**A systematic investigation into the structure-activity relationships of a benzothiazolone series of β2-adrenoceptor agonists bearing 2-benzyloxycyclopentyl *N*-substituents**

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**ABSTRACT**: A previous optimisation of a 4-hydroxybenzothiazolone series of β2-adrenoceptor agonists identified the diastereoisomers **10** and **11** as a high-efficacy slow-binding agonist and clinical candidate respectively. …

**Introduction**

B2A history and current areas of interest

Clinical applications: bronchodilators



Figure 1. Structures of the adrenaline and selected β2-adrenoceptor agonists approved as inhaled bronchodilators.

Our own interest in GPCR pharmacology and in particular B2A agonists (refs?)

4-HBT experience: from the screening of a wide range of N-substituents within the 4-hydroxybenzothiazolone β2-AR series the 2-benzyloxycyclopentyl was identified as particularly interesting based upon the diastereoisomers QAN746 and C26



Figure 2. Structures of 4-hydroxybenzothiazolone containing β2-adrenoceptor agonists.

Unique opportunity to explore B2A SAR with stereoisomers to make subtle changes in receptor interactions with minimal impacts upon physical properties. In this article we describe the preparation of the remaining 6 stereoisomers corresponding to the constitutional isomer encompassing **10** and **11** and assess their B2A profiles.

In addition the slow-binding nature of the (*R*,*R*)-benzyloxycyclopentylamine made **10** an interesting analogue to explore modification to the B2A pharmacophore, as previously done with indacaterol (simpler readout / lower affinity starting point) to explore in particular signaling bias.

**Results and discussion**

**Synthesis of the β2-agonists**: The structures of the eight stereoisomers corresponding to and including C26 **10** and NVP-QAN746 **11** are shown in Figure 3.



|  |  |  |
| --- | --- | --- |
| **Compound** | **Configuration** | |
| **benzylic alcohol** | **N-substituent** |
| **10** C26 QAN745 | 1*R* | 1'*R*,2'*R* |
| **11** NVP-QAN746 | 1*R* | 1'*S*,2'*S* |
| **13** QAS008 | 1*S* | 1'*R*,2'*R* |
| **14** QAS007 | 1*S* | 1'*S*,2'*S* |
| **15** QAT568 | 1*R* | 1'*R*,2'*S* |
| **16** QAT565 | 1*R* | 1'*S*,2'*R* |
| **17** QAT569 | 1*S* | 1'*R*,2'*S* |
| **18** QAT567 | 1*S* | 1'*S*,2'*R* |

Figure 3 structures of all the stereoisomers corresponding to the ? of C26 and NVP-QAN746.

To synthesise the benzothiazolone β2-AR agonists, variations of the benzyne-mediated cyclisation approach developed to prepare the natural product **9** were used. The *trans*-substituted cyclopentylamines were commercially available and the *cis*-isomers were readily prepared in 3 steps from the *cis*-2-aminocyclopentanol enantiomers, as outlined in Scheme 1.

Selective mono-alkylation of the 2-aminobenzyloxycyclopentanols with the chiral epoxides **15** was then readily achieved following in situ N-silylation as previously described. Acid-catalysed cleavage of the two ether protecting groups then gave the targeted compounds **13**-**18**.

Scheme 1. Synthesis of the 4-hydroxybenzothiazolone analogues **13**-**18**, exemplified by the preparation of **15**.



Reagents and conditions: (a) 1.0 equiv phthalic anhydride, 1.2 equiv Hünig’s base, 130 °C, 3 h (42-45%); (b) BnBr, NaH, DMF, room temperature, 18 h (55-57%), (c) N2H4.H2O, AcOH, EtOH, reflux, 2 h (55-70%); (d) **21**, BTMSA, DMF, 80 °C, 18 h (44-46%); 1M HCl(aq), *i*PrOH, 80 °C, 5 h (74-90%).

With a high affinity/efficacy N-substituent, what is the impact when key elements of the classic β2-AR pharmacophore are modified? To assess this prepared rescorsinol analogue **24**, des-phenol **25**, des-hydroxy **26** and the quaternary methyl analogue **27**, Figure 4.



Figure 4. Structures of the N-(1'*R*,2'*R*)-benzyloxycyclopenyl substituted benzothiazolone analogues **24**-**27** with modifications to the B2A pharmacophore.

To prepare the 5-hydroxybenzothiazolone analogue **24** an analogous benzyne-mediated cyclisation approach was applied as used for the synthesis of the 4-hydroxy isomers, Scheme 2. Starting from 3,5-difluoronitrobenzene, an SNAr reaction with *tert*.butoxide efficiently delivered the ether **28** by displacing one of the two equivalent fluorine atoms. A subsequent Buchwald coupling with **28** and benzophenone imine was followed by hydrolysis to provide the aniline **29**. Conversion to the thiocarbamate cyclisation precursor **30** was then achieved in two steps via isothiocyanate formation and the subsequent addition of isopropanol. Treatment of **30** with 2.5 equivalents of *tert*.buytl lithium generated the 7-lithio benzothiazole intermediate which was acylated with DMF to give the aldehyde **31**. The *tert*.butyl group in **30** was again found to play a key role in suppressing competing deprotonations *ortho* to the aryl ether and as a result facilitate the desired benzyne-mediated cyclisation to the 7-lithiated benzothiazole intermediate.x Wittig methylenation of **31** followed by asymmetric dihydroxylation generated the diol **32** with the desired (*R*)-configuration at the benzylic center. Activation of the primary alcohol of **32** via tosylation then enabled a displacement reaction at this position with the primary amine **33**, either directly or via the epoxide intermediate. Attempts to obtain the epoxide for this system as an isolable intermediate suggested it to be of higher reactivity when compared to **22**. Finally, formic acid catalysed cleavage of both aryl ethers provided the targeted 5-hydroxybenzothiazolone **24**.

Scheme 2. Synthesis of the 5-hydroxybenzothiazolone analogue **24**.



Reagents and conditions: (a) *tert*.BuOH, NaH, DMA, room temperature, 3 days; (b) (Ph)2CNH2, NaOMe, Pd2(dba)3, BINAP, toluene, 2 days 80 °C; (c) H2NOH.H2O, NaOAc, MeOH, room temperature, 3 h; (d) C(S)Cl2, CHCl3, water, room temperature, 18 h (55%, 4 steps); (e) *i*PrOH, Et3N, reflux 18 h (87%); (f) 2.5 equiv. *tert*.BuLi, THF, -78 to 0 °C, then DMF, -78 to 0 °C, (53-62%); (g) Ph3PMe.Br, nBuLi, THF, 0 °C to room temperature, (56%); (h) (DHQD)2PHAL, K3Fe(CN)6, K2CO3, OsO4, *tert*.BuOH, water, 0 °C, 3 days (67-76%); (i) TsCl, pyridine, 0 °C (82%); (j) (1*R*,2*R*)-2-(benzyloxy)cyclopentan-1-amine **33**, toluene, 90 °C, 18 h (62%); (k) HCO2H, room temperature, 7 h (69%).

Similarly, the des-phenol **25** and des-hydroxy **26** analogues were prepared through the benzyne-mediated cyclisation approach as highlighted in Schemes 3 and 4.

Scheme 3. Synthesis of the des-phenol analogue **25**.



Reagents and conditions: (a) *i*PrOH, Et3N, reflux 18 h (73%); (b) 2.8 equiv. *tert*.BuLi, THF -78 to -30 °C, 1 h, then DMF, -78 to -30 °C, 1 h (64%); (c) Ph3PMe.Br, nBuLi, THF, 0 °C to room temperature, 5 h (82%); (d) AD-mix-β, *tert*.BuOH, water, 0 °C to room temperature, 3 days (95%); (e) TsCl, pyridine 0 °C to room temperature, 4 h (61%); (f) **33**, *tert*.BuOH, 80 °C, 18 h (63%); (g) HCO2H, room temperature, 48 h (56%).

To prepare the *des*-hydroxy analogue **26** a modified version of the above route was developed which used ethylene oxide as the electrophile to ract with the 7-lithiated benzothiazole intermediate, Scheme 4.

Scheme 4. Synthesis of the des-hydroxy analogue **26**.



Reagents and conditions: (a) 2.5 equiv. *tert*.BuLi, THF -78 to -20 °C, 1 h, then ethylene oxide in THF, -20 °C to room temperature, 1 h (75%); (b) MeSO2Cl, pyridine, CH2Cl2, 0 °C to room temperature, 2 h (78%); (c) **33**, Hünigs base, water, 95 °C, 14 h; (d) 48% HBr, *i*PrOH, 60 °C, 4 h (46%, 2 steps).

The quaternary methyl α-amino analogue **27** was prepared from the racemic cyclopentylamine **40** which also yielded the diastereoisomer **42**, the methylated analogue of the clinical candidate **11**, Scheme 5.

Scheme 5. Synthesis of the quaternary methyl α-amino analogues **27** and **42**.



Reagents and conditions: (a) NaN3, AcOH, H2O, room temperature, 12 h; (b) BnBr, NaH, toluene, 2 days (37%, 2 steps); (c) PPh3, THF, H2O, 65 °C, 6 days (19%); (d) BTMSA, DMF, room temperature, then (*R*)-**22** in DMF, 90 °C for 40 h (53%); (e) 1M HCl(aq), *i*PrOH, 75 °C, 12 h; (f) chiral SFC separation (**27** 34%, **42** 30%, 2 steps).

\*racemic cyclopentyl moiety with *trans*-N,O configuration, (1*R*,2*R*)-shown.

Chiral analysis and purification statement. 5 log units between most and least potent stereoisomers.

logD7.4 and KIAM values to support comparable lipophilicity interactions / rebinding potential

Stereoisomers (and single atom changes) minimise any differences in membrane partitioning, so a similar level of rebinding.

Table 2. Lipophilicity and pKa measurements for the β2-adrenoceptor agonists .

|  |  |  |  |
| --- | --- | --- | --- |
| Compound | pKa | logD7.4 | KIAM |
| **10, C26** | 8.8, 10.6 (A) |  | 1.23 |
| **11, QAN746** | 8.8, 10.6 (A) |  | 1.25 |
| 1**3,** QAS008 | 7.4, 8.2, 10.6 |  |  |
| 1**4,** QAS007 |  |  | 1.19 |
| 1**5,** QAT568 |  | 3.00 |  |
| 1**6,** QAT565 |  | 3.00 |  |
| 1**7,** QAT569 |  | 3.00 |  |
| 1**8,** QAT567 |  | 3.00 |  |
| **24,** QAS553 |  |  |  |
| **25,** CIE316 |  |  |  |
| **26,** FLT582 |  | 3.40 |  |
| **27,** QPJ533 |  |  |  |
| **42,** EWX937 |  |  |  |

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Compound | chirality | | binding pKi | miniG | | GsCASE | | arrestin-2 | |
| benzylic alcohol | cyclo-pentane | pEC50 | Bmax | pEC50 | Bmax | pEC50 | Bmax |
| Formoterol | *rac* | - | 7.44 ± 0.12 | 8.74 ± 0.08 | 102.9 ± 1.9 | 8.96 ± 0.11 | 100 ± 6 .4 | 8.61 ± 0.09 | 94.3 ± 2.3 |
| Cmpd **10**  **C26** | *R* | 1*R*,2*R* | 9.43 ± 0.16 | 8.59 ± 0.03 | 108.5 ± 2.6 | 8.48 ± 0.10 | 92.8 ± 7.0 | 8.50 ± 0.05 | 109.7 ± 5.6 |
| Cmpd **11**  **QAN746** | *R* | 1*S*,2*S* | 7.20 ± 0.12 | 8.78 ± 0.06 | 105.4 ± 1.6 | 8.92 ± 0.09 | 82.6 ± 6.6 | 8.50 ± 0.09 | 124.7 ± 4.8 |
| Cmpd 1**3**  QAS008 | *S* | 1*R*,2*R* | 7.36 ± 0.14 | 7.50 ± 0.13 | 96.3 ± 3.0 | 7.45 ± 0.08 | 93.5 ± 7.5 | 6.40 ± 0.14 | 66.5 ± 5.5 |
| Cmpd 1**4**  QAS007 | *S* | 1*S*,2*S* | 6.01 ± 0.13 | 7.37 ± 0.13 | 99.6 ± 1.6 | 7.25 ± 0.14 | 90.4 ± 7.5 | 7.05 ± 0.14 | 107.8 ± 7.1 |
| Cmpd 1**5**  QAT568 | *R* | 1*R*,2*S* | 7.93 ± 0.18 | 8.75 ± 0.11 | 101.2 ± 1.6 | 8.23 ± 0.11 | 100.9 ± 9.7 | 8.62 ± 0.13 | 90.3 ± 6.5 |
| Cmpd 1**6**  QAT565 | *R* | 1*S*,2*R* | 6.86 ± 0.15 | 8.54 ± 0.08 | 91.3 ± 1.3 | 8.51 ± 0.18 | 97.4 ± 7.3 | 7.93 ± 0.13 | 56.3 ± 6.1 |
| Cmpd 1**7**  QAT569 | *S* | 1*R*,2*S* | 5.91 ± 0.16 | 6.85 ± 0.14 | 99.1 ± 0.9 | 6.28 ± 0.14 | 92 ± 7.7 | 6.66 ± 0.17 | 86.1 ± 5.1 |
| Cmpd 1**8**  QAT567 | *S* | 1*S*,2*R* | 4.33 ± 0.22 | 5.67 ± 0.06 | 92.3 ± 2.0 | 5.72 ± 0.12 | 87.5 ± 10.8 | 4.90 ± 0.12 | 63.5 ± 8.4 |
| Cmpd **24**  QAS553 | *R* | 1*R*,2*R* | 7.92 ± 0.22 | 8.78 ± 0.08 | 73.5 ± 1.0 | 7.47 ± 0.21 | 104.2 ± 11.8 | 8.59 ± 0.40 | 16.7 ± 2.4 |
| Cmpd **25**  CIE316 | *R* | 1*R*,2*R* | 9.03 ± 0.09 | 8.90 ± 0.12 | 60.1 ± 1.1 | 6.94 ± 0.25 | 82.4 ± 12.4 | n.d. | < 20% |
| Cmpd **26**  FLT582 | *-* | 1*R*,2*R* | 7.99 ± 0.21 | 8.33 ± 0.06 | 59.6 ± 1.1 | 7.85 ± 0.22 | 71.4 ± 9.3 | n.d. | < 20% |
| Cmpd **27**  QPJ533 | *R* | 1*R*,2*R* | 9.93 ± 0.18 | 8.66 ± 0.13 | 105.9 ± 3.3 | 8.01 ± 0.14 | 91.7 ± 8.1 | 8.58 ± 0.12 | 107.2 ± 5.9 |
| Cmpd **42**  EWX937 | *R* | 1*S*,2*S* | 7.91 ± 0.15 | 8.71 ± 0.14 | 103.9 ± 1.5 | 8.29 ± 0.23 | 88.0 ± 9.6 | 8.40 ± 0.09 | 111.4 ± 4.7 |

**Data analysis**

The benzothiazolone derivatives were tested in a series of assays to evaluate their affinities and functional effects towards the B2A with formoterol as the reference compound, Table 3.

Affinity for the B2A was measured using ….

Functional activity was measured using ….

SAR level

* Discuss role of chirality center contributing to β2-AR activity trans-cyclopentyl (10, 11, 13, 14) higher affinity compared to cis cyclopentyl isomers (15-18).
* R-benzylic alcohol and 1-R-cyclopentyl optimal for affinity in both cis and trans.
* 2-R-cyclopentyl optimal for affinity in trans. In contrast, 2-S-cyclopentyl optimal for affinity in cis.
* Compounds **10** and **11** show highest Bmax values in miniG and more clearly in arrestin-2

Receptor pharmacology level

Correlation between affinity and IE between 8 isomers and **10** and 4 pharmacophore modified analogues (**11** and **42**).



How best to represent compounds on plots: colour / numbering? Include all in manuscript, or split with SI?



Correlation bias and IE

Bias plots for selected compounds

10 and 11





Rest of bias plots in SI? Original plots for editing compound names?





Correlation kinetics and signaling bias / IE



**Correlations of mG *k*off and binding affinity but not mG assay measured efficacy (as predicted from Harwood et al., 2024)!**

**Binding affinity and mG pEC50 correlated**



* 5-hydroxybenzothiazolone **24**, des-phenyl **25** and des-hydroxy **26** show reduced but significant Bmax in miniG but minimal weak arrestin-2 Bmax.
* Addition of α-methyl produces an increase in affinity for **27** versus **10** and **42** versus **11**, but not as marked as for indacaterol (<http://dx.doi.org/10.1016/j.bmcl.2012.07.096>): 26-fold (15.6 vs. 0.6 nM).
* Compounds **24**, **25** and **26** show a clear G-protein versus arrestin-2 bias, albeit with lower G-protein intrinsic efficacy.
* *Time dependency of C26 from http://dx.doi.org/10.1124/mol.115.101253, is this accounted for in the miniG and GsCASE assays?*
* *Slow koff for C26, how do the assays capture this (initial low-rate of cAMP increasing to super agonist)? Dissociation half-life for C26 32.7 min and α-methyl indacaterol 28.9 min*
* *With the A431 cAMP assay we saw ‘super agonism’ for C26, how best to rationalize these differences?*

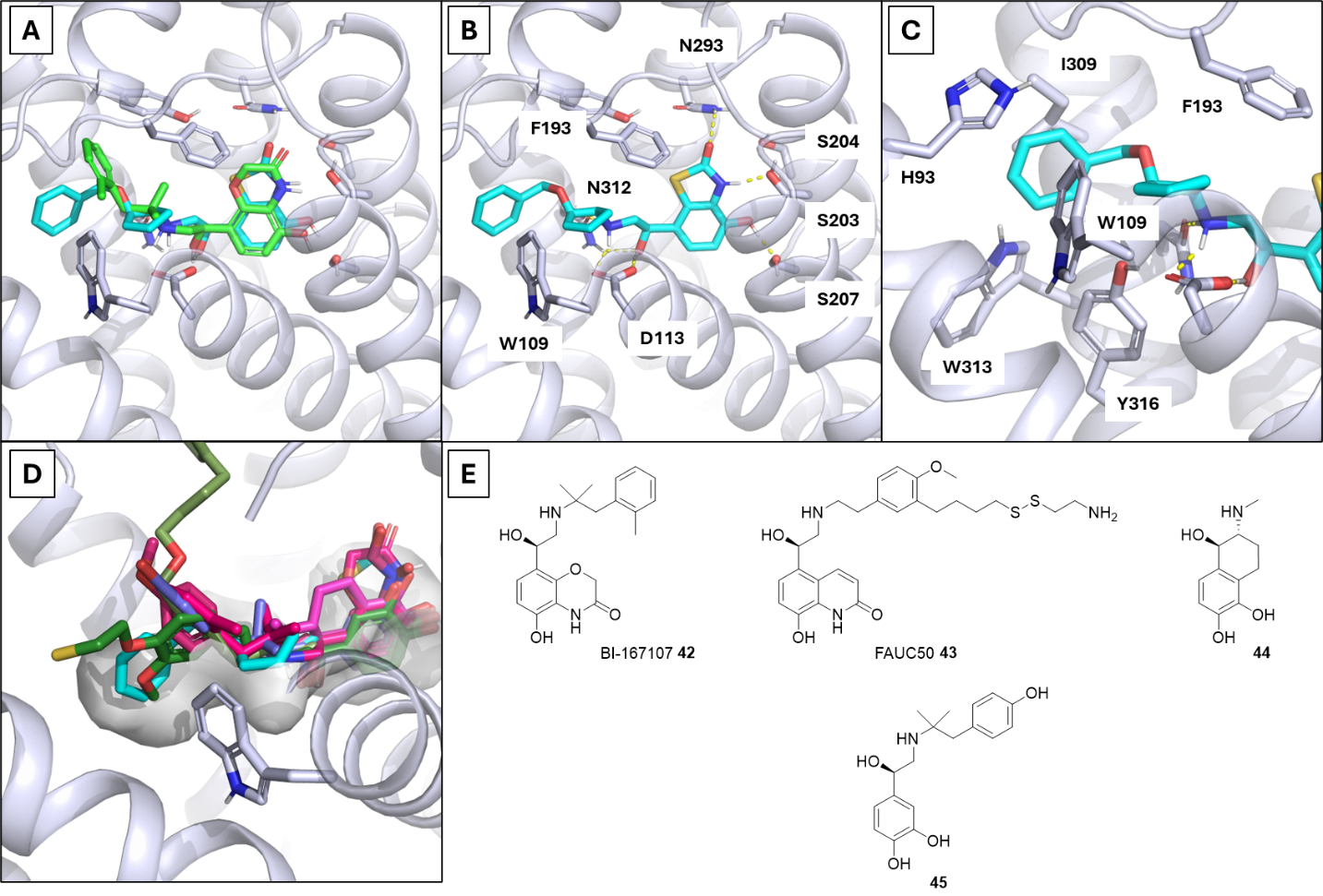
Possible to predict IE / bias from structure?

**CADD rationale**

***N-Substituent extends into a hydrophobic sub-pocket at Trp109***

The benzothiazolones in Table 1 (**10**, **11**, **13**-**18**, **24**-**27**, **42**) were docked into the x-ray crystal structure of active state β2-adrenergic receptor (β2-AR) coupled to Gs protein, solved with the full agonist BI-167107 **42** in the orthosteric pocket.1 We then validated each docking pose with triplicate molecular dynamics simulations of 100 ns each. Inspection of the docking poses indicate that all key polar interactions formed by BI‑167107 **42** in the ligand binding pocket are retained by the benzothiazolones in Table 1; specifically, hydrogen bonding interactions to S203 and S207 and a network of salt-bridge/hydrogen bonding interactions between the ethanolamine linker and D113 / N312 (see Figure 1 A and Supporting Information Table S1). These polar contacts are also seen in the cryo-EM structure of the β2-AR Gs protein complex coupled to the agonist formoterol and represent the established interactions for the β2-AR pharmacophore.2, 3 In addition to the retained polar interactions, we find that the binding pose of the most potent analog, compound **10**, is able to extend the terminal phenyl ring into a hydrophobic sub-pocket at W109, potentially accounting for the exquisite efficacy of this agonist (see Figure 1 C). The terminal phenyl ring of compound **10** is surrounded by hydrophobic contacts from W109, I309, W313, Y316 and H93 in this sub-pocket region.

We used GPCRdb4 to examine all published agonist bound active state structures of β2-AR finding that this ability to occupy the hydrophobic sub-pocket at W109 is novel; only the covalent (nor)adrenaline analog FAUC50 **43** places a methoxy in this region when covalently bound to the rationally introduced cysteine in the β2-AR H93C variant (see Figure 1 D).5



**Figure 1** – Overlay of the compound **10** agonist binding pose (cyan) with A) BI‑167107 **42** (green) showing conservation of the key polar interactions, after docking of compound **10** to x-ray crystal structure of β2-AR Gs protein complex (3SN61) and short molecular dynamics refinement. B) Compound **10** binding pose at β2-AR with key residues labelled and C) close-in view of the W109 sub-pocket. D) Compound **10** (cyan) is found to occupy a novel hydrophobic sub-pocket at W109 when comparing to all published agonist bound structures of β2-AR, with only the covalent FAUC50 **43** (dark green) found to place a methoxy group in this region when covalently attached to H93C mutant of β2‑AR.5 The structures in D) are depicted in E) BI‑167107 **42** 3SN61, formoterol **5** 7BZ22, FAUC50 **43** 4QKX5, conformationally constrained epinephrine c-Epi **44** 8GG06, salmeterol **4** 6MXT7 and hydroxybenzyl isoproterenol **45** 4LDL8.

***Weaker stereochemical analogs have sub-optimal docking poses***

Further inspection of the docking poses for the benzothiazolones in Table 1 indicate that although each can be accommodated into the pocket to form key polar interactions and place the terminal phenyl in/near the W109 sub-pocket, the fit is non-optimal compared to compound **10**. After short molecular dynamics refinement, each of the ligand-receptor complexes underwent production simulations totaling 2.4 μs, from which MM-PBSA binding affinity scores and dynamic protein-ligand interaction fingerprints were calculated. Correlating the MM-PBSA binding affinity scores to measured binding pKi values, we see a good trend with binding affinity (Pearson R=-0.73 p‑value=0.0046), indicating that differences in ligand binding affinity between stereochemical analogs originate from non‑covalent interactions as sampled by the simulations (see Figure 2 B and Table 1).

The subset of compounds **10**, **11**, **15** and **16** have the same *R-* stereochemistry at the benzylic alcohol position and differ in terms of their cyclopentane configurations. Inspecting the docking poses after molecular dynamics refinement for this subset (see Figure 2 A), each molecule is able to position the terminal phenyl ring into the W109 sub-pocket, however, the cyclopentane ring must adopt different orientations to accommodate this. The MM-PBSA binding affinity score does not discriminate within this subset, given that each achieves a similar affinity estimate. However, there are differences in the D113 hydrogen bond fraction, in particular compound **16** achieves an average count of 1.86 across simulations compared to 2.0 for compound **10** (see Supporting Information Table S1). This may be attributed to the different cyclopentane orientation of compound **16**, which no longer shields the ethanolamine linker – D113 interaction from solvent.

Conversely, compounds **13**, **14**, **17** and **18** have the opposite (*S*)-configuration at the benzylic alcohol compared to compound **10** (*R-*). In this case, the MM-PBSA binding affinity reasonably discriminates these analogs, with compounds **10** and **13** having similar scores and **14**, **17** and **18** all having poorer MM-PBSA scores. Inspection of the docking poses for compounds **10** and **13** (see Figure 2 C) indicates that although the benzylic alcohol can find a similar orientation to reach D113, this comes at the expense of benzothiazolone and may therefore disrupt fit into the pocket.

Compounds **24**, **25** and **26** shift or remove key functional groups that contribute to the β2-AR pharmacophore: compound **24** moves the alcohol at the benzothiazolone from the 4- to 5-position, compound **25** removes this phenol while compound **26** removes the benzylic alcohol on the ethanolamine linker. Compound **26** comes at the greatest expense of binding pKi, mini-G and arrestin-2 pEC50 with significantly reduced D113 hydrogen bonding and poorer MM-PBSA score (see Table 1 and Supporting Information Table S1).

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**Figure 2** – A) Docking poses after molecular dynamics refinement for compounds **10** (cyan), **11** (salmon), **15** (brown) and **16** (green) which share the same R- stereochemistry at the benzylic alcohol position yet differing stereochemistry at the cyclopentane. B) Correlation between measured β2-AR binding pKi and MM‑PBSA binding affinity score from molecular dynamics for the benzothiazolones in Table 1. C) Docking poses after molecular dynamics refinement for compounds **10** (cyan) and **13** (yellow) which share the same 1R,2R- stereochemistry at the cyclopentane yet differing stereochemistry at the benzylic alcohol.

***Asn293 hydrogen bond is key for functional agonist efficacy of the stereoisomers 10, 11, 13-18***

We studied the protein-ligand interaction fingerprints derived from the production molecular dynamics simulations for correlation with measured efficacy. Compounds **10**, **11**, **13**-**18** represent stereochemically pure isomers of the same constitution and therefore an interesting subset, since all the molecules have the same functional groups to interact with the receptor with similar physical properties. We found a strong correlation between the ability of the diastereosiomers to form a hydrogen bond to N293 of β2*‑*AR and mini-G pEC50 with Pearson R=0.87 p-value=0.00497 (see Figure 3 A). Concurrently, we find an inverse correlation between mini-G pEC50 and root-mean-square deviation (RMSD) of the PIF microswitch away from the active state with Pearson R=‑0.783 p-value=0.02153 (see Figure 3 B). The PIF motif, formed by P211/I121/F282 in β2*‑*AR, sits directly below the orthosteric ligand binding pocket Figure 4 whose re-arrangement is implicated in the activation/de-activation process of class A GPCRs.9 This suggests that the N293 hydrogen bond to the carbonyl of the benzothiazolone stereoisomers stabilizes the agonist binding mode; loss of this hydrogen bond by the weaker analogs results in movement of the ligand in the pocket and destabilization of the PIF motif. This is further evidenced by the shifts in pi-stacking of the benzothiazolone stereoisomers from F290 to F289 Figure 4.

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**Figure 3** – A) Correlation between measured mini-G pEC50 of the stereosiomers **10**, **11**, **13**-**18** and fraction of N293 hydrogen bond formed during molecular dynamics simulation. B) Correlation between measured mini-G pEC50 of the diastereoisomers and root-mean-square deviation (Å) of the PIF motif of β2‑AR away from the active state (3SN61) during molecular dynamics simulation.

A collage of different types of molecules

Description automatically generated

**Figure x** – Movement of potent analogs from pi-stacking to F290 towards pi-stacking to F289 for weaker analogs. A) F290 pi-stacking dynamic protein-ligand interaction fingerprints from molecular dynamics simulations for the benzothazolone stereoisomers **10**, **11**, **13**-**18**. B) F289 pi-stacking dynamic protein-ligand interaction fingerprints from molecular dynamics simulations for **10**, **11**, **13**-**18**. C) The PIF motif, formed by P211/I121/F282, is directly beneath the orthosteric ligand binding pocket (compound **10** shown in cyan). D) Compound **10** (cyan) pi-stacks to F290 (grey), whereas compound **18** (dark blue) shifts to pi-stack to F289 (also dark blue).

***Methyl substitution at cyclopentane increases pose stability***

Methyl substitution at the cyclopentane position improves β2-AR binding pKi for the stereochemically matched molecular pairs of compound **27** vs compound **10** and compound **42** versus compound **11**. However, this only translates into a modest improvement in mini-G functional efficacy for compound **27**, with compound **42** and **11** having similar mini-G efficacy. Comparing the ligand binding poses of compound **11** and **42** (see Figure 5A), the methyl substitution of compound **42** causes a disruption of the binding pose, with the cyclopentane adopting an orientation away from D113, similar to the pose observed for compound **16**. However, in the case of compound **27** the methyl is well accommodated into the pocket, with compounds **10** and **27** sharing identical binding poses (see Figure 4 C). In the case of compound **27**, the methyl is proximal to residue F193, however, the dynamic protein-ligand interaction fingerprints do not reflect a large difference between van der Waals contacts between the compounds **10** / **27** and F193 (see Figure 4 B). We do see an improved MM-PBSA binding affinity score and lower overall RMSD of compound **27** in the pocket compared to compound **10** (see Supporting Information Table S3), indicating a more stable binding pose and increased non-covalent interactions. This indicates the additional methyl of **27** may occupy this region of the pocket with hydrophobicity to stabilize the ligand binding pose – this will also slightly increasing lipophilicity of the agonist, which typically improves biomolecular recognition due to the hydrophobic effect.10 Finally, the methyl substitution may rigidify the bound conformer, reflected in a lower strain energy derived from conformer sampling and quantum mechanics (see Supporting Information Table S3).11

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Description automatically generated

**Figure 5** – A) Docking poses after molecular dynamics refinement for des-methyl compound **11** (salmon) and methylated compound **42** (magenta). B) Dynamic protein-ligand interaction fingerprints for van der Waals contact between compounds **10**, **27** and **42** to F193 residue. C) Docking poses after molecular dynamics refinement for des-methyl compound **10** (cyan) and methylated compound **27** (dark blue).

***Biased analogs display a change in serine hydrogen bonding contacts***

Origins for ligand bias are of high interest to the GPCR community and continue to be studied. Compared to compound **10**, which has full efficacy on both G protein and arrestin-2, there is clear ligand bias for the subset of compounds **16**, **24**, **25** and **26**, although this is somewhat convoluted by differing efficacy on the G protein pathway. We studied the dynamic protein-ligand interaction fingerprints of this subset for correlations with ligand bias; however, we did not find a statistically significant correlation between specific interactions and bias. We do however observe differing serine interactions, with biased ligands **24** & **25** displaying lower serine 207 hydrogen bonding and higher serine 203 hydrogen bond interactions (see Table 2) compared to unbiased, full agonist compound **10**. However, this pattern is not observed with biased compounds **16** & **26**. Structural observations of formoterol bound to the β1-AR arrestin-1 complex indicate weakened interactions to these two serine residues on helix-V of β1-AR compared to the G protein coupled state,12 suggesting these interactions may be important determinants of ligand bias.

***To update: in general, ligand bias follows miniG Bmax. Input David/Dmitry? MD simulations do not capture the origins of ligand bias***

**Table 2** – Pharmacology data and serine 203 and 207 hydrogen bond (HB) fractions from molecular dynamics simulations for the subset of biased compounds **16**, **24**, **25**, **26** and unbiased full agonist **10**.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Id** | **binding pKi** | **miniG pEC50** | **miniG Bmax** | **GsCASE pEC50** | **GsCASE Bmax** | **arrestin-2 pEC50** | **arrestin-2 Bmax** | **SER203 HB fraction** | **SER207 HB fraction** |
| **10** | 9.43 | 8.59 | 108.5 | 8.48 | 92.8 | 8.5 | 109.7 | 0.55 | 0.88 |
| **16** | 6.86 | 8.54 | 91.3 | 8.51 | 97.4 | 7.93 | 56.3 | 0.61 | 0.90 |
| **24** | 7.92 | 8.78 | 73.5 | 7.47 | 104.2 | 8.59 | 16.7 | 0.76 | 0.75 |
| **25** | 9.03 | 8.9 | 60.1 | 6.94 | 82.4 | - | <20 | 0.79 | 0.02 |
| **26** | 7.99 | 8.33 | 59.6 | 7.85 | 71.4 | - | <20 | 0.68 | 0.89 |

**Conclusion**

A range of potencies and signalling biases were determined across all the ligands. By characterising all 8 diastereoisomers possible to better understand the interactions driving activation.

Interaction of the N-substituent benzyl group with W109 sub-pocket key interaction and retained through alternative positioning enthanolamine linker and benzothaizolone.

5-hydroxybenzothiazolone 12 found to be a high affinity and relatively low IE agonist. Observation formed the starting point for the identification of the oral β2-AR agonist 5-HOB for the treatment of muscle-wasting conditions.

Relationships between IE and G-protein IE

Lower IE ligands show higher levels of β-arrestin bias, no obvious differences in the way the ligands interact the B2AR to activate Gs and arrestin-2 signalling: orthosteric agonist stabilize a similar ensemble of conformations activating both G-proteins and β-arrestin, G-protein activation is more efficient.

**Experimental Section**

General paragraph

Step 1: Preparation of QAQ486

A 12 L 3-neck flask was charged with K2(CO)3 (813.3 mmol, 112.4 g), K3Fe(CN)6 (813.3 mmol, 267.8 g) and (DHQD)2PHAL (10.84 mmol, 8.45g), anhydrous t-BuOH (2 L) and water (2.2 L). The reaction mixture was stirred vigorously for 30 min at ambient temp. The mixture was then cooled to 5 °C (internal temp) and a solution of OsO4 (2.5 wt% in *tert*.BuOH) (5.42 mmol, 55.13 mL) was added drop-wise over 15 min followed by a solution of PLB003577 (271.1 mmol, 1.0 equiv, 79g) in t-BuOH (200 mL) over 45 min. The resulting mixture was allowed to warm to ambient temp and stirred for 22h. The TLC and LC/MS analysis confirmed complete consumption of starting material. The reaction was quenched by adding sodium bisulfite (260 g) in portions at ambient temp over 30 min and stirred for 1h. Then the mixture was extracted with EtOAc (5L), organic layer was separated and washed with brine (1 L). The organic layer was dried over Na2SO4, filtered and concentrated under vacuum to give ~90 g of crude QAQ486. The crude product was purified by silica gel column by eluting with 0 – 60% EtOAc/Hexane mixture. The product containing fractions were combined and concentrated under reduced pressure to afford 58g of QAQ486 (66.7% yield, >98% purity by LC/MS) as a sticky solid. The above solid was triturated with hexane (300 ML) to afford the product QAQ486 as a white solid.

***Ligand docking***

To dock compounds 1-12, first the coordinates of the active state β2-AR coupled to Gs protein with full agonist BI-167107 bound (3SN61) were downloaded from the OPM database.16 The β2‑AR (chain R) and BI-167107 coordinates only were kept, removing G protein and antibody regions. The protein was then prepared using CCG MOE to add hydrogens, assign standard amino acid protonation states at pH 7.4, and optimize the hydrogen bonding network.17 Schrodinger Glide was then used to prepare a docking grid, with the coordinates of BI-167107 used to define the grid center and a grid was generated with inner box size of 10 Å and outer box size of 25 Å.18 Compounds 1-12 were then docked using Schrodinger Glide SP before manual inspection and further minimization with CCG MOE. Strain analysis was performed on the docking poses using the ReSCoSS program.14

***Molecular dynamics simulations***

To prepare for molecular dynamics simulations, missing loops in ECL2 (A176-H178) and ICL2 (F240-F264) were filled using the AlphaFold2-multimer prediction of β2-AR coupled to Gs protein from GPCRdb.3 The acidic residues D79 (sodium binding pocket), E122 (membrane exposed) and D130 (DRY motif) were modelled as charge neutral, protonation states assumed in the active state of β2-AR.19 The receptor was then embedded into a solvated POPC lipid bilayer specifying a minimum distance of 10 Å between the receptor and box edge and a water layer thickness of 15 Å, with 0.15 M NaCl salt solution added such that the system was charge neutralized using the PACKMOL‑memgen utility from AmberTools24.20-22

Each of the complexes consisting of compounds 1-12, the prepared β2-AR receptor and POPC bilayer simulation box then underwent the same equilibration protocol in triplicate using Amber24 and PMEMD CUDA on GPU cards.21, 23 The β2-AR receptor was modelled with ff19SB,24 lipids with Lipid21,25 water with TIP3P,26 salt ions with JC parameters27 and ligands with gaff228 and conformationally averaged AM1-BCC charges.29 First, the system was minimized for 1000 steps using sander, with the first 500 steps using the steepest descent method and the remaining steps using the conjugate gradient method. Next, a longer minimization was performed with PMEMD CUDA of 10,000 steps; the first 5000 steps used the steepest descent method and the remaining steps used the conjugate gradient method. The system was then heated  from 0 to 100 K in a 5 ps constant volume run using Langevin dynamics,30 with restraints of 10 kcal/mol/Å2 applied to all atoms, excluding waters and salt ions. The volume was then allowed to change freely, and temperature increased to 303 K with a Langevin collision frequency of γ = 1 ps–1, with anisotropic Berendsen control of the pressure around 1 atm applied by coupling the periodic box with a time constant of 2 ps for 100 ps.31 The simulation continued in the NPT ensemble with semi-isotropic Berendsen pressure coupling, with the X- and Y-dimensions coupled and the Z-dimension allowed to change freely for 1 ns, with restraints on 5 kcal/mol/Å2 applied to receptor backbone atoms and ligand atoms only. An additional 1 ns of NPT simulation followed, with 5 kcal/mol/Å2 restraints on receptor carbon-alpha and ligand atoms only. Finally, all restraints were removed and the system was simulated in the NPT ensemble for 100 ns.

Upon equilibration, triplicate 800 ns production simulations were performed for each of the compounds 1-12 using identical NPT settings, with exception that the Monte Carlo barostat was applied for pressure coupling,32 and Hydrogen Mass Repartitioning was applied to allow a 4 fs time-step.33

***Simulation analysis protocols***

MM-GBSA and MM-PBSA ligand binding affinity scores were calculated with the MMPBSA.py utility in AmberTools24 on the production trajectories with a frame rate of every 10 ns.22, 34 Key polar interactions in the ligand binding pocket were counted with the CPPTRAJ utility in AmberTools24.22, 35 RMSD analysis of the PIF motif to the 3SN6 active state conformation was also determined using CPPTAJ. Dynamic protein-ligand interaction fingerprints were calculated on each production trajectory using the ProLIF package,36 using a frame rate of 1 ns and a radius of 12 Å from the ligand to consider interactions.

All values are reported as bulk averages over the triplicate production runs with standard deviation. Figures were generated with PyMOL and matplotlib.37

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**References**

1.

**Supporting Information**

Preparation of 1-tert-butoxy-4-fluoro-2-nitro-benzene:

A solution of potassium tert-butoxide (69.4 g) in THF (500 ml) is added over a period of 1 h to a solution of 2,5-difluoronitrobenzene (81.2 g, 500 mmol) in THF (500 ml) at -5 °C under nitrogen. The reaction mixture is stirred for 1 h at this temperature and a part of the THF (890 ml) is distilled off. Ethyl acetate (600 ml) is added for better phase separation and the organic phase is extracted with a saturated NH4Cl solution (2 x 375 ml) and finally with water (400 ml). The organic layer is concentrated in vacuo to give 129.2 g (121%) of the title compound (91 area% by HPLC) as oil.

Preparation of 2-tert-butoxy-5-fluoro-aniline:

A solution of crude 1-tert-butoxy-4-fluoro-2-nitro-benzene (129.2 g, 500 mmol) in ethanol (1 l) is treated with palladium (10%) on carbon (12.9 g). The resulting suspension is then heated to 45 °C and hydrogen is applied with a pressure of 0.2 bar for a period of 3.5 h until the hydrogen uptake is complete. The reaction mixture is cooled to room temperature and the catalyst is filtered off. Ethanol is used to wash the catalyst. The solution is concentrated in vacuo to give 92.6 g (101%) 2-tert-butoxy-5-fluoro-aniline (96 area% by HPLC) as oil.

Preparation of 1-tert-butoxy-4-fluoro-2-isothiocyanato benzene:

A solution of thiophosgene (24 g, 209 mmol) in dichloromethane (18 ml) is added to a biphasic mixture of 2-tert-butoxy-5-fluoro-aniline (38.15 g, content 91.4%, 190 mmol), dichloromethane (175 ml) and 2 N NaOH (285 ml) at 0-2 °C over a period of 1 h. The mixture is stirred for 1 h at this temperature and then heated to 25 °C within 1 h and stirred for an additional hour. The phases are separated and the organic layer is washed with water (200 ml) in two portions and is concentrated in vacuo to give 44.7 g (105%) of the crude product. Purification by flash-chromatography (SiO2, heptane/ethyl acetate 95:5) yielded 36.64 g of 1-tert-butoxy-4-fluoro-2-isothiacyanato benzene (85.6%) as pale yellow oil (97.8 area% by HPLC).

Preparation of (2-tert-butoxy-5-fluoro-phenyl)-thiocarbamic acid O-isopropyl ester

Isopropyl alcohol (7.5 g, 125 mmol) is added to a suspension of sodium hydride (3 g, 60% dispersion in mineral oil) in toluene (175 ml) at 20 °C within 30 min. The mixture is cooled to 0 °C and a solution of 1-tert-butoxy-4-fluoro-2-isothiacyanato benzene (11.5 g, 50 mmol) in toluene (50 ml) is added over a period of 1 h maintaining a temperature of 0-5 °C. The resulting suspension is aged for 1.5 h while warming to 20 °C. The mixture is cooled to 0 °C again and an aqueous saturated solution of NH4Cl (50 ml) is added. After warming to 20 °C the phases are separated and washed with water (2 x 30 ml). The aqueous phases are back-extracted with toluene (2 x 30 ml). The organic phases are combined and concentrated in vacuo to give 15.1 g (93 area% by HPLC) of the crude product as pale yellow crystals. The crude product is recrystallised from isopropanol to yield 11.9 g (99.5 area% HPLC) of the title compound (83.2%) as off white crystals.

Preparation of 1-(4-tert-butoxy-2-isopropoxy-benzothiazo-7-yl)-2-chloro-ethanone:

A solution of tert-butyllithium (24.7 g, 15% in pentane, 57.75 mmol) is added over a period of 40 min to a solution of (2-tert-butoxy-5-fluoro-phenyl)-thiocarbamic acid O-isopropyl ester (15 g, 52.5 mmol) in tetrahydrofuran (150 ml) at -70 °C under an atmosphere of nitrogen. The mixture is than warmed to -30 °C and the second portion of tert-butyllithium (31.4 g, 15% in pentane, 73.5 mmol) is added over a period of 35 min. The reaction mixture is aged for 1.5 h, before being cooled to -70°C. A solution of N-methyl-N-methoxy chloro acetamide (9.4 g, 68.25 mmol) in tetrahydrofuran (9.4 g) is added over a period of 0.5 h to the reaction mixture. After stirring for 3 h the pH is adjusted to 3-4 with 1 M hydrochloric acid (105 ml) at -30 °C. The organic phase is separated and washed with water (2 x 55 ml) and the aqueous phases are back-extracted with ethyl acetate (2 x 55 ml). The organic phases are combined, dried over MgSO4 and concentrated in vacuo to give 20.59 g (80 area% by HPLC) of the crude product as an oil. The crude product is recrystallised from isopropanol to yield 10.8 g (96.5% area HPLC) of the title compound (60%) as off white crystals.

Preparation of 1-(4-tert-butoxy-2-isopropoxy-benzothiazo-7-yl)-2-chloro-ethanol:

(1R,2S)-(+)-cis-1-Amino-2-indanol (0.44 g, 2.95 mmol), is added to a solution of 1-(4-tert-butoxy-2-isopropoxy-benzothiazo-7-yl)-2-chloro-ethanone (10 g, 29.2 mmol) in tetrahydrofuran (100 ml). The mixture is aged at 25 °C for 15 min followed by the addition of borane (29.2 ml, 29.2 mmol) in tetrahydrofuran over a period of 1 h under argon. After ageing for 15 min 0.1 N sulphuric acid (100 ml) is added for quenching. Ethyl acetate (200 ml) is added and the phases are separated after 5 min. The organic layer is washed with water (30 ml) and the aqueous phases are back-extracted with ethyl acetate (50 ml). The organic phases are combined, dried over MgSO4 and concentrated in vacuo to give 10.4 g (90.2 area% by HPLC) of the crude product as an oil. The crude product is recrystallised from heptane to yield 7.7 g (96.8% area HPLC, 98.2 % (e/e)) of the title compound (77 %) as white crystals.

Preparation of 4-tert-butoxy-2-isopropoxy-7-(R)-oxiranyl-benzothiazol:

Potassium carbonate (20.1 g, 145.4 mmol) and water (10 ml) are added to a solution of 1-(4-tert-butoxy-2-isopropoxy-benzothiazo-7-yl)-2-chloro-ethanol (20 g, 58.16 mmol) in acetone at 23-25 °C. The suspension is heated to 52 °C and aged for 1 d. The salts are filtered off and the filtrate is dried in vacuo to give 18.22 g of the title compound (102% of theory) as an colorless oil (99.0% area by HPLC).

Preparation of (R)-2-((1S,2S)-2-Benzyloxy-cyclopentylamino)-1-(4-tert-butoxy-2-isopropox-benzothiazol-7-yl)-ethanol:

(1S,2S)-2-Benzyloxycyclopentylamine (8.44 g, 44.1 mmol) is added to a solution of 4-tert-butoxy-2-isopropoxy-7-(R)-oxiranyl-benzothiazol (13.13 g, 42 mmol) in acetonitrile 64 ml) at 25 °C. The reaction mixture is then heated at 82 °C over a period of 22 h. 24 ml of acetonitrile is removed by distillation under slightly reduced pressure at 80 to 65 °C. The solution is then cooled to 35-30 °C and seeded with the title compound. The resulting suspension is cooled to 0- -5 °C, the suspension is filtered, washed with acetonitrile (10 ml) and dried in vacuo at 60 °C to yield 13.4 g of the title compound (64.1%) as white crystals, (97.6% area by HPLC).

Preparation of 7-[(R-2-((1S,2S)-2-benzyloxy-cyclopentylamino)-1-hydroxy-ethyl]-4-hydroxy-3H-benzothiazol-2-one

A suspension of (R)-2-((1S,2S)-2-benzyloxy-cyclopentylamino)-1-(4-tert-butoxy-2-isopropox-benzothiazol-7-yl)-ethanol (25 g, 50.1 mmol) in isopropanol (94 ml) and 75 ml water is treated with 1 N hydrochloric acid (150 ml) within 15 min at 25 °C. The mixture is then heated at 70°C for 2.5 h to become a clear solution. The reaction mixture is cooled to 60 °C and 2 N sodium hydroxide (90 ml) is added over a period of 0.5 h. The resulting suspension is then cooled to 0 °C. The product is filtered off, washed with isopropanol/water (1:1 2 x 25 ml) and dried in vacuo at 60°C. Yield 17.09 g of the product (85.3%) as white crystals (99.2% area by HPLC, 98.4 % (e/e)).

Bias plots







**Table S?** – Measured β2-AR binding pKi values and MM-PBSA binding affinity scores from molecular dynamics simulations for the benzothiazolones in Table 1.

|  |  |  |
| --- | --- | --- |
| **Compound** | **Binding pKi** | **MM-PBSA affinity score (kcal/mol)** |
| **10** | 9.43 ± 0.16 | -66.42 ± 1.07 |
| **11** | 7.2 ± 0.12 | -67.08 ± 1.81 |
| **13** | 7.36 ± 0.14 | -68.37 ± 2.11 |
| **14** | 6.01 ± 0.13 | -56.06 ± 0.37 |
| **15** | 7.93 ± 0.18 | -72.86 ± 0.67 |
| **16** | 6.86 ± 0.15 | -63.60 ± 1.81 |
| **17** | 5.91 ± 0.16 | -56.63 ± 4.31 |
| **18** | 4.33 ± 0.22 | -58.81 ± 0.68 |
| **24** | 7.92 ± 0.22 | -72.07 ± 3.40 |
| **25** | 9.03 ± 0.09 | -71.77 ± 0.64 |
| **26** | 7.99 ± 0.21 | -58.67 ± 1.98 |
| **27** | 9.93 ± 0.18 | -87.69 ± 0.51 |
| **42** | 7.91 ± 0.15 | -77.41 ± 1.47 |

**Table S?** - Hydrogen bond (HB) fractions from molecular dynamics simulations for compounds 1-12 and PIF RMSD (Å) from the active state 3SN6 over the production simulations.1

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Id** | **ASP113 HB Fraction** | **ASN312 HB Fraction** | **ASN293 HB Fraction** | **SER203 HB Fraction** | **SER204 HB Fraction** | **SER207 HB Fraction** | **PIF RMSD active (Å)** |
| Formoterol **5** | 2.00 | 1.76 | 0.20 | 0.19 | 0.00 | 0.78 | 1.14 |
| **10** | 2.00 | 1.70 | 0.27 | 0.55 | 0.00 | 0.88 | 1.18 |
| **11** | 2.05 | 1.65 | 0.45 | 0.51 | 0.00 | 0.87 | 1.24 |
| **13** | 1.99 | 1.37 | 0.18 | 0.66 | 0.00 | 0.94 | 1.35 |
| **14** | 1.92 | 1.52 | 0.13 | 0.52 | 0.02 | 0.60 | 1.34 |
| **15** | 1.98 | 1.91 | 0.32 | 0.61 | 0.00 | 0.94 | 1.38 |
| **16** | 1.86 | 1.74 | 0.19 | 0.61 | 0.00 | 0.90 | 1.32 |
| **17** | 1.99 | 1.47 | 0.09 | 0.57 | 0.00 | 0.78 | 1.42 |
| **18** | 1.97 | 1.21 | 0.03 | 0.54 | 0.00 | 0.89 | 1.49 |
| **24** | 2.00 | 1.71 | 0.31 | 0.76 | 0.00 | 0.75 | 1.19 |
| **25** | 1.99 | 1.65 | 0.46 | 0.79 | 0.00 | 0.02 | 1.38 |
| **26** | 1.02 | 1.69 | 0.49 | 0.68 | 0.00 | 0.89 | 1.10 |
| **27** | 1.99 | 1.66 | 0.55 | 0.64 | 0.00 | 0.94 | 1.50 |
| **42** | 1.97 | 1.59 | 0.29 | 0.55 | 0.00 | 0.95 | 1.29 |

**Table S?** – Binding affinity scores from docking and production molecular dynamics simulations, ligand RMSD (Å) during simulations and strain energy derived from a conformational sampling procedure with quantum mechanical energies of the posed conformer versus the global minima identified.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Id** | **docking score (kcal/mol)** | **MM-GBSA (kcal/mol)** | **stdev MM-GBSA** | **MM-PBSA (kcal/mol)** | **stdev MM-PBSA** | **Lig RMSD (A)** | **stdev Lig RMSD** | **Strain (kcal/mol)** |
| Formoterol **5** | -10.07 | -62.36 | 0.18 | -57.81 | 0.67 | 1.21 | 0.18 | 2.81 |
| **10** | -9.36 | -71.89 | 0.90 | -66.42 | 1.07 | 1.88 | 0.82 | 6.65 |
| **11** | -9.35 | -69.61 | 2.15 | -67.08 | 1.81 | 1.69 | 0.40 | 13.00 |
| **13** | -9.91 | -74.39 | 1.81 | -68.37 | 2.11 | 2.88 | 0.08 | 7.88 |
| **14** | -9.34 | -63.81 | 0.82 | -56.06 | 0.37 | 2.21 | 0.75 | 7.88 |
| **15** | -9.64 | -75.15 | 1.34 | -72.86 | 0.67 | 2.76 | 0.10 | 6.40 |
| **16** | -9.21 | -68.62 | 0.71 | -63.60 | 1.81 | 1.64 | 0.08 | 6.54 |
| **17** | -9.28 | -63.50 | 2.80 | -56.63 | 4.31 | 2.59 | 0.05 | 7.79 |
| **18** | -9.76 | -67.04 | 0.75 | -58.81 | 0.68 | 1.96 | 0.16 | 10.07 |
| **24** | -11.29 | -76.70 | 1.34 | -72.07 | 3.40 | 2.41 | 0.28 | 4.18 |
| **25** | -10.95 | -74.82 | 0.69 | -71.77 | 0.64 | 2.01 | 0.61 | 6.49 |
| **26** | -8.93 | -66.53 | 0.82 | -58.67 | 1.98 | 1.67 | 0.35 | 4.97 |
| **27** | -9.90 | -89.83 | 0.49 | -87.69 | 0.51 | 0.94 | 0.01 | 5.59 |
| **42** | -9.06 | -83.72 | 0.43 | -77.41 | 1.47 | 1.81 | 0.16 | 6.52 |