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Identification of potent CNS-penetrant thiazolidinones as novel CGRP receptor antagonists

Pramod Joshi [a], Corey Anderson [a], Hayley Binch [a], Sabine Hadida [a], Sanghee Yoo [b], Danielle Bergeron [b], Caroline Decker [c], Ernst terHaar [d], Jonathan Moore [d], Miguel Garcia-Guzman [e], Andreas Termin [a,*]

[a] Vertex Pharmaceuticals Inc., Department of Chemistry and Drug Innovation, 11010 Torreyana Road, San Diego, CA 92121, United States
[b] Vertex Pharmaceuticals Inc., Department of Biology, 11010 Torreyana Road, San Diego, CA 92121, United States
[c] Vertex Pharmaceuticals Inc., Department of Drug Metabolism and Pharmacokinetics, 11010 Torreyana Road, San Diego, CA 92121, United States
[d] Vertex Pharmaceuticals Inc., Department of Structural Biology, 130 Waverly Street, Cambridge, MA 02139, United States
[e] Aspyrian Therapeutics Inc., 11189 Sorrento Valley Rd, San Diego CA 92121, United States

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ABSTRACT

Calcitonin gene-related peptide (CGRP) has been implicated in acute migraine pathogenesis. In an effort to identify novel CGRP receptor antagonists for the treatment of migraine, we have discovered thiazolidinone **49**, a potent (K_i = 30 pM, IC_{50} = 1 nM), orally bioavailable, CNS-penetrant CGRP antagonist with good pharmacokinetic properties.

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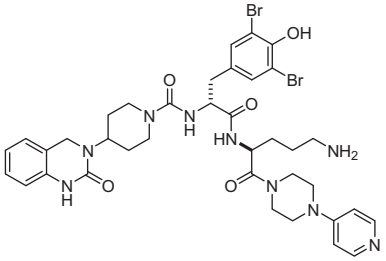
CGRP is a 37-amino acid neuropeptide that is expressed and released by the trigeminal ganglia nerve fibers, and plays a critical role in migraine.[1a] CGRP signals through a heteromeric receptor composed of a G protein-coupled receptor called calcitonin receptor-like receptor (CRLR) and a receptor activity modifying protein (RAMP1). CGRP is one of the most potent endogenous vasodilators known.[1b] Increased levels of CGRP are observed during migraine attacks and intravenous administration of CGRP can induce migraines.[1c]

An early clinical proof of concept study showed that intravenous dosing of BIBN4096 (olcegepant), a potent CGRP receptor antagonist, was efficacious in alleviating pain during migraine headaches.[1a] Olcegepant showed comparable efficacy to triptans, and showed no serious cardiovascular effects.[2] Olcegepant has poor oral bioavailability (% F < 1), and limited CNS exposure; therefore its development was discontinued due to the rapid clearance and poor physical properties of this high molecular weight (MW 855) peptidic molecule.[1a] We initiated a program to identify a potent, orally bioavailable, CNS-penetrant CGRP-receptor antagonist with good physicochemical properties. More recently, Merck[3–5] has reported positive Phase III clinical trial data for telcagepant



BIBN4096BS (Olcegepant) MW 855

Figure 1. Structure of olcegepant (BIBN4096).

Table 1

Compound	IC_{50}[a] (μM)	K_i[b] (μM)
Olcegepant	0.004 ± 0.063	0.00001 ± 0.00002
1	0.74 ± 0.29	0.282
2	0.37 ± 0.16	0.032 ± 0.026

[a] IC_{50} β-lactamse assay[7] ($n \geqslant 3$) with cells treated with CGRP, compound and cAMP antibody and represents cellular inhibition of cAMP production.
[b] K_i Binding assay ($n \geqslant 3$) SK-N-MC membrane treated with ^{125}I-CGRP, compound and $K_i = IC_{50}/(1 + [\text{radioligand}]/K_d)$ was calculated.[6]

* Corresponding author.
E-mail addresses: Miguel_Guzman@aspyriantherapeutics.com (M. Garcia-Guzman), andreas_termin@vrtx.com (A. Termin).

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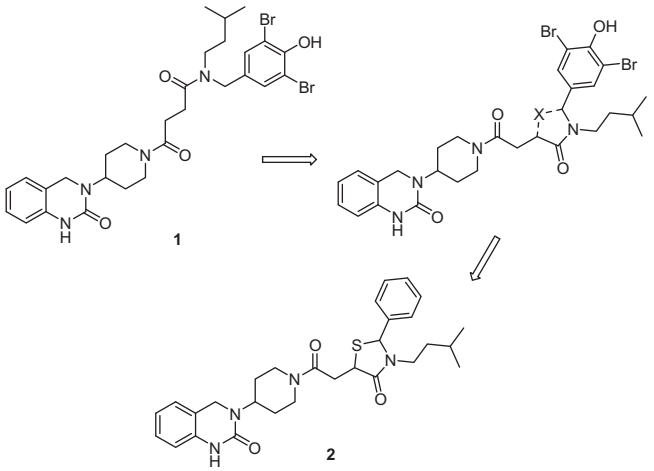


Figure 2. Conformational constraint provided potent thiazolidinone **2**.

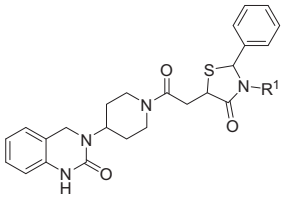
(MK-0974), a CGRP receptor antagonist with improved oral bioavailability but limited CNS exposure (Fig. 1).

Examining the structure of olcegepant, we hypothesized that reduction of its peptidic nature and lowering the molecular weight and PSA could improve oral bioavailability and provide an antagonist with better physicochemical properties. Our initial approach involved screening of smaller fragments of olcegepant to assess their CGRP receptor binding. This effort led to the identification of **1** (Table 1) which showed modest competitive binding in a [^{125}I]CGRP receptor radioligand binding assay and had significantly reduced molecular weight (MW 664).[6] Compound **1** was further tested for its functional ability to inhibit CGRP-stimulated cellular cAMP production using a reporter assay[7] and was found to be moderately potent.

We subsequently hypothesized that applying a degree of conformational constraint to **1** could potentially improve the potency of this molecule. Several cyclic cores incorporating these features were tested (data not shown) and thiazolidinone **2**, which showed a three-fold gain in CGRP receptor binding compared to **1**, was selected for further SAR exploration (Fig. 2).

The thiazolidinone scaffold was especially well suited for parallel synthesis using mercaptosuccinic acid (MSA) and a large pool of aldehydes and amines as reagents. This three-component synthesis involved formation of the imine and treatment of the reaction mixture with MSA in one pot to give the racemic thiazolidinone acetic acid.[8] Thus compound **2** was prepared (Scheme 1) from the reaction of MSA and the imine generated from benzaldehyde and isopentyl amine, and subsequent coupling of the resulting thiazolidinone acetic acid with dihydroquinazolinone using amide bond coupling conditions (HATU and DIEA in DMF at ambient temperature).

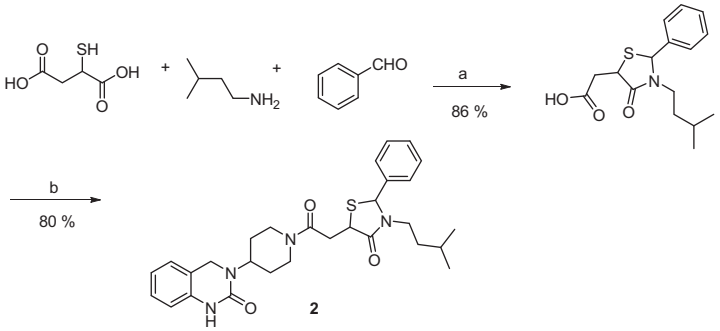
Table 2
Effect of substitution (R^1), on the amine of thiazolidinone ring



Compound	R^1	IC_{50}[a] (μM)	K_i[b] (μM)
3	Methyl	4.47 ± 3.23	0.203
4	iso-Propyl	1.5 ± 1.15	0.115 ± 0.011
5	Cyclopropyl	5.15	0.865 ± 0.416
6	Methylcyclopropyl	0.84 ± 0.08	0.045
7	Butyl	0.32 ± 0.033	0.064
8	iso-Butyl	0.37+0.16	0.032 ± 0.026
9	tert-Butyl	7.6	1.88
10	2,2,2-Trifluoroethyl	4.54 ± 1.19	0.763
11	Methoxy ethyl	1.22 ± 0.54	0.124
12	2-Diethylaminoethyl	4.88	0.303
13	1-Phenethyl	0.94 ± 0.32	0.297
14	Cyclohexyl	0.71 ± 0.31	0.088
15	3-((Tetrahydrofuran-2-yl)methyl)	0.52 ± 0.13	0.068
16	neo-Pentyl	0.25 ± 0.18	0.009 ± 0.006
17	Phenyl	2.94	0.806
18	4-Fluorobenzyl	0.75 ± 0.1	0.056
19	Methyl-3-pyridyl	0.80 ± 0.39	0.26
20	Ethyl-2-pyridyl	1.37	0.202

[a] IC_{50} β-lactamse assay ($n \geqslant 3$) with cells treated with CGRP, compound and cAMP antibody and represents cellular inhibition of cAMP production.
[b] K_i Binding assay ($n \geqslant 3$) SK-N-MC membrane treated with ^{125}I-CGRP, compound and $K_i = IC_{50}/(1+[\text{radioligand}]/K_d)$ was calculated.

The encouraging data for compound **2** prompted our initial SAR exploration of the thiazolidinone ring focusing on the amide nitrogen and phenyl ring substituents, in an effort to improve CGRP receptor binding and functional cAMP activity. Several thiazolidinones with *N*-alkyl and aromatic moieties were prepared. SAR of this series showed that aliphatic hydrophobic groups on the thiazolidinone core improved CGRP binding. Long chain and branched alkyl groups such as **8** improved binding while *N*-aryl substituted thiazolidinones such as **17** showed a loss in CGRP receptor binding compared to **2**. Compound **16**, with a *neo*-pentyl side-chain showed a seven-fold gain in binding along-with a two-fold gain in functional cAMP inhibition. We speculate that this side-chain of **16** may be accessing the same hydrophobic pocket where the side-chain of the lysine residue of olcegepant resides in the CGRP



Scheme 1. Synthesis of thiazolidinones from MSA, benzaldehyde and isopentylamine. (a) DMF, 80 °C; (b) 3-(piperidin-4-yl)-3,4-dihydroquinazolin-2(1*H*)-one, HATU, DIEA, DMF, RT.

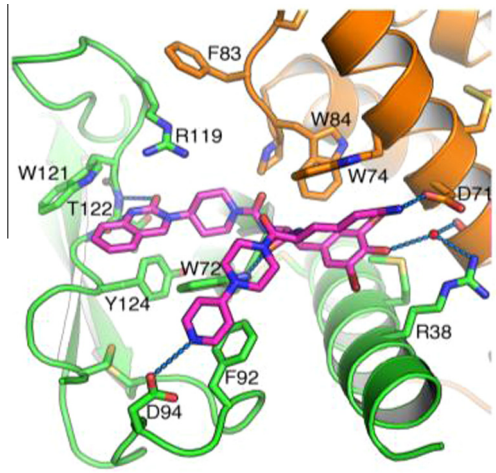
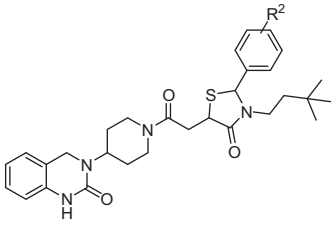


Figure 3. Structure of olcegepant in the N-terminal ectodomain of CLR-RAMP1 complex.[9]

receptor. Polar side chains as exemplified by methoxy methyl ether **11** and diethylamino thiazolidinone **12** showed three to five-fold loss in CGRP receptor binding (Table 2).

The structure of olcegepant with the N-terminal ectodomain complex of CGRP receptor is published.[9] The drug binds in an extended conformation stretching ~18 Å from a hydrogen bond donor-site at Thr122 of CLR, across the interface with RAMP-1 and deep into a hydrophobic pocket formed by helix αC1 of CLR and helix αR2 of RAMP1. RAMP1 residues Trp74 (helix αR2) and Trp84 (in the loop connecting RAMP1 helices αR2 and αR3) form the ceiling and back surface of the hydrophobic pocket. The quinazolone moiety of olcegepant forms a hydrogen bond donor–acceptor pair with the backbone NH and carbonyl Thr 122. The lysine amino group of olcegepant forms a salt-bridge with the RAMP1 Asp71 side chain carboxyl, while the indole of RAMP1 Trp74 stacks with the aliphatic portion. Finally, the terminal pyridyl stacks with the CRLR Phe92 and also makes a hydrogen bond with the side chain carboxyl of CRLR Asp94 (Fig. 3).

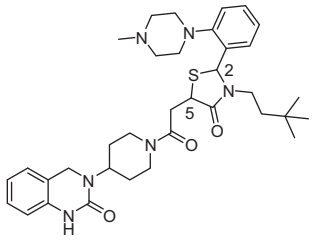
Table 3
Effect of substitution (R^2) on the phenyl group of thiazolidinone ring



Compound	R^2	IC_{50}[a] (μM)	K_i[b] (μM)
21	2-Fluoro	0.18 ± 0.05	0.054
22	2,3-Difluoro	0.21 ± 0.07	0.118
23	2,4-Difluoro	0.19 ± 0.09	0.03
24	2,6-Difluoro	0.17 ± 0.11	0.059
25	2-Piperazine	0.06 ± 0.02	0.002 ± 0.001
26	2-Morpholine	0.16	0.052 ± 0.022
27	2-*N*-Methylpiperazine	0.04 ± 0.02	0.002 ± 0.002
28	2-(4-Hydroxypiperidine)	0.044 ± 0.005	0.018 ± 0.006
29	2-(1*H*-Imidazol-1-yl)	0.086 ± 0.034	0.007 ± 0.0009
30	2-(1*H*-Pyrazol-1-yl)	0.062 ± 0.018	0.04 ± 0.008
31	2-(2-(Pyridin-3-yl)	0.088 ± 0.06	0.022 ± 0.013
32	2-(2-(Pyridin-4-yl)	0.099 ± 0.001	0.02
33	2-(2-(4-Butylpiperazin-1-yl)	0.044 ± 0.005	0.003 ± 0.002
34	2-(4-(2-Hydroxyethyl)piperazin-1-yl)	0.036 ± 0.004	0.008 ± 0.011

[a] IC_{50} β-lactamse assay ($n \geqslant 3$) with cells treated with CGRP, compound and cAMP antibody and represents cellular inhibition of cAMP production.
[b] K_i Binding assay ($n \geqslant 3$) SK-N-MC membrane treated with ^{125}I-CGRP, compound and $K_i = IC_{50}/(1+[\text{radioligand}]/K_d)$ was calculated.

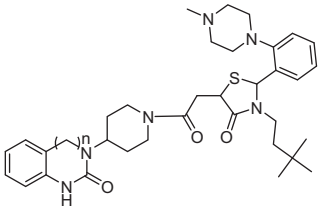
Table 4
Effect of stereochemistry on thiazolidinone ring



Compound	Diastereomer	IC_{50}[a] (μM)	K_i[b] (μM)
35	(2*R*, 5*S*)	0.004 ± 0.002	0.002 ± 0.001
36	(2*R*, 5*R*)	0.098 ± 0.114	0.01 ± 0.011
37	(2*S*, 5*R*)	0.01 ± 0.006	0.0009 ± 0.0005
38	(2*S*, 5*S*)	13.9 ± 12.5	1.8 ± 0

[a] IC_{50} β-lactamse assay ($n \geqslant 3$) with cells treated with CGRP, compound and cAMP antibody and represents cellular inhibition of cAMP production.
[b] K_i Binding assay ($n \geqslant 3$) SK-N-MC membrane treated with ^{125}I-CGRP, compound and $K_i = IC_{50}/(1+[\text{radioligand}]/K_d)$ was calculated.

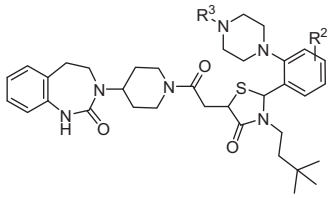
Table 5
Effect of replacement of quinazolinone ($n = 1$) with benzodiazepinone ($n = 2$)



Compound		IC_{50}[a] (μM)	K_i[b] (μM)
27	$n = 1$	0.042 ± 0.02	0.002 ± 0.002
39	$n = 2$	0.019 ± 0.013	0.002 ± 0.0002

[a] IC_{50} β-lactamse assay ($n \geqslant 3$) with cells treated with CGRP, compound and cAMP antibody and represents cellular inhibition of cAMP production.
[b] K_i Binding assay ($n \geqslant 3$) SK-N-MC membrane treated with ^{125}I-CGRP, compound and $K_i = IC_{50}/(1+[\text{radioligand}]/K_d)$ was calculated.

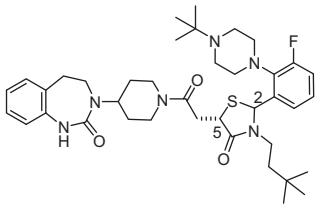
Table 6
Effect of substitutions R^2 and R^3 on thiazolidinone ring



Compounds	R^2	R^3	IC_{50},[a] (μM)	K_i[b] (μM)
40	H	H	0.033 ± 0.005	0.001 ± 0.00001
41	H	Me	0.019 ± 0.013	0.002 ± 0.0002
42	3′-Fluoro	Me	0.003 ± 0.002	0.0005 ± 0.0005
43	4′-Fluoro	Me	0.084 ± 0.005	0.014 ± 0.018
44	5′-Fluoro	Me	0.072 ± 0.023	0.014 ± 0.018
45	6′-Fluoro	Me	0.065 ± 0.021	0.001 ± 0.001
46	3′-Fluoro	Et	0.002 ± 0.0002	0.00007 ± 0.00001
47	3′-Fluoro	^{i}Pr	0.0003 ± 0.0001	0.00002 ± 0.00002
48	3′-Fluoro	^{t}Bu	0.0006 ± 0.0003	0.0001 ± 0.00007

[a] IC_{50} β-lactamse assay ($n \geqslant 3$) with cells treated with CGRP, compound and cAMP antibody and represents cellular inhibition of cAMP production.
[b] K_i Binding assay ($n \geqslant 3$) SK-N-MC membrane treated with ^{125}I-CGRP, compound and $K_i = IC_{50}/(1+[\text{radioligand}]/K_d)$ was calculated.

Table 7
Effect of stereochemistry on thiazolidinone ring for **48**



Compound	IC_{50}[a] (μM)	K_i[b] (μM)
49	0.001 ± 0.0009	0.00003 ± 0.00003
50	0.046 ± 0.01	0.001 ± 0.001

[a] IC_{50} β-lactamse assay ($n \geqslant 3$) with cells treated with CGRP, compound and cAMP antibody and represents cellular inhibition of cAMP production.
[b] K_i Binding assay ($n \geqslant 3$) SK-N-MC membrane treated with ^{125}I-CGRP, compound and $K_i = IC_{50}/(1+[\text{radioligand}]/K_d)$ was calculated.

With the structural information, we next focused on the effect of phenyl ring substitution (R^2) of the thiazolidinone (Table 3) by incorporating amine groups on the phenyl ring to mimic the lysine amino group of olcegepant. Several amines at the 2′ position were tested and were found to have similar binding affinity but improved cAMP inhibitory activity compared to **16**. Thus 2-(4-methylpiperazin-1-yl)phenyl substitution of the thiazolidinone (**27**) and the *tert*-butyl analog **33** (Table 3) retained potent CGRP receptor binding and showed a five-fold improvement in the cAMP assay compared to **16**.

Compound **27** (Table 3) was chosen to assess the stereochemical preference of thiazolidinone diastereomers for binding to the CGRP receptor. All four diastereomers (**35**–**38**) were separated by chiral SFC and tested in both the binding and cAMP inhibition assays (Table 4). The (2*R*, 5*S*) diastereomer **35** was found to be the most potent isomer in both the assays. The relative stereochemical assignment of **35** was based on NOE experiments which showed a strong NOE from the proton at the C-2 position to the proton at the C-5 position, thus proving their *cis*-relationship.

During the course of this study, we observed that the compounds bearing the dihydroquinazolinone moiety underwent benzylic oxidation over time at ambient temperatures to give the quinazolinone, as previously reported.[4] Therefore, we replaced the six-membered dihydroquinazolinone core with the seven membered benzodiazepinone system as exemplified by compound **39**. Compound **39** retained CGRP receptor binding and functional activity compared to dihydroquinazolinone **27**. (Table 5). The benzodiazepinone series did not suffer from oxidative degradation and was used for subsequent SAR explorations.

Probing alkyl substitution of the piperazine nitrogen atom showed that the *tert*-butyl group was preferred as increased lipophilicity on the piperazine increased binding to the CGRP receptor ($R^1 = {}^t\text{Bu} > {}^i\text{Pr} > \text{Et} > \text{Me}$).

Although the initial fluoro-substitution of the aromatic ring of thiazolidinones (**21**) showed only a modest gain in receptor binding, combining the fluoro- substituent with the 2′-*tert*-butyl piperazine group showed marked improvement in CGRP receptor binding and cAMP inhibition. This effort led to thiazolidinone **48** (Table 6).

Based on our earlier observations that the (2*S*, 5*S*) diastereomer was the most potent thiazolidinone core, we needed to prepare the enantiomerically pure (*S*)-MSA isomer. Thus MSA was resolved using a literature procedure to yield enantiomerically pure (*S*)-enantiomer.[11] (*S*)-MSA was subjected to the thiazolidinone reaction (Scheme 1) to give a mixture of diastereomeric acids. This acid mixture was coupled with benzodiazepinone and the resulting products were separated using reverse phase preparative chromatography using acetonitrile and water mixtures to give the desired products.

The pure (2*R*, 5*S*) and (2*S*, 5*S*) diastereomers **49** and **50** were tested in both the binding and cAMP inhibition assays. It was observed that the (2*R*, 5*S*) enantiomer **49** was the more potent isomer in both the binding and cAMP assay (Table 7). This was consistent with our earlier studies which showed that the preferred configuration at C-5 position was (*S*).

Compound **27**, **47**, **49**, olcegepant and telcagepant were evaluated in rat PK studies (Table 8), telcagepant had low iv clearance, whereas olcegepant, **49** and **47** had similar intravenous clearance values (approximately equal to hepatic blood flow) and **27** had higher clearance. Telcagepant, **49**, **47** and **29** had better oral exposure and bioavailability (45, 13, 32, and 53%, respectively) than olcegepant (bioavailability of <1% observed). In addition, the brain to plasma ratio for **47** and **49** (1.51 and 0.53, respectively) are significantly higher than that of the clinically efficacious olcegepant (<0.03) or telcagepant (0.03). The better brain/plasma ratios for thiazolidonones **47** and **49** can be attributed to the significant

Table 8
Rat PK data for select compounds

Compound	%F	AUC (mg.hr/mL)	C_{max} (mg/mL)	Cl (mL/min/kg)	Brain/Plasma ratio	PSA
Olcegepant	<1	0.26	0.87	65	<0.03	176.5
Telcagepant	45	3.32	0.55	27	0.03	97.4
49	13	0.53	0.41	47	0.53	79.4
47	32	0.65	0.12	65	1.51	79.4
27	53	1.6	0.26	127	ND	79.4

Mean of $n = 3$; Telcagepant: 10 mg/kg in 40% PEG400/10% TPGS (α-tocopherol vitamin E succinate)/water; **49** and **47**: 10 mg/kg po in suspension vehicle; **27**: 30 mg/kg in 10% TPGS in suspension; brain to plasma ratios determined following an iv bolus at 0.25 or 1 h post-dose.

reduction in PSA (79.4) (Table 8) compared to telcagepant (PSA 97.4) and olcegepant (PSA 176.5).[12] Although several features influence brain penetration, we believe that lower molecular weight and PSA led to improved permeability for thiazolidinone series. This led us to speculate that the thiazolidinone series offered potential for development of potent, CNS-penetrant CGRP receptor antagonists for the treatment of migraine.

In summary the thiazolidinone series afforded very potent CGRP receptor antagonist with lower molecular weights (693 and 707 for compounds **47** and **49**, respectively) vs. 855 for olcegepant. In rat PK/tissue distribution studies, increased brain exposure for **47** and **49** compared to olcegepant and telcagepant and improved oral bioavailability for **47**, **49** and **27** compared to Olcegepant was observed.

Acknowledgment

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6. Binding assay: SK-N-MC non-recombinant membrane solution was diluted 1:40 in CGRP binding solution and homogenized for about 1 min. Then 40 μL of this solution was plated onto 96-well round bottom plate containing 20 μL of [5×] test compound and incubated for 30 min at room temperature. 40 μL of 0.125 nM 125I-CGRP solution was added to each well (final concentration of 50 pM) and incubated for 2 h at room temperature. The binding reaction was stopped by rapid filtration over 0.5% PEI-treated GF/C filter and washed four times with cold buffer solution. The plate was dried at 65 °C for 1 h. Each well was treated with 50 μL of Microsinct-PS and sealed with TopSeal-A. The plate was read on a Packard Instruments Topcount. Ki was calculated using Cheng–Prusoff equation[10] ($K_i = IC_{50}/(1 + [radioligand]/K_d)$, where $K_d = 55$ pM and IC_{50} are experimentally determined values.
7. β-Lactamase assay: SK-N-MC cell line endogenously expressing CGRP was transduced with a retroviral vector containing a transcription reporter, β-lactamase gene downstream of cAMP responsive promoter. β-Lactamase activity was measured using a fluorescence energy transfer (FRET) dye, CCF4/AM (Zlokarnik, G.; Negulescu, P. A.; Knapp, T. E.; Mere, L.; Burres, N.; Feng, L.; Whitney, M.; Roemer, K.; Tsien, R. Y. *Science* **1998**, *279*, 84). 30,000 cells/well were plated in growth medium (MEM+10% FBS) in poly-D-lysine coated black well and clear bottom, 384 well plate (Greiner) and allowed to grow overnight in regular growth conditions (37 °C, 5% CO_2). The following day, growth medium was removed and replaced with 30 μL/well of assay media (MEM+1% FBS) + compound and pre-incubated for 30 min. After pre-incubation, 10 μL/well of [4×] 0.8-nM of CGRP peptide was added (final concentration of 200 pM). The plate was incubated for 3 h (37 °C, 5% CO_2) and 10 °μL of CCF4/AM was added across plate. The plate was incubated for additional 2 hrs at room temperature and read at tcPR, a Vertex proprietary fluorescence plate reader.
8. Representative experimental procedure: To a mixture of benzaldehyde (20.1 mg, 0.2 mmol) and isopentyl amine (16 mg, 0.2 mmol) in 500 μL of DMF was added mercaptosuccinic acid (45 mg, 0.3 mmol) and the mixture was heated at 80 °C for 16 h. The reaction mixture was cooled and partitioned between ethyl acetate and water. The organics were separated and dried and solvent removed to give the thiazoldinone acetic acid. The acid (31 mg, 0.1 mmol) was coupled with 3-(piperidin-4-yl)-3,4-dihydroquinazolin-2(1*H*)-one (23 mg, 0.1 mmol) using HATU (38 mg, 0.1 mmol) in the presence of diisopropyl ethyl amine (44 μL, 0.25 mmol) in 300 μL of DMF at ambient temperature, and was purified with 10–90% acetonitrile/water to give 3-isopentyl-5-(2-oxo-2-(4-(2-oxo-1,2-dihydroquinazolin-3(4*H*)-yl)piperidin-1-yl)ethyl)-2-phenylthiazolidin-4-one as the desired product.
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