*m*-Cl	7.98 ± 0.04	11.20 ± 0.17	3.22
C09	*o*-Cl	8.76 ± 0.08	10.74 ± 0.24	1.98
C10	*p*-OH	7.65 ± 0.21	10.44 ± 0.31	2.79
C11	*m*-OH	8.62 ± 0.06	10.74 ± 0.12	2.12
(*R*)-C07	*p*-Cl	9.07	10.75	1.68
(*R*)-C08	*m*-Cl	9.22	10.65	1.43
XY-2		5.00 ± 0.00[c]	10.68 ± 0.28[c]	5.68[c]
XY-3		8.85 ± 0.45[d]	10.62 ± 0.04[d]	1.77[d]
Isoprenaline		8.90	9.67	0.77
Salmeterol		6.43	9.87	3.44
Olodaterol		6.91	10.06	3.15

[a] Data represent mean ± SEM obtained from at least four independent experiments.
[b] Selectivity is defined as pEC_{50} at the β_2-adrenoceptor - pEC_{50} at the β_1-adrenoceptor.
[c] Adopted from Ref. 21.
[d] Adopted from Ref. 22.

compounds **B01**–**B03**. Compounds **B01**–**B03** displayed similar potency and selectivity. Remarkable potency and selectivity were also observed in compounds bearing the methoxy motif, as illustrated in compounds **B04** and **B05**, and especially **B06**. The β_2/β_1-selectivity of **B06** was highest in this series which was similar to that of salmeterol or olodateol. It seems that the position of the methoxy group on the terminal benzene ring had little influence on activity. Addition of a fluorine group in the *m*- or *p*-positions yielded compounds **B07** and **B08**, with similar activities (β_2 pEC_{50} values equal to 10.63 and 10.55, respectively). The methyl-substituted compounds **B09**, **B10** and **B11** displayed higher activities compared with the references. However, they possessed a lower selectivity compared with salmeterol and olodateol. The *p*- and *m*-hydroxyl-substituted compounds **B12** and **B13** was substantially equipotent and were among the most potent compounds in this series. The 3,4,5-trimethoxy-substituted compound **B14** offered a moderate β_2-agonistic activity comparable to compounds **B04**–**B06**.

It has been documented that olodaterol analogues with two geminal methyl groups on the α carbon could be potent β_2-agonists [16,17,31]. Next, we investigated a series of compounds with dimethyl substitution on the α carbon of the aminopropylbenzene scaffold thus completing structure-activity relationship analysis of substitution at this position with different number of the methyl group. The high β_2 pEC_{50} values of the compounds in Table 3 suggest that a wide range of substitutions on the terminal benzene ring could be tolerated, including methoxy substitution (**C01**–**C03**), methyl substitution (**C04**–**C06**), chlorine substitution (**C07**–**C09**) and hydroxyl substitution (**C10**, **C11**). In particular, compounds **C04** and **C08** exhibited selectivity on par with salmeterol and olodaterol,

Table 4
Intrinsic activities of selected compounds on HEK293 cells endogenously expressing human β_2-adrenoceptors.

Compound	IA	Compound	IA	Compound	IA
A01	63.60	B06	85.48	C02	74.49
A02	70.20	B07	62.38	C03	84.63
A03	75.63	B08	78.11	C04	81.30
A04	75.41	B09	83.14	C05	65.48
A05	66.98	B10	82.73	C06	70.02
A06	71.18	B11	72.31	C07	76.95
A07	70.85	B12	72.65	C08	72.87
A08	53.78	B13	85.32	C09	78.84
B04	64.67	B14	78.85	C10	67.62
B05	69.93	C01	74.24	C11	73.39
salmeterol	48.36	olodaterol	50.62	isoprenaline	100

with potency exceeding the latter. Their selectivity was between that of the 5-hydroxy-4*H*-benzo [1,4]oxazin-3-one analogue XY-3 [22] and the 8-hydroxyquinolin-2(*1H*)-one analogue XY-2 [21], and their potency was similar to or higher than that of XY-2 and XY-3. The data further indicate that methyl substitution was superior to methoxy substitution in terms of potency. Chlorine substitution was also favorable as suggested by the β_2 pEC_{50} values of **C07**, **C08** and **C09** (10.90, 11.20 and 10.74, respectively). The hydroxyl analogues **C10** and **C11** also had high potency comparing with the references but their selectivity, similar to the methoxy analogues **C01**–**C03**, was lower than that of salmeterol and olodaterol. There was no obviously improvement in potency and selectivity in **(*R*)-C07** and **(*R*)-C08** compared with **C07** and **C08**.

Our strategy was to maintain the 5-(2-amino-1-hydroxyethyl)-8-hydroxyquinolin-2(1*H*)-one moiety which maximally preserve affinity and selectivity of the compounds to the β_2-AR, and then varied the number of the methyl group on the α carbon relative to the central amino accompanied with different substitutions on the tail benzene ring. It was demonstrated that dimethyl or methyl substitution on the α carbon as in **B09**–**B10** and **C04**–**C06** promoted activity as compared with compounds **A01-A03** without substitution (compare Table 2 to Table 1, and Table 3 to Table 1). A similar feature was also found among the fluorinated compounds **B07**–**B08** and **A04-A05** (compare Tables 2 and 1). In general, **B** and **C** series compounds with one or two methyl substitution had similar activity and selectivity (compare Tables 2 and 3). To investigate the role of stereochemistry on the compounds' activity, we synthesized the (*R*) enantiomers of the highly potent compounds **B04**, **C07** and **C08** with respect to the chiral center with the β-hydroxyl group. The (*R*) enantiomers were found to have similar β_2-agonistic activities compared with the racemates (Tables 2 and 3). Despite of the fact that **(*R*,*R*)-B04**, **(*R*)-C07** and **(*R*)-C08** had higher β_2-agonistic activities compared with salmeterol and olodaterol, they also had higher β_1-agonistic activities rendering their selectivity one order of magnitude lower than that of salmeterol or olodaterol.

2.3. Intrinsic activity study

A subset of the test compounds were then tested for their abilities to stimulate cAMP accumulation in HEK293 cells endogenously expressing human β_2-ARs, and intrinsic activities (IAs) of the compounds were determined where the IA of isoprenaline was defined as 100%. These compounds exhibited IAs ranging from 50% to 86% where the IAs of the long-acting β_2-agonists salmeterol and olodaterol were found to be 48.36% and 50.62%, respectively (Table 4). It was concluded that all the new compounds tested were partial β_2-agonists in this system.

2.4. The smooth muscle relaxant activities on isolated tracheal strips

The smooth muscle relaxant effect is an important indicator of the biological activity of β_2-agonists *in vivo*. The test compounds were profiled through a smooth muscle relaxation assay on isolated guinea pig tracheal strips (Table 5). Isoprenaline, salmeterol and olodaterol were used as reference compounds. In order to compare the effects of different compounds assaying on different tissue preparations, relaxant responses of the compounds were expressed as percentages of the maximal contractile effects induced by histamine. Concentration - relaxation curves were constructed using GraphPad Prism, version 8.0. E_{max} (maximal relaxant effect) represents the maximal relaxant effect obtained with the maximal concentrations of the compounds. The potency (pD_2) value

Table 5
Summary of the values of pD2 and Emax of compounds and the reference β2-agonists on isolated guinea pig trachea.

Compound	pD_2	E_{max}	Compound	pD_2	E_{max}
A01	7.72 ± 0.14	125.03 ± 6.35* #a	B13	7.61 ± 0.17	163.50 ± 6.39
A02	7.66 ± 0.15	144.08 ± 12.24* #a	B14	7.22 ± 0.15* #a	141.24 ± 11.15* #a
A03	8.21 ± 0.30	138.26 ± 10.64* #a	(*R*,*R*)-B04	7.35 ± 0.13* #a	94.33 ± 5.01* #a
A04	7.50 ± 0.14[a]	153.51 ± 10.48*	C01	7.86 ± 0.21	160.72 ± 9.63
A05	7.39 ± 0.17*[a]	153.33 ± 7.28* #a	C02	7.45 ± 0.09*[a]	112.14 ± 13.71* #a
A06	8.83 ± 0.38* #a	76.71 ± 7.25* #a	C03	6.86 ± 0.41* #a	141.17 ± 8.33* #a
A07	6.93 ± 0.07* #a	95.51 ± 3.91* #a	C04	7.35 ± 0.09* #a	92.66 ± 7.22* #a
A08	7.74 ± 0.23	111.22 ± 7.67* #a	C05	7.37 ± 0.34[a]	151.90 ± 16.19
B01	7.87 ± 0.16	95.26 ± 6.12* #a	C06	5.87 ± 0.05* #a	122.43 ± 5.65* #a
B02	7.16 ± 0.10* #a	104.57 ± 9.51* #a	C07	7.69 ± 0.26	111.58 ± 4.01* #a
B03	8.31 ± 0.24#	86.61 ± 6.42* #a	C08	5.29 ± 0.41* ba	213.75 ± 49.28
B04	7.62 ± 0.14[a]	129.54 ± 15.45* #a	C09	7.58 ± 0.16[a]	123.83 ± 11.62* #a
B05	7.33 ± 0.13* #a	196.39 ± 9.69	C10	6.90 ± 0.05* #a	82.74 ± 2.71[a] #a
B06	7.48 ± 0.13[a]	103.92 ± 11.34* #a	C11	6.37 ± 0.03* #a	102.89 ± 5.56* #a
B07	6.61 ± 0.56* #a	163.31 ± 11.12	(R)-C07	7.56 ± 0.20[a]	95.33 ± 4.37* #a
B08	6.57 ± 0.05* #a	100.32 ± 12.63* #a	(R)-C08	5.97 ± 0.21* #a	134.31 ± 9.76* #a
B09	7.39 ± 0.25[a]	92.13 ± 2.60* #a	salmeterol	7.71 ± 0.14	172.45 ± 9.61
B10	7.58 ± 0.10[a]	95.87 ± 10.65* #a	olodaterol	8.15 ± 0.27	169.76 ± 6.01
B11	7.08 ± 0.52[a]	119.02 ± 10.11* #a	isoprenaline	7.84 ± 0.19	180.57 ± 9.20
B12	7.97 ± 0.24	71.26 ± 16.90* #a			

The pharmacodynamic parameters were determined in isolated guinea-pig tracheal slices pre-contracted with histamine (5 × 10−5 M). Data are expressed as means ± S.D. (n = 4).
*p < 0.05, compared with that of Iso.
#p < 0.05, compared with that of Sal.
ap < 0.05, compared with that of Olo.

represents the negative logarithm of the concentration of the drug, at which a relaxation is equal to 50% of the drug's own maximal effect (EC_{50}). EC_{50} was determined by non-linear regression analysis using GraphPad Prism, version 8.0. The probability of differences between the mean data of two groups was calculated by paired Student's *t*-test with a statistical software SPSS 26.0 and $p < 0.05$ was considered significant.

As shown in Fig. 3 and Table 5, the E_{max} values of compounds **B05** and **C08** were higher than those of the other test compounds and were comparable to those of isoprenaline, salmeterol and olodaterol. Compounds **B05** and **C08** had lower pD_2 values relative to the references. Compounds **B07**, **B13** and **C01** were found to produce prominent smooth muscle relaxant effects. Their E_{max} values were fair (>160%) as compared with references (170–180%). The pD_2 values of **B13**, **C01**, **A04** and **A05** were similar to those of isoprenaline and salmeterol. The E_{max} values of compounds **A04** and **A05** were lower than those of the references. The rest of the test compounds had much lower E_{max} values relative to the references.

2.5. The measurement of onset time and duration of action on tracheal muscles

On the basis of the activity data from the cAMP assay and the smooth muscle relaxation assay, the single methyl-substituted

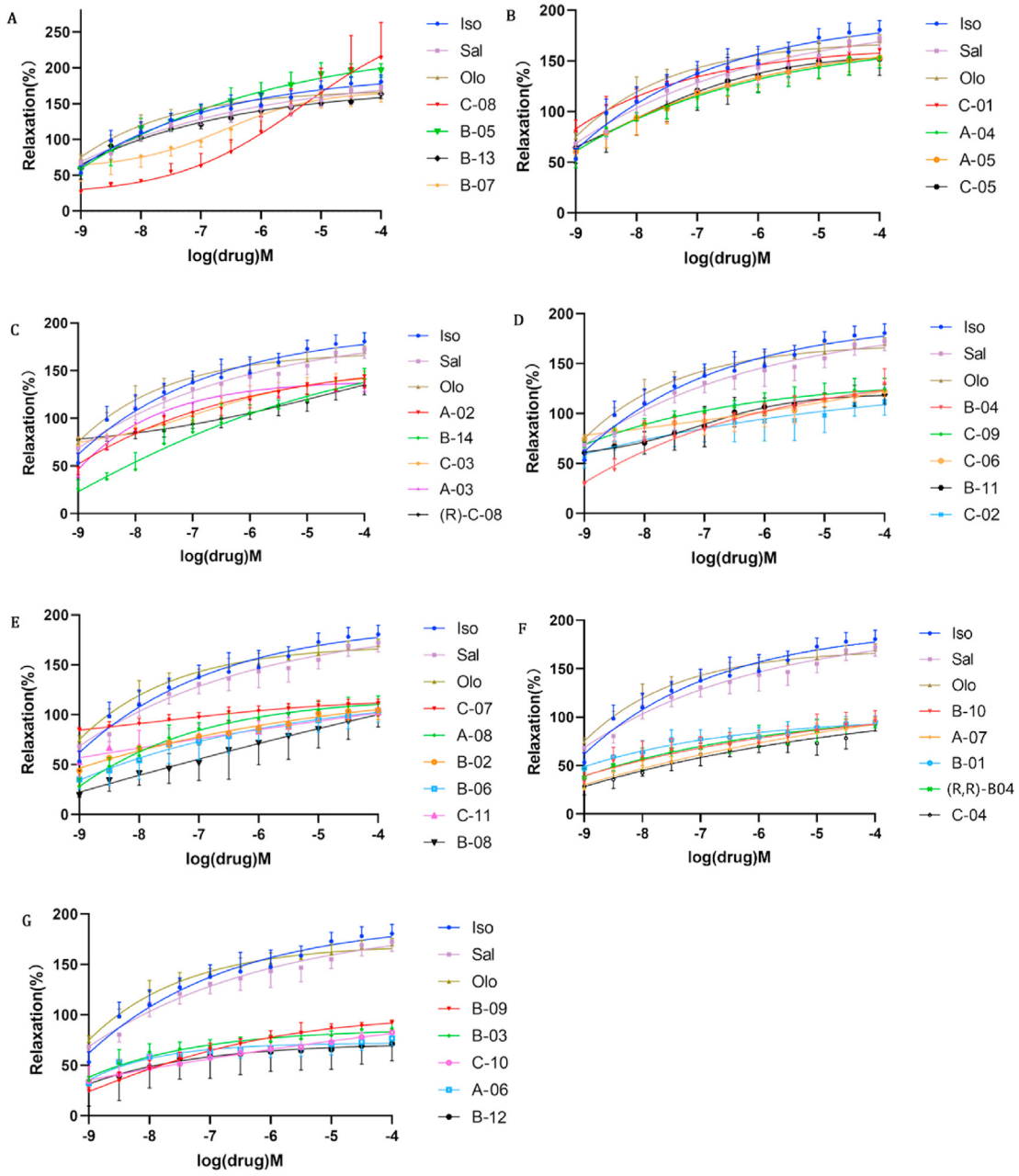


Fig. 3. Summary of concentration – relaxation curves of selected compounds on contracted guinea pig trachea. Isoprenaline, salmeterol and olodaterol were served as references. Data were expressed as relaxation percentages of the maximum contraction induced by histamine (5×10^{-5} M) and as means ± S.D. (n = 4). (A) Concentration – relaxation curves of compounds **B05**, **B07**, **B13**, and **C08** on contracted guinea pig trachea. (B) Concentration – relaxation curves of compounds **A04**, **A05**, **C01**, and **C05** on contracted guinea pig trachea. (C) Concentration – relaxation curves of compounds **A02**, **A03**, **B14** and **(*R*)-C08** on contracted guinea pig trachea. (D) Concentration – relaxation curves of compounds **B04**, **B11**, **C02**, **C06**, and **C09** on contracted guinea pig trachea. (E) Concentration – relaxation curves of compounds **A08**, **B02**, **B06**, **B08**, **C07** and **C11** on contracted guinea pig trachea. (F) Concentration – relaxation curves of compounds **A07**, **B01**, **B10**, **C04** and **(*R,R*)-C04** on contracted guinea pig trachea. (G) Concentration – relaxation curves of compounds **A06**, **B03**, **B09**, **B12** and **C10** on contracted guinea pig trachea.

analogue **B05** and the double methyl-substituted analogue **C08**, with high potency and selectivity for the β_2-AR and high tracheal relaxant efficacy, were then evaluated for onset time and duration of action on isolated tracheal muscles. As shown in Table 6, rapid onset of action and long duration of action were observed for compounds **B05** and **C08**. Compound **B05** exhibited a slightly longer onset time compared with isoprenaline, salmeterol and olodaterol. The onset time of **C08** was similar to that of isoprenaline. In addition to their potential fast action onset, compounds **B05** and **C08** were also found to produce long-lasting smooth muscle relaxant effects for over 12 h. These data suggest that compounds **B05** and **C08** might behave as long-acting β_2-agonists *in vivo*.

2.6. Molecular docking analysis of compounds B05 and C08

To gain a deeper insight into the superior biological activities of compounds **B05** and **C08**, the binding poses of the compounds on the human β_2-AR (PDB: 4LDL) were obtained by molecular docking using the Schrödinger Maestro software package. The 8-hydroxyquinolin-2(1*H*)-one moiety of **B05** and **C08** exhibited multiple interactions with the β_2-AR (Fig. 4). The phenolic hydroxyl group formed hydrogen bond interactions with Ser207. Ser203 formed two hydrogen bonds with the oxygen atom and the nitrogen atom on the lactam. In addition, we also observed $\pi-\sigma$ interactions and $\pi-\pi$ interactions between the 8-hydroxyquinolin-2(1*H*)-one moiety and residues Val114 and Phe290. The amino group of the 2-amino-1-phenylethanol core was charged at pH 7.4 leading to a salt bridge with Asp113. Residues Asp113 and Asn312 also interacted with the oxygen atom of the β-OH group via two hydrogen bonds. Consequently, these key interactions might contribute to the stable binding of the 5-(2-amino-1-hydroxyethyl)-8-hydroxyquinolin-2(1*H*)-one scaffold to the β_2-AR and the remarkable biological activities of the 5-(2-amino-1-hydroxyethyl)-8-hydroxyquinolin-2(1*H*)-one analogues. The propylbenzene moiety fitted well into the hydrophobic pocket making hydrophobic interactions with Ile309 and other hydrophobic residues. Interestingly, the oxygen atom on the terminal methoxy group in **B05** and the chlorine atom in **C08** did not engage in hydrogen bond interactions with any amino acid residues in the binding pocket. The emphasis of hydrophobic interactions in the tail region could be the reason for the small differences in the experimental β_2 pEC_{50} values for the 5-(2-amino-1-hydroxyethyl)-8-hydroxyquinolin-2(1*H*)-one derivatives with different substitutions on the terminal benzene ring (Tables 1–3).

Table 6
Onset time (Ot_{50}) and duration of action (DoA) of B05, C08, isoprenaline, salmeterol and olodaterol.

Compound	Ot_{50} (min)	DoA (h)
B05	4.97 ± 0.11	>12
C08	3.43 ± 0.31	>12
isoprenaline	3.10 ± 0.42	–
salmeterol	4.23 ± 0.34	>12
olodaterol	2.31 ± 0.49	>12

3. Conclusion

We have designed and synthesized a new cohort of 5-(2-amino-1-hydroxyethyl)-8-hydroxyquinolin-2(1*H*)-one derivatives as β_2-AR agonists and studied their β_2-agonistic activities. Activity data suggest that α-dimethyl or α-methyl substitution on the amino tail group mildly improved activity. Compounds **B05** and **C08** were found to be potent (EC_{50} < 20 pM) and selective β_2-agonists. They behaved as partial β_2-agonists in non-overexpressed HEK293 cells and possessed rapid smooth muscle relaxant actions and long duration of action in isolated guinea pig tracheal strip preparations. Experimental data and molecular docking once again verify the high β_2-agonistic activity of the 5-(2-amino-1-hydroxyethyl)-8-hydroxyquinolin-2(1*H*)-one analogues. Collectively, the present data support that **B05** and **C08** could be better β_2-agonists for the treatment of chronic respiratory diseases.

4. Experimental section

4.1. Chemistry

NMR spectroscopic measurement was performed for compounds dissolved in DMSO-d_6 on a 400 MHz or 600 MHz Bruker spectrometer. The high-resolution mass spectra (HRMS) of the target compounds were produced by an Agilent 6530 accurate-mass Q-TOF LC-MS system. All the final compounds were purified by column chromatography through silica gel (300–400 mesh). Thin layer chromatography (TLC) on silica gel plates was used to monitor the progress of the reaction. All starting materials were purchased from commercial suppliers.

4.1.1. General procedures for the preparation of compounds *4–01* to *4–08*

To a solution of NaH (2.39 g, 99.0 mmol) in anhydrous THF (100 mL) chilled on ice, diethyl (cyanomethyl)phosphonate (14.0 g, 79.3 mmol) in anhydrous THF (50 mL) was added. The reaction was stirred at room temperature for 50 min. Then, a solution of substituted benzaldehyde (66.2 mmol) was added dropwise to the reaction mixture. The reaction was stirred at room temperature for 1 h. Removal of the solvent gave a residue. The residue was washed with H_2O (300 mL) and filtered dried to give compound **2**. To a

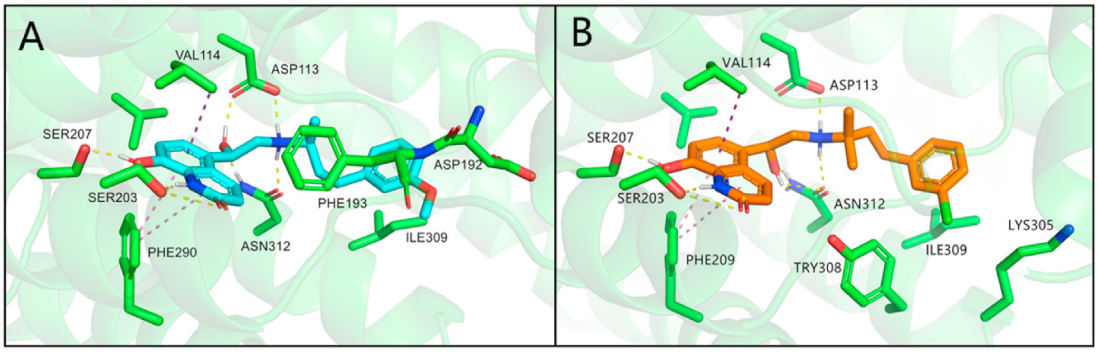


Fig. 4. Binding modes of compounds **B05** (A) and **C08** (B) at the β_2-adrenoceptor active site.

solution of **2** in MeOH, 10% Pd/C (0.1 equiv.) was added and the mixture was stirred at room temperature for 1 h under 2 atm of hydrogen. The Pd/C was filtered and the solvent was evaporated to yield compound **3**. To a solution of **3** in anhydrous THF, BH_3·THF was added under nitrogen atmosphere. The solution was stirred at 60 °C for 40 min. The reaction was quenched with MeOH and evaporated in vacuum to produce compounds **4**–**01** to **4**–**08**.

*4.1.2. General procedures for the preparation of compounds **8**–**01** to **8**–**14***

To a solution of substituted benzaldehyde (80.6 mmol) in anhydrous acetone (100 mL), 1 M NaOH (80.6 mmol) in water was added. The reaction was stirred at room temperature for 2 h. Removal of the solvent gave a residue. H_2O (300 mL) was added to the residue. The mixture was extracted with EtOAc (3 × 50 mL), washed with brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure to obtain compound **6**. To a solution of **3** in EtOAc, 10% Pd/C (0.1 equiv.) was added. The mixture was stirred at room temperature for 1 h under 2 atm of hydrogen. The Pd/C was filtered and the solvent was removed to yield compound **7**. $HCOONH_4$ was added to a solution of **7** and 10% Pd/C (0.1 equiv.) in MeOH (100 mL). The reaction mixture was heated to 75 °C for 5 h. The mixture was cool to room temperature and the solvent was evaporated to yield a residue. H_2O (100 mL) was added to the residue. The mixture was extracted with EtOAc (3 × 50 mL), washed with brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure to obtain compounds **8**–**01** to **8**–**14**.

*4.1.3. General procedures for the preparation of compounds **13**–**01** to **13**–**11***

Compounds **13**–**01** to **13**–**11** were synthesized from different substituted benzaldehydes as previously reported [21].

*4.1.4. General procedures for the preparation of compounds **A01-A08**, **B01**–**B14**, and **C01**–**C11***

Compounds **A01-A08**, **B01**–**B14**, and **C01**–**C11** were obtained by combining intermediate **14** with different primary amines (**4**–**01** to **4**–**08**, **8**–**01** to **8**–**14** and **13**–**01** to **13**–**11**) in a 2-step reaction as previously reported [21].

*4.1.5. 8-Hydroxy-5-(1-hydroxy-2-((3-(o-tolyl)propyl)amino)ethyl) quinolin-2(1H)-one hydrochloride (**A01**)*

White solid; yield 97%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.53 (s, 2H), 9.48 (s, 1H), 8.76 (s, 1H), 8.31 (d, J = 9.9 Hz, 1H), 7.32–7.07 (m, 2H), 7.02 (dd, J = 10.3, 5.0 Hz, 3H), 6.55 (d, J = 9.8 Hz, 1H), 5.48 (dd, J = 10.0, 2.2 Hz, 1H), 3.18–2.78 (m, 4H), 2.61 (td, J = 7.5, 3.2 Hz, 2H), 2.40–2.11 (m, 3H), 2.07–1.81 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.10, 142.82, 140.06, 136.79, 135.89, 128.32, 128.02, 127.85, 127.68, 126.06, 124.70, 121.40, 119.09, 116.06, 113.46, 64.60, 52.86, 46.12, 31.40, 26.31, 20.41. Calcd. for $C_{21}H_{24}N_2O_3$ $[M+H]^+$ 353.1787; found 351.1961.

*4.1.6. 8-Hydroxy-5-(1-hydroxy-2-((3-(m-tolyl)propyl)amino)ethyl) quinolin-2(1H)-one hydrochloride (**A02**)*

White solid; yield 96%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.53 (s, 2H), 9.48 (s, 1H), 8.76 (s, 1H), 8.31 (d, J = 9.9 Hz, 1H), 7.32–7.07 (m, 2H), 7.02 (dd, J = 10.3, 5.0 Hz, 3H), 6.55 (d, J = 9.8 Hz, 1H), 5.48 (dd, J = 10.0, 2.2 Hz, 1H), 3.18–2.78 (m, 4H), 2.61 (td, J = 7.5, 3.2 Hz, 2H), 2.40–2.11 (m, 3H), 2.07–1.81 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.10, 142.82, 140.06, 136.79, 135.89, 128.32, 128.02, 127.85, 127.68, 126.06, 124.70, 121.40, 119.09, 116.06, 113.46, 64.60, 52.86, 46.12, 31.40, 26.31, 20.41. Calcd. for $C_{21}H_{24}N_2O_3$ $[M+H]^+$ 353.1787; found 353.1886.

*4.1.7. 8-Hydroxy-5-(1-hydroxy-2-((3-(p-tolyl)propyl)amino)ethyl) quinolin-2(1H)-one hydrochloride (**A03**)*

White solid; yield 93%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.53 (s, 2H), 9.48 (s, 1H), 8.76 (s, 1H), 8.31 (d, J = 9.9 Hz, 1H), 7.32–7.07 (m, 2H), 7.02 (dd, J = 10.3, 5.0 Hz, 3H), 6.55 (d, J = 9.8 Hz, 1H), 5.48 (dd, J = 10.0, 2.2 Hz, 1H), 3.18–2.78 (m, 4H), 2.61 (td, J = 7.5, 3.2 Hz, 2H), 2.40–2.11 (m, 3H), 2.07–1.81 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.10, 142.82, 140.06, 136.79, 135.89, 128.32, 128.02, 127.85, 127.68, 126.06, 124.70, 121.40, 119.09, 116.06, 113.46, 64.60, 52.86, 46.12, 31.40, 26.31, 20.41. Calcd. for $C_{21}H_{24}N_2O_3$ $[M+H]^+$ 353.1787; found 351.1884.

*4.1.8. 5-(2-((3-(3-fluorophenyl)propyl)amino)-1-hydroxyethyl)-8-hydroxyquinolin-2(1H)-one hydrochloride (**A04**)*

White solid; yield 94%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.52 (s, 2H), 9.50 (s, 1H), 8.76 (s, 1H), 8.31 (d, J = 9.9 Hz, 1H), 7.34 (dd, J = 14.4, 7.8 Hz, 1H), 7.15 (d, J = 8.1 Hz, 1H), 7.10–6.96 (m, 4H), 6.55 (d, J = 9.8 Hz, 1H), 5.48 (d, J = 8.3 Hz, 1H), 3.26–2.82 (m, 4H), 2.78–2.60 (m, 2H), 2.00 (dd, J = 15.2, 8.2 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.94, 161.53, 161.16, 144.27, 144.20, 143.86, 136.86, 130.77, 130.69, 129.07, 128.98, 124.94, 124.92, 122.59, 120.16, 117.12, 115.57, 115.36, 114.50, 113.41, 113.20, 65.71, 53.86, 47.00, 32.09, 27.04. Calcd. for $C_{20}H_{21}FN_2O_3$ $[M+H]^+$ 357.1536; found 357.1538.

*4.1.9. 5-(2-((3-(4-fluorophenyl)propyl)amino)-1-hydroxyethyl)-8-hydroxyquinolin-2(1H)-one hydrochloride (**A05**)*

White solid; yield 91%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.52 (s, 2H), 9.38 (s, 1H), 8.74 (s, 1H), 8.29 (d, J = 9.9 Hz, 1H), 7.27 (dd, J = 8.5, 5.6 Hz, 2H), 7.20–7.08 (m, 3H), 7.01 (d, J = 8.1 Hz, 1H), 6.55 (d, J = 9.9 Hz, 1H), 5.46 (d, J = 9.9 Hz, 1H), 3.13–2.85 (m, 4H), 2.69–2.57 (m, 2H), 2.01–1.87 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.00, 160.93, 160.08, 159.33, 142.78, 136.29, 136.27, 135.73, 129.46, 129.41, 127.97, 127.89, 121.53, 119.08, 116.03, 114.53, 114.39, 113.39, 64.62, 59.15, 52.75, 45.96, 30.54, 26.41, 20.16, 13.48. Calcd. for $C_{20}H_{21}FN_2O_3$ $[M-H]^-$ 355.1536; found 355.1510.

*4.1.10. 5-(2-((3-(3,5-difluorophenyl)propyl)amino)-1-hydroxyethyl)-8-hydroxyquinolin-2(1H)-one hydrochloride (**A06**)*

White solid; yield 93%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.53 (s, 2H), 9.56 (s, 1H), 8.76 (s, 1H), 8.32 (d, J = 9.9 Hz, 1H), 7.16 (d, J = 8.2 Hz, 1H), 7.11–6.91 (m, 4H), 6.55 (d, J = 9.8 Hz, 1H), 5.49 (d, J = 9.8 Hz, 1H), 3.06 (t, J = 10.5 Hz, 1H), 3.02–2.87 (m, 3H), 2.79–2.63 (m, 2H), 2.10–1.94 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 163.70, 163.61, 162.07, 161.98, 161.18, 145.97, 143.88, 136.92, 129.09, 128.95, 122.53, 120.17, 117.14, 114.51, 112.05, 112.02, 111.92, 111.89, 102.19, 102.02, 101.85, 65.69, 53.86, 46.79, 31.97, 26.67. Calcd. for $C_{20}H_{20}F_2N_2O_3$ $[M+H]^+$ 375.1442; found 375.1539.

*4.1.11. 8-Hydroxy-5-(1-hydroxy-2-((3-(3-(trifluoromethyl)phenyl) propyl)amino)ethyl)quinolin-2(1H)-one hydrochloride (**A07**)*

White solid; yield 93%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.50 (d, J = 7.5 Hz, 2H), 9.29 (s, 1H), 8.71 (s, 1H), 8.27 (d, J = 9.9 Hz, 1H), 7.60 (s, 1H), 7.56 (t, J = 7.1 Hz, 2H), 7.15 (d, J = 8.1 Hz, 1H), 7.00 (d, J = 8.2 Hz, 1H), 6.55 (d, J = 9.8 Hz, 1H), 6.15 (d, J = 3.6 Hz, 1H), 5.44 (d, J = 10.0 Hz, 1H), 3.07 (s, 1H), 2.99 (d, J = 24.7 Hz, 3H), 2.81–2.70 (m, 2H), 2.01 (qd, J = 13.6, 7.2 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.17, 143.86, 142.80, 136.76, 133.04, 129.92, 129.71, 129.50, 129.02, 125.68, 125.30, 125.28, 123.88, 123.35, 122.66, 120.18, 117.11, 114.45, 65.74, 53.76, 46.99, 32.09, 27.23. Calcd. for $C_{21}H_{21}F_3N_2O_3$ $[M+H]^+$ 407.1504; found 407.1629.

*4.1.12. 8-Hydroxy-5-(1-hydroxy-2-((3-(3,4,5-trimethoxyphenyl) propyl)amino)ethyl)quinolin-2(1H)-one hydrochloride (**A08**)*

White solid; yield 97%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.49 (d,

J = 8.4 Hz, 2H), 9.31 (s, 1H), 8.70 (s, 1H), 8.28 (d, J = 9.9 Hz, 1H), 7.15 (d, J = 8.2 Hz, 1H), 7.00 (d, J = 8.2 Hz, 1H), 6.55 (d, J = 9.9 Hz, 1H), 6.53 (s, 1H), 6.15 (d, J = 3.6 Hz, 1H), 5.45 (d, J = 10.0 Hz, 1H), 3.76 (s, 6H), 3.62 (s, 3H), 3.06 (d, J = 8.2 Hz, 1H), 3.04−2.98 (m, 1H), 2.97−2.90 (m, 2H), 2.60 (dd, J = 15.0, 7.5 Hz, 2H), 2.06−1.88 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.16, 153.25, 143.86, 136.87, 136.78, 136.16, 129.05, 128.99, 122.66, 120.16, 117.10, 114.46, 105.98, 65.74, 60.42, 56.26, 53.84, 47.09, 32.79, 27.35. Calcd. for $C_{23}H_{28}N_2O_6$ $[M+H]^+$ 429.1947; found 429.2046.

4.1.13. 5-(2-((4-(2-chlorophenyl)butan-2-yl)amino)-1-hydroxyethyl)-8-hydroxyquinolin-2(1H)-one hydrochloride (**B01**)

White solid; yield 91%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.47 (s, 2H), 9.63−9.24 (m, 1H), 8.59 (s, 1H), 8.30 (dd, J = 9.9, 3.5 Hz, 1H), 7.37−7.26 (m, 2H), 7.22 (dd, J = 10.0, 4.2 Hz, 2H), 7.18 (dd, J = 8.4, 3.7 Hz, 1H), 7.01 (dd, J = 8.2, 1.2 Hz, 1H), 6.55 (d, J = 9.9 Hz, 1H), 5.56−5.29 (m, 1H), 3.22 (d, J = 3.8 Hz, 1H), 3.11−2.91 (m, 2H), 2.71 (tt, J = 22.0, 11.3 Hz, 1H), 2.63−2.52 (m, 1H), 2.21−2.03 (m, 1H), 1.90−1.65 (m, 1H), 1.31 (dd, J = 15.1, 6.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.14, 143.85, 141.38, 141.35, 136.80, 129.14, 128.98, 128.89, 128.86, 128.75, 128.72, 126.49, 122.65, 120.23, 117.15, 114.50, 65.87, 65.69, 53.94, 53.89, 51.21, 51.04, 49.06, 34.64, 33.71, 31.54, 31.38, 16.20, 15.58. Calcd. for $C_{21}H_{23}ClN_2O_3$ $[M+H]^+$ 387.1397; found 387.1890.

4.1.14. 5-(2-((4-(3-chlorophenyl)butan-2-yl)amino)-1-hydroxyethyl)-8-hydroxyquinolin-2(1H)-one hydrochloride (**B02**)

White solid; yield 92%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.47 (s, 2H), 9.53 (s, 1H), 8.61 (s, 1H), 8.32 (d, J = 10.0 Hz, 1H), 7.31 (ddd, J = 12.1, 7.6, 3.2 Hz, 2H), 7.23 (d, J = 6.8 Hz, 2H), 7.18 (dd, J = 8.0, 3.8 Hz, 1H), 7.02 (d, J = 8.2 Hz, 1H), 6.55 (d, J = 9.8 Hz, 1H), 5.52 (d, J = 9.5 Hz, 1H), 3.23 (d, J = 6.8 Hz, 1H), 3.09−2.93 (m, 2H), 2.71 (ddd, J = 14.7, 10.0, 4.8 Hz, 1H), 2.65−2.53 (m, 1H), 2.19−2.05 (m, 1H), 1.79 (ddd, J = 18.1, 13.0, 4.6 Hz, 1H), 1.31 (dd, J = 15.5, 6.5 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.08, 158.74, 158.72, 142.78, 141.85, 141.83, 135.64, 128.83, 128.80, 128.03, 128.01, 127.89, 121.64, 119.92, 119.89, 119.14, 119.11, 116.05, 115.63, 115.59, 113.44, 113.39, 112.84, 110.78, 110.75, 64.79, 64.62, 54.81, 54.80, 54.32, 52.81, 52.74, 50.08, 49.86, 33.43, 32.43, 30.45, 30.30, 15.13, 14.45. Calcd. for $C_{21}H_{23}ClN_2O_3$ $[M+H]^+$ 387.1397; found 387.1471.

4.1.15. 5-(2-((4-(4-chlorophenyl)butan-2-yl)amino)-1-hydroxyethyl)-8-hydroxyquinolin-2(1H)-one hydrochloride (**B03**)

White solid; yield 93%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.48 (s, 2H), 9.60 (s, 1H), 8.62 (d, J = 6.1 Hz, 1H), 8.34 (dd, J = 9.9, 3.2 Hz, 1H), 7.34 (d, J = 4.3 Hz, 1H), 7.30−7.27 (m, 1H), 7.23 (dd, J = 15.3, 8.5 Hz, 2H), 7.18 (dd, J = 5.4, 2.8 Hz, 1H), 7.07−6.97 (m, 1H), 6.55 (d, J = 9.9 Hz, 1H), 5.59−5.45 (m, 1H), 3.22 (d, J = 3.6 Hz, 1H), 3.02 (d, J = 5.8 Hz, 2H), 2.72 (ddd, J = 18.6, 9.9, 4.6 Hz, 1H), 2.59 (ddd, J = 14.0, 11.0, 7.0 Hz, 1H), 2.13 (tdd, J = 10.6, 7.1, 3.8 Hz, 1H), 1.88−1.68 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.16, 143.87, 141.40, 141.37, 140.43, 136.91, 131.08, 130.67, 130.64, 129.17, 128.95, 128.88, 128.85, 128.79, 128.75, 128.72, 126.48, 122.58, 120.22, 117.16, 114.53, 65.84, 65.66, 53.94, 53.89, 53.79, 51.29, 51.12, 49.05, 34.61, 34.37, 33.72, 33.52, 31.55, 31.38, 30.77, 30.62, 16.17, 15.58. Calcd. for $C_{21}H_{23}ClN_2O_3$ $[M+H]^+$ 387.1397; found 387.1489.

4.1.16. 8-Hydroxy-5-(1-hydroxy-2-((4-(2-methoxyphenyl)butan-2-yl)amino)ethyl)quinolin-2(1H)-one hydrochloride (**B04**)

White solid; yield 96%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.49 (s, 2H), 9.47 (d, J = 55.1 Hz, 1H), 8.57 (s, 1H), 8.32 (dd, J = 10.0, 1.2 Hz, 1H), 7.28−7.10 (m, 3H), 7.07−6.99 (m, 1H), 6.95 (dd, J = 7.8, 3.5 Hz, 1H), 6.87 (td, J = 7.4, 3.5 Hz, 1H), 6.55 (dd, J = 9.9, 2.2 Hz, 1H), 5.60−5.41 (m, 1H), 3.75 (d, J = 2.6 Hz, 3H), 3.23 (s, 1H), 3.09−2.90 (m, 2H), 2.72−2.60 (m, 1H), 2.60−2.52 (m, 1H), 2.14−1.92 (m, 1H), 1.86−1.64 (m, 1H), 1.31 (dd, J = 14.0, 6.5 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.09, 156.34, 156.32, 142.80, 135.77, 128.94, 128.91, 128.10, 128.08, 128.06, 128.03, 127.86, 126.89, 121.51, 119.75, 119.73, 119.11, 116.06, 113.42, 110.11, 110.09, 64.71, 64.57, 54.63, 52.89, 52.81, 50.03, 49.89, 32.03, 31.18, 25.05, 24.86, 15.21, 14.54. Calcd. for $C_{22}H_{26}N_2O_4$ $[M+H]^+$ 383.1893; found 383.1981.

4.1.17. 8-Hydroxy-5-(1-hydroxy-2-((4-(3-methoxyphenyl)butan-2-yl)amino)ethyl)quinolin-2(1H)-one hydrochloride (**B05**)

White solid; yield 94%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (s, 2H), 9.28 (s, 1H), 8.56 (s, 1H), 8.27 (d, J = 9.8 Hz, 1H), 7.29−7.10 (m, 2H), 7.06−6.96 (m, 1H), 6.90−6.66 (m, 3H), 6.55 (d, J = 9.8 Hz, 1H), 5.47 (d, J = 9.5 Hz, 1H), 3.72 (d, J = 4.6 Hz, 3H), 3.21 (s, 1H), 3.02 (d, J = 5.7 Hz, 2H), 2.75−2.63 (m, 1H), 2.57 (dd, J = 12.0, 7.9 Hz, 1H), 2.09 (d, J = 9.6 Hz, 1H), 1.83−1.67 (m, 1H), 1.29 (dd, J = 14.8, 6.5 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.08, 158.74, 158.72, 142.78, 141.85, 141.83, 135.64, 128.83, 128.80, 128.03, 128.01, 127.89, 121.64, 119.92, 119.89, 119.14, 119.11, 116.05, 113.44, 113.39, 110.78, 110.75, 64.79, 64.62, 54.81, 54.80, 54.32, 52.81, 52.74, 50.08, 49.86, 33.43, 32.43, 30.45, 30.30, 30.20, 28.40, 15.24, 15.13, 14.56, 14.45. Calcd. for $C_{22}H_{26}N_2O_4$ $[M+H]^+$ 383.1893; found 383.1999.

4.1.18. 8-Hydroxy-5-(1-hydroxy-2-((4-(4-methoxyphenyl)butan-2-yl)amino)ethyl)quinolin-2(1H)-one hydrochloride (**B06**)

White solid; yield 98%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (s, 2H), 9.42 (s, 1H), 8.58 (s, 1H), 8.30 (dd, J = 9.9, 3.2 Hz, 1H), 7.28−7.08 (m, 3H), 7.02 (d, J = 8.2 Hz, 1H), 6.93−6.78 (m, 2H), 6.56 (d, J = 9.9 Hz, 1H), 6.14 (s, 1H), 5.57−5.39 (m, 1H), 3.72 (d, J = 3.0 Hz, 3H), 3.19 (d, J = 8.4 Hz, 1H), 3.02 (s, 2H), 2.65 (ddd, J = 14.3, 9.6, 4.8 Hz, 1H), 2.60−2.53 (m, 1H), 2.21−1.97 (m, 1H), 1.92−1.59 (m, 1H), 1.30 (dd, J = 15.0, 6.5 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.17, 158.04, 143.86, 136.77, 133.15, 133.11, 129.70, 129.67, 129.14, 128.97, 122.69, 120.20, 120.17, 117.12, 114.46, 114.29, 114.26, 65.85, 65.67, 55.45, 53.87, 53.80, 51.22, 51.02, 34.88, 33.90, 30.63, 30.46, 16.20, 15.54. Calcd. for $C_{22}H_{26}N_2O_4$ $[M+H]^+$ 383.1893; found 383.1988.

4.1.19. 5-(2-((4-(3-fluorophenyl)butan-2-yl)amino)-1-hydroxyethyl)-8-hydroxyquinolin-2(1H)-one hydrochloride (**B07**)

White solid; yield 97%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.47 (s, 2H), 9.51 (s, 1H), 8.60 (s, 1H), 8.31 (dd, J = 10.0, 2.4 Hz, 1H), 7.33 (dtd, J = 8.1, 6.4, 4.7 Hz, 1H), 7.18 (dd, J = 8.2, 2.5 Hz, 1H), 7.13−7.06 (m, 2H), 7.05−6.98 (m, 2H), 6.55 (d, J = 9.9 Hz, 1H), 5.51 (td, J = 9.1, 4.5 Hz, 1H), 3.22 (s, 1H), 3.01 (t, J = 10.5 Hz, 2H), 2.75 (dt, J = 16.4, 5.8 Hz, 1H), 2.68−2.56 (m, 1H), 2.21−2.08 (m, 1H), 1.87−1.70 (m, 1H), 1.30 (dd, J = 15.1, 6.5 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.74, 162.45, 160.84, 160.09, 143.32, 143.29, 143.27, 142.79, 135.74, 129.69, 129.67, 129.65, 129.59, 128.07, 127.87, 123.87, 123.86, 123.84, 123.82, 123.80, 123.78, 121.56, 119.13, 119.11, 116.07, 116.06, 114.50, 114.47, 114.42, 114.36, 114.33, 114.28, 113.39, 112.27, 112.13, 64.74, 64.58, 59.15, 52.68, 50.12, 49.97, 45.68, 34.93, 33.13, 32.21, 30.04, 29.90, 29.81, 20.16, 17.43, 15.10, 14.48, 13.48. Calcd. for $C_{21}H_{23}FN_2O_3$ $[M+H]^+$ 371.1693; found 371.1792.

4.1.20. 5-(2-((4-(4-fluorophenyl)butan-2-yl)amino)-1-hydroxyethyl)-8-hydroxyquinolin-2(1H)-one hydrochloride (**B08**)

White solid; yield 96%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (s, 2H), 9.45 (s, 1H), 8.59 (s, 1H), 8.30 (d, J = 9.9 Hz, 1H), 7.27 (dd, J = 8.4, 5.6 Hz, 2H), 7.18 (dd, J = 8.2, 3.3 Hz, 1H), 7.11 (ddd, J = 8.8, 7.7, 4.7 Hz, 2H), 7.01 (d, J = 8.2 Hz, 1H), 6.55 (d, J = 9.8 Hz, 1H), 5.51 (d, J = 9.1 Hz, 1H), 3.23 (d, J = 6.8 Hz, 1H), 3.10−2.92 (m, 2H), 2.70 (ddd, J = 10.6, 7.8, 3.5 Hz, 1H), 2.65−2.54 (m, 1H), 2.17−2.03 (m, 1H), 1.85−1.69 (m, 1H), 1.30 (dd, J = 15.2, 6.5 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.90, 160.08, 159.30, 142.78, 136.43, 136.41, 136.39, 135.71, 129.47, 129.44, 129.42, 129.39, 128.06, 127.88, 121.58, 119.12, 119.10, 116.05, 114.55, 114.51, 114.43, 114.42, 114.41, 114.37, 113.38,

64.76, 64.58, 55.49, 52.73, 52.71, 50.13, 49.96, 47.97, 45.70, 33.61, 32.66, 29.56, 29.40, 15.11, 14.48. Calcd. for $C_{21}H_{23}FN_2O_3$ $[M+H]^+$ 371.1693; found 371.1785.

*4.1.21. 8-Hydroxy-5-(1-hydroxy-2-((4-(o-tolyl)butan-2-yl)amino) ethyl)quinolin-2(1H)-one hydrochloride (**B09**)*

White solid; yield 94%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (s, 2H), 9.45 (s, 1H), 8.59 (s, 1H), 8.30 (d, J = 9.9 Hz, 1H), 7.27 (dd, J = 8.4, 5.6 Hz, 2H), 7.18 (dd, J = 8.2, 3.3 Hz, 1H), 7.11 (ddd, J = 8.8, 7.7, 4.7 Hz, 2H), 7.01 (d, J = 8.2 Hz, 1H), 6.55 (d, J = 9.8 Hz, 1H), 5.51 (d, J = 9.1 Hz, 1H), 3.23 (d, J = 6.8 Hz, 1H), 3.10−2.92 (m, 2H), 2.70 (ddd, J = 10.6, 7.8, 3.5 Hz, 1H), 2.65−2.54 (m, 1H), 2.17−2.03 (m, 1H), 1.85−1.69 (m, 1H), 1.30 (dd, J = 15.2, 6.5 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.90, 160.08, 159.30, 142.78, 136.43, 136.41, 136.39, 135.71, 129.47, 129.44, 129.42, 129.39, 128.06, 127.88, 121.58, 119.12, 119.10, 116.05, 114.55, 114.51, 114.43, 114.42, 114.41, 114.37, 113.38, 64.76, 64.58, 55.49, 52.73, 52.71, 50.13, 49.96, 47.97, 45.70, 33.61, 32.66, 29.56, 29.40, 15.11, 14.48. Calcd. for $C_{22}H_{26}N_2O_3$ $[M+H]^+$ 367.1943; found 367.2031.

*4.1.22. 8-Hydroxy-5-(1-hydroxy-2-((4-(m-tolyl)butan-2-yl)amino) ethyl)quinolin-2(1H)-one hydrochloride (**B10**)*

White solid; yield 95%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (s, 2H), 9.50 (s, 1H), 8.59 (s, 1H), 8.31 (d, J = 9.9 Hz, 1H), 7.17 (td, J = 7.9, 4.1 Hz, 2H), 7.01 (dd, J = 14.4, 6.5 Hz, 4H), 6.55 (d, J = 9.8 Hz, 1H), 5.52 (d, J = 8.8 Hz, 1H), 3.22 (s, 1H), 3.01 (d, J = 5.4 Hz, 2H), 2.67 (qd, J = 10.1, 4.7 Hz, 1H), 2.56 (dd, J = 11.2, 6.4 Hz, 1H), 2.27 (d, J = 4.2 Hz, 3H), 2.10 (dd, J = 13.3, 10.0 Hz, 1H), 1.77 (dqd, J = 14.9, 10.1, 5.2 Hz, 1H), 1.30 (dd, J = 15.2, 6.5 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.08, 142.78, 140.20, 140.17, 136.81, 136.78, 135.73, 128.35, 128.32, 128.06, 127.87, 127.70, 127.67, 126.04, 124.72, 124.69, 121.57, 119.11, 119.09, 116.05, 113.39, 64.75, 64.58, 52.84, 52.78, 50.13, 49.95, 33.57, 32.63, 30.40, 30.24, 20.41, 20.40, 15.12, 14.48. Calcd. for $C_{22}H_{26}N_2O_3$ $[M+H]^+$ 367.1943; found 367.2064.

*4.1.23. 8-Hydroxy-5-(1-hydroxy-2-((4-(p-tolyl)butan-2-yl)amino) ethyl)quinolin-2(1H)-one hydrochloride (**B11**)*

White solid; yield 93%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.40 (s, 1H), 9.11 (s, 2H), 8.30 (dd, J = 9.9, 3.2 Hz, 1H), 7.16 (dd, J = 8.2, 3.8 Hz, 1H), 7.12−7.05 (m, 4H), 7.01 (dd, J = 8.1, 2.0 Hz, 1H), 6.53 (t, J = 7.9 Hz, 1H), 5.42 (s, 1H), 3.12 (s, 1H), 2.97 (s, 2H), 2.70−2.58 (m, 1H), 2.55 (dd, J = 9.9, 6.7 Hz, 3H), 2.03 (s, 1H), 1.79−1.61 (m, 1H), 1.26 (dd, J = 12.4, 6.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.16, 143.76, 138.35, 136.83, 135.32, 129.43, 129.40, 128.96, 128.62, 128.59, 122.60, 120.21, 117.18, 114.45, 53.62, 31.13, 31.00, 21.07. Calcd. for $C_{22}H_{26}N_2O_3$ $[M+H]^+$ 367.1943; found 367.2031.

*4.1.24. 8-Hydroxy-5-(1-hydroxy-2-((4-(3-hydroxyphenyl)butan-2-yl)amino)ethyl)quinolin-2(1H)-one hydrochloride (**B12**)*

White solid; yield 94%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.29 (s, 1H), 8.27 (dd, J = 9.9, 3.4 Hz, 1H), 7.16 (dd, J = 8.1, 4.1 Hz, 1H), 7.03 (dtd, J = 9.4, 8.0, 1.5 Hz, 2H), 6.69−6.57 (m, 3H), 6.54 (d, J = 9.8 Hz, 1H), 5.37 (s, 1H), 3.08 (s, 1H), 2.95 (s, 2H), 2.57 (dd, J = 10.0, 4.8 Hz, 1H), 2.45 (d, J = 14.5 Hz, 1H), 1.99 (s, 1H), 1.69 (s, 1H), 1.27−1.20 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.15, 157.87, 143.68, 136.93, 129.71, 128.96, 122.51, 120.23, 119.31, 119.28, 117.24, 115.64, 115.62, 114.49, 113.38, 60.21, 53.53, 31.64, 31.51, 21.23, 14.56. Calcd. for $C_{21}H_{24}N_2O_4$ $[M+H]^+$ 369.1736; found 369.1817.

*4.1.25. 8-Hydroxy-5-(1-hydroxy-2-((4-(4-hydroxyphenyl)butan-2-yl)amino)ethyl)quinolin-2(1H)-one hydrochloride (**B13**)*

White solid; yield 91%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.45 (s, 1H), 9.19 (s, 1H), 8.25 (dd, J = 9.3, 5.3 Hz, 1H), 7.25−7.10 (m, 1H), 6.99 (d, J = 6.9 Hz, 3H), 6.68 (s, 2H), 6.55 (d, J = 9.7 Hz, 1H), 5.38 (s, 1H), 3.10 (s, 1H), 2.97 (s, 2H), 2.57 (s, 1H), 2.45 (d, J = 6.6 Hz, 1H), 1.99 (s, 1H), 1.66 (s, 1H), 1.24 (dd, J = 16.3, 5.2 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.15, 156.01, 143.78, 136.80, 131.35, 129.56, 129.53, 128.99, 122.62, 120.24, 117.18, 115.64, 115.61, 114.48, 55.39, 53.68, 30.72, 30.58. Calcd. for $C_{21}H_{24}N_2O_4$ $[M+H]^+$ 369.1736; found 369.1820.

*4.1.26. 8-Hydroxy-5-(1-hydroxy-2-((4-(3,4,5-trimethoxyphenyl) butan-2-yl)amino)ethyl)quinolin-2(1H)-one hydrochloride (**B14**)*

White solid; yield 95%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (s, 2H), 9.49 (s, 1H), 8.59 (s, 1H), 8.32 (dd, J = 10.0, 3.8 Hz, 1H), 7.18 (dd, J = 8.2, 2.3 Hz, 1H), 7.01 (d, J = 8.2 Hz, 1H), 6.54 (t, J = 4.9 Hz, 2H), 6.13 (s, 1H), 5.50 (dd, J = 15.0, 8.2 Hz, 1H), 3.75 (d, J = 5.3 Hz, 6H), 3.61 (d, J = 3.6 Hz, 3H), 3.19 (d, J = 12.8 Hz, 1H), 3.01 (t, J = 6.5 Hz, 2H), 2.74−2.59 (m, 1H), 2.57 (s, 1H), 2.25−2.04 (m, 1H), 1.86−1.65 (m, 1H), 1.30 (dd, J = 15.1, 6.5 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.09, 152.17, 152.16, 142.76, 135.97, 135.96, 135.72, 135.06, 128.11, 127.88, 121.59, 119.10, 119.07, 116.04, 113.38, 104.92, 104.88, 64.83, 64.64, 59.33, 55.19, 52.73, 52.70, 50.25, 49.98, 33.49, 32.63, 30.73, 30.65, 15.20, 14.54. Calcd. for $C_{24}H_{30}N_2O_6$ $[M+H]^+$ 443.2104; found 443.2210.

*4.1.27. 8-Hydroxy-5-(1-hydroxy-2-((4-(4-methoxyphenyl)-2-methylbutan-2-yl)amino)ethyl)quinolin-2(1H)-one hydrochloride (**C01**)*

White solid; yield 96%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48−8.20 (m, 1H), 7.12 (dt, J = 16.7, 8.2 Hz, 4H), 6.83 (d, J = 8.6 Hz, 2H), 6.50 (d, J = 9.9 Hz, 1H), 5.34 (s, 1H), 3.71 (s, 3H), 3.39 (d, J = 1.7 Hz, 2H), 2.86 (d, J = 5.7 Hz, 2H), 1.87−1.60 (m, 2H), 1.29−1.21 (m, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.67, 161.32, 157.88, 143.81, 137.36, 134.15, 129.60, 128.84, 122.23, 120.17, 117.24, 114.57, 114.22, 114.15, 103.49, 71.15, 70.78, 55.45, 49.32, 29.03, 21.84, 19.65, 18.71. Calcd. for $C_{23}H_{28}N_2O_4$ $[M+H]^+$ 397.2049; found 397.2147.

*4.1.28. 8-Hydroxy-5-(1-hydroxy-2-((4-(3-methoxyphenyl)-2-methylbutan-2-yl)amino)ethyl)quinolin-2(1H)-one hydrochloride (**C02**)*

White solid; yield 97%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (s, 2H), 9.39 (s, 1H), 8.41 (d, J = 10.0 Hz, 1H), 8.25 (d, J = 10.0 Hz, 1H), 7.18 (t, J = 8.1 Hz, 2H), 6.98 (d, J = 8.2 Hz, 1H), 6.83−6.67 (m, 3H), 6.54 (d, J = 9.9 Hz, 1H), 5.43 (d, J = 8.7 Hz, 1H), 3.71 (d, J = 4.3 Hz, 3H), 2.98 (t, J = 13.2 Hz, 2H), 2.68−2.52 (m, 2H), 1.96−1.82 (m, 2H), 1.44−1.24 (m, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.07, 158.72, 142.80, 142.32, 135.63, 128.84, 128.80, 128.04, 127.89, 121.67, 119.88, 119.79, 119.13, 116.06, 113.41, 113.36, 113.30, 110.72, 64.74, 58.45, 54.32, 47.38, 28.76, 24.37, 22.01, 21.94. Calcd. for $C_{23}H_{28}N_2O_4$ $[M+H]^+$ 397.2049; found 397.2180.

*4.1.29. 8-Hydroxy-5-(1-hydroxy-2-((4-(2-methoxyphenyl)-2-methylbutan-2-yl)amino)ethyl)quinolin-2(1H)-one hydrochloride (**C03**)*

White solid; yield 93%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.47 (s, 2H), 9.53 (s, 1H), 8.48 (t, J = 9.9 Hz, 1H), 8.32 (d, J = 9.9 Hz, 1H), 7.29−7.09 (m, 3H), 7.03 (d, J = 8.2 Hz, 1H), 6.93 (d, J = 8.0 Hz, 1H), 6.86 (t, J = 7.3 Hz, 1H), 6.55 (d, J = 9.9 Hz, 1H), 5.50 (d, J = 9.1 Hz, 1H), 3.65 (s, 3H), 3.11−2.86 (m, 2H), 2.59 (dd, J = 10.5, 5.0 Hz, 2H), 1.89−1.72 (m, 2H), 1.38 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.08, 156.26, 142.80, 135.61, 128.97, 128.34, 128.10, 127.89, 126.88, 121.67, 119.75, 119.12, 116.06, 113.39, 110.07, 64.73, 58.40, 54.48, 47.40, 36.72, 23.59, 22.18, 22.15. Calcd. for $C_{23}H_{28}N_2O_4$ $[M+H]^+$ 397.2049; found 397.2137.

*4.1.30. 8-Hydroxy-5-(1-hydroxy-2-((2-methyl-4-(p-tolyl)butan-2-yl)amino)ethyl)quinolin-2(1H)-one hydrochloride (**C04**)*

White solid; yield 92%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.41 (s, 2H), 9.59 (s, 1H), 8.45 (d, J = 9.1 Hz, 1H), 8.32 (d, J = 9.9 Hz, 1H), 7.21

(d, J = 8.2 Hz, 1H), 7.09 (s, 4H), 7.01 (d, J = 8.2 Hz, 1H), 6.55 (d, J = 9.9 Hz, 1H), 5.49 (d, J = 9.1 Hz, 1H), 3.18–2.86 (m, 2H), 2.59 (dd, J = 9.9, 5.0 Hz, 2H), 2.25 (s, 3H), 1.96–1.79 (m, 2H), 1.37 (d, J = 6.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.12, 143.85, 138.65, 136.76, 135.33, 129.41, 129.13, 129.00, 128.59, 122.70, 120.24, 117.18, 114.51, 65.86, 59.58, 48.53, 29.37, 23.11, 23.04, 21.06. Calcd. for $C_{23}H_{28}N_2O_3$ [M − H]$^-$ 379.2100; found 379.2387.

*4.1.31. 8-Hydroxy-5-(1-hydroxy-2-((2-methyl-4-(m-tolyl)butan-2-yl)amino)ethyl)quinolin-2(1H)-one hydrochloride (**C05**)*

White solid; yield 90%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.47 (s, 2H), 9.64 (s, 1H), 8.46 (d, J = 9.8 Hz, 1H), 8.33 (d, J = 10.0 Hz, 1H), 7.28–7.08 (m, 2H), 7.09–6.94 (m, 4H), 6.55 (d, J = 9.9 Hz, 1H), 5.57–5.43 (m, 1H), 3.12–2.89 (m, 2H), 2.68–2.54 (m, 2H), 2.26 (s, 3H), 2.01–1.80 (m, 2H), 1.37 (d, J = 5.2 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.13, 143.87, 141.70, 137.88, 136.79, 129.39, 129.14, 128.98, 128.76, 127.06, 125.79, 122.68, 120.24, 117.17, 114.49, 65.83, 59.55, 48.54, 29.74, 23.10, 23.03, 21.45. Calcd. for $C_{23}H_{28}N_2O_3$ [M+H]$^+$ 381.2100; found 381.2206.

*4.1.32. 8-Hydroxy-5-(1-hydroxy-2-((2-methyl-4-(o-tolyl)butan-2-yl)amino)ethyl)quinolin-2(1H)-one hydrochloride (**C06**)*

White solid; yield 97%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (s, 2H), 9.68 (d, J = 8.5 Hz, 1H), 8.45 (t, J = 9.8 Hz, 1H), 8.34 (d, J = 9.9 Hz, 1H), 7.21 (d, J = 8.2 Hz, 1H), 7.17–7.07 (m, 4H), 7.01 (d, J = 8.2 Hz, 1H), 6.54 (d, J = 9.9 Hz, 1H), 5.50 (d, J = 9.2 Hz, 1H), 3.11–2.89 (m, 2H), 2.61 (dd, J = 9.3, 6.6 Hz, 2H), 2.28 (s, 3H), 1.84 (dd, J = 11.1, 6.4 Hz, 2H), 1.41 (d, J = 9.4 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.12, 143.85, 139.91, 136.81, 135.96, 130.56, 129.20, 129.15, 128.98, 126.57, 126.48, 122.66, 120.23, 117.17, 114.50, 65.84, 65.37, 59.58, 48.52, 38.30, 27.41, 22.89, 19.27, 15.63. Calcd. for $C_{23}H_{28}N_2O_3$ [M+H]$^+$ 381.2100; found 381.2192.

*4.1.33. 5-(2-((4-(4-chlorophenyl)-2-methylbutan-2-yl)amino)-1-hydroxyethyl)-8-hydroxyquinolin-2(1H)-one hydrochloride (**C07**)*

White solid; yield 96%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.47 (s, 2H), 9.67 (d, J = 8.9 Hz, 1H), 8.46 (d, J = 9.0 Hz, 1H), 8.33 (d, J = 9.9 Hz, 1H), 7.44–7.12 (m, 5H), 7.02 (d, J = 8.2 Hz, 1H), 6.55 (d, J = 9.9 Hz, 1H), 5.50 (d, J = 9.1 Hz, 1H), 3.00 (t, J = 12.6 Hz, 2H), 2.64 (dd, J = 9.4, 4.1 Hz, 2H), 1.93 (dd, J = 15.6, 7.0 Hz, 2H), 1.37 (dd, J = 6.7, 4.1 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.14, 143.87, 140.86, 136.80, 131.03, 130.64, 129.13, 128.98, 128.77, 122.68, 120.25, 117.19, 114.50, 65.82, 59.57, 59.51, 48.53, 29.10, 23.09, 23.01. Calcd. for $C_{22}H_{25}ClN_2O_3$ [M+H]$^+$ 401.1554; found 401.1687.

*4.1.34. 5-(2-((4-(3-chlorophenyl)-2-methylbutan-2-yl)amino)-1-hydroxyethyl)-8-hydroxyquinolin-2(1H)-one hydrochloride (**C08**)*

White solid; yield 97%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (s, 2H), 9.59 (s, 1H), 8.44 (s, 1H), 8.31 (dd, J = 10.0, 1.7 Hz, 1H), 7.34–7.16 (m, 5H), 7.01 (d, J = 8.2 Hz, 1H), 6.55 (d, J = 9.8 Hz, 1H), 5.49 (d, J = 8.9 Hz, 1H), 3.01 (t, J = 17.8 Hz, 2H), 2.66 (dt, J = 13.6, 6.4 Hz, 2H), 1.98–1.85 (m, 2H), 1.37 (dd, J = 6.4, 3.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.13, 144.49, 143.87, 136.79, 133.46, 130.70, 128.86, 128.73, 128.64, 127.56, 126.44, 122.69, 120.25, 117.18, 114.50, 65.84, 59.51, 48.54, 29.40, 23.14, 23.03, 14.56. Calcd. for $C_{22}H_{25}ClN_2O_3$ [M+H]$^+$ 401.1554; found 401.1652.

*4.1.35. 5-(2-((4-(2-chlorophenyl)-2-methylbutan-2-yl)amino)-1-hydroxyethyl)-8-hydroxyquinolin-2(1H)-one hydrochloride (**C09**)*

White solid; yield 94%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (s, 2H), 9.61 (d, J = 9.5 Hz, 1H), 8.49 (dd, J = 15.5, 10.4 Hz, 1H), 8.32 (d, J = 10.0 Hz, 1H), 7.39 (ddd, J = 15.5, 7.5, 1.7 Hz, 1H), 7.32–7.17 (m, 4H), 7.01 (d, J = 8.2 Hz, 1H), 6.55 (d, J = 9.9 Hz, 1H), 5.49 (d, J = 9.2 Hz, 1H), 3.01 (dd, J = 19.4, 9.8 Hz, 2H), 2.80–2.58 (m, 2H), 1.89 (ddd, J = 13.6, 12.5, 7.1 Hz, 2H), 1.39 (dd, J = 12.8, 5.9 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.13, 143.86, 139.13, 136.77, 133.20, 131.30, 129.78, 129.13, 128.86, 128.73, 127.94, 122.70, 120.25, 117.17, 114.49, 65.87, 59.57, 59.44, 48.50, 37.86, 27.91, 23.00, 14.56. Calcd. for $C_{22}H_{25}ClN_2O_3$ [M+H]$^+$ 401.1554; found 401.1645.

*4.1.36. 8-Hydroxy-5-(1-hydroxy-2-((4-(4-hydroxyphenyl)-2-methylbutan-2-yl)amino)ethyl)quinolin-2(1H)-one hydrochloride (**C10**)*

White solid; yield 96%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.47 (d, J = 6.5 Hz, 2H), 9.27 (d, J = 60.3 Hz, 2H), 8.42 (d, J = 9.5 Hz, 1H), 8.26 (d, J = 9.9 Hz, 1H), 7.21 (d, J = 8.2 Hz, 1H), 7.00 (dd, J = 8.3, 3.0 Hz, 3H), 6.67 (dd, J = 8.8, 2.3 Hz, 2H), 6.56 (d, J = 9.9 Hz, 1H), 6.16 (s, 1H), 5.44 (d, J = 9.3 Hz, 1H), 3.12–2.90 (m, 2H), 2.50 (dt, J = 3.5, 1.7 Hz, 2H), 1.92–1.78 (m, 2H), 1.35 (d, J = 5.9 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.74, 160.07, 154.90, 142.80, 135.50, 130.59, 128.45, 127.99, 127.91, 121.73, 119.17, 116.06, 114.52, 113.33, 64.82, 58.46, 47.24, 27.82, 22.01, 21.94. Calcd. for $C_{22}H_{26}N_2O_4$ [M+H]$^+$ 383.1893; found 383.2017.

*4.1.37. 8-Hydroxy-5-(1-hydroxy-2-((4-(3-hydroxyphenyl)-2-methylbutan-2-yl)amino)ethyl)quinolin-2(1H)-one hydrochloride (**C11**)*

White solid; yield 91%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (s, 2H), 9.51 (s, 1H), 9.30 (s, 1H), 8.43 (s, 1H), 8.29 (d, J = 10.0 Hz, 1H), 7.21 (d, J = 8.2 Hz, 1H), 7.12–6.93 (m, 2H), 6.74–6.48 (m, 4H), 6.16 (s, 1H), 5.47 (d, J = 8.9 Hz, 1H), 3.00 (s, 2H), 2.55 (dd, J = 8.2, 4.3 Hz, 2H), 2.01–1.77 (m, 2H), 1.36 (d, J = 7.4 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.15, 157.89, 143.86, 143.14, 136.66, 129.75, 129.10, 128.99, 122.78, 120.23, 119.28, 117.16, 115.63, 114.44, 113.40, 65.87, 65.38, 59.54, 48.41, 29.79, 23.04, 22.99, 15.64. Calcd. for $C_{22}H_{26}N_2O_4$ [M+H]$^+$ 383.1893; found 383.1967.

*4.1.38. 8-Hydroxy-5-((1R)-1-hydroxy-2-((4-(2-methoxyphenyl) butan-2-yl)amino)ethyl)quinolin-2(1H)-one hydrochloride ((**R,R**)-**B04**)*

(*R,R*)-B04 was obtained by semipreparative HPLC.

White solid; yield 95%; ee > 99%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.50 (s, 2H), 9.55–9.33 (m, 1H), 8.65–8.52 (m, 1H), 8.31 (dd, J = 10.0, 2.2 Hz, 1H), 7.20–7.15 (m, 3H), 7.02 (dd, J = 8.1, 2.0 Hz, 1H), 6.97–6.93 (m, 1H), 6.90–6.85 (m, 1H), 6.55 (dd, J = 9.9, 3.5 Hz, 1H), 5.54–5.46 (m, 1H), 3.75 (d, J = 4.5 Hz, 3H), 3.27–3.19 (m, 1H), 3.07–2.95 (m, 2H), 2.69–2.61 (m, 1H), 2.59–2.52 (m, 1H), 2.07–2.00 (m, 1H), 1.80–1.68 (m, 1H), 1.31 (dd, J = 21.5, 6.5 Hz, 3H). Calcd. for $C_{22}H_{26}N_2O_4$ [M − H]$^-$ 381.1893; found 381.1989.

*4.1.39. General process for preparation of compounds **(R)-C07**, and **(R)-C08***

Compounds ***(R)*-C07**, and ***(R)*-C08** were obtained as previously reported [21,22].

*4.1.40. (R)-5-(2-((4-(4-chlorophenyl)-2-methylbutan-2-yl)amino)-1-hydroxyethyl)-8-hydroxyquinolin-2(1H)-one hydrochloride ((**R**)-**C07**)*

White solid; yield 93%; ee > 99%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.49 (s, 2H), 9.62 (s, 1H), 8.45 (t, J = 10.9 Hz, 1H), 8.32 (d, J = 9.9 Hz, 1H), 7.29 (t, J = 7.5 Hz, 2H), 7.22 (d, J = 6.5 Hz, 3H), 7.03–7.00 (m, 1H), 6.55 (d, J = 9.8 Hz, 1H), 5.49 (d, J = 9.6 Hz, 1H), 3.04–2.95 (m, 2H), 2.66–2.62 (m, 2H), 1.93 (t, J = 8.4 Hz, 2H), 1.38 (d, J = 10.2 Hz, 6H). Calcd. for $C_{22}H_{25}ClN_2O_3$ [M+H]$^+$ 401.1554; found 401.1576.

*4.1.41. (R)-5-(2-((4-(3-chlorophenyl)-2-methylbutan-2-yl)amino)-1-hydroxyethyl)-8-hydroxyquinolin-2(1H)-one hydrochloride ((**R**)-**C08**)*

White solid; yield 90%; ee > 99%; ^{1}H NMR (600 MHz, DMSO-d_6)

δ 10.49 (s, 2H), 9.64 (s, 1H), 8.44 (d, J = 10.6 Hz, 1H), 8.32 (dd, J = 9.9, 2.3 Hz, 1H), 7.35–7.27 (m, 2H), 7.23–7.19 (m, 3H), 7.02 (d, J = 8.1 Hz, 1H), 6.55 (d, J = 9.8 Hz, 1H), 5.49 (d, J = 9.7 Hz, 1H), 3.06–2.93 (m, 2H), 2.70–2.60 (m, 2H), 1.93 (t, J = 8.6 Hz, 2H), 1.38 (d, J = 10.3 Hz, 6H). Calcd. for $C_{22}H_{25}ClN_2O_3$ $[M+H]^+$ 401.1554; found 401.1562.

4.2. Biological tests

4.2.1. Cellular cAMP assay

The cellular cAMP assay was performed as previously described [32].

4.2.2. Isolated Guinea pig trachea relaxation assay

A detailed method for the isolated guinea pig trachea relaxation assay has been reported [32].

4.3. Molecular modeling

Molecular docking was conducted using a reported method [32].

Declaration of competing interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113697.

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