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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 $\beta\gamma$, 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. $G\beta\gamma$-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 $G\beta\gamma$-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 $\beta\gamma$ 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.

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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].

UBO-QIC is a close analog of a known $G\alpha_q$ inhibitor YM-254890 (Fig. 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 were 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 $G\alpha_{15}$.

Abbreviations: 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, [[(1R,2R,3S,4R,5S)-4-[6-amino-2-(methylthio)-9H-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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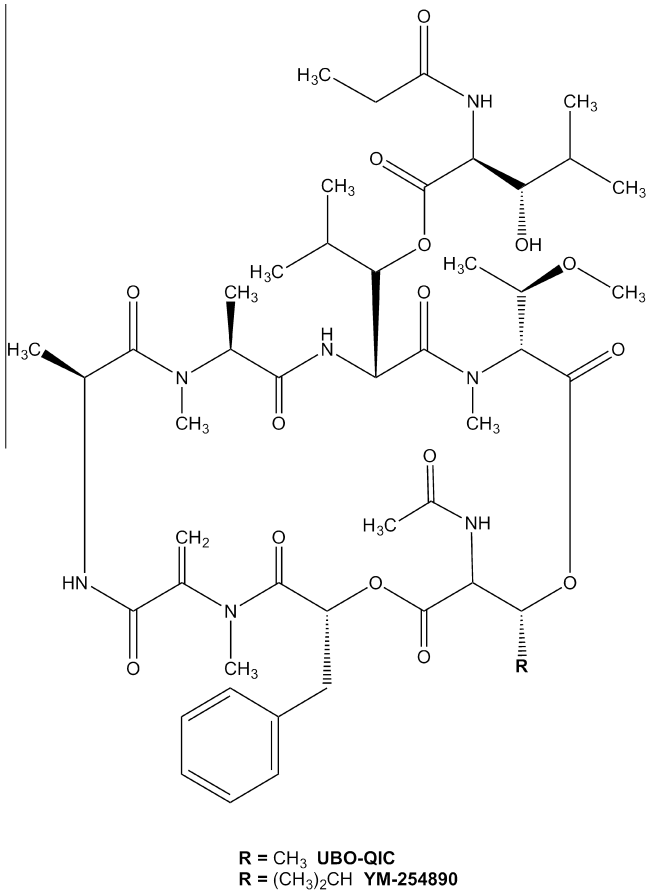

Fig. 1. Chemical structures of naturally-occurring cyclic depsipeptides UBO-QIC (FR900359) and YM-254890.

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 into 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 $G\alpha_q$ over other G proteins and their subunits, especially the $\beta\gamma$ 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 $G\alpha_q$ 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

[[(1R,2R,3S,4R,5S)-4-[6-Amino-2-(methylthio)-9H-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-Akt1/2/3 (p-Ser473) Assay Kit were purchased from PerkinElmer (Waltham, MA). HEK293 and DDT1-MF2 were from ATCC (Manassas, 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 $P2Y_1R$ 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 pretreatment with UBO-QIC (100 nM) for 30 min and before the addition of agonists followed by an additional 30 min of 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 to 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-Akt1/2/3 (p-Ser473) Assay Kit (PerkinElmer, Waltham, MA).