2-Amino-4-benzyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridines: Novel Selective β_3-Adrenoceptor Agonists

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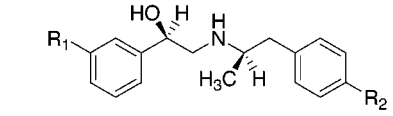
Trimetoquinol (TMQ, **7**) is a potent nonselective β-adrenoceptor (AR) agonist. Replacement of the catechol moiety of TMQ with a 2-aminothiazole group resulted in novel thiazolopyridine derivatives **9**−**11** which have been synthesized and evaluated for biological activity on human β_1-, β_2-, and β_3-AR. The Bischler−Napieralski reaction has been employed as a novel approach to construct the 2-amino-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine ring system. Although in radioligand binding studies analogues **9** and **10** did not show selectivity toward β_3-AR, they exhibited a high degree of selective β_3-AR agonist activity in functional assays. Moreover, the β_3-AR agonist activity of the 2-aminothiazole derivatives is abolished by N-acetylation (analogue **11**) or ring opening (analogue **25**). This illustrates the importance of the intact 2-amino-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine ring for β_3-AR activity.

Introduction

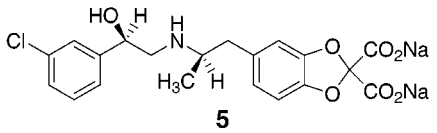
Following the classification of β-adrenergic receptors (AR) into β_1- and β_2-subtypes in 1967,[1] a third member of this family was pharmacologically identified in 1984[2] and was designated as the β_3-AR. The subsequent cloning and characterization of β_3-AR from genomic libraries of several species including human,[3] mouse,[4] and rat[5] provided conclusive evidence for its existence. Human β_3-AR and its mRNA have been found in the GI tract (e.g., small intestine, gall bladder) and adipose tissue (e.g., omental, retroperitoneal, mammary subcutaneous, abdominal subcutaneous fat).[6−10]

The β_3-AR has been shown to mediate various pharmacological and physiological effects such as lipolysis, thermogenesis, and intestinal smooth muscle relaxation in rodents.[11] It has also been thought to play an important role in glucose homeostasis and energy balance in humans.[12] Additional evidence for this role was provided by the discovery of a Trp64Arg mutation in the human β_3-AR that is associated with the early onset of non-insulin-dependent diabetes mellitus (NIDDM) in Pima Indians,[13] a greater dynamic capacity to gain weight in French obese patients,[14] and an increased tendency to develop NIDDM in Finnish obese patients.[15] Therefore, a number of laboratories[12,16] have pursued selective β_3-AR agonists that have the potential for treating obesity, NIDDM, and intestinal hypermotility disorders. These agonists shall also be able to help further define the physiological roles played by the β_3-AR. The current β_3-AR agonists belong to two general structural classes (Chart 1): i.e., phenethanolamines, e.g., BRL compounds (**1**−**4**) and CL 316243 (**5**), and aryloxypropanolamines, e.g., CGP-12177 (**6**). Despite these reported endeavors, none of the compounds investigated have been reported to produce good therapeutic effects in humans due to a lack of selectivity or potency.[17] Hence, efforts are underway in our and other laboratories to synthesize novel, potent, selective β_3-AR agonists.

Chart 1



	R_1	R_2
1	H	CO_2CH_3
2	H	CO_2H
3	Cl	$OCH_2CO_2CH_3$
4	Cl	OCH_2CO_2H



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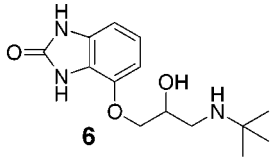

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Trimetoquinol (TMQ, **7**; Chart 2) is a known potent, nonselective β-AR agonist, used clinically in Japan as a bronchorelaxant.[18−21] Our laboratory has focused on TMQ structural modifications in order to obtain selective β-AR agonists. Our previous structural modifications of TMQ have generated potent, selective β_2-AR agonists.[22] In this study, we report novel structural modifications of TMQ, with the goal of synthesizing potent and selective β_3-AR agonists. It has been discovered that the catechol moiety of TMQ is indispensable for the agonist activities at both human β_1- and β_2-AR,[23] whereas its degree of contribution to TMQ's β_3-AR

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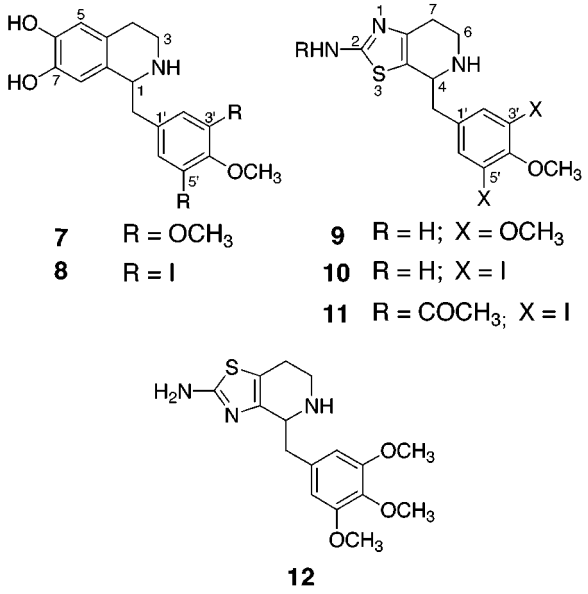
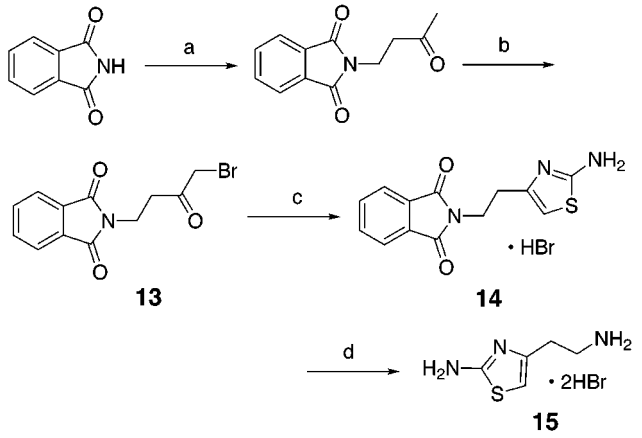
Chart 2



activity has not been evaluated. On the basis of the observation that many previously claimed β_3-AR agonists lack a catechol moiety, we hypothesized that the catechol of TMQ is not necessary for the observed activity at β_3-AR, and selective β_3-AR agonists will thus be achieved by modifying this catechol group. Compound **9** (Chart 2) has been designed by replacing catechol with 2-aminothiazole. It is also expected that this replacement will lead to chemically and enzymatically more stable compounds, because the stability problems associated with the catechol have been circumvented. Indeed, 2-aminothiazole has been employed to replace the catechol or phenolic group to afford orally active dopamine autoreceptor agonists.[24] We have previously found that substitution of the 3′- and 5′-methoxy groups of TMQ with iodine atoms (i.e., **8**, Chart 2) is well-tolerated at β_1- and β_2-AR.[25] Furthermore, compound **8** exhibits greater binding affinities at all three β-AR subtypes (especially at β_3-AR) than TMQ (Table 1); a similar modification was thus applied to analogue **9** to give analogue **10**. Compounds **11** and **25** were also synthesized and their activities evaluated at the three

Scheme 1[a]



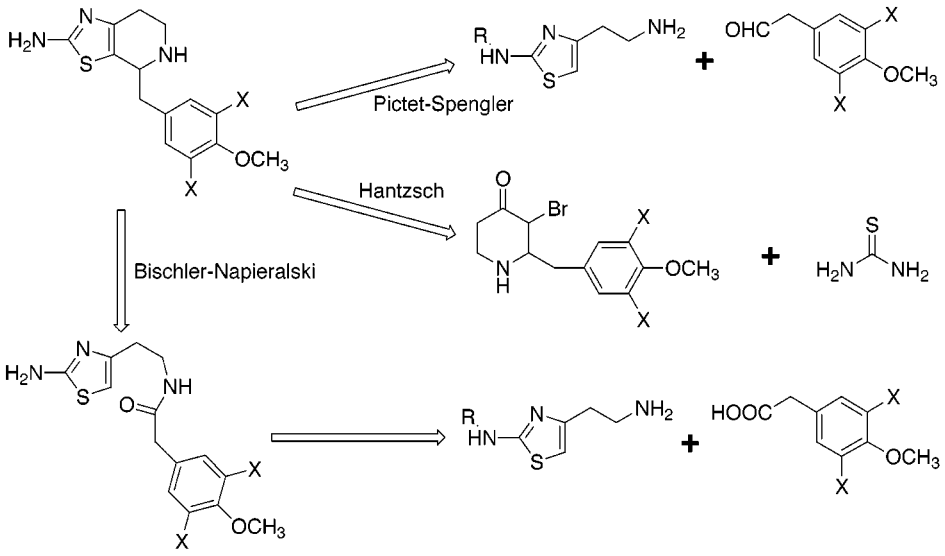
[a] (a) Methyl vinyl ketone, NaOEt, EtOH, EtOAc, rt → reflux; (b) (i) Br_2, MeOH, 0 °C → rt, (ii) 10.0 M H_2SO_4, rt; (c) thiourea, acetone, rt; (d) 30% HBr, reflux.

β-AR subtypes to determine the effects of N-acetylation or ring opening, respectively, on biological activities. Analogue **12** was designed to determine the effect of switching positions of S and N of the thiazole ring in analogue **9**. However, several attempts to prepare **12** were unsuccessful.

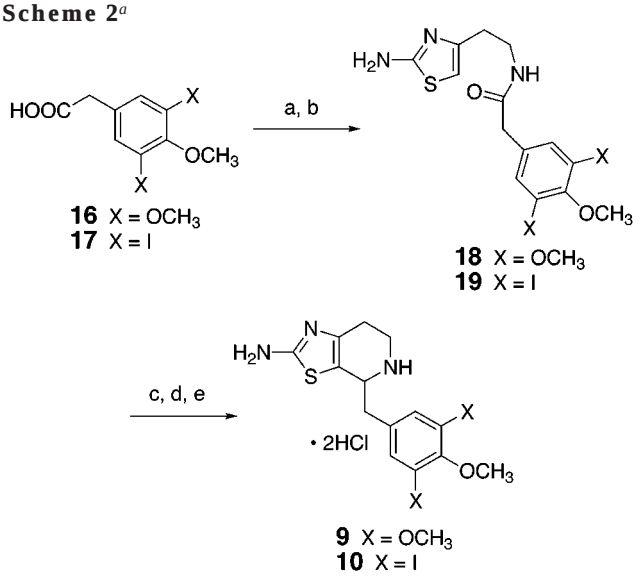
Chemistry

The preparation of 2-amino-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridines has been well-documented in the literature.[26−28] They are constructed by two approaches: (a) Pictet−Spengler reaction—the condensation and subsequent cyclization between an aldehyde and 2-(2′-amino-4′-thiazolyl)ethylamine derivatives; (b) Hantzsch thiazole synthesis—the condensation and subsequent cyclization between an α-bromopiperidone derivative and thiourea (Chart 3). However, it was envisioned that both of these approaches would not meet our synthetic challenge. The Pictet−Spengler reaction would necessitate the use of an inherently unstable substituted phenylacetaldehyde, whereas the required α-bromopiperidone for Hantzsch thiazole synthesis is not readily accessible synthetically. Alternatively, the Bischler−Napieralski reaction (Chart 3) is an attractive

Chart 3

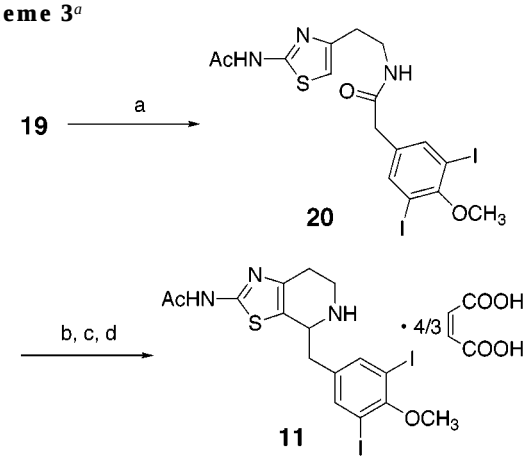


Scheme 2[a]



[a] (a) Oxalyl chloride, dry benzene, 0 °C → rt → reflux; (b) **14**, NaOH, $CHCl_3$, H_2O, rt; (c) $POCl_3$, CH_3CN, reflux; (d) $NaBH_4$, MeOH, 0 °C → rt; (e) 1.0 M HCl in Et_2O.

Scheme 3[a]



[a] (a) Acetic anhydride, dry benzene, dry CH_3CN, reflux; (b) $POCl_3$, CH_3CN, reflux; (c) $NaBH_4$, MeOH, 0 °C → rt; (d) maleic acid, CH_3CN.

approach for the synthesis of our designed TMQ analogues, because the required substituted phenylacetic acids are stable and readily accessible. The Bischler–Napieralski reaction is routinely used to prepare 3,4-dihydroisoquinolines;[29] however, its application to the preparation of 2-amino-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridines via the corresponding dihydro intermediate is novel.

According to Timmerman's synthetic scheme,[30] compound **15**, which is the starting amine for the Bischler–Napieralski approach, has been prepared (Scheme 1). However, in our hands, the transformation from **13** to **14** did not proceed in good yield as described. Instead, the procedure described by Sprague et al.[31] converted **13** to **14** almost quantitatively.

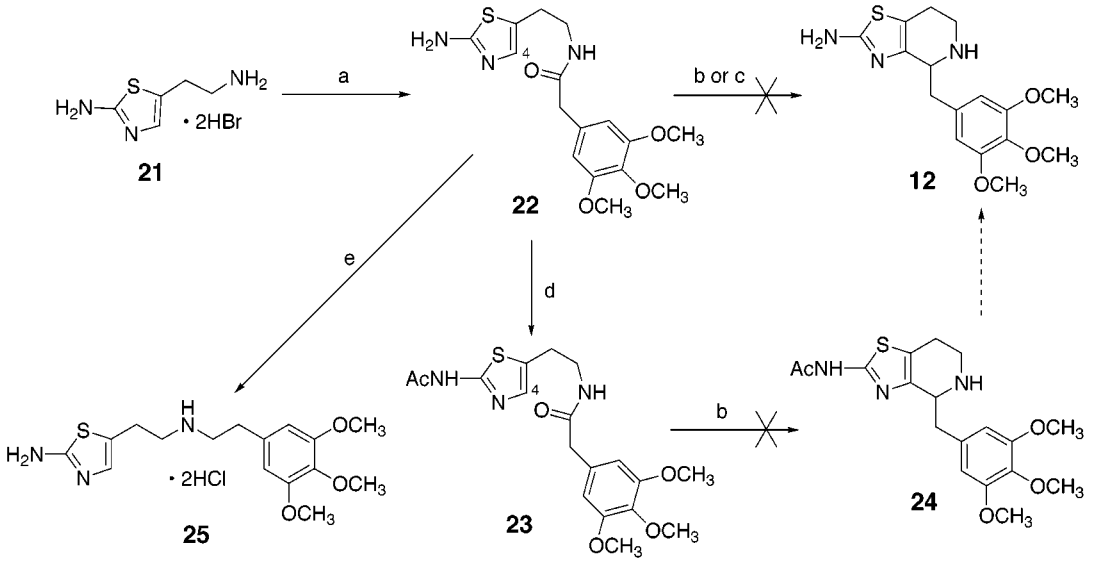
Scheme 2 depicts the synthesis of analogues **9** and **10**. Under the Schotten–Baumann conditions, substituted phenylacetic acid **16** or **17**[32] was allowed to react with compound **15**. The amide precursors **18** and **19** were obtained, and each was treated with $POCl_3$ in refluxing acetonitrile. The dihydro intermediate was reduced in situ with $NaBH_4$, giving final compounds **9** and **10** which were purified by crystallization or column chromatography on silica gel.

The acetamido analogue **11** was prepared according to Scheme 3. Acetylation of **19** with acetic anhydride gave precursor **20** that was then subjected to $POCl_3$ and $NaBH_4$. Compound **11** was isolated as its maleic acid salt. Attempts to make the salt of **11** with stronger acids (HCl, oxalic acid) failed because of spontaneous deacetylation.

Analogue **12** (Scheme 4) is a derivative of 2-amino-4,5,6,7-tetrahydrothiazolo[4,5-*c*]pyridine that is an unknown heterocyclic system. Amide precursor **22** has been prepared from compound **21** and 3,4,5-trimethoxyphenylacetic acid under Schotten–Baumann conditions.

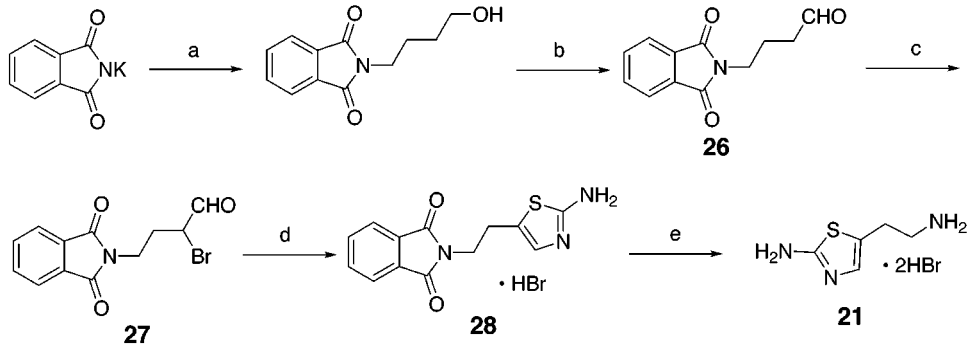
Compound **21** was synthesized according to a literature scheme (Scheme 5);[30] however, the conversion from the α-bromoaldehyde **27** to 2-aminothiazole **28** was again achieved effectively by using the procedure of Sprague et al.[31] Using various conditions[29,33,34] of the

Scheme 4[a]



[a] (a) 3,4,5-Trimethoxyphenylacetyl chloride, NaOH, $CHCl_3$, H_2O, rt; (b) (i) $POCl_3$, CH_3CN, reflux, (ii) $NaBH_4$, MeOH, 0 °C → rt; (c) P_2O_5, celite, dry $CHCl_3$, dry benzene, reflux; (d) acetic anhydride, dry benzene, dry CH_3CN, reflux; (e) (i) BH_3·THF, THF, 0 °C → rt → reflux, (ii) 1.0 M HCl in Et_2O.

Scheme 5[a]



[a] (a) 4-Chloro-1-butanol, dry DMF, 70 °C; (b) (i) oxalyl chloride, dry CH_2Cl_2, dry DMSO, −60 °C → −50 °C, (ii) Et_3N, H_2O, −50 °C → rt; (c) Br_2, dry CCl_4, rt; (d) thiourea, acetone, rt; (e) 30% HBr, reflux.

Table 1. Binding Affinities of TMQ Analogues on Human β-AR Subtypes

	pK_i ± SEM[a]		
	human β_1-AR	human β_2-AR	human β_3-AR
ISO	5.80 ± 0.07	6.17 ± 0.12	4.73 ± 0.25
TMQ	6.49 ± 0.06	7.36 ± 0.23	5.43 ± 0.28
8	7.10 ± 0.06	8.69 ± 0.16	7.67 ± 0.24
9	5.21 ± 0.08	6.21 ± 0.12	4.17 ± 0.05
10	6.14 ± 0.08	6.37 ± 0.08	5.83 ± 0.15

[a] Human β_1-, β_2-, and β_3-AR were expressed in CHO cells. [^{125}I]ICYP was used as the radioligand. K_i values were calculated using the following equation: $K_i(M) = IC_{50}(1 + [L]/K_d)$, wherein IC_{50} is the molar concentration of an analogue at which the radioligand binding was reduced by 50%, [L] is the radioligand concentration used, and K_d is the radioligand equilibrium dissociation constant. p$K_i = -\log K_i$; SEM, standard error of mean; $n = 3-9$.

Bischler−Napieralski reaction, attempts to cyclize the amide precursor **22** proved to be unsuccessful. On the basis of the published mechanism[35] of the Bischler−Napieralski reaction, in which the cyclization step is achieved by aromatic electrophilic substitution via a nitrilium intermediate, the failed cyclizations in our studies could be due to the low negative charge density at C4 of precursors **22** and **23**, which has been predicted by semiempirical charge density calculations (W. Zheng, J. E. De Los Angeles, D. D. Miller, unpublished results). However, **22** has been reduced by BH_3 to give the open-chain analogue **25** that has also been examined in all three human β-AR subtypes. Similarly, the acetamido amide precursor **23** also failed to give the cyclized product under the reaction conditions described.

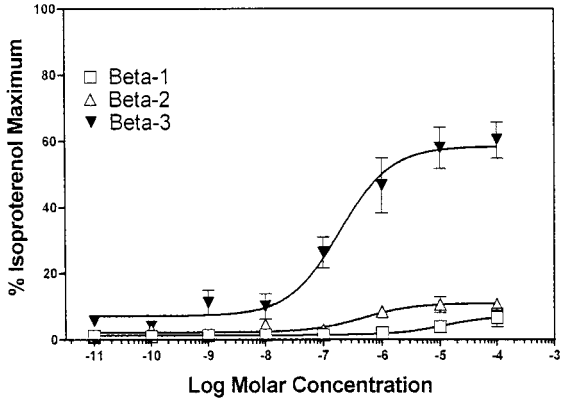


Figure 1. Concentration−response curves of compound **10** on cAMP accumulations in CHO cells expressing human β_1-, β_2-, and β_3-AR. Drug-induced changes in cellular cAMP were determined by the CRE−LUC assay (see Experimental Section). Data are the mean ± SEM of $n = 8$.

Biological Results and Discussion

Chinese hamster ovary (CHO) cells expressing human β_1-, β_2-, or β_3-AR were used to evaluate the newly designed and synthesized TMQ analogues. Table 1 compares the binding affinities of analogues **9** and **10** at human β_1-, β_2-, or β_3-AR to those of the reference compounds, (*R*)-(−)-isoproterenol (ISO), TMQ, and **8**. Receptor activation potencies and intrinsic activities of the novel TMQ analogues, together with those for reference compounds (ISO, TMQ, **8**), at human β_1-, β_2-, or β_3-AR are given in Table 2. Both cAMP radioimmunoassay and cAMP response element (CRE)−luciferase

Table 2. Functional Activities of TMQ Analogues on Human β-AR Subtypes

	human β_1-AR		human β_2-AR		human β_3-AR	
	pK_{act} ± SEM[a]	IA ± SEM[b]	pK_{act} ± SEM	IA ± SEM	pK_{act} ± SEM	IA ± SEM
			A. cAMP-RIA Assay[c]			
ISO	8.75 ± 0.14	100	8.40 ± 0.17	100	7.37 ± 0.11	100
TMQ	8.70 ± 0.11	109 ± 10	8.33 ± 0.24[d]	95 ± 3	8.60 ± 0.15	95 ± 3
8	8.11 ± 0.13	103 ± 4	8.47 ± 0.12	56 ± 9	8.76 ± 0.2	120 ± 9
9	NA[e]	<10	NA	20 ± 1	5.06 ± 0.01	54 ± 1
10	NA	<5	NA	<5	6.95 ± 0.11	67 ± 3
11	NA	<10	NA	<10	NA	<10
			B. CRE−LUC Assay[c]			
10	NA	<10	NA	<15	6.71 ± 0.18	62 ± 3
25	NA	<10	NA	<10	NA	<10

[a] Human β_1-, β_2-, and β_3-AR were expressed in CHO cells. K_{act} is the molar drug concentration which produces a cAMP response equal to 50% of its maximal response, p$K_{act} = -\log K_{act}$. [b] IA, intrinsic activity, expressed as the percentage of a maximal analogue response relative to the maximal response (100%) of (*R*)-(−)-isoproterenol (ISO). [c] See Experimental Section. [d] Data for *S*-(−)-TMQ. [e] NA, not active at 100 μM; $n = 4-12$.

(LUC) reporter gene (CRE−LUC) assays (see Experimental Section) were employed to determine the receptor activities of the compounds.

Replacement of the catechol of TMQ with a 2-aminothiazole group (analogue **9**) resulted in a moderate decrease (14−19-fold) in receptor binding affinities for all three human β-AR subtypes (Table 1). However, while **9** is inactive (up to 100 μM) at human β_1- and β_2-AR, it was observed to be a partial agonist for human β_3-AR despite a 3467-fold decrease in potency as compared to TMQ (Table 2). These results suggest that the catechol group of TMQ is essential for its functional activity at human β_1- and β_2-AR, but not for its binding affinity at these two β-AR subtypes. Findings consistent with this observation were provided by previous site-directed mutagenesis studies on human β_1- and β_2-AR.[23] A similar moderate decrease in affinity observed for the human β_3-AR suggests that the catechol moiety is also not critical for binding to β_3-AR. Perturbation of catechol interactions with all the β-AR subtypes may well have been compensated for by a strong binding interaction between the 1-trimethoxybenzyl group of TMQ and β-AR, and this provides a possible explanation for lack of loss of receptor affinity. However, our results from functional assays suggest that, unlike at β_1- and β_2-AR, the catechol moiety of TMQ is not indispensable for β_3-AR activation.

The 2-aminothiazole group has been demonstrated to be a viable bioisostere for catechol/phenolic groups in developing orally active dopamine agonists.[24,36] However, this replacement in our studies with TMQ led to dichotomous results. While a moderate decrease in receptor binding affinity was observed at all three human β-AR subtypes, the functional activity was abolished (up to 100 μM) at both human β_1- and β_2-AR, but functional activity was retained at β_3-AR, thus providing the desired selectivity for β_3-AR.

Replacing the 3′- and 5′-methoxy group of **9** with iodine atoms (analogue **10**) resulted in a significant increase (46-fold) in binding affinity at β_3-AR, compared to minor increases (8.5- and 1.4-fold) at β_1- and β_2-AR, respectively (Table 1). Similar observations were noted for compound **8** which exhibited significant (174-fold) and moderate increases (4- and 21-fold) in binding affinities at β_3- and β_1-, and β_2-AR, respectively, as compared to TMQ (Table 1). Interestingly, while compound **10** is inactive (up to 100 μM) at human β_1- and β_2-AR, it activates β_3-AR with a 78-fold greater potency than **9**, and its intrinsic activity (67%) is also greater than that of **9** (54%) (Table 2). These results indicate that the decreases in potency and intrinsic activity at human β_3-AR following replacement of the catechol moiety of TMQ with a 2-aminothiazole group can be reversed to a certain extent by increasing the receptor binding affinity resulting from modification of the 4-trimethoxybenzyl group of analogue **9**. In fact, while **9** is >3000-fold less potent than TMQ at activating β_3-AR, **10** is only 45-fold less potent than TMQ. However, in contrast to TMQ, compound **10** displays a very high selectivity for activation of β_3-AR. Consistent functional activities for **10** were obtained by both the cAMP radioimmunoassay and the CRE−LUC assays (Table 2, Figure 1).

We were also interested in examining the possible effects of functionalizing the 2-aminothiazole moiety of **10** on its activities at β-AR. Introduction of an acetyl group on **10** resulted in compound **11** that is inactive (up to 100 μM) at β_1- and β_2-AR. Surprisingly, this modification of **10** completely abolishes β_3-AR activity (up to 100 μM) (Table 2). This result indicates that an intact 2-aminothiazole moiety is essential for maintaining functional activity at β_3-AR. This phenomenon is likely due to steric perturbation at the receptor site corresponding to the 2-amino group of **10**, and hence a functionally active β_3-AR conformation cannot be achieved.

The open-chain compound **25** was also evaluated at human β_1-, β_2-, and β_3-AR by the CRE−LUC assay (Table 2). It is functionally inactive (up to 100 μM) at all three human β-AR subtypes. Similar biological results have been previously reported[37] on some other TMQ open-chain analogues.

Conclusions

In this study, we have shown that replacing the catechol moiety of TMQ with the 2-aminothiazole group completely abolished the β_1- and β_2-AR activities while retaining activity at β_3-AR. Thus, highly selective β_3-AR agonists (**9**, **10**) have been identified. It has been demonstrated that the β_3-AR activity of the 2-aminothiazole analogues is abolished by N-acetylation or ring opening. A parallel increase in β_3-AR binding affinity and functional activity has also been observed in proceeding from analogue **9** to **10**. This information should prove to be very useful for identifying highly selective and more potent β_3-AR agonists that could be useful in the treatment of obesity, NIDDM, and intestinal hypermotility disorders. These agonists, including **10** identified in this study, shall also be helpful in further defining the physiological roles of β_3-AR.

Experimental Section

Chemistry. A Thomas-Hoover capillary melting point apparatus was used to measure all melting points that are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AX 300 spectrometer (300 and 75.5 MHz, respectively), and chemical shift values (δ) are expressed as parts per million (ppm) relative to tetramethylsilane (TMS). Peaks are abbreviated as follows: s, singlet; d, doublet; t, triplet; q, quartet; bs, broad singlet. IR spectra were recorded on a Perkin-Elmer System 2000-FTIR. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA 30091), and the values found are within ±0.4% of the theoretical values. Silica gel (Merck, 230−400 mesh, 60 Å) was used for flash column chromatography. THF was dried by distillation from sodium benzophenone ketyl, anhydrous benzene was from Aldrich, and anhydrous ethyl ether was from Fischer. All starting materials were from Aldrich and used without further purification.

2-Amino-4-(2-phthalimidoethyl)thiazole Hydrobromide (14). To a solution of **13**[30] (2.08 g, 7.0 mmol) in acetone (45 mL) was added a solution of thiourea (0.535 g, 7.0 mmol) in acetone (25 mL) with rapid rate at room temperature. Just after the addition was complete, a precipitate appeared; the suspension was stirred overnight at room temperature and filtered to afford 2.45 g (99%) of **14** as a colorless powder: mp 258−260 °C dec (lit.[30] mp 195 °C dec starting point); ^{1}H NMR (DMSO-d_6) δ 2.82 (t, J = 6.3 Hz, 2H), 3.83 (t, J = 6.2 Hz, 2H), 6.56 (s, 1H), 7.81−7.88 (m, 4H), 9.01 (bs, 2H); IR (KBr) 3214, 3092, 1767, 1721, 1633, 1573, 1402 cm^{-1}. Anal. ($C_{13}H_{11}N_3O_2S$·HBr) C, H, N.

***N*-2-[(2-Amino-4-thiazolyl)ethyl]-3,4,5-trimethoxyphenylacetamide (18). (a)** To a suspension of 3,4,5-trimethoxyphenylacetic acid (4.52 g, 0.02 mol) in dry benzene (200 mL) was added dropwise oxalyl chloride (20 mL, 0.23 mol) at 0 °C. After the addition was complete, the reaction mixture was stirred at room temperature until a clear solution was obtained (about 1 h). The solution was then heated at reflux for 2.5 h. The reaction mixture was cooled to room temperature and evaporated to give a yellow oil. It was dissolved in benzene (~50 mL) and evaporated again (repeated for two more times); 4.9 g (100%) of the acid chloride was obtained as a viscous yellow oil after drying in vacuo: ^{1}H NMR ($CDCl_3$) δ 3.83 (s, 3H), 3.84 (s, 6H), 4.05 (s, 2H), 6.45 (s, 2H); IR (neat) 300, 2941, 2840, 1799, 1593 cm^{-1}.

(b) To a well-stirred suspension of **15**[30] (976 mg, 3.2 mmol), NaOH (512 mg, 12.8 mmol) in $CHCl_3$ (7 mL), and H_2O (5 mL) was added slowly a solution of above-obtained 3,4,5-trimethoxyphenylacetyl chloride (784 mg, 3.2 mmol) in $CHCl_3$ (6 mL) at room temperature. After the addition was complete, the reaction mixture was stirred vigorously at room temperature for 1.5 h. The $CHCl_3$ layer was separated, and the H_2O layer was extracted with $CHCl_3$. The combined organics were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The oily residue was dissolved in $CHCl_3$ (40 mL), to which HCl (1.0 M solution in dry Et_2O) (10 mL) was added at 0 °C. The whole mixture was concentrated; the oily residue was dissolved in H_2O (10 mL). The aqueous solution was washed successively with EtOAc, Et_2O, and $CHCl_3$ and basified with 20% NaOH. The product was extracted with $CHCl_3$, the combined organics were washed with brine, dried over anhydrous Na_2SO_4, and concentrated, and the resulting solid residue was crystallized from EtOAc to afford 803 mg (71%) of **18** as a colorless crystal: mp 148−149.5 °C; ^{1}H NMR ($CDCl_3$) δ 2.57 (t, J = 6.2 Hz, 2H), 3.42 (q, J = 5.8 Hz, 2H), 3.46 (s, 2H), 3.79 (s, 3H), 3.80 (s, 6H), 5.16 (bs, 2H), 5.96 (s, 1H), 6.43 (s, 2H), 6.50 (bs, 1H); ^{13}C NMR ($CDCl_3$) δ 30.6, 38.9, 44.1, 56.1, 60.8, 103.1, 106.8, 130.5, 136.9, 150.1, 153.3, 167.9, 170.7; IR (KBr) 3433, 3266, 3080, 2939, 1646, 1624, 1590 cm^{-1}. Anal. ($C_{16}H_{21}N_3O_4S$) C, H, N.

2-Amino-4-(3,4,5-trimethoxybenzyl)-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine Dihydrochloride (9). A mixture of **18** (70.3 mg, 0.2 mmol) and phosphorus oxychloride (0.27 mL, 2.9 mmol) in CH_3CN (4 mL) was stirred and heated at reflux for 5 h. After the reaction mixture was cooled and concentrated under reduced pressure, the residue was dissolved in MeOH (2 mL), and the solution was heated at reflux for 30 min. After evaporation, the residue was dissolved in MeOH and evaporated again (repeated three more times). To the stirred solution of the resulting residue in MeOH (10 mL) was added $NaBH_4$ (757 mg, 20 mmol) in portions cautiously at 0 °C. After the addition was complete, the reaction mixture was stirred overnight at room temperature. After the reaction mixture was evaporated to dryness under reduced pressure, the residue was dissolved in H_2O (5 mL), cooled with an ice−water bath, and basified with 20% NaOH. The basic solution was extracted with EtOAc; the combined organics were washed with brine, dried over anhydrous Na_2SO_4, and concentrated to give a viscous yellow oil. The oily residue was dissolved in $CHCl_3$ (5 mL), to which HCl (1.0 M solution in dry Et_2O) (2 mL) was added at 0 °C. The precipitate was filtered off, washed successively with Et_2O, EtOAc, and $CHCl_3$, and crystallized from MeOH−Et_2O to afford 36.5 mg (45%) of **9** as a pale-yellow powder: mp 230−231 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.71−2.90 (m, 2H), 2.96−3.03 (m, 1H), 3.17−3.23 (m, 3H), 3.64 (s, 3H), 3.75 (s, 6H), 4.77 (bs, 1H), 6.67 (s, 2H), 8.48 (bs, 1H), 9.68 (bs, 1H), 9.85 (bs, 1H); ^{13}C NMR (CD_3OD) δ 21.8, 39.8, 41.0, 54.8, 56.8, 61.2, 108.3, 112.9, 130.7, 133.9, 139.2, 155.2, 171.5; IR (KBr) 3392, 2940, 2839, 2771, 1632, 1593 cm^{-1}. Anal. ($C_{16}H_{21}N_3O_3S\cdot 2HCl\cdot 0.5H_2O$) C, H, N.

***N*-2-[(2-Amino-4-thiazolyl)ethyl]-3,5-diiodo-4-methoxyphenylacetamide (19).** In the same manner as **18**, the title compound was prepared from **15** (590.3 mg, 1.94 mmol) and 3,5-diiodo-4-methoxyphenylacetyl chloride (844.8 mg, 1.94 mmol) which in turn was obtained by treating its corresponding acid **17**[32] with oxalyl chloride as described above for 3,4,5-trimethoxyphenylacetic acid. Recrystallization from EtOAc gave 642.8 mg (61%) of **19** as a colorless crystal: mp 175−176 °C; ^{1}H NMR (CD_3OD) δ 2.63 (t, J = 6.8 Hz, 2H), 3.35 (s, 2H), 3.41 (t, J = 6.8 Hz, 2H), 3.79 (s, 3H), 6.05 (t, J = 0.8 Hz, 1H), 7.72 (s, 2H); ^{13}C NMR (CD_3OD) δ 31.9, 39.7, 41.8, 61.1, 90.9, 103.4, 137.1, 141.6, 149.9, 159.4, 171.5, 172.9; IR (KBr) 3431, 3272, 3083, 2933, 1643, 1623, 1579, 1524 cm^{-1}. Anal. ($C_{14}H_{15}I_2N_3O_2S$) C, H, N.

2-Amino-4-(3,5-diiodo-4-methoxybenzyl)-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine Dihydrochloride (10). In the same manner as **9**, the title compound was prepared from **19** (380.1 mg, 0.7 mmol). After flash column chromatography on silica gel, eluting with MeOH/$CHCl_3$ (1:30), 72 mg (20%) of the free base form of the product was obtained as a white solid, and it was treated with HCl (1.0 M solution in dry Et_2O) to afford **10** as a pale-yellow solid: mp 201−203 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.67−2.77 (m, 2H), 3.02−3.11 (m, 2H), 3.15−3.35 (m, 2H), 3.74 (s, 3H), 4.74 (bs, 1H), 7.90 (s, 2H), 8.31 (bs, 2H), 9.59 (bs, 1H), 9.79 (bs, 1H); ^{13}C NMR δ 22.5, 38.0, 41.5, 54.9, 61.2, 91.9, 112.6, 135.1, 135.5, 142.6, 160.7, 171.4; IR (KBr) 3421, 2964, 2937, 2775, 1628, 1577 cm^{-1}. Anal. ($C_{14}H_{15}I_2N_3OS\cdot 2HCl$) C, H, N.

***N*-2-[(2-Acetamido-4-thiazolyl)ethyl]-3,5-diiodo-4-methoxyphenylacetamide (20).** To a stirred suspension of **19** (434.5 mg, 0.8 mmol) in dry CH_3CN (1.8 mL) was added dropwise a solution of acetic anhydride (o.16 mL, 1.7 mmol) in dry benzene (0.6 mL). After the addition was complete, the reaction mixture was heated at reflux for 2.5 h. After the reaction mixture was cooled to room temperature and concentrated under reduced pressure to remove solvents completely, H_2O (5 mL) was added to the residue and the mixture was basified with saturated $NaHCO_3$ aqueous solution to pH 7.5−8.0. The solid material was filtered off and crystallized from CH_3CN to afford 430 mg (92%) of **20** as a colorless crystal: mp 231−232 °C; ^{1}H NMR(DMSO-d_6) δ 2.10 (s, 3H), 2.69 (t, J = 7.0 Hz, 2H), 3.29−3.32 (m, 4 H), 3.71 (s, 3H), 6.70 (s, 1H), 7.67 (s, 2H), 8.08 (t, J = 5.4 Hz, 1H), 12.04 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 22.5, 31.1, 38.2, 40.1, 60.2, 90.9, 108.0, 136.7, 139.9, 148.3, 157.0, 157.5, 168.2, 169.3; IR (KBr) 3429, 3273, 3062, 1644, 1554, 1537 cm^{-1}. Anal. ($C_{16}H_{17}I_2N_3O_3S$) C, H, N.

2-Acetamido-4-(3,5-diiodo-4-methoxybenzyl)-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine Maleate (11). In the same manner as **9**, the title compound was prepared from **20** (300 mg, 0.51 mmol). After flash column chromatography on silica gel, eluting with acetone/hexane (1:3), 150 mg (52%) of the free base form of **11** was obtained as a white powder: mp 170−172 °C; ^{1}H NMR (DMSO-d_6) δ 2.09 (s, 3H), 2.53−2.60 (m, 2H), 2.66−2.73 (m, 1H), 2.77−2.86 (m, 1H), 2.89−2.95 (m, 1H), 3.09−3.15 (m, 1H), 3.72 (s, 3H), 4.12−4.13 (m, 1H), 7.80 (s, 2H), 11.91 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 22.5, 27.0, 40.4, 40.8, 53.9, 60.2, 90.9, 124.2, 139.3, 140.4, 143.7, 155.5, 156.7, 168.0; IR (KBr) 3428, 3252, 2914, 1675, 1636, 1565. Anal. ($C_{16}H_{17}N_3SO_2I_2$) C, H, N.

11 was obtained as a white powder by treating the above-obtained free base with maleic acid in CH_3CN: mp 121 °C dec; ^{1}H NMR (CD_3OD) δ 2.18 (s, 3H), 2.93−3.09 (m, 4H), 3.38−3.44 (m, 1H), 3.63−3.71 (m, 1H), 4.93−4.96 (m, 1H), 6.26 (s, 8/3H), 7.85 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 22.4, 23.4, 37.7, 40.4, 53.7, 60.3, 91.6, 118.2, 134.7, 135.4, 140.7, 142.1, 157.5, 157.8, 167.1, 168.6; IR (KBr) 3436, 3049, 2968, 2946, 1700, 1686, 1624, 1571 cm^{-1}. Anal. ($C_{16}H_{17}I_2N_3O_2S\cdot {}^4/_3C_4H_4O_4\cdot {}^1/_3Et_2O$) C, H, N.

2-Amino-5-(2-phthalimidoethyl)thiazole Hydrobromide (28). In the same manner as **14**, the title compound was prepared from thiourea (2.75 g, 36.1 mmol) and **27**[30] (crude, 10.69 g, 36.1 mmol). Recrystallization from MeOH/EtOH (1:10) gave 6.19 g (46% based on aldehyde **26**[30]) of **28** as colorless plates: mp 244−246 °C dec (lit.[30] mp 180 °C dec starting point); ^{1}H NMR (DMSO-d_6) δ 2.96 (t, J = 6.2 Hz, 2H), 3.77 (t, J = 6.3 Hz, 2H), 7.08 (s, 1H), 7.82−7.89 (m, 4H), 9.01 (bs, 2H); IR (KBr) 3308, 3222, 3108, 2975, 1764, 1711, 1618, 1608, 1554, 1402 cm^{-1}. Anal. ($C_{13}H_{11}N_3O_2S\cdot HBr$) C, H, N.

***N*-2-[(2-Amino-5-thiazolyl)ethyl]-3,4,5-trimethoxyphenylacetamide (22).** In the same manner as **18**, the title compound was prepared from **21**[30] (1.95 g, 6.4 mmol) and 3,4,5-trimethoxyphenylacetyl chloride (1.57 g, 6.4 mmol). Recrystallization from $CHCl_3$/hexanes gave 1.42 g (63%) of **22** as colorless crystals: mp 121−122 °C; ^{1}H NMR ($CDCl_3$) δ 2.74 (t, J = 6.0 Hz, 2H), 3.34 (q, J = 6.3 Hz, 2H), 3.43 (s, 2H), 3.79 (d, 9H), 5.22 (bs, 2H), 5.78 (t, J = 5.6 Hz, 1H), 6.39 (s, 2H), 6.59 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.8, 40.4, 44.0, 56.1, 60.8, 106.4, 124.4, 130.3, 135.8, 137.1, 153.5, 167.3, 170.9; IR (KBr) 3294, 3114, 2995, 2936, 1654, 1636, 1588 cm^{-1}. Anal. ($C_{16}H_{21}N_3O_4S$) C, H, N.

***N*-2-[(2-Amino-5-thiazolyl)ethyl]-3,4,5-trimethoxyphenethylamine Dihydrochloride (25).** To a suspension of **22** (176 mg, 0.5 mmol) in dry THF (0.5 mL) was added dropwise slowly BH_3·THF (1.0 M in THF, 3.5 mL). After the addition was complete, the reaction mixture was stirred at room temperature for 30 min and then was heated at reflux for 1 h. The reaction mixture was cooled to room temperature and treated cautiously with 10% HCl aqueous solution (3 mL), and the solution was heated at reflux for 30 min. After removal of THF, H_2O (10 mL) was added to the residue. The acidic solution was basified with 10% NaOH aqueous solution at 0 °C. The product was extracted with $CHCl_3$ (20, 20, 10 mL); the combined organic was washed with brine (20 mL) and dried over anhydrous Na_2SO_4. After filtration and evaporation, a viscous oil was obtained, and it was dissolved in $CHCl_3$ (8 mL) and treated with HCl (1.0 M in dry Et_2O). The whole mixture was evaporated to dryness, and the resulting white solid was crystallized in MeOH/Et_2O to give 109 mg (53%) of **25** as white crystals: mp 235 °C dec; ^{1}H NMR (CD_3OD) δ 2.98−3.03 (m, 2H), 3.11−3.16 (m, 2H), 3.29−3.34 (m, 4H), 3.72 (s, 3H), 3.84 (s, 6H), 6.63 (s, 2H), 7.18 (s, 1H); ^{13}C NMR (CD_3OD) δ 24.6, 33.5, 48.4, 50.3, 56.7, 61.1, 107.3, 121.7, 125.3, 133.7, 138.3, 154.9, 172.0; IR (KBr) 3427, 2947, 2767, 1630, 1590 cm^{-1}. Anal. ($C_{16}H_{23}N_3O_3S$·2HCl) C, H, N.

Pharmacological Studies. 1. Radioligand Binding Studies. Following digestion with trypsin, CHO cells expressing human β_1-, β_2-, or β_3-AR were harvested into Ham's F-12 solution. Cells were pelleted and washed three times with Tris-EDTA buffer (pH 7.4; TRIZMA HCl, 50 mM; NaCl, 150 mM; disodium EDTA·$2H_2O$, 20 mM). Cells were then suspended in Tris-EDTA buffer after centrifugation**.** Competition binding assays were performed at 37 °C by incubating cells with varying concentrations of drugs in the presence of [^{125}I]-iodocyanopindolol ((1.5−5) × 10^4 cells/18−70 pM for human β_1- or β_2-AR, (3−5) × 10^5 cells/200−500 pM for human β_3-AR). Nonspecific binding was determined in the presence of (−)-propranolol (1 μM for human β_1- or β_2-AR, 100 μM for human β_3-AR). The incubations were terminated by rapid filtration over Whatman GF/B (for human β_1- or β_2-AR) or Whatman GF/C (for human β_3-AR, presoaked in 0.1% poly(ethylenimine)) glass fiber filters using a Brandel model 12-R cell harvester. The filters were washed three times with the Tris-EDTA buffer (4 °C) and dried under cell harvester vacuum. The radioactivity in the filters was measured by gamma scintillation counting using a Beckman gamma counter model 8000. K_i values were calculated from the obtained IC_{50} values by the method of Cheng and Prusoff.[38]

2. cAMP Radioimmunoassay (cAMP-RIA Assay). CHO cells expressing either human β_1-, β_2-, or β_3-AR subtypes were used as previously described.[39] These cells were grown to confluence in 60-mm dishes, washed with Hank's balanced salt solution, and then incubated with Hank's balanced salt solution (pH 7.4) containing 20 mM HEPES, 1 mM 3-isobutyl-1-methylxanthine (IBMX), and 1 mM L-ascorbic acid for 30 min at 37 °C. Varying concentrations (10^{-11}−10^{-4} M) of the drugs were added with an additional 30 min of incubation. After removal of the Hank's buffer, the cAMP generated within the cells was extracted by the addition of trichloroacetic acid (6% w/v). cAMP content was determined as the amount of ^{125}I-labeled succinyl-cAMP tyrosine methyl ester/antibody precipitated, as described by Brooker et al.[40] The precipitated protein was dissolved in 0.1 N NaOH. Protein content was determined by the method of Lowry et al.,[41] using bovine serum albumin as the standard.

3. cAMP Response Element (CRE)−Luciferase (LUC) Reporter Gene (CRE−LUC) Assay. CHO cells stably expressing human β_1-, β_2-, or β_3-AR subtypes were transfected with a 6 CRE−LUC plasmid (gift from Dr. A. Himmler, Vienna, Austria) using electroporation with a single 70-ms, 150-V pulse.[42] The transfected CHO cells were seeded at a density of 40 000/well in 96-well microtiter plates (Culturplate, Packard) and allowed to grow for 20 h. After 20 h, the cells were treated with varying drug concentrations (10^{-11}−10^{-4} M) for 4 h. Following drug exposures, the cells were lysed, and luciferase activity was measured using the LucLite assay kit (Packard). Changes in light production were measured by a Topcount luminometer (Packard).

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