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On the Selectivity of the $G\alpha_q$ Inhibitor UBO-QIC: A Comparison with the $G\alpha_i$ Inhibitor Pertussis Toxin

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Abstract

$G\alpha_q$ inhibitor UBO-QIC (FR900359) is becoming an important pharmacological tool, but its selectivity against other G proteins and their subunits, especially βγ, has not been well characterized. We examined UBO-QIC's effect on diverse signaling pathways mediated via various G protein-coupled receptors (GPCRs) and G protein subunits by comparison with known $G\alpha_i$ inhibitor pertussis toxin. As expected, UBO-QIC inhibited $G\alpha_q$ signaling in all assay systems examined. However, other non-$G\alpha_q$-events, e.g. Gβγ-mediated intracellular calcium release and inositol phosphate production, following activation of G_i-coupled A_1 adenosine and M_2 muscarinic acetylcholine receptors, were also blocked by low concentrations of UBO-QIC, indicating that its effect is not limited to $G\alpha_q$. Thus, UBO-QIC also inhibits Gβγ-mediated signaling similarly to pertussis toxin, although UBO-QIC does not affect $G\alpha_i$-mediated inhibition or $G\alpha_s$-mediated stimulation of adenylyl cyclase activity. However, the blockade by UBO-QIC of GPCR signaling, such as carbachol- or adenosine-mediated calcium or inositol phosphate increases, does not always indicate inhibition of $G\alpha_q$-mediated events, as the βγ subunits released from G_i proteins following the activation of G_i-coupled receptors, e.g. M_2 and A_1Rs, may produce similar signaling events. Furthermore, UBO-QIC completely inhibited Akt signaling, but only partially blocked ERK1/2 activity stimulated by the Gq-coupled $P2Y_1R$. Thus, we have revealed new aspects of the pharmacological interactions of UBO-QIC.

Keywords

G protein; Gαq inhibitor; GPCR; Gβγsubunits; FR900359; UBO-QIC

1. Introduction

UBO-QIC (FR900359), a cyclic depsipeptide from the flowering plant *Ardisia crenata sims*, was found to inhibit platelet aggregation and to decrease blood pressure [1]. UBO-QIC has been recently used as a selective inhibitor of $G\alpha_q$ signaling [2-7].

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UBO-QIC is a close analog of a known $G\alpha_q$ inhibitor YM-254890 (Figure 1) [8-10], which is relatively well, albeit not thoroughly, characterized at G protein signaling and has been used more widely as a $G\alpha_q$ inhibitor [10-13]. The two depsipeptides differ only in the presence of an isopropyl group in YM-254890 in place of methyl. Nishimura et al. [14] demonstrated in an X-ray crystal structure of the complex of YM-254890 with $G\alpha_q/\beta\gamma$ that the depsipeptide binds to an interdomain region of the $G\alpha_q$ subunit, and showed that it inhibits the release of GDP from $G\alpha_q$. YM-254890 has been used as a selective $G\alpha_q$ inhibitor, although only limited data on the selectivity of YM-254890 for $G\alpha_q$ over $G\beta\gamma$ or $G\alpha_{15}$ subunits was reported by Takasaki et al. [9]. YM-254890 (10 μM, 5 min pretreatment) was shown to inhibit formyl peptide (fMLP)-induced Ca^{2+} mobilization (via $G\beta\gamma$) in differentiated HL60 cells to a lesser extent than pertussis toxin (PTX, 50 ng/ml for 6 h), although it produced a much larger inhibition in UTP-mediated (via $G\alpha_q$) Ca^{2+} release [9]. YM-254890 was also shown to have only a small effect on $G\alpha_{15}$-mediated Ca^{2+} release in Chinese hamster ovary (CHO) cells expressing recombinant human fMLP receptor and $G\alpha_{15}$. Based on that study, it was concluded that YM-254890 is selective for $G\alpha_q$ over $G\beta\gamma$ and $G\alpha_{15}$.

The $G\alpha_q$ inhibitors have clearly greatly enabled the investigation of G_q-coupled receptor signaling, and UBO-QIC is becoming a widely-used tool in pharmacology due to its recent commercial availability [2-7]. However, the selectivity of UBO-QIC for Gαq over other G proteins and their subunits, especially the βγ subunit-mediated signaling following the activation of G_i-coupled receptors, has not been well characterized. Considering the fact that in many cases $G\alpha_q$ and $G\beta\gamma$ mediate similar signaling events, e.g. both Gαq and $G\beta\gamma$ subunits mediate phospholipase C activation or Ca^{2+} release [9,15-19], it is important to examine the effect of UBO-QIC on $G\beta\gamma$ signaling pathways. The present study explored this possibility by comparing UBO-QIC with a known $G\alpha_i$ inhibitor PTX.

2. Materials and Methods

2.1. Materials

[[(1*R*,2*R*,3*S*,4*R*,5*S*)-4-[6-Amino-2-(methylthio)-9*H*-purin-9-yl]-2,3-dihydroxybicyclo[3.1.0]hex-1-yl]methyl] diphosphoric acid monoester trisodium salt (MRS2365) was from Tocris (St. Louis, MO). N^6-Cyclopentyladenosine (CPA), carbachol, PTX and 2-methylthioadenosine 5′-diphosphate trisodium salt (2MeSADP), adenosine-5′-*N*-ethyluronamide (NECA) were from Sigma (St. Louis, MO). UBO-QIC was purchased from University of Bonn (Germany). IP-One Tb HTRF kit was from Cisbio Bioassays (Bedford, MA). AlphaScreen cAMP kit, SureFire p-ERK1/2 (Thr202/Tyr204) Assay Kit and AlphaScreen SureFire p-Akt 1/2/3 (p-Ser473) Assay Kit were purchased from PerkinElmer (Waltham, MA). HEK293 and DDT1-MF2 were from ATCC (Mannasas, VA); CHO cell lines stably expressing the human A_1AR, A_{2A}AR, and A_{2B}AR, and human M_3 and M_2 muscarinic acetylcholine receptors were made at the Laboratory of Bioorganic Chemistry, NIDDK (Bethesda, MD). 1321N1 astrocytoma cells expressing either the human $P2Y_1$R or $P2Y_{12}$R were from T. K. Harden (University of North Carolina, Chapel Hill, NC); all other reagents were from standard commercial sources and of analytical grade.

2.2. Inositol 1-phosphate Assay

Inositol 1-phosphate (IP-1), a metabolite of inositol trisphosphate, which is downstream of signaling by $G\alpha_q$ or $G\beta\gamma$ subunits, was detected using the IP-One Tb HTRF kit (Cisbio Bioassays, Bedford, MA), as described elsewhere earlier [20]. Cells were grown in 96-well plates overnight before the pretreatment with UBO-QIC (100 nM) for 30 min before the addition of agonists followed by additional 30 min incubation. Assay plates were read on a Mithras LB940 reader (Berthold Technologies, Oak Ridge, TN) or a PerkinElmer (Waltham, MA) EnSpire plate reader using a time-resolved fluorescence ratio (665/620 nm).

2.3. Intracellular calcium mobilization

Cells were grown overnight in 100 μl of media in 96-well black plates at 37°C at 5% CO_2. Cells were pretreated with various concentrations of UBO-QIC or GO6983 (10 μM) for 30 min or PTX (200 ng/ml) overnight before the addition of agonists. The calcium assay kit was used as directed without washing cells, and with probenecid added to the loading dye at a final concentration of 2.5 mM to increase dye retention. Cells were incubated with 100 μl dye/probenecid for 60 min at room temperature. The compound plate was prepared using dilutions of various compounds in Hank's Buffer (pH 7.4). Samples were run in duplicate or triplicate using a FLIPR TETRA High Throughput Cellular Screening System (Molecular Devices, Sunnyvale, CA) at room temperature. Cell fluorescence (excitation at 485 nm; emission at 525 nm) was monitored following exposure to the compound. Increases in intracellular calcium are reported as the maximum fluorescence value after exposure minus the basal fluorescence value before exposure.

2.4. Activation of extracellular-signal-regulated kinase 1/2 (ERK1/2) and Akt1/2/3

For the stimulation of ERK1/2 activity, the method used was as previously described [21,22]. Briefly, CHO or 1321N1 astrocytoma cells (30,000 cells/100 μl) were seeded in a 96-well plate in complete growth medium. After cell attachment, medium was removed and cells were serum-starved overnight in medium without fetal bovine serum. Cells were pretreated with UBO-QIC (100 nM) or GO6983 (10 μM) for 30 min or PTX (200 ng/ml) overnight before the addition of agonists. Agonists were prepared in Hank's buffered salt solution, and cells were stimulated for 5 min. Medium was removed and cells were lysed with 1x Lysis Buffer (20 μl) (PerkinElmer AlphaScreen SureFire p-ERK1/2 (Thr202/Tyr204) Assay Kit) (PerkinElmer, Waltham, MA). Lysate (4 μl/well) was transferred to a 384-well ProxiPlate Plus (PerkinElmer). Reagents were added according the manual from the manufacturer, and the plate was measured using an EnVision multilabel reader using standard AlphaScreen settings. For the stimulation of Akt1/2/3 activity, the procedures were essentially the same as that of the ERK1/2 activity, except that the stimulation time for the $P2Y_1$ receptor is 20 min. The Akt activity was measured using AlphaScreen SureFire p-Akt 1/2/3 (p-Ser473) Assay Kit (PerkinElmer, Waltham, MA).

2.5. Cyclic AMP accumulation assay

Cells were cultured in DMEM medium containing 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin and 2 μmol/ml glutamine. For the assay of 3′,5′-cyclic adenosine monophosphate (cAMP), cells were plated in 96-well plates in 100 μl medium

overnight and were first treated with UBO (300 nM) for 30 min or PTX (200 ng/ml) overnight, before the treatment with agonists and/or test compounds in the presence of phosphodiesterase inhibitor rolipram (10 μM). The reaction was terminated by removal of the supernatant, and cells were lysed upon the addition of 50 μl of lysis buffer (0.3% Tween-20). For determination of production of cAMP, an AlphaScreen cAMP kit was used according to manufacturer's instructions (PerkinElmer, Waltham, MA).

2.6. Statistical and data analyses

Functional parameters were calculated using Prism 6.0 software (GraphPAD, San Diego, CA). Data were expressed as mean ± standard error. Statistical significance of the differences was assessed using a Student's t test (between two conditions) or a One-Way Analysis of Variance (ANOVA) followed by Bonferroni's multiple comparison tests (between multiple conditions). Differences yielding P values < 0.05 were considered as statistically significant.

3. Results

We first tested the effect of UBO-QIC on $G\alpha_s$-mediated events. UBO-QIC did not show any effect on cAMP accumulation induced by nonselective adenosine receptor (AR) agonist NECA in CHO cells stably expressing the G_s-coupled human A_{2B}AR (Figure 2a). The EC_{50} values (nM) of NECA in the absence and presence of UBO-QIC (300 nM) were 156 ± 27 and 169 ± 18 nM, respectively, which were not significantly different (P>0.05, Student's test). The $G\alpha_i$ inhibitor PTX also did not substantially affect the concentration-response curve of NECA (Figure 2a). The EC_{50} value of NECA in the presence of 200 ng/ml PTX (overnight incubation) was 172 ± 36 nM, which was not significantly different from that in the absence (P>0.05, Student's t-test). UBO-QIC also did not affect cAMP accumulation induced by A_{2A}AR-selective agonist CGS21680 in CHO cells stably expressing the recombinant human A_{2A}AR (data not shown).

In addition to its effect on G_s coupling, we also examined the effect of UBO-QIC on $G\alpha_i$ coupling. Figure 2b shows that UBO-QIC has no effect on NECA-induced inhibition of forskolin-stimulated cAMP accumulation in CHO cells stably expressing the recombinant human A_1 ARs. The EC_{50} values of NECA in the presence and absence of UBO-QIC (300 nM) were 4.9 ± 1.6 and 5.6 ± 1.7 nM, respectively, which were not significantly different (P>0.05, Student's test). As a control, PTX (200 ng/ml) blocked the effect of NECA (Figure 2b). It is noted that, in the presence of PTX, NECA actually stimulates cAMP accumulation, which is due to the fact that A_1ARs can also couple to G_s protein in addtition to G_i protein coupling, a phenomenon observed previously by Cordeaux et al. [26]. In addition to the A_1AR, we also tested the effect of UBO on another G_i-coupled receptor, $P2Y_{12}$. Figure 2c shows that UBO (300 nM) did not produce any effect on 2MeSADP-induced inhibition of forskolin-stimulated cAMP accumulation, although PTX (200 ng/ml) completely blocked the effect of 2MeSADP. The EC_{50} values of 2MeSADP in the presence and absence of UBO are 1.6 ± 0.3 and 1.7 ± 0.5 nM, respectively, which are not significantly different (P>0.05, Student's test).

We then examined the effect of UBO-QIC on M_3 muscarinic receptor-mediated $G\alpha_q$ coupling. Figure 2D shows that carbachol concentration-dependently induced intracellular calcium mobilization in CHO cells stably expressing the recombinant human M_3 receptor corresponding to an EC_{50} value of 56.8 ± 12.7 nM. Unlike its lack of effect on the stimulation or inhibition of cAMP production, UBO-QIC (300 nM) completely blocked the effect of carbachol on Ca^{2+} transients. By contrast, PTX 200 ng/ml has no effect (EC_{50} = 46.6 ± 15.9 nM). Thus, the above experiments confirmed the selectivity of UBO-QIC for $G\alpha_q$ in comparison to the $G\alpha_i$ and $G\alpha_s$ proteins.

Since both $G\alpha_q$ and $G_{\beta\gamma}$ subunits can mediate calcium mobilization [15,16], we further examined the potential effect of UBO-QIC on one G_q-coupled receptor, the $P2Y_1$ receptor ($P2Y_1R$) for extracellular nucleotides, and on three G_i-coupled receptors, the A_1AR, $P2Y_{12}R$ and M_2 muscarinic receptor. Surprisingly, it was found that UBO-QIC, in a concentration-dependent manner, inhibited calcium mobilization induced by all receptors tested (Figure 3). At 10 nM, UBO-QIC produced a substantial inhibition, and at 100 nM, the calcium responses were completely blocked (Figure 3). Higher concentrations (300 nM and 1000 nM) also completely blocked agonist effects (data not shown). As a comparison, PTX (200 ng/ml) also completely blocked responses to stimulation of M_2, $P2Y_{12}R$, and A_1AR, but not $P2Y_1R$-mediated calcium mobilization. In CHO cells expressing the human A_1AR, the EC_{50} value of NECA to induce calcium transients was 10.6 ± 2.9 nM. UBO-QIC at the concentration of 10 nM diminished the maximum effect of NECA by ~60%. Carbachol concentration-dependently elicited calcium mobilization in CHO cells expressing the M_2 receptor, corresponding to an EC_{50} value of 252 ± 43 nM. UBO-QIC (10 nM) reduced the maximal effect of carbachol by ~50%. Similarly, UBO-QIC (10 nM) blocked by ~50% of the maximum effect of 2MeSADP-induced calcium transients in 1321N1 astrocytoma cells expressing either the $P2Y_1R$ (EC_{50}=1.1 ± 0.3 nM) or $P2Y_{12}R$ (EC_{50}=21.6 ± 6.4 nM) (Figure 3). Thus, UBO-QIC blocks other intracellular signaling, in addition to $G\alpha_q$.

In addition to the effect of UBO-QIC on calcium mobilization, we also performed the assays of IP1 production to confirm that UBO-QIC blocks the function mediated via both G_q- and G_i-coupled receptors. It has been reported that production of inositol phosphates or calcium mobilization are mediated both via G_q-coupled receptors and G_i-coupled receptors (via $G\beta\gamma$ subunits) [15,17-19, 23]. Figure 4 shows that, at 100 nM, UBO-QIC almost completely blocked the agonist responses mediated via two G_q-coupled receptors (M_3 and $P2Y_1$) and two G_i-coupled receptors (A_1 and M_2). The EC_{50} values (nM) of NECA (A_1), carbachol (M_2), carbachol (M_3), and 2MeSADP ($P2Y_1$) were 15.8 ± 4.2, 351 ± 86, 11.6 ± 2.1, and 162 ± 18, respectively.

Activation of most $G\alpha_q$- and $G\alpha_i$-coupled receptors results in stimulation of ERK1/2 activity [21,22,24,25]. We tested the effect of UBO-QIC on ERK1/2 activation mediated through various GPCRs. In an initial experiment, it was found that UBO-QIC, at the concentration of 10 nM, which substantially diminished calcium mobilization, produced little if any effect on ERK1/2 activation. Figure 5 shows that at 100 nM, UBO-QIC only partially (around 30% or less) blocks the maximal agonist effect in ERK1/2 phosphorylation mediated via $P2Y_1$, A_1AR, or M_2 receptor. In the case of M_3 receptor, even at the concentration of 300 nM, UBO-QIC did not diminish the maximum agonist response by more than 30%. In all cases,

UBO-QIC produced a somewhat non-parallel right shift of the agonist concentration-response curves, which was at odds with its effects in calcium mobilization or IP1 production. The broad-spectrum inhibitor of protein kinase C (PKC) GO6983 [27] completely blocked ERK1/2 phosphorylation mediated via M_3 and $P2Y_1$ receptors (Figure 5c,d). As expected, treatment with PTX inhibited ERK1/2 phosphorylation mediated via G_i-coupled A_1 and M_2 but not G_q-coupled $P2Y_1$ and M_3 receptors (Figure 5).

In addition to stimulation of ERK1/2 activity, both G_q- and G_i-coupled receptors are known to modulate PI3K-Akt signaling pathway [28]. Therefore, we examined the effect of UBO-QIC on Akt activity following the activation by both the G_q-coupled $P2Y_1R$ and the G_i-coupled A_1AR or $P2Y_{12}R$. Figure 6 shows that 2MeSADP-induced Akt1/2/3 phosphorylation in 1321N1 cells exressing the $P2Y_1R$ was completely inhibited by UBO-QIC (100 nM). By contrast, UBO-QIC (100 nM) had no effect on A_1AR or the $P2Y_{12}R$, although PTX (200 ng/ml) completely blocked the Akt1/2/3 activity stimulated by both receptors. At a higher concentration (1.0 μM), UBO-QIC showed a modest but significant inhibition of the maximum effect of 2MeSADP-stimulated Akt activity ($P<0.05$ compared with corresponding values in the absence of UBO; Figure 6c). Thus, the pattern of inhibition of Akt activity by UBO-QIC is different from that in other signaling pathways.

To further demonstrate the inhibitory effect of UBO-QIC on both $G\alpha_q$ and non-$G\alpha_q$-mediated events in a relatively natural system, we examined its effect on calcium mobilization induced by a selective A_1AR agonist CPA in DDT1-MF2 (Syrian hamster smooth muscle) cells known to express an A_1AR endogenously that is sensitive to PTX [29]. Figure 7 shows that CPA-induced intracellular calcium mobilization in DDT1-MF2 cells is completely blocked by both UBO-QIC and PTX. By comparison, UBO-QIC but not PTX antagonizes calcium transients mediated by a $P2Y_1R$-selective agonist MRS2365 [30] in HEK293 cells that endogenously express the human $P2Y_1R$ (Figure 7).

4. Discussion

The present study clearly demonstrated that UBO-QIC is a potent inhibitor of $G\beta\gamma$-mediated signaling events following the activation of G_i-coupled receptors, although UBO-QIC is selective for $G\alpha_q$ in comparison to $G\alpha_i$- or $G\alpha_s$-mediated inhibition or stimulation of cAMP accumulation (Figure 8). Thus, caution is needed when using UBO-QIC to define $G\alpha_q$ coupling of certain GPCRs since $G\alpha_q$ and $G\beta\gamma$ often mediate similar downstream signaling events.

The $G\alpha_q$ coupling of GPCRs has been probed in several recent publications using UBO-QIC to block production of inositol phosphates [6], calcium release [5,7,31] or GTPγS binding [2]. Inamdar et al. [4] reported that UBO-QIC is a selective $G\alpha_q$ inhibitor based on studies using $G\alpha_q$ knockout murine platelets. The authors concluded that UBO-QIC has no effect on $G\alpha_i$ subunits based on the results that UBO-QIC has no effect on phosphorylation of protein kinase B (Akt), which is downstream of $G\alpha_i$. The authors also concluded that the $G\alpha_{12/13}$ pathway was not involved, because platelet shape remained intact in $G\alpha_q$ knockout mice. However, the effect of UBO-QIC on $G\beta\gamma$-mediated events was not explored in those previous studies. In addition to clear demonstration of the blockade by UBO-QIC of both

Gβγ and $G\alpha_q$-mediated events, results from the present study also indicate that the weak inhibition or even lack of inhibition by UBO-QIC of some pathways, such as ERK1/2 and Akt, is not an evidence that $G\alpha_q$ is not involved as these events are often mediated via multiple upstream signaling molecules. For example, Figure 5 shows that, even at 300 nM, UBO-QIC only diminished about 30% of the maximal effect of carbachol, although it shifted the concentration-response curve to the right. The concentrations of the agonists used in this assay were critical for the interpretation of the percentage inhibition by UBO-QIC. Consistent with the present study, Wauson et al. [3] also showed that UBO-QIC only partially reduced ERK1/2 stimulation mediated via $G\alpha_q$-coupled T1R1/T1R3 heterodimeric taste receptor in the MIN6 pancreatic β-cell derived line. It is noted that in addition to Gα, βγ subunits released both from $G_{q/11}$ and G_i classes of G proteins can stimulate ERK1/2 although via a different phosphorylation site and lead to different downstream events [32]. It is intriguing to know if UBO-QIC can distinguish downstream events mediated by different βγ subunits. This could be examined by using βγ G_i over-expression system or using G_i protein mutants which may obtain more direct and less disputable results. It should be interesting to elucidate the identity of βγ subunit complexes involved in the signaling cascade that are sensitive to UBO-QIC. However, this may not be an easy task given the fact that at least 5 β-subunits and 11 γ-subunits have been identified. We do not have evidence as to how UBO-QIC affects only βγ subunits released from G_i, but not the $G\alpha_i$ protein. It has been reported that a small molecule 12155 binds directly the $G_{\beta\gamma}$, but without affecting $G\alpha_i$ subunit in the G_i heterotrimer [33].

The present study demonstrated a different pattern of inhibition by UBO-QIC for ERK1/2 and Akt activity mediated by the same class of receptors. For example, UBO-QIC completely blocked $P2Y_1R$-mediated Akt activity but only partially inhibited ERK1/2 activity. The reason for this difference could be due to the fact that Akt and ERK1/2 activity are mediated via different upstream mechanisms directed under the same receptor. Murga et al. [28] showed that PKC did not seem to link $G\alpha_q$ to Akt but PI3 kinase could link a receptor to Akt phosphorylation, although the conclusion was only based on results from individual G_q-coupled receptors. GPCR-mediated ERK1/2 activity has been extensively explored, which could be mediated via various Gα, Gβγ, and β-arrestins [24], and PKC is often involved in ERK1/2 activity meditated via various G protein subunits. On the other hand, it has been shown that blockade of G protein signaling by PKC inhibitors such as GO6983 may enhance β-arrestin-mediated signaling, and the inhibition of arrestin-mediated signaling, such as by siRNA, may promote signaling mediated via G protein subunits [34]. It is not clear, if the activity of other G protein subunits or β-arrestins are changed following the blockade of $G\alpha_q$ by UBO-QIC, which could be similar or different for some signaling events blocked by inhibitors of other signaling molecules, such as PKC and PI3 kinase.

A number of G protein inhibitors have been reported previously. For example, PTX inhibits the α subunit of G_i proteins by locking subunits in a GDP-bound inactive state [35], but also affected release of βγ subunits [17,20,36]. GRK2i, a peptide analog of G-protein receptor kinase (GRK), blocks only Gβγ signaling thus rendering it a useful tool to discern between Gα and βγ pathways [4,15]. However, it is not clear if UBO-QIC interacts with Gβγ in the same way as GRK2i or not. It is also not clear if YM-254890 or UBO-QIC inhibits signaling events mediated by βγ subunits released from $G\alpha_q$ [32], although YM-254890 was proposed

to inhibit G_q proteins by inhibiting the release of GDP from the α subunits [14]. In addition to the G protein inhibitors, G protein activators were also found to activate multiple G proteins or G protein subunits. For example, Pasteurella multocida toxin (PMT) was initially found to activate G_q and $G_{12/13}$, but it was found later that it also activates $G\alpha_i$, and $G\beta\gamma$ subunits are needed for Gα signaling [37]. We do not have a structural explanation of the interaction of UBO-QIC with Gβγ, as it was noted that although the binding region is close to Gβγ in the X-ray structure, the related depsipeptide YM-254890 bound to Gα in the absence of the Gβγ subunits [14].

As mentioned in the Introduction section, compared with UBO-QIC, YM-254890 is a relatively well-characterized $G\alpha_q$ inhibitor [8,10-13]. However, even for YM-254890, only very limited data are available concerning its selectivity against some G proteins, such as G_{15} and Gβγ [9].

Our findings are supported by a very recent report by Kukkonen [38], who raised similar doubts about the $G\alpha_q$ selectivity and showed that UBO-QIC at the concentration of 1 μM could inhibit $G\alpha_{16}$ in addition to $G\alpha_q$ [35]. The author cautioned that “further studies are required to establish its profile with respect to the different G_q-family proteins”, and we agree.

In summary, UBO-QIC is an extremely useful inhibitor of $G\alpha_q$ signaling, but has additional effects that need to be considered in interpreting pharmacological data. The present study clearly demonstrated that UBO-QIC blocked both $G\alpha_q$ and Gβγ-mediated signaling following the activation of G_q- and G_i-coupled receptors.

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Abbreviations

BAY60-6583 (LUF6210)	2-[[6-amino-3,5-dicyano-4-[4-(cyclopropylmethoxy)phenyl]-2-pyridinyl]thio]-acetamide
cAMP	3′,5′-cyclic adenosine monophosphate
CGS21680	2-[p-(2-carboxyethyl)phenyl-ethylamino]-5′-*N*-ethylcarboxamidoadenosine
CHO	Chinese hamster ovary
CPA	N^6-cyclopentyladenosine
DMEM	Dulbecco's modified Eagle's medium
ERK	extracellular-signal-regulated kinase
fMLP	formyl peptide

GO6983	3-[1-[3-(dimethylamino)propyl]-5-methoxy-1*H*-indol-3-yl]-4-(1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione
GPCR	G protein-coupled receptor
HEK	human embryonic kidney
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
IP1	inositol 1-phosphate
2MeSADP	2-methylthioadenosine 5′-diphosphate trisodium salt
MRS2365	[[(1*R*,2*R*,3*S*,4*R*,5*S*)-4-[6-amino-2-(methylthio)-9*H*-purin-9-yl]-2,3-dihydroxybicyclo[3.1.0]hex-1-yl]methyl] diphosphoric acid monoester
NECA	adenosine-5′-*N*-ethyluronamide
PTX	pertussis toxin
UBO-QIC	L-threonine, (3*R*)-*N*-acetyl-3-hydroxy-L-leucyl-(α*R*)-α-hydroxybenzenepropanoyl-2, 3-didehydro-*N*-methylalanyl-L-alanyl-*N*-methyl-L-alanyl-(3*R*)-3-[[(2*S*,3*R*)-3-hydroxy-4-methyl-1-oxo-2-[(1-oxopropyl)amino]pentyl]oxy]-L-leucyl-*N*,*O*-dimethyl-, (7→1)-lactone

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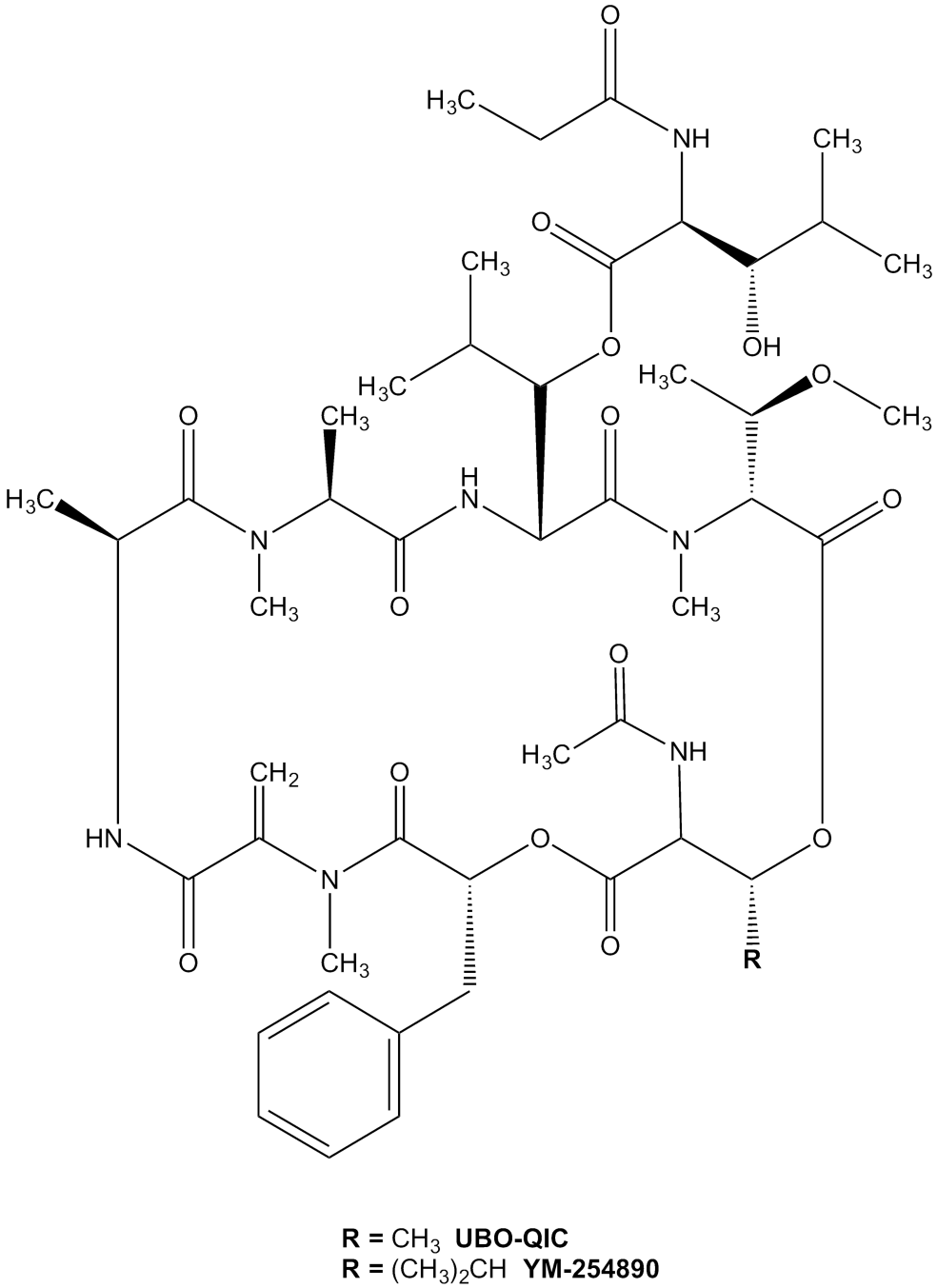


Figure 1.
Chemical structures of naturally-occuring cyclic depsipeptides UBO-QIC (FR900359) and YM-254890.

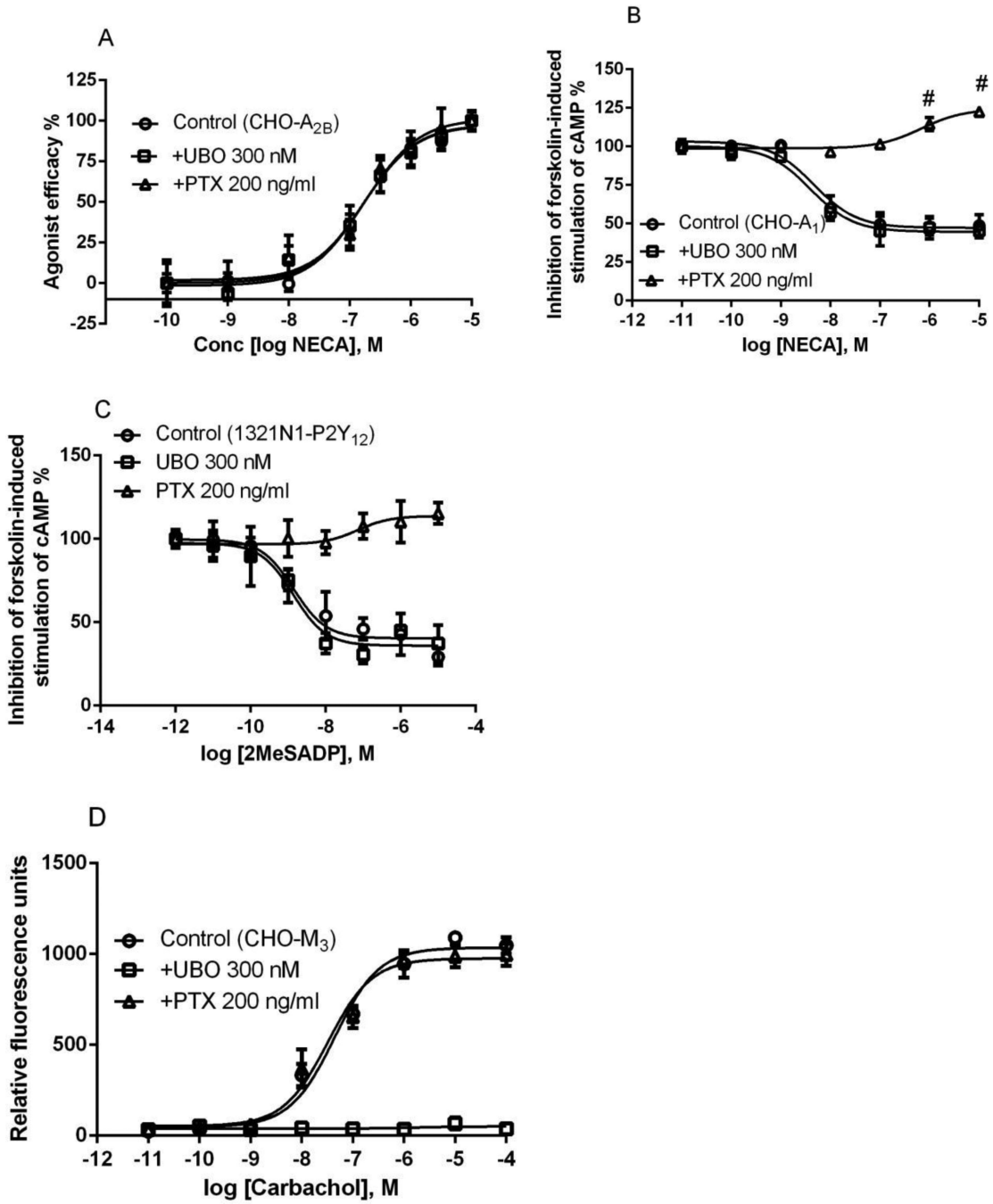


Figure 2.

Selectivity of UBO-QIC for $G\alpha_q$- versus $G\alpha_s$- and $G\alpha_i$-mediated signaling. A. NECA-induced cAMP accumulation in CHO cells expressing the recombinant human A_{2B}AR ($G\alpha_s$). B. NECA-induced inhibition of forskolin-stimulated (10 μM) accumulation of cAMP in CHO cells expressing the recombinant human A_1AR ($G\alpha_i$). Cells were treated with agonist for 20 min and forskolin for 10 min. C. 2MeSADP-induced inhibition of forskolin-stimulated (10 μM) accumulation of cAMP in 1321N1 astrocytoma cells expressing the human $P2Y_{12}$ receptors. D. Carbachol-inducd intracellular calcium mobilization in CHO

cells expressing the recombinant human M3 muscarinic receptor ($G\alpha_q$). For all assays, cells were pretreated with UBO-QIC (300 nM) for 30 min or PTX (200 ng/ml) overnight before the addition of agonists. Results are expressed as mean ± SEM and are from at least three independent experiments performed in duplicate or triplicate. The EC_{50} values from agonist response curves are listed in the text. #Significantly different from control or lower concentrations of NECA in the presence of PTX ($P<0.05$, One-Way ANOVA with post-hoc test).

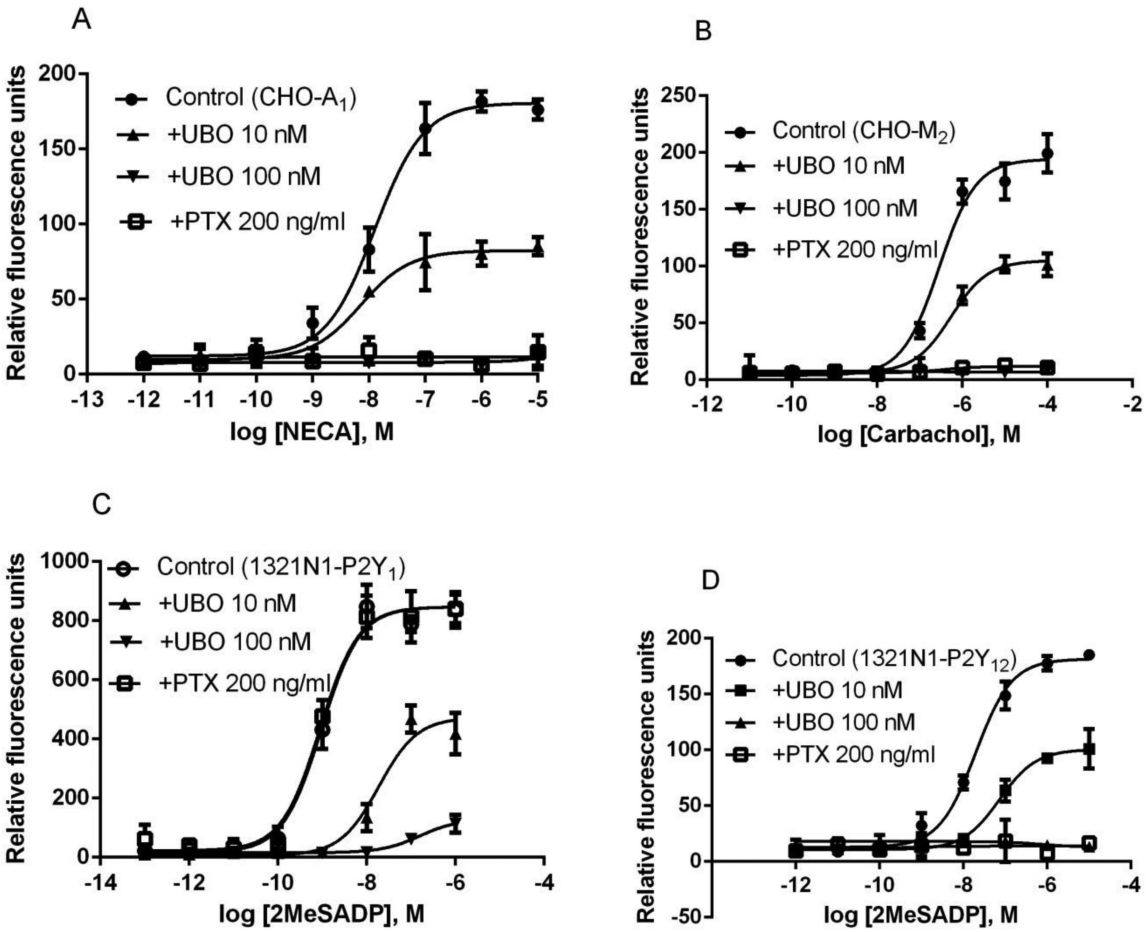


Figure 3.

Intracellular calcium mobilization mediated via the G_i-coupled A_1AR (A) and M_2 muscarinic receptor (B), both expressed in CHO cells, and the G_q-coupled $P2Y_1$ (C) and G_i-coupled $P2Y_{12}$ nucleotide receptors (D) expressed in 1321N1 astrocytoma cells. Cells were pretreated with UBO-QIC (10 and 100 nM) for 30 min or PTX (200 ng/ml) overnight before the addition of agonists. Results were expressed as mean ± SEM from 3 independent experiments performed in duplicate.

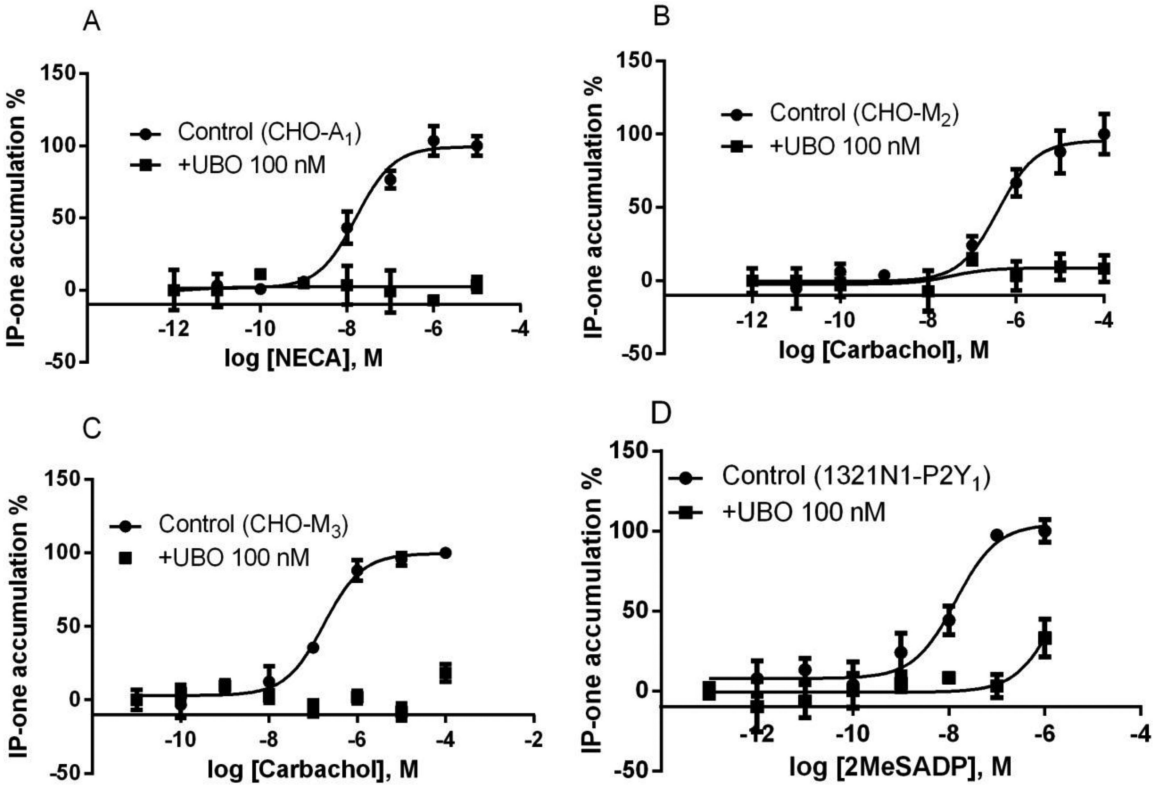


Figure 4.

Stimulation of IP1 production via activation of G_i-coupled A_1AR (A) and M_2 muscarinic receptor (B) expressed in CHO cells, or G_q-coupled M_3 muscarinic receptors in CHO cells (C) and G_q-coupled $P2Y_1$ nucleotide receptor in 1321N1 astrocytoma cells (D). Cells were pretreated with UBO-QIC (100 nM) for 30 min and PTX (200 ng/ml) overnight before the addition of agonists. Results were expressed as mean ± SEM from 3-4 separate experiments performed in duplicate.

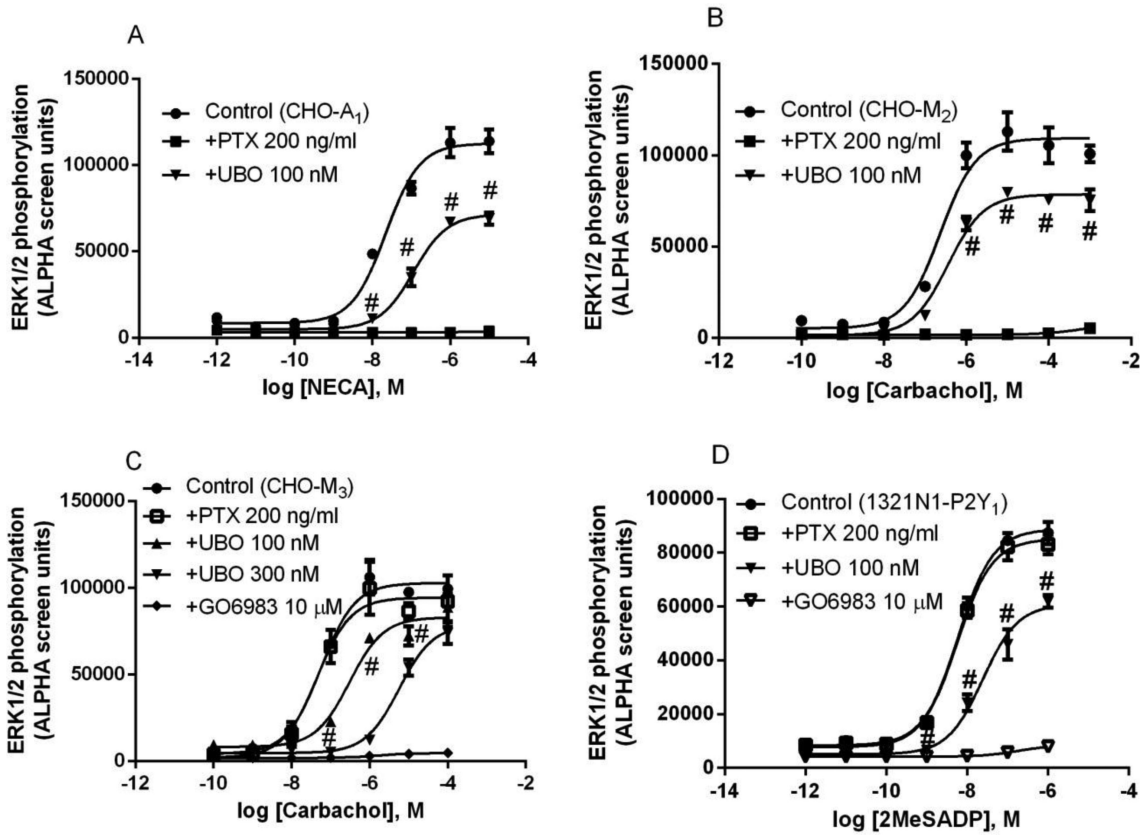
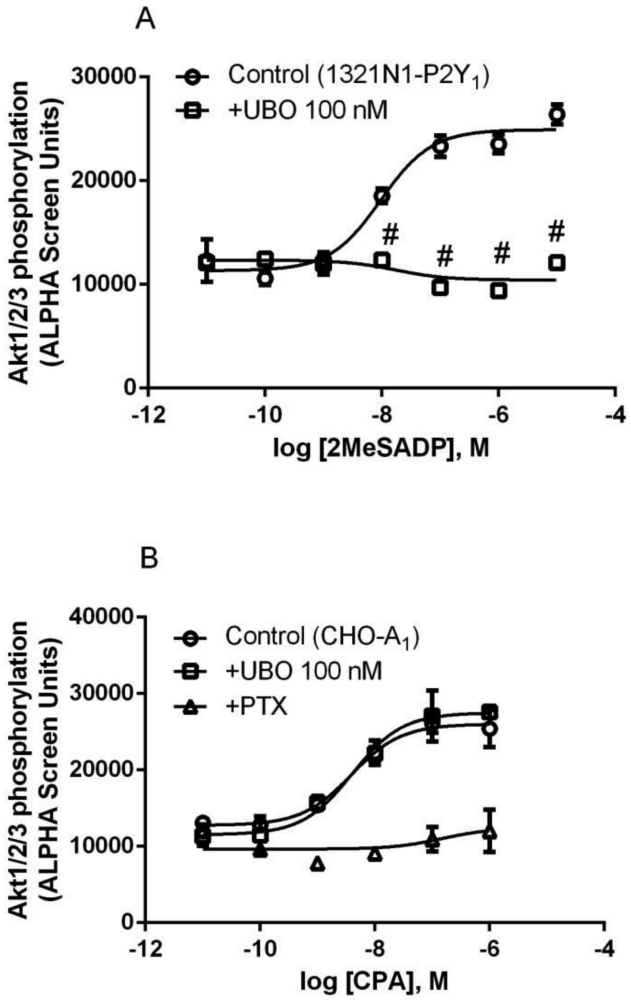


Figure 5.

Stimulation of ERK1/2 activity in CHO cells expressing the A_1AR (A), M_2 (B) or M_3 (D) muscarinic receptor, and in 1321N1 astrocytoma cells expressing the $P2Y_1$R (C). Cells were pretreated with UBO-QIC (100 nM) or GO6983 (10 μM) for 30 min or PTX (200 ng/ml) overnight before the addition of agonists (5 min incubation). Data are mean ± SEM from three experiments performed in duplicate. #Significantly different from the corresponding Control values in the absence of UBO ($P<0.05$).



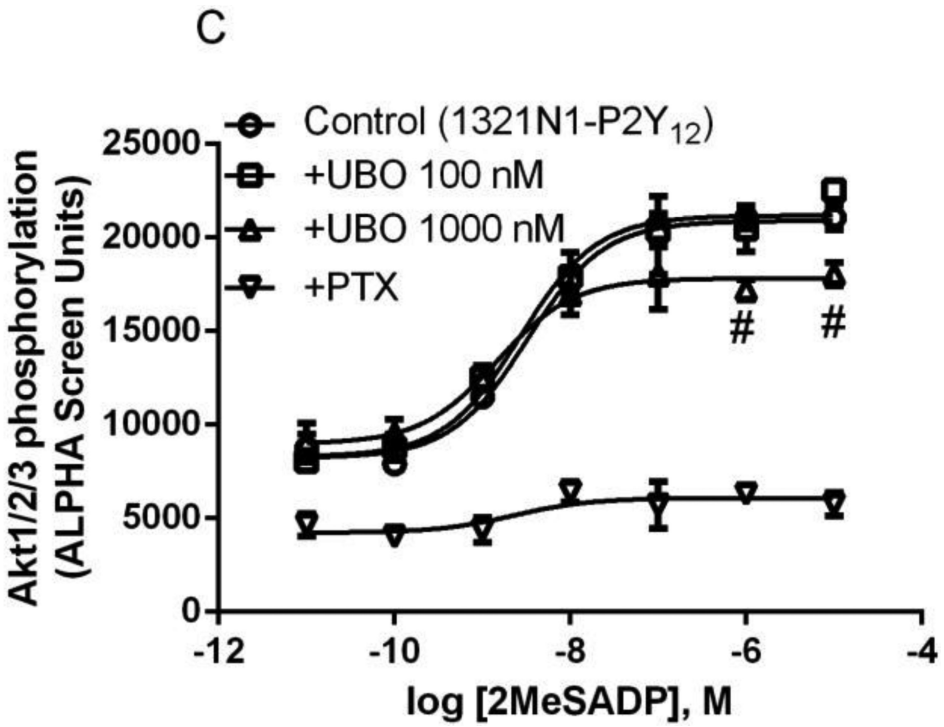


Figure 6.

Effect of UBO-QIC on the Akt1/2/3 phosphorylation stimulated by Gαq-coupled $P2Y_1R$ (A, 1321N1 cells) or Gi-coupled A_1AR (B, CHO cells) or $P2Y_{12}R$ (C, 1321N1 cells). A. Cells were pretreated with UBO-QIC (100 nM) for 30 min before the addition of agonists. B. Cells were pretreated with UBO-QIC (100 nM) for 30 min or PTX (200 ng/ml) overnight. C. Cells were pretreated with UBO-QIC (100 nM and 300 nM) for 30 min or PTX (200 ng/ml) overnight before the addition of agonist. Results are expressed as mean ± SEM from 3 independent experiments performed in duplicate. #Significantly different from corresponding control values ($P<0.05$).

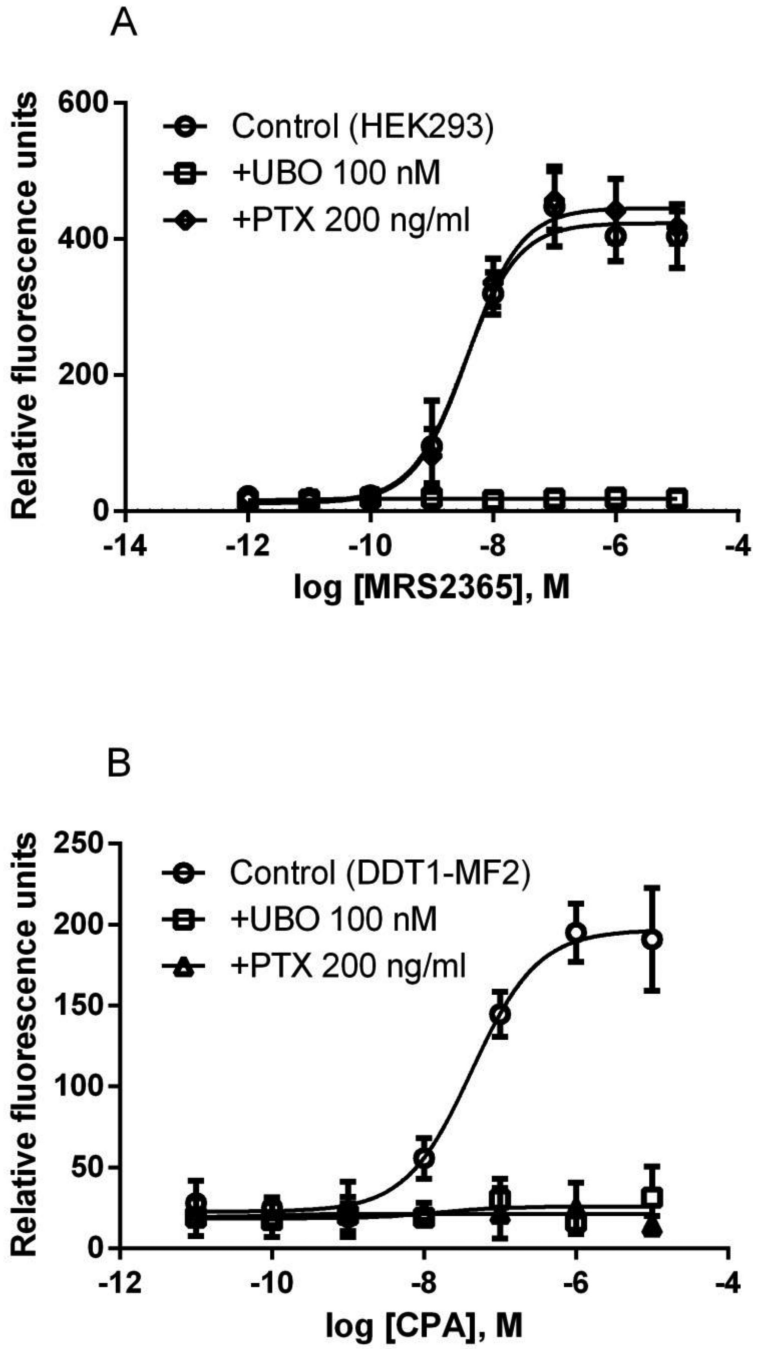


Figure 7.

MRS2365-induced intracellular calcium mobilization in HEK293 cells expressing an endogenous $P2Y_1R$ (A) and CPA- induced intracellular calcium mobilization in DDT1-MF2 cells with an endogenous A_1AR (B). Cells were pretreated with UBO-QIC (100 nM) for 30 min or PTX (200 ng/ml) overnight before the addition of agonist. All data are in relative fluorescence units and are expressed as mean ± SEM from 3 experiments performed in triplicate.

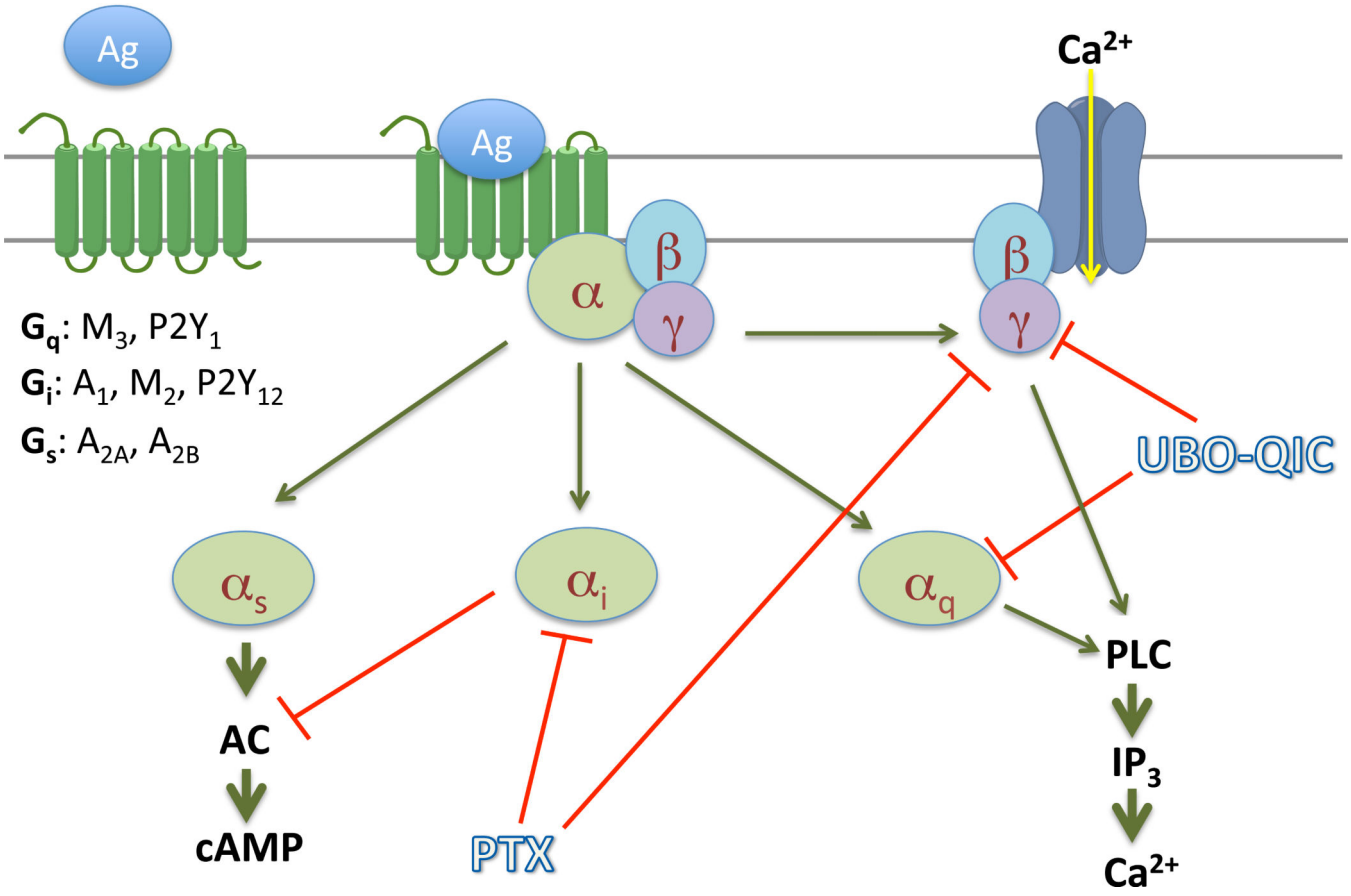


Figure 8.
GPCR signaling pathways and their interaction with inhibitors.