Bioorg. Med. Chem. Lett. 41 (2021) 128008

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structure activity relationship of 3-nitro-2-(trifluoromethyl)-2*H*-chromene derivatives as $P2Y_6$ receptor antagonists

Young-Hwan Jung, Shanu Jain, Varun Gopinatth, Ngan B. Phung, Zhan-Guo Gao, Kenneth A. Jacobson *

Molecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA

ARTICLE INFO

Keywords:
P2Y receptor
Antagonist
Purinergic
Calcium mobilization
Structure activity relationship

ABSTRACT

Various 6-alkynyl analogues of a known 3-nitro-2-(trifluoromethyl)-2*H*-chromene antagonist **3** of the G_q-coupled $P2Y_6$ receptor ($P2Y_6R$) were synthesized using a Sonogashira reaction to replace a 6-iodo group. The analogues were tested in a functional assay consisting of inhibition of calcium mobilization in $P2Y_6R$-expressing astrocytoma cells elicited by native $P2Y_6R$ agonist UDP. 6-Ethynyl and 6-cyano groups were installed, and the alkynes were extended through both alkyl and aryl spacers. The most potent antagonists, with IC_{50} of ~1 μM, were found to be trialkylsilyl-ethynyl **7** and **8** (3–5 fold greater affinity than reference **3**), *t*-butyl prop-2-yn-1-ylcarbamate **14** and *p*-carboxyphenyl-ethynyl **16** derivatives, and **3** and **8** displayed surmountable antagonism of UDP-induced production of inositol phosphates. Other chain-extended terminal carboxylate derivatives were less potent than the corresponding methyl ester derivatives. Thus, the 6 position in this chromene series is suitable for derivatization with flexibility of substitution, even with sterically extended chains, without losing $P2Y_6R$ affinity. However, a 3-carboxylic acid or 3-ester substitution did not serve as a nitro bioisostere, as the affinity was eliminated. These compounds provide additional ligand tools for the underexplored $P2Y_6R$, which is a target for inflammatory, neurodegenerative and metabolic diseases.

The $P2Y_6$ receptor ($P2Y_6R$) is activated by UDP and is localized in immune cells as well as small intestine, blood, heart, blood vessels and the central nervous system.[1,2] $P2Y_6R$ is a G_q protein-coupled member of the purinergic metabotropic P2Y receptor family and is associated with proinflammatory effects.[2] In microglial cells, the $P2Y_6R$ induces phagocytosis of debris such as amyloid proteins, and its agonists have been explored for treatment of Alzheimer's disease and brain ischemia.[3–5] A $P2Y_6R$ antagonist was shown to reduce chronic neuropathic pain by counteracting UDP-induced microglial and inflammatory responses.[6] Furthermore, a $P2Y_6R$ agonist prodrug was found to be beneficial in an in vivo asthma model.[7] $P2Y_6R$ antagonists have been explored for cancer treatment with mixed results.[8,9] The vasoconstricting effects of $P2Y_6R$ agonists have also been explored, suggesting a role for antagonists in treating hypertension.[10] The $P2Y_6R$ is proposed to form a heterodimer with the angiotensin (AT) II type-1 receptor (AT_1R), leading to an undesired effect of $P2Y_6R$ agonists to promote vascular inflammation. A recent study supported the concept of using $P2Y_6R$ antagonists for treatment of obesity and diabetes based on the mouse phenotype on a high fat diet when the receptor is knocked out in adipocytes.[11] UDP analogues with increased affinity have been reported, including those with substituted uracil rings and with rigid, bicyclic substitutions of the ribose ring to achieve a receptor-preferred conformation.[12,13] The latter ribose modification is termed South (S)-methanocarba (bicyclo[3.1.0]hexane), which maintains a constrained (S) conformation. A UDP analogue with an isomeric North (N)-methanocarba modification was completely inactive at the human (h) $P2Y_6R$.

Only a few series of $P2Y_6R$ antagonists have been reported,[35,14–16] and none have achieved nM affinity. A uridylyl phosphosulfate derivative weakly antagonized $P2Y_6R$ with IC_{50} 112 μM.[16] A bicyclic diketopiperazine scaffold was also found to inhibit $P2Y_6R$ effects with IC_{50} ~ 10 μM, but it was not receptor subtype selective.[17] Thus, there is a pressing need for more potent, competitive and drug-like antagonists. Aryl di-isothiocyanate derivatives are moderately potent antagonists, but because of the requirement of the chemically reactive -NCS groups are likely irreversibly receptor-bound antagonists, also consistent with the insurmountable antagonism observed.[14] Widely used $P2Y_6R$

* Corresponding author at: Molecular Recognition Section, Bldg. 8A, Rm. B1A-19, NIH, NIDDK, LBC, Bethesda, MD 20892-0810, USA.
E-mail address: kennethj@niddk.nih.gov (K.A. Jacobson).

https://doi.org/10.1016/j.bmcl.2021.128008
Received 26 February 2021; Received in revised form 24 March 2021; Accepted 25 March 2021
Available online 6 April 2021
0960-894X/Published by Elsevier Ltd.

antagonist MRS2578 **1** (Chart 1) having two phenyl 3-isothiocyanato groups inhibited $P2Y_6R$ signaling in two species homologues with IC_{50} values of 37 nM (h) or 98 nM (rat, r), with selectivity compared to other P2YRs. Curiously, the potency and selectivity were highly dependent on the length of the alkyl chain and the positions of the symmetric aryl isothiocyanate groups. A shorter symmetric homologue bearing phenyl 4-isothiocyanato groups, compound **2**, only inhibited $hP2Y_6R$ (although selectively), but not $rP2Y_6R$.[14] A step forward in the search for potentially competitive antagonists was achieved several years ago, when a hit from library screening, 3-nitro-2-(trifluoromethyl)-2*H*-chromene **3**, was reported by Ito et al. to be a selective $P2Y_6R$ antagonist of μM affinity.[15] At a 30 μM concentration, it displayed no antagonism of calcium mobilization induced by the native agonists of $P2Y_1R$ (10 nM ADP), $P2Y_2R$ (1 μM UTP), $P2Y_4R$ (100 nM UTP) and $P2Y_{11}R$ (10 μM ATP) expressed in 1321N1 astrocytoma cells. Its 6-bromo analogue **4** was also a $P2Y_6R$ antagonist of moderate affinity, but 2-phenyl substitution reduced the affinity by an order of magnitude. The binding sites on the $P2Y_6R$ of both of these chemical classes, isothiocyanates and chromenes, are unexplored, using either mutagenesis or molecular modeling. In this study, we have chosen to empirically probe the structure activity relationship (SAR) of the chromene series using the 6 position as the site for installation of alkynes through a Sonogashira reaction.

The synthesis of 3-nitro-2-(trifluoromethyl)-2*H*-chromene derivatives based on **3** and **4** was performed as shown in Scheme 1 (procedures in Supporting information). The newly synthesized analogues tested for $hP2Y_6R$ antagonism (**5**–**25**) are shown in Table 1. Substitution of the 6 position of the 3-nitro-2-(trifluoromethyl)-2*H*-chromene scaffold was chosen as the principal site of derivatization. The 6-bromo analogue **4** was previously reported as similarly potent as the 6-H compound,[15] suggesting that other halo atoms might be suitable at this position. A 6-iodo group was selected as the first novel halo derivative (**5**) in our series. Subsequently, a Sonogashira reaction was used to replace a 6-iodo group with various alkynes (**7**–**24**). The TMS group was removed from **7** to provide alkyne **6**. A variety of functionalized terminal alkyne intermediates (Schemes 2A and B) were utilized to prepare these 2*H*-chromene derivatives. For example, *t*-butyl prop-2-yn-1-ylcarbamate reacted with 6-iodo derivative **5** to yield the Boc-amino derivative **14**. Also, a 6-cyano derivative **25** was prepared from **30**. To introduce carboxylate groups at the 3 position of **5**, the 3-ethyl ester **26** was synthesized, followed by hydrolysis with LiOH to yield **27** (Scheme 2C).[18] The preparation of tetramethylrhodamine derivative **43** by a Sonogashira reaction using the alkyne derivatized fluorophore is shown in Supporting information (Scheme S1).

The potency of the 3-nitro-2-(trifluoromethyl)–2*H*-chromene derivatives was determined in an assay of calcium mobilization in $hP2Y_6R$-expressing 1321N1 astrocytoma cells,[12–14] in a 96-well format. A fixed concentration (100 nM, $2.4XEC_{50}$) of the native agonist UDP was used, and the IC_{50} of each chromene derivative determined (Fig. 1). There is a wide spread of IC_{50} values depending on the distal functionalization of the alkynyl group, differing by up to 2 orders of magnitude. The bromo derivative **4** displayed an IC_{50} of 3.49 μM, although it was reported as 4-fold more potent in Ito et al.[15] Pharmacological procedures are described in the Supporting information.

The 6-iodo derivative **5** had an IC_{50} similar to the known 6-bromo compound **4**. Due to the synthetic accessibility, we prepared a series of 6-ethynyl derivatives that tended to preserve the $P2Y_6R$ affinity. The simplest substitution in the 6-ethynyl derivative **6** increased the $hP2Y_6R$ antagonist by 2-fold compared to the lead compound **3**. Trimethylsilyl **7** and triethylsilyl **8** derivatives were found to be among the most potent $hP2Y_6R$ antagonists in this functional assay with $IC_{50} < 1$ μM. Therefore, we replaced the Si atom with C in *t*-butyl derivative **9** and examined closely related alkynes (c-Pr **10** and hydroxymethyl **11** derivatives). The silicon-to-carbon switch of an ibuprofen analogue, conceptually similar to compound **7** compared to **9**, was exploited and was suggested as a general modification in medicinal chemistry.[20] However, here the carbon analogue **9** was roughly an order of magnitude weaker than the silicon derivative **7**.

An acylated propargylamine moiety, present in acetyl **12** and Boc **14** derivatives, mostly preserved the $P2Y_6R$ affinity. A neutral Boc-amino derivative **14** was equipotent to the ethynyl compound **6**. Therefore, there appeared to be no immediate steric limitation to extending the group at the 6 position. Thus, a propargylamine moiety was included as a spacer in subsequent derivatives, including **12**–**14** and **18**–**22**. Terminal methyl ester groups (**15**, **18**, **20**, **23** and **24**) or carboxylates (**13**, **16**, **19** and **21**) were present in various chain elongated derivatives. However, no terminal amino derivatives were included. The attempted synthesis of an unacylated propargylamine derivative (not shown) related to **14** failed, because this chemical series is unstable to strong acidic (i.e. Boc deprotection of **14**) and basic conditions needed for removal of common protecting groups (i.e. phthaloyl in **22**). The lack of steric constraints in this region of the chromene scaffold was indicated by the substitution of the alkyne with a phthaloylamino group in **22** leading to slightly higher affinity than the *N*-acetyl analogue **12**. Curiously, a benzoic acid derivative **16** was equipotent to **14**, but its methyl ester derivative **15** was ~6-fold less potent. Therefore, an early preference for an anionic group at the terminal position of the chain appeared, at least with a *p*-substituted phenylethynyl group in **16**. Initially, this seemed consistent with a hypothetical proximity to the highly positively charged extracellular loop regions typical of P2YRs. However, in the case of extended aliphatic amino acid derivatives, a terminal carboxylate group led to much weaker interactions with the receptor. For example, methyl ester **18** displayed roughly an order of magnitude higher affinity than its corresponding carboxylate **19**, and methyl ester **20** had 13-fold higher affinity than its carboxylate **21**. Similarly, anionic succinate derivative **13** was 30-fold weaker in its interaction with the $P2Y_6R$ than its neutral, truncated *N*-acetyl derivative **12**. The presence of a water-solubilizing, terminal urea group in **17** lowered affinity.

A 6-cyano derivative **25** had 4-fold lower affinity than the corresponding 6-ethynyl derivative **6**. An attempt to identify a nitro

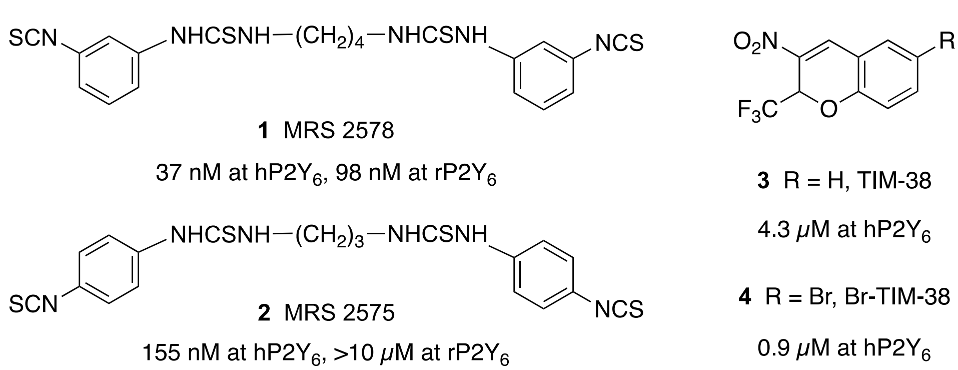
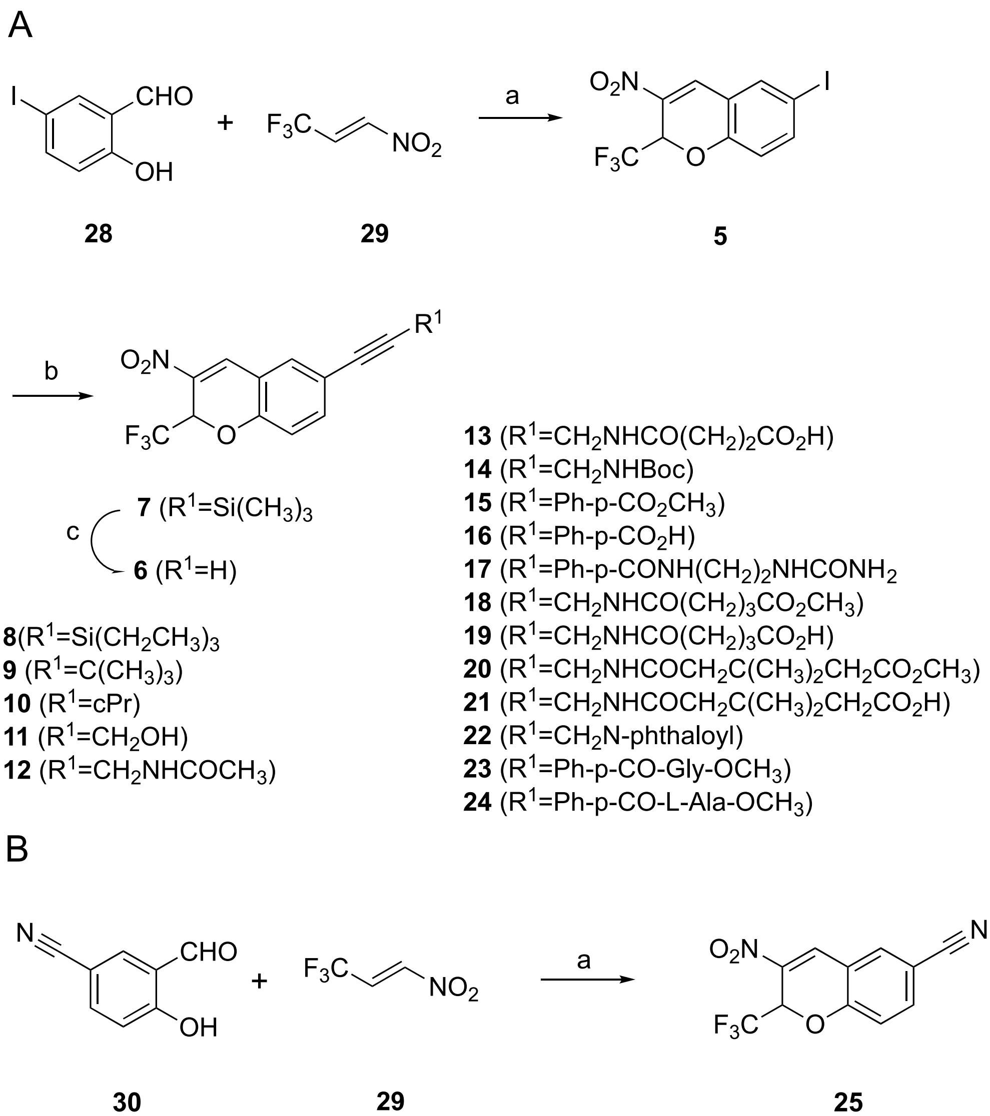


Chart 1. Structures of reported selective $P2Y_6R$ antagonists.[14,15]



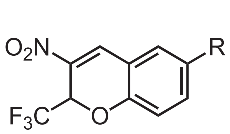
Scheme 1. Synthesis of compounds **5**–**25**. Reagents and Conditions: A. (a) TEA, DCM, rt, 5 h, 65–77%; (b) $PdCl_2(PPh_3)_2$, CuI, TEA, THF, rt, 12 h, 47–96%; (c) K_2CO_3, MeOH, rt, 1.5 h, 93%. B. (a) TEA, DCM, rt, 5 h, 65%.

bioisostere in the form of a carboxyl group present in anionic derivative **27** and its ethyl ester **26** failed. Sterically bulky fluorescent tetramethylrhodamine derivative **43** only weakly bound to the P2Y$_6$R. Although some of the compounds with improved affinity, e.g. **8**, were more hydrophobic than the lead compound **3**, the inhibitory potency was not a function of hydrophobicity. Carboxylate **16** was both more polar and more potent than **15**. The cLogP values (ChemDraw, v. 19) are in parentheses: **3** (3.00), **8** (3.00), **15** (5.60), **16** (5.38) and **23** (2.43).

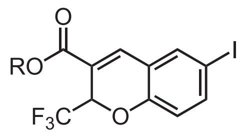
Compound **3** has been shown to inhibit UDP-induced calcium mobilization in 1321N1 cells expressing the human P2Y$_6$R in an insurmountable manner.[15] In testing the generality of this characteristic within the chemical series, we also found that increasing concentrations of the triethylsilyl analogue **8** right-shifted the UDP activation curve in P2Y$_6$R expressing 1321N1 astrocytoma cells with a reduction in the maximal effect, as well (Fig. 2). Since calcium mobilization is not an assay under equilibrium conditions, it is not possible to conclusively determine whether **3** and **8** are surmountable or insurmountable antagonists. Thus, we further examined the ability of **3** and **8** to inhibit UDP-induced IP-1 accumulation ($G_{q/11}$-mediated production of inositol phosphates).[43] Fig. 3 shows that at the concentration of 10 μM, **3** and **8** shifted the concentration–response curve to the right 4.6- and 10.9-fold, respectively, without affecting the maximum effect of UDP. Thus, both **3** and **8** are surmountable antagonists, and **8** is more potent than **3** in both functional assays. Unlike **3** and **8**, MRS2578 **1** has been demonstrated to be an insurmountable antagonist in a similar assay of inositol phosphates.[14]

Off-target activity of selected, relatively potent analogues (**5**, **7**, **8**, **12**, **14** and **22**) was determined at 46 different receptors and channels by the Psychoactive Drug Screening Program (PDSP) at University of North Carolina.[21] A few significant off-target interactions were found for these derivatives in the μM range, particularly at some biogenic amine receptors. They included: **5** (receptor, K_i in μM or *% inhibition at* 10 μ*M*): adrenergic α_{2A}, 4.8; dopamine D_5, 1.6; GABA$_A$ *65%*; σ_1, 2.1; **7**: α_{2A}, 4.7; D_5, 4.2; σ_1, 1.4; **8**: α_{2A}; **9**: D_5, 2.1; **12**: α_{2A}, 2.2; α_{2B}, 6.7; D_5, 1.1; σ_1, 4.4; **14**: α_{2A}, 0.94 ± 0.33; D_3, 1.7 ± 1.0; D_5, 1.25 ± 0.16; **16**: D_1, 1.1; D_5, 2.1; opioid μ, 4.2; α_{2A}, 1.8; α_{2B}, 1.0; α_{2C}, 2.6; **22**: α_{2A}, 0.65; α_{2B}, 4.7; D_5, 1.4. Thus, *t*-butyl derivative **9** displayed only one weak off-target activity. Other receptors reported <50% inhibition at 10 μM, and most of these interactions displayed a negligible % inhibition in the primary comprehensive screen, as shown for compound **7** (Supporting Information). Thus, these compounds are not promiscuous receptor binders. Nevertheless, the relatively few off-target interactions in the μM range may in some cases interfere with the use of these compounds as pharmacological probes, and the need for additional SAR study to eliminate

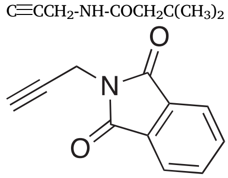
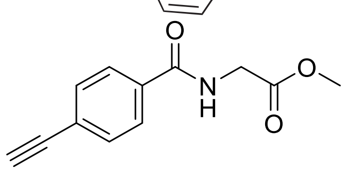
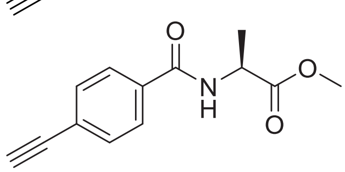
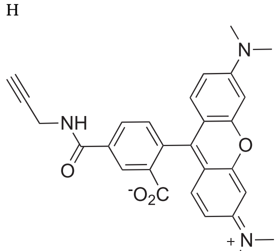
Table 1
Inhibition of UDP-induced Ca^{2+} mobilization in a $hP2Y_6R$-expressing 1321N1 human astrocytoma cell line.[a]



3 – 25, 43



26, 27

Compound	R =	IC_{50}, $hP2Y_6R$ (μM, mean ± SEM)[c]
Known compounds		
3, TIM-38[b]	H	2.91 ± 1.21
4, Br-TIM-38[b]	Br	3.49 ± 1.54
New compounds		
5	I	4.00 ± 1.59
6	C≡CH	1.33 ± 0.31
7[d]	C≡C-$Si(CH_3)_3$	0.785 ± 0.058
8[d]	C≡C-$Si(CH_2CH_3)_3$	0.604 ± 0.239
9[d]	C≡C-$C(CH_3)_3$	6.59 ± 1.95
10	C≡C-(c-Pr)	4.87 ± 1.96
11	C≡C-CH_2OH	7.31 ± 1.03
12	C≡CCH_2-NH-$COCH_3$	7.67 ± 2.22
13	C≡CCH_2-NH-$CO(CH_2)_2$-CO_2H	121 ± 72
14[d]	C≡CCH_2-NH-Boc	1.20 ± 0.08
15	C≡C-Ph-p-CO_2CH_3	7.13 ± 3.14
16[d]	C≡C-Ph-p-CO_2H	1.09 ± 0.42
17	C≡C-Ph-p-$CONH(CH_2)_2$-$NHCONH_2$	10.6 ± 1.3
18	C≡CCH_2-NH-$CO(CH_2)_3$-CO_2CH_3	5.23 ± 1.74
19	C≡CCH_2-NH-$CO(CH_2)_3$-CO_2H	69.1 ± 20.7
20	C≡CCH_2-NH-$COCH_2C(CH_3)_2$ CH_2-CO_2CH_3	5.90 ± 1.38
21	C≡CCH_2-NH-$COCH_2C(CH_3)_2$ CH_2-CO_2H	77.9 ± 60.7
22		4.65 ± 1.17
23		6.33 ± 0.90
24		6.84 ± 0.57
25	C≡N	5.33 ± 1.83
26	CH_3CH_2	>100
27	H	>100
43		34.6 ± 8.9

[a] Measured in Ca^{2+} assays in whole $hP2Y_6R$-expressing 1321N1 cells (gift of T. K. Harden, Univ. of North Carolina, Chapel Hill, NC).[19,12–14] UDP (100 nM) was used as agonist.
[b] Compound reported by Ito et al., 2017.[15] IC_{50} values shown were determined here.
[c] 3–4 independent determinations, each in triplicate.
[d] **7**, MRS4695; **8**, MRS4774; **9**, MRS4773; **14**, MRS4706; **16**, MRS4656.

these activities is suggested.

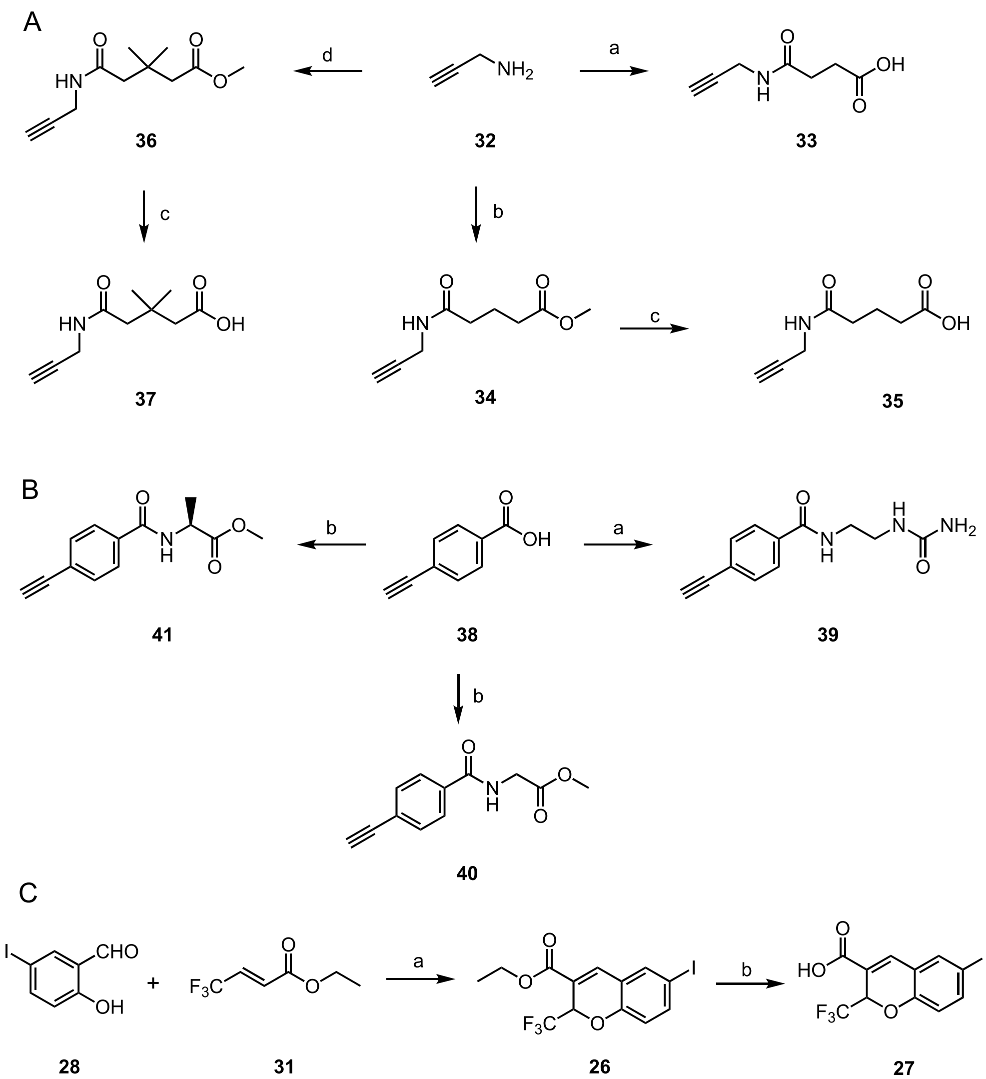
The stability of a representative $P2Y_6R$ antagonist toward nucleophiles was examined. There was no evidence of reaction of compound **7** with 2-phenylethanethiol as a nucleophile during aqueous incubation in the presence of LiOH as a base (Supporting Information).[22]

The discovery of drug-like antagonists would enable the proof of concept of various therapeutic applications of blocking this receptor. $P2Y_6R$ antagonists have been considered in the context of possible anticancer, cerebroprotective, antihypertensive, anti-inflammatory, antidiabetic and anti-ischemic activity, or for use in heart failure and chronic pain.[6,23–27] For example, a heterodimer of two GPCRs, AT_1R and $P2Y_6R$, was disrupted by MRS2578, a $P2Y_6R$-selective inhibitor, to reduce the risk of angiotensin-induced hypertension in an animal model.[23] $P2Y_6R$ activation also promotes the appearance of membrane protrusions and migration of lung and colon cancer cells.[28] Thus, there is sufficient justification to discover novel $P2Y_6R$ antagonists. However, curiously the deletion of $P2Y_6R$ promoted a more inflammatory phenotype in alveolar macrophages.[29] Also, the mouse $P2Y_6R$ knockout increased inflammation and intestinal recruitment of Th17/Th1 lymphocytes in the inflammatory bowel disease (dextran sodium sulfate, DSS) model.[30] Recently, Salem et al. showed that activation of the colonic epithelial cell $P2Y_6R$ induced intestinal inflammation, which was reduced by native nucleoside triphosphate diphosphohydrolase 8 (NTPDase8) through cleaving endogenous UDP.[31] Consistently, MRS2578 protected against intestinal inflammation in colitis induced by DSS.

The effect of pharmacological inhibition and complete genetic deletion of the receptor might not be equivalent, but there is still justification to test newly-reported $P2Y_6R$ antagonists in various disease models.[32] In a high-fat diet model of obesity, the knockout of $P2Y_6R$ in both whole body and selectively in adipocytes, but not in skeletal muscle alone, improved metabolic parameters.[11] The adipocyte knockout mice resembled the whole-body knockout of $P2Y_6R$, and therefore systemic antagonism is predicted to be beneficial in diabetes. Recent studies also suggest the application of $P2Y_6R$ antagonists for chronic pain treatment,[6,27,33] although an earlier study showed no protective effect by MRS2578 (10 mg/kg, i.p.) in the mouse spared nerve injury model, even though the antagonist was bioavailable over at least 30 min.[34] MRS2578 administration (i.t.) relieved neuropathic pain in the rat chronic constriction injury (CCI) model or following UDP (i.t.) administration.[24] $P2Y_6R$ antagonists might also prove useful in treating persistent bladder storage symptoms in male benign prostatic hyperplasia (BPH).[35] $P2Y_6R$ activation led to bladder overactivity via increased mucosal ATP release, an effect blocked by blocked by 50 nM MRS2578.

However, tractable leads for novel high affinity $P2Y_6R$ antagonists are lacking. Furthermore, there is no X-ray or cryo-EM structure of the $P2Y_6R$. It is apparent that the structures of the $P2Y_6R$ and other P2YR already determined are sufficiently divergent to make an *in silico* screening approach to identify novel chemotypes as $P2Y_6R$ antagonists uncertain. It would be most efficient for receptor modeling to already have diverse classes of competitive antagonists as a modeling test set, but such is lacking. Although both $P2Y_1R$ and $P2Y_6R$ are nucleoside-5′-diphosphate receptors of the G_q-coupled subfamily, and models of the $P2Y_6R$ based on the X-ray structure of G_q-coupled $P2Y_1R$ (and on the chemokine CXCR4 receptor) have been reported,[13,36] there appear to be major structural differences affecting ligand SAR. For example, it was not possible to derive $P2Y_6R$ antagonists by generalizing from bisphosphates $P2Y_1R$ antagonists, such as MRS2179,[14] to the uridine family. The corresponding 2′-hydroxy- or 2′-deoxy-uridine-3,5-bisphosphates did not antagonize the $P2Y_6R$.[37]

Thus, we chose to explore a reported lead despite its unfavorable physicochemical characteristics, e.g. 3-nitro-2-(trifluoromethyl)-2*H*-chromene derivatives.[15] The related chromone scaffold has also been applied to diverse drug classes,[38] and several chromenes were found to inhibit ecto-nucleotidase CD73.[39] One of the disadvantages of compound **3** as a lead molecule is that the analogues are racemic. Another



Scheme 2. Synthesis various functionalized alkyne intermediates and 3-carboxy derivatives **26** and **27**. A. (a) succinic anhydride, 4-DMAP, DCM, rt, 1 h, 87%; (b) glutaric acid monomethyl ester chloride, 4-DMAP, DCM, rt, 5 h, 72%; (c) KOH, MeOH: water = 2:1, rt, 0.5 h, 68–98%; (d) 5-methoxy-3,3-dimethyl-5-oxopentanoic acid, HATU, 4-DMAP, DCM, rt, 12 h, 77%. B. (a) (2-amino-ethyl)-urea hydrochloride, DIPEA, HATU, DMF, 0 °C, 1 h, 71%; (b) glycine methyl ester hydrochloride for **40** or L-alanine methyl ester hydrochloride for **41**, HATU, 4-DMAP, DCM, rt, 12 h, 84–87%. C. (a) K_2CO_3, DMF, 80 °C, 12 h, 46%; (b) LiOH, water:MeOH = 1:1, 50 °C, 5 h, 85%.

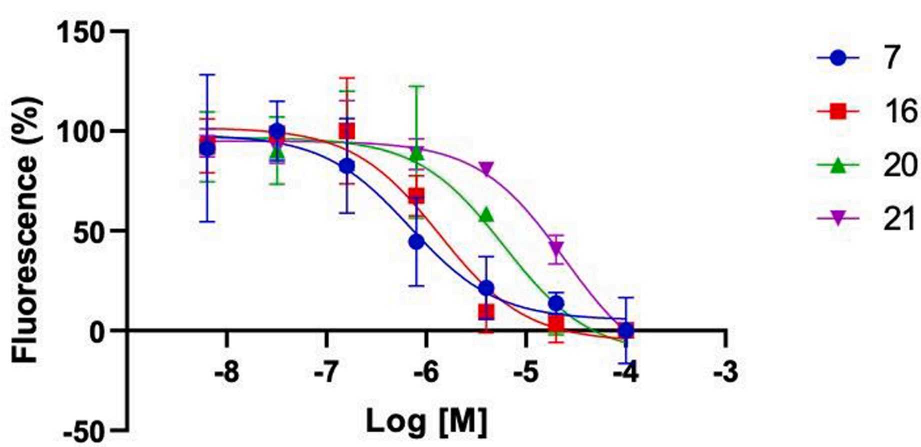


Fig. 1. Antagonism by representative antagonists (silyl derivative **7**, benzoic acid derivative **16**, and extended ester **20** and corresponding carboxylate **21**) of UDP-induced Ca^{2+} mobilization in $P2Y_6R$-expressing 1321N1 cells.[19,12–14] 100 nM UDP was used as agonist.

disadvantage is the presence of a nitro group in **3**, which is a structural alert in medicinal chemistry,[40] although nitro-bearing drugs and drug candidates have been developed.[41] Although various functional groups have been found to serve as nitro group bioisoteres at various targets,[42] replacement with a 3-carboxylate or 3-ester failed to preserve affinity in the present series. Nevertheless, we have established that the SAR of **3** can be explored, leading to a family of alkynyl congeners with variable μM $P2Y_6R$ affinity. A site on the scaffold for chain extension has been identified: the 6 position, which is suitable for derivatization with flexibility of substitution, even with sterically extended chains, without

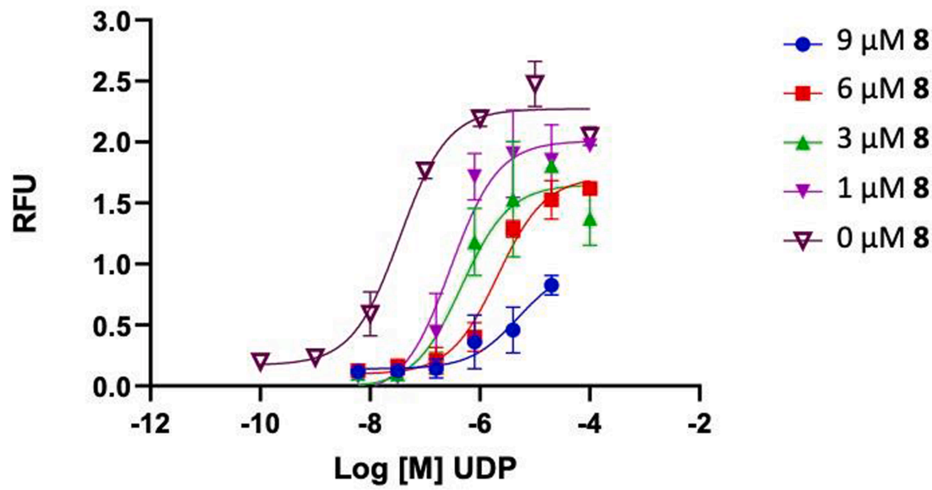


Fig. 2. Antagonism by fixed concentrations of compound **8** of concentration response curves for UDP-induced Ca^{2+} mobilization in $P2Y_6R$-expressing 1321N1 cells (representative curves). EC_{50} values (μM, mean ± SEM, N = 3) for UDP are: 0.287 ± 0.036 (1 μM); 0.59 ± 0.27 (3 μM); 1.03 ± 0.56 (6 μM); 2.37 ± 1.28 (9 μM). EC_{50} value for UDP alone was 41.6 nM ± 7.0 nM.

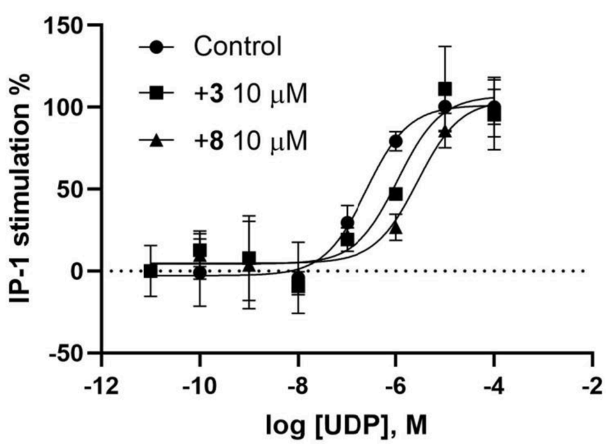


Fig. 3. Antagonism of inhibit UDP-induced IP-1 accumulation by compounds **3** and **8** (10 μM) in $P2Y_6R$-expressing 1321N1 cells (N = 3). EC_{50} values (μM, mean ± SEM) for UDP (conc. **8**) are: 0.295 ± 0.088 (UDP alone); 1.35 ± 0.45 (**3**); 3.21 ± 0.86 (**8**).

losing $P2Y_6R$ affinity.

In conclusion, substitution of 3-nitro-2-(trifluoromethyl)-2*H*-chromenes with linear alkynes and extending amide chains provided considerable $P2Y_6R$ affinity in some analogues. Relatively potent trimethylsilyl-ethynyl **7** and triethylsilyl-ethynyl **8** derivatives displayed 3-fold greater antagonistic affinity (IC_{50} < 1 μM) than reference chromene **3**. There are other derivatives also which may be worth considering, for further screening. The surmountable antagonism by **3** and **8** of UDP-induced production of inositol phosphates is encouraging for the future utility of this compound series. P2YR ligands often benefit from the presence of anionic groups, consistent with the negative charge on the native nucleotide ligands. However, only one terminal carboxylate derivative, a carboxy-phenyl-ethynyl analogue **16** retained comparable affinity, while extended carboxylates were considerably weaker in binding. Thus, we have expanded to range of analogues of **3** that block a $P2Y_6R$ functional effect with a consistent SAR pattern observed for 6-alkynyl derivatives. Although we do not have a three-dimensional model of the $P2Y_6R$ binding of this series, the empirical SAR exploration suggested that the binding region on the receptor surrounding the extended 6-alkynyl groups allows much steric freedom of substitution, possibly suggestive of facing the extracellular loops. For example, substitution of the alkyne with a phthaloylamino group in **22** or phenyl groups preserves affinity. We were unsuccessful in an effort to find a bioisostere of the 3-nitro group or to maintain affinity in a fluorescent analogue. These identification of position 6 in this chromene series as a versatile site for derivatization will provide additional ligand tools for the underexplored $P2Y_6R$, which was shown to be involved in various animal models of disease.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank the NIDDK Intramural Research Program (ZIADK31116) for support. We thank Bryan L. Roth, National Institute of Mental Health's Psychoactive Drug Screening Program (Univ. North Carolina at Chapel Hill, Contract # HHSN-271-2008-00025-C) for screening data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128008.

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