GPCR ligand–dendrimer (GLiDe) conjugates: Future smart drugs?

ARTICLE *in* TRENDS IN PHARMACOLOGICAL SCIENCES · OCTOBER 2010

Impact Factor: 11.54 · DOI: 10.1016/j.tips.2010.09.002 · Source: PubMed

1 AUTHOR:

Kenneth A. Jacobson
National Institutes of Health
754 PUBLICATIONS **30,083** CITATIONS

SEE PROFILE

Published in final edited form as:
Trends Pharmacol Sci. 2010 December ; 31(12): 575–579. doi:10.1016/j.tips.2010.09.002.

GPCR ligand-dendrimer (GLiDe) conjugates: future smart drugs?

Kenneth A. Jacobson
Molecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes, Digestive and Kidney Diseases, NIH, Bethesda, MD 20892

Abstract

Unlike nanocarriers that are intended to release their drug cargo at the site of action, biocompatibile polyamidoamine (PAMAM) conjugates are designed to act at cell surface G protein-coupled receptors (GPCRs) without drug release. These multivalent GPCR Ligand-Dendrimer (GLiDe) conjugates display qualitatively different pharmacological properties in comparison to the monomeric drugs and might be useful as novel tools to study GPCR homo- and heterodimers and higher aggregates. The structure of the conjugate determines the profile of biological activity, receptor selectivity, and physical properties such as water solubility. Prosthetic groups for receptor characterization and imaging can be introduced without a loss of affinity. The feasibility of targeting multiple adenosine and P2Y receptors for synergistic effects has been shown. Testing *in vivo* will now be needed to explore the effects on pharmacokinetics and tissue targeting.

Introduction

The mechanism of action of at least one quarter of the drugs on the market involves modulation of rhodopsin-like GPCRs on the cell surface [1]. A recurrent issue in the discovery and clinical development of new GPCR ligands, agonists and antagonists is the occurrence of a given receptor in multiple tissues, leading to side effects. The same receptor might occur in a target tissue, in which a desired pharmacological effect can be demonstrated, and in off-target tissues, in which the effect can be less than beneficial or even harmful. A means of designing GPCR modulators that function as “smart drugs” is needed [2]. This need is especially evident for receptor classes such as the purine receptors, which encompasses both adenosine receptors (ARs) [3] and purinergic P2Y receptors [4] for extracellular nucleotides that are expressed at varying levels and in diverse combinations in nearly every organ. Even with the promise of structure-based design of GPCR ligands [5,6] (now with an increasing number of X-ray crystallographic structures available), tissue- or organ-selectivity would provide a great advantage therapeutically.

Pro-drug approaches have sought to achieve this goal of organ-selective delivery of GPCR ligands [7]. However, their success is dependent on the presence of a specific enzyme for the release of the free drug species in the target tissue, which is often unfeasible. Instead, the field has adopted the approach of using covalent conjugates of chemically functionalized GPCR ligands tethered from macromolecular carriers, which emerged from the early work of Ringsdorf and others [8]. The polyamidoamine (PAMAM) dendrimers form a particularly versatile and biocompatible class of polymeric drug carriers. First introduced by Tomalia

Correspondence to: Jacobson, K.A. (kajacobs@helix.nih.gov), Chief, Laboratory of Bioorganic Chemistry and Molecular Recognition Section, Bldg. 8A, Rm. B1A-19, NIH, NIDDK, LBC, Bethesda, MD 20892-0810 USA. *Tel: 301-496-9024. Fax: 301-480-8422.*

[9], these tree-like polymers have found use in diagnostic imaging and in research for delivering nucleic acids and peptides to cells for internalization [10,11]. Charge and size parameters that affect internalization of PAMAM dendrimers have been studied [12]. The PAMAM dendrimers can be tailored to a wide range of molecular weights by adding outer layers that bifurcate in successive “generations” that multiply the number of terminal groups geometrically, and the nature of the terminal functionality can be altered for conjugation purposes or for tuning pharmacokinetic properties in vivo. Enhanced stability of ligands when conjugated to PAMAM dendrimers has been noted [13]. Most of the examples of use of PAMAM dendrimers as drug carriers involved internalization of the complex followed by dissociation or cleavage of the drug cargo to act intracellularly. In one example, arginine-glycine-aspartic acid (RGD) peptides were conjugated to PAMAM dendrimers for binding to cell-surface integrin receptors prior to internalization [14]. With GPCR Ligand-Dendrimer (GLiDe) conjugates [15–21], we have taken a different approach that requires strategic functionalization of the small molecular drug such that it displays a desired biological property extracellularly while still tethered covalently to peripheral groups of the dendrimer. A peptide hormone was also coupled to a PAMAM dendrimer to serve as a targeting moiety through binding to its GPCR [22].

PAMAM conjugates as multivalent ligands of GPCRs

GLiDe conjugates have been described recently as interacting with a variety of adenosine and P2Y receptors (Table 1). These are the first covalent conjugates of PAMAM dendrimers with small molecular GPCR agonists and antagonists that display high potency at a target receptor (nM to μM range). Dissociation of the drug and/or internalization of the complex are generally not necessary or desirable by this approach, which maintains or enhances the binding affinity of the functionalized congeners while covalently bound [23]. Thus, it is now possible to have a multivalent display of potent tethered ligands, typically attached at the peripheral amino or carboxylic groups through amide linkages. Although the chemical structures are based on stoichiometrically-defined substitutions on well-defined polymeric carriers, i.e. averaged structures, the pharmacology of these conjugates can be characterized consistently and reproducibly. Nevertheless, there is a need for the preparation of more chemically defined or monodisperse GLiDe conjugates.

A rationale for the GLiDe conjugate approach is shown in Figure 1. The application of nanocarriers for selective tissue delivery in cancer and other diseases, including in the brain, has been reviewed [24,25]. Similarly, GLiDe conjugates may provide advantages over monomeric drugs. However, the biocompatibility of GLiDe conjugates *in vivo* and the feasibility of these drugs to cross the blood-brain barrier (and other membrane barriers) in order to treat central nervous system (CNS)-associated diseases remain unexplored.

Dimers of GPCRs might be homomeric or heteromeric, and this association, as well as higher order aggregation, can have a major effect on the pharmacological behavior [26]. The multivalent GLiDe conjugates might prove to be useful tools for investigation of GPCR aggregates, given the predicted ability of these conjugates to bridge multiple binding sites within the transmembrane region. Functional negative allostery between protomers in dimeric GPCRs has been detected [27]; this raises the issue of how dendrimer-bound agonists occupying neighboring binding sites would compare with agonist/antagonist combinations. Furthermore, the design of potent GLiDe conjugates is suggestive as a means of drug targeting, perhaps on the basis of an antibody complex. It is also possible that conjugates containing two different pharmacophores would show enhanced affinity for the corresponding heterodimers or permit co-activation of two separate receptors on the same cell. Reporter groups, intended for imaging or diagnostic purposes, might also be incorporated in the conjugates without loss of the high affinity in binding to the receptor.

The first example of a GLiDe conjugate in the purine receptor family is a nearly fully substituted third generation (G3) PAMAM conjugate of a potent, carboxy functionalized A_{2A} AR agonist, CGS21680 [15]. This conjugate not only binds to the receptor but also activates it to inhibit platelet aggregation (a known action of this AR subtype), thus serving as a prototypical example of a novel antithrombotic agent. At moderate (μM) concentrations, no internalization in the platelets was observed using flow cytometry, thus serving the overall strategy of direct action at cell surface receptors.

Another means of inhibiting platelet aggregation pharmacologically is to antagonize the action of the native agonist ADP at either $P2Y_1$ or $P2Y_{12}$ receptors, the latter being the protein target of the blockbuster drug Plavix® (clopidogrel) [4]. We recently overcame synthetic difficulties to demonstrate that a $P2Y_1$ receptor antagonist, an adenine nucleotide derivative, could be incorporated into a GLiDe conjugate that resulted in an anti-aggregatory effect on human platelets [28]. The inability to maintain in a PAMAM conjugate the nM affinity of the parent monomeric $P2Y_1$ receptor antagonist MRS2500 was ascribed using ligand docking to a conformational flip of the adenine moiety in the receptor-bound form in this strucutural series. Nevertheless, the moderate antithrombotic activity demonstrated that the GLiDe conjugate approach is equally applicable to nucleotides as well as nucleosides, to receptor antagonists as well as agonists.

The polymeric multivalency of GLiDe conjugates provides unusual pharmacodynamic and pharmacokinetic properties. One of the objectives of this approach is to enhance receptor potency and selectivity in the multivalent conjugates in comparison to the monomers, and such qualitative differences in selectivity were already observed. A nucleoside conjugate of an N^6-derivatized adenosine attached to a G2.5 PAMAM dendrimer, intended for selectivity at the A_1AR, instead displayed unexpectedly high affinity at the A_3AR [18]. A_3AR agonists are already in clinical trials for treating inflammatory conditions and cancer [29]. This nM affinity permitted the detection by fluorescence microscopy of the punctate occurrence of the A_3AR expressed in CHO cells (Figure 1d). A larger molecular weight variation of this structure was shown to be protective in HL-1 mouse cardiomyocytes exposed to H_2O_2 for induction of apoptosis [21]. The GLiDe conjugate was at least 100-fold more potent than the monomeric nucleoside in protection, which was dependent on the transfection of the cells with the human A_3AR. This enhanced protective potency was not manifested by increased binding affinity but might result from functional amplification of the activation signal.

If multiple anchor points exist for the GLiDe conjugates, a large increase in affinity in comparison with the monomers would be expected, which was demonstrated for agonists of the A_3AR and the $P2Y_{14}$ receptors [19,20]. The affinity enhancement of the A_3AR GLiDe conjugates depended on the chemical linkage used. Thus, a functionalized nucleoside was thethered through a triazole ring formed using Cu^I-catalyzed click chemistry, which is a convenient and efficient method for joining molecules that contain azido ($-N_3$) and terminal alkyne (-C≡CH) groups [30]. The resulting conjugates displayed nM or sub-nM affinity, whereas amide-linked conjugates of the same pharmacophore were considerably less potent. In the case of the Gi-activating $P2Y_{14}$ receptor, amide-linked GLiDe conjugates of the agonist UDP-glucuronic acid [19] were sufficient for high potency in inhibition of cyclic AMP in transfected HEK cells, which varied widely as a function of the percent substitution of the PAMAM dendrimer and the generation (Figure 2). This potency enhancement is suggestive of simultaneous binding to more than one $P2Y_{14}$ receptor, but this aspect must be further explored for an unequivocal demonstration. Nevertheless, the nM potency of these nucleotide conjugates allows the incorporation of reporter groups in the drug-bearing dendrimers of fluorescent, metal-chelating, or biotin moieties, without a loss of biological activity.

PEGylated unimolecular micellar conjugates

A difficulty encountered with the initial amide- or thiourea-linked A_{2A}AR agonists was reduced aqueous solubility, likely due to H-bonding-induced aggregation of the dendrimer [15,16]. Aggregation can reduce bioavailability and increase nonspecific membrane binding. Therefore, we tested whether appending polyethylene glycol (PEG) chains to free terminal groups of the carrier would still allow efficient binding of the nucleosides to their binding sites [17]. The effects of increasing PEG chain length and the degree of substitution on the receptor-binding affinity and activation were systematically probed. Some of the conjugates were modestly enhanced in potency compared to the monomeric agonist. Although there was a small reduction in affinity with the longer (2000 MW) PEG chains, solubility could be enhanced without losing receptor binding. Therefore, these antithrombotic PEGylated GLiDe conjugates can be considered unimolecular micelles, in which the outer hydrophilic layer determines the interface for interaction with medium, membrane surfaces, and biopolymers. The degree of internalization will generally depend largely on the cell type and other conditions, but in platelets it was promoted by amine terminal groups on the carriers, which could be reduced by acetylation of the remaining peripheral amines. Therefore, physicochemical properties of the conjugates, such as size, water solubility and charge, could be modulated leading to predictable changes in the biological activity.

Multiple pharmacophores on the same carrier

These two examples of agonists of the A_3AR and the $P2Y_{14}$ receptor were used as a test of the ability to combine two different pharmacophores into the same GLiDe conjugate [20]. Thus, the conjugate shown in Figure 3 was able to bind to and activate either receptor with high potency. We recently reported that the RBL-2H3 mast cell line, known previously to express functional A_3ARs, also expresses the $P2Y_{14}$ receptor, and both receptors facilitate the release of inflammatory mediators [31]. It is not yet known whether $A_3AR/P2Y_{14}$ heterodimers occur or whether this bi-functional multivalent conjugate acts in a synergistic fashion in RBL-2H3 cells. Nevertheless, the ability to potently activate both receptors suggests that disease treatment using specific combinations of GPCR ligands is possible.

Bivalent ligands have been reported as specific pharmacological tools for homo- and heterodimeric GPCRs [32]. However, tethering two pharmacophores in a manner that is expected to achieve the precise geometry needed to reach both binding sites is a risky endeavor, i.e. difficult to predict a priori due to uncertainty in spatial characteristics of receptor dimers in relation to conformation of the tether. A GLiDe conjugate that contains a statistical distribution of geometries separating two pendant ligands is more likely than a pure substance with a single chain length to contain a suitable combination to bridge two binding sites on adjoining GPCRs protomers. Also, many of the GPCR biological systems have “switchlike” properties that are dictated by interacting ligands. These properties of GPCRs might make predicting a priori GLiDe conjugate actions difficult.

Conclusions

We have introduced a general methodology for designing in stages multivalent GLiDe conjugates that might display qualitatively different pharmacological properties in comparison with the monomeric drugs. Although the ability of these polymeric derivatives to cross biological membranes is likely reduced and structural heterogeneity is present, there are advantages over the monomeric drugs. Nevertheless, large hurdles must be addressed before significant therapeutic advances are achieved. The potential of this approach for use in disease models in animals must still be demonstrated, and basic questions remain concerning cellular uptake, the pharmacological effects of ligands binding to neighboring

GPCR proteins, the alteration of selectivity based on composition of the conjugates, and side effects. This methodology has been applied to study adenosine and P2Y receptors, but potentially it could be used for any GPCR. Potent biological activity has been observed *in vitro*, and the receptor selectivity can be modulated by the structure of the conjugate. The physical properties of these biocompatibile PAMAM conjugates, such as surface polarity, which affects aggregation and internalization, can also be readily modulated by structural changes. Prosthetic groups for receptor characterization and imaging can be introduced without loss of affinity. Novel tools are needed to study GPCR aggregates and these conjugates are also of interest in this context. Testing *in vivo* will now be needed to explore the effects on pharmacokinetics and tissue targeting, in the hope that future "smart drugs" will arise from the GLiDe conjugate approach.

Acknowledgments

Support by the NIDDK Intramural Research Program, NIH is acknowledged.

Glossary

AR	adenosine receptor (family of 4 GPCRs activated by extracellular adenosine and its nucleoside analogues)
CGS21680	2-[p-(2-carboxyethyl)phenyl-ethylamino]-5′-*N*-ethylcarboxamidoadenosine
GLiDe	covalently conjugated GPCR Ligand-Dendrimer
GPCR	G protein-coupled receptor
Ligand docking	process of computational identification of an energetically favorable binding mode of a small molecule in its receptor site
MRS2500	(1′R,2′S,4′S,5′S)-4-(2-iodo-6-methylamino-purin-9-yl)-1-[(phosphato)-methyl]-2-(phosphato)-bicyclo[3.1.0]hexane
PAMAM	polyamidoamine
P2Y	family of 8 GPCRs by various extracellular adenine and uracil nucleotides
RBL	rat basophilic leukemia

References

1. Overington JP, et al. How many drug targets are there? Nat. Rev. Drug Disc 2006;5:993–996.
2. Oh YK, et al. Intelligent drug delivery systems. Bioconjugate Chem 2009;20:1813–1815.
3. Jacobson KA, Gao ZG. Adenosine receptors as therapeutic targets. Nature Rev. Drug Disc 2006;5:247–264.
4. Abbracchio MP, et al. International Union of Pharmacology LVIII. Update on the P2Y G protein-coupled nucleotide receptors: From molecular mechanisms and pathophysiology to therapy. Pharmacol. Rev 2006;58:281–341. [PubMed: 16968944]
5. Katritch V, et al. Structure-based discovery of novel chemotypes for adenosine A_{2A} receptor antagonists. J. Med. Chem 2010;53:1799–1780. [PubMed: 20095623]
6. Carlsson J, et al. Structure-based discovery of adenosine A_{2A} receptor ligands. J. Med. Chem 2010;53:3748–3755. [PubMed: 20405927]
7. Müller CE. Prodrug approaches for enhancing the bioavailability of drugs with low solubility. Chem. Biodivers 2009;6:2071–2083. [PubMed: 19937841]

8. Duncan R, et al. Polymer-drug conjugates: towards a novel approach for the treatment of endrocine-related cancer. Endocrine-Related Cancer 2005;12:S189–S199. [PubMed: 16113096]
9. Menjoge AR, et al. Dendrimer-based drug and imaging conjugates: design considerations for nanomedical applications. Drug Disc. Today 2010;15:171–185.
10. Villaraza AJ, et al. Macromolecules, dendrimers, and nanomaterials in magnetic resonance imaging: the interplay between size, function, and pharmacokinetics. Chem. Rev 2010;110:2921–2959. [PubMed: 20067234]
11. Lim YB, et al. Self-assembled ternary complex of cationic dendrimer, cucurbituril, and DNA: noncovalent strategy in developing a gene delivery carrier. Bioconjug. Chem 2002;13:1181–1185. [PubMed: 12440851]
12. Albertazzi L, et al. Dendrimer internalization and intracellular trafficking in living cells. Mol. Pharm 2010;7:680–688. [PubMed: 20394437]
13. Yellepeddi VK, et al. Surface modified poly(amido)amine dendrimers as diverse nanomolecules for biomedical applications. Expert Opin. Drug Deliv 2009;6:835–850. [PubMed: 19637972]
14. Hill E, et al. Synthetic PAMAM-RGD conjugates target and bind to odontoblast-like MDPC 23 cells and the predentin in tooth organ cultures. Bioconjugate Chem 2007;18:1756–1762.
15. Kim Y, et al. Toward multivalent signaling across G protein-coupled receptors from poly(amidoamine) dendrimers. Bioconjugate Chem 2008;19:406–411.
16. Kim Y, et al. Application of the functionalized congener approach to dendrimer-based signaling agents acting through A_{2A} adenosine receptors. Purinergic Signalling 2009;5:39–50. [PubMed: 18600474]
17. Kim Y, et al. PEGylated dendritic unimolecular micelles as versatile carriers for ligands of G protein-coupled receptors. Bioconjugate Chem 2009;20:1888–1898.
18. Klutz AM, et al. Enhanced A_3 adenosine receptor selectivity of multivalent nucleoside-dendrimer conjugates. J. Nanobiotechnol 2008;6:12.
19. Das A, et al. Enhanced potency of nucleotide-dendrimer conjugates as agonists of the $P2Y_{14}$ receptor: Multivalent effect in G protein-coupled receptor recognition. Bioconjugate Chem 2009;20:1650–1659.
20. Tosh DK, et al. Polyamidoamine (PAMAM) dendrimer conjugates of “clickable” agonists of the A_3 adenosine receptor and coactivation of the $P2Y_{14}$ receptor by a tethered nucleotide. Bioconjugate Chem 2010;21:372–384.
21. Keene AM, et al. Multivalent dendrimeric and monomeric adenosine agonists attenuate cell death in HL-1 mouse cardiomyocytes expressing the A_3 adenosine receptor. Biochem. Pharmacol 2010;80:188–196. [PubMed: 20346920]
22. Bi X, et al. Synthesis, characterization and stability of a luteinizing hormone-releasing hormone (LHRH)-functionalized poly(amidoamine) dendrimer conjugate. J. Biomater Sci. Polym. Ed 2008;19:131–142. [PubMed: 18177559]
23. Jacobson KA. Functionalized congener approach to the design of ligands for G protein–coupled receptors (GPCRs). Bioconjugate Chem 2009;20:1816–1835.
24. Peer D, et al. Nanocarriers as an emerging platform for cancer therapy. Nat. Nanotechnol 2007;2:751–760. [PubMed: 18654426]
25. Juillerat-Jeanneret L. The targeted delivery of cancer drugs across the blood-brain barrier: chemical modifications of drugs or drug-nanoparticles? Drug Disc. Today 2008;13:1099–1106.
26. Ferré S, et al. Building a new conceptual framework for receptor heteromers. Nat. Chem. Biol 2009;5:131–134. [PubMed: 19219011]
27. Han Y, et al. Allosteric communication between protomers of dopamine class A GPCR dimers modulates activation. Nature Chem. Biol 2009;5:688–695. [PubMed: 19648932]
28. de Castro S, et al. Functionalized congeners of $P2Y_1$ receptor antagonists: 2-Alkynyl (N)-methanocarba 2’-deoxyadenosine 3’,5’-bisphosphate analogues and conjugation to a polyamidoamine (PAMAM) dendrimer carrier. Bioconjugate Chem 2010;21:1190–1205.
29. Fishman P, et al. Adenosine receptors and cancer. Handb. Exp. Pharmacol 2009;193:399–441. [PubMed: 19639290]

30. Kolb HC, Sharpless KB. The growing impact of click chemistry on drug discovery. Drug Discov. Today 2003;8:1128–1137. [PubMed: 14678739]

31. Gao ZG, et al. UDP-glucose acting at $P2Y_{14}$ receptors is a mediator of mast cell degranulation. Biochem. Pharmacol 2010;79:873–879. [PubMed: 19896471]

32. Liu Z, et al. Design of multivalent ligand targeting G-protein-coupled receptors. Curr. Pharm. Des 2009;15:682–718. [PubMed: 19199990]

33. Ivanov AA, Jacobson KA. Molecular modeling of a PAMAM-CGS21680 dendrimer bound to an A_{2A} adenosine receptor homodimer. Bioorg. Med. Chem. Lett 2008;18:4312–4315. [PubMed: 18639453]

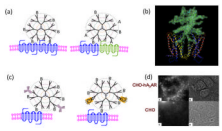

Figure 1.
Rationale for exploring GPCR Ligand-Dendrimer (GLiDe) conjugates. (a) Bridging of two adjacent protomers in homo- or heterodimeric GPCR aggregates. A single strategically tethered ligand B might span two binding sites. By incorporating multiple ligands A and B, a preference for the heteromer may be anticipated. Thus, there may be selective binding in tissues expressing combinations of GPCRs. (b) Molecular model of a GLiDe conjugate (e.g. having A_{2A}AR agonist properties in Kim *et al.* [15]) bridging the two protomers of a homodimeric A_{2A}AR [reprinted from ref. [33] with permission from Elsevier]. (c) Prosthetic groups may be incorporated for targeting (e.g. an antibody) or therapeutics or as reporter groups for diagnostics (e.g. a fluorescent or luminescent group) without loss of receptor binding. (d) Fluorescent microscopic image of A_3AR expressed in CHO cells [18]. The left panels are fluorescent images, and right panels are light microscopic images of the same fields; upper panels – in cells expressing the human A_3AR, lower – untransfected cells.

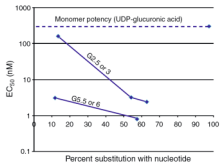

Figure 2.

Nucleotide dendrimer agonists of the $P2Y_{14}$ receptor: Dependence of potency on the generation of the PAMAM dendrimer carrier and its percent substitution [19]. G2.5 and G5.5 PAMAM dendrimers have a total of 64 and 256 terminal carboxylic acid groups prior to ligand coupling. G3 and G6 dendrimers have a total of 64 and 256 terminal primary amino groups prior to ligand coupling.

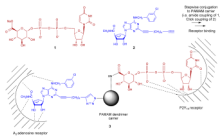

Figure 3.

A nucleoside/nucleotide conjugate of a PAMAM G4 dendrimer, which functions as a dual agonist of the $P2Y_{14}$ receptor and the A_3AR [20]. The synthetic sequence required the preparation of the bifunctional GLiDe conjugate (**c**). First, the nucleotide UDP-glucuronic acid (**a**) was linked to peripheral amino groups on the dendrimer by amide bond formation, followed by conjugating the nucleoside (**b**) to peripheral azido groups by facile click chemistry [30]. The latter step involved cyclization of a terminal acetylene moiety on the prefunctionalized nucleoside (b) with a terminal azido group on the dendrimer in the presence of cuprous sulfate to form a stable triazole ring. The alkyne group that is adjacent to the adenine ring in **(b)** maintains the A_3AR affinity, but does not participate in the click reaction. The stoichiometry of substitution was on average ~31 nucleotide moieties and ~33 adenosine moieties per 64 possible terminal positions. The bifunctional conjugate (c) potently activated the human A_3AR expressed in CHO cells and the human $P2Y_{14}$ receptor expressed in HEK-293 cells.

Table 1

Examples of GLiDe conjugates for adenosine and P2Y receptors.

Receptor(s) (ligand linkage and PAMAM generation)	Cell type	Effect	Potency (nM)[a]	Ref.
A_{2A} agonist (amide, G3)	Human platelet HEK-293 cells	Antithrombotic	151	15,17
		cAMP generation	11.2, 151[b]	
A_{2A} agonist (thiourea, G3)	HEK293 cells	cAMP generation	96–152[b]	16
A_3 agonist (amide, G2.5)	Cardiomyocytes	Antiapoptotic protection	35	21
A_3 agonist (triazole, G4)	CHO cells	Inhibition of cAMP	0.14[b]	20
$P2Y_1$ antagonist (triazole, G4)	Human platelet	Antithrombotic	1000–4000[c]	28
$P2Y_{14}$ agonist (amide, G6)	HEK293 cells	Inhibition of cAMP	0.8	19
$P2Y_{14}$ agonist (amide, G4) + A_3 agonist (triazole)[d]	HEK293 and CHO cells	Inhibition of cAMP	2.24 ($P2Y_{14}$), 1.36 (A_3), 39.5[b] (A_3)	20

[a] Calculated on the basis of the concentration of the conjugate.

[b] Binding affinity at human AR.

[c] At 4 μM inhibited 94% of platelet aggregation induced by 5 μM ADP.

[d] Structures of the terminal groups of this bifunctional conjugate are shown in Figure 3.