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Synthesis and SAR of 2′,3′-bis-*O*-substituted N^6, 5′-bis-ureidoadenosine derivatives: Implications for prodrug delivery and mechanism of action

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ABSTRACT

A series of 2′,3′-bis-*O*-silylated or -acylated derivatives of lead compound **3a** (2′,3′-bis-*O*-*tert*-butyldimethylsilyl-5′-deoxy-5′-(*N*-methylcarbamoyl)amino-N^6-(*N*-phenylcarbamoyl)adenosine) were prepared and evaluated for antiproliferative activity against a panel of murine and human cancer cell lines (L1210, FM3A, CEM, and HeLa). 2′,3′-*O*-Silyl groups investigated included triethylsilyl (**10a**), *tert*-butyldiphenylsilyl (**10b**), and triisopropylsilyl (**10c**). 2′,3′-*O*-Acyl groups investigated included acetyl (**13a**), benzoyl (**13b**), isobutyryl (**13c**), butanoyl (**13d**), pivaloyl (**13e**), hexanoyl (**13f**), octanoyl (**13g**), decanoyl (**13h**), and hexadecanoyl (**13i**). IC_{50} values ranged from 3.0 ± 0.3 to >200 μg/mL, with no improvement relative to lead compound **3a**. Derivative **10a** was approximately equipotent to **3a**, while compounds **13e–g** were from three to fivefold less potent, and all other compounds were significantly much less active. A desilylated derivative (5′-deoxy-5′-(*N*-methylcarbamoyl)amino-N^6-(*N*-phenylcarbamoyl)adenosine; **5**) and several representative derivatives from each subgroup (**10a–10c**, **13a–13c**) were screened for binding affinity for bone morphogenetic protein receptor **1b** (BMPR1b). Only compound **5** showed appreciable affinity (K_d = 11.7 ± 0.5 μM), consistent with the inference that **3a** may act as a prodrug depot form of the biologically active derivative **5**. Docking studies (Surflex Dock, Sybyl X 1.3) for compounds **3a** and **5** support this conclusion.

As part of research directed toward the design, synthesis, and biological evaluation of potential inhibitors of HIV integrase, we discovered potent antiproliferative activities associated with a new class of N^6,5′-bis-ureidoadenosine derivatives exemplified by compounds **1–3** (Fig. 1).[1] IC_{50} values for **1–3a** (R = Ph) ranged from approximately 1–8 μM against a majority of the human cancer cell lines in the NCI-60. IC_{50} values for **3b–i** ranged from 3–182 μg/mL against a panel of tumor cell lines consisting of murine leukemia (L1210), murine mammary carcinoma (FM3A), human T-lymphocyte (CEM), and human cervix carcinoma (HeLa). Preliminary SAR studies revealed that for optimal cytostatic activities (low μM), the N^6- and 5′-urea moieties are required, and substitution with at least one 2′(3′) *tert*-butyldimethylsilyl (TBS) group is also necessary. Interestingly, compounds **5** and **6** were essentially inactive against the NCI-60 screen at 10 μM concentrations. Similarly, 5′-carbamates **4a–i** were significantly less active than the analogous 5′-ureas (**3a–i**) against L1210, FM3A, CEM, and HeLa—in spite of the fact that **4a–i** possess nearly identical substitutions as the 5′-ureas.[1a]

The above observations support the conclusion that the 2′,3′-*O*-TBS groups are necessary, but not sufficient, for biological activity and have prompted us to investigate the role of the 2′,3′-*O*-substitution in this class of compounds. Herein we report the synthesis and antiproliferative activities for a series of variously substituted 2′,3′-*O*-derivatives of the most potent of these compounds (**3a**), and draw preliminary conclusions from the mechanistic implications of this SAR study.

The synthesis begins with 5′-azido-5′-deoxyadenosine (**7**) and gives 2′,3′-bis-*O*-silylated or 2′,3′-bis-*O*-acylated products in good to excellent yields (Scheme 1). The synthesis is very straightforward and is amenable to scale-up. Silylation of **7** with triethylsilylchloride, *tert*-butyldiphenylsilylchloride, or triisopropylsilylchloride gave compounds **8a–c** in 42–60% yield. Acylation of compounds **8a–c** with phenylisocyanate gave N^6-phenylurea derivatives **9a–c** (54–82%). A one-pot, two-step reaction sequence involving reduction of the 5′-azido group of compounds **9a–c** followed by acylation with the relatively safe and innocuous methylisocyanate surrogate, *N*-methyl *p*-nitrophenylcarbamate,[2] gave **10a–c** in 66–77% yield. 2′,3′-Bis-*O*-acylated compounds **13a–c** and **13d–i** were obtained via two different routes. Compounds **13a–c** were obtained in good yields via a five-step protocol analogous to the one employed in preparing **10a–c**. However, the more lipophilic 2′,3′-bis-*O*-acylated compounds **13g–i** were obtained in very low yields following this procedure. An alternative route involving one step from compound **5** was investigated. This route was generally much more efficient,

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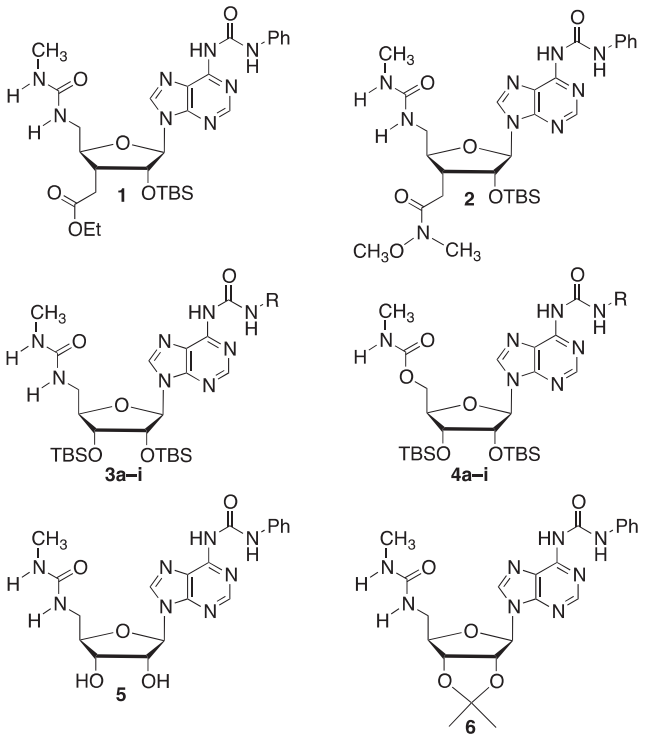


Figure 1.

and yields for **13d–i** ranged from 46–63% (the highest yield for **13e** was 26%, even with this more efficient method, presumably due to the steric bulk of the pivaloyl esters). As a point of comparison, only trace amounts of **13i** were obtained when the five-step sequence—steps e, f, b, c, and d—was attempted. Finally, compounds **14a–c** were obtained in moderate to good yields (31–66%) by treating **11a–c** with the aforementioned one-pot, two-step reduction/acylation (steps c and d). The antiproliferative activities for compounds **3a**, **4a**, **10a–c**, **13a–i**, and **14a–c** are shown in Table 1. Interestingly, the IC_{50} values for 2′,3′-bis-*O*-triethylsilyl derivative **10a** were very similar to those for the 2′,3′-bis-*O*-TBS derivative **3a**. In contrast, IC_{50} values for 2′,3′-bis-*O*-*tert*-butyldiphenylsilyl and/or 2′,3′-bis-*O*-triisopropylsilyl derivatives (**10b** and **10c**, respectively), were significantly inferior to **3a**. Acyl derivatives **13a–i** were generally much less active than **3a**, especially the *O*-benzoyl, *O*-decanoyl, and *O*-hexadecanoyl derivatives (**13b**, **13h**, and **13i**, respectively). The *O*-pivaloyl, *O*-hexanoyl, and *O*-octanoyl derivatives (**13e**, **13f**, and **13g**, respectively) exhibited nearly equivalent antiproliferative activities, but IC_{50} values for these compounds were from three to fivefold higher than those for compound **3a**. Compounds **14a–c** (each of which lacks the N^6-phenylurea) showed generally lower antiproliferative activity than their corresponding N^6-substituted analogues (**13a–c**).

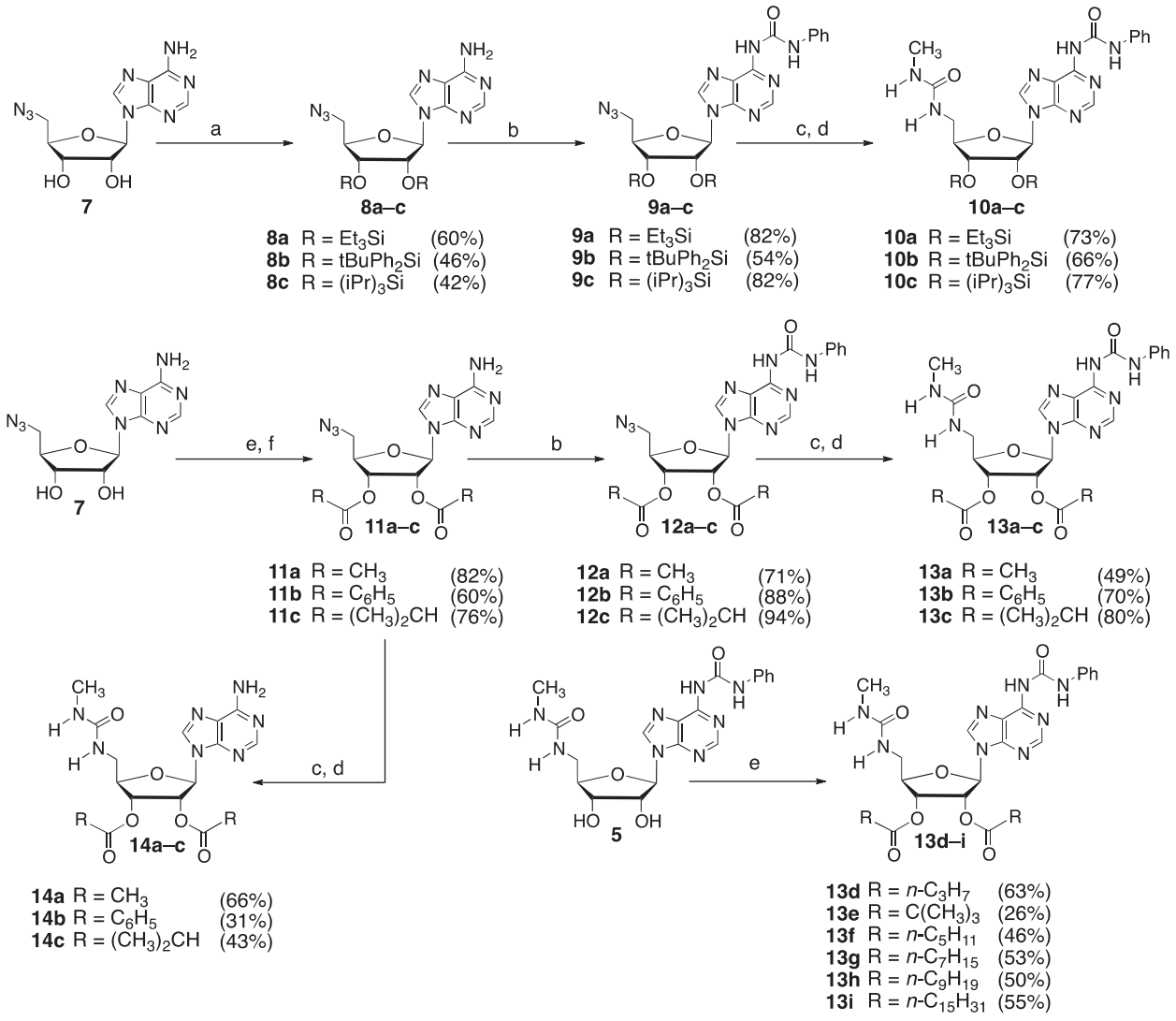
Recently, we demonstrated that compound **5** (Fig. 1) binds to the ATP-binding site of bone morphogenetic protein receptor 1b (BMPR1b) with low μM affinity ($K_d = 11.7 \pm 0.5$ μM).[1a] When screened against a panel of 441 protein kinases, compound **5** exhibited its greatest activity against BMPR1b, inhibiting binding of BMPR1b to an ATP-binding site ligand by approx. 50% at 10 μM concentration. Compound **3a**, in contrast, did not bind to BMPR1b at concentrations as high as 30 μM.[1a] BMPR1b is a transmembrane receptor with serine/threonine protein kinase activity. The ATP-binding domain lies within the cytoplasm and phosphorylates downstream targets (SMADs 1, 5, and 8), which in turn regulate expression of inhibitor of differentiation gene 1 (Id1).[3] Overexpression of Id1 has been reported in a number of cancers, including lung,[4] breast,[5] colon,[6] ovarian,[7] pancreas,[8] prostate,[9] and renal cancers.[10] Downregulation, inhibition, and/or inactivation of Id1 have been shown to induce apoptosis in several of these cancers.[11] Inhibition of BMPR1b by the desilylated analogue of **3a**, compound **5**, could constitute a plausible mechanism for the broad-spectrum antiproliferative activity exhibited by compound **3a**.[12] In this context, compound **3a** would most likely serve as a prodrug form of the active species, desilylated derivative compound **5**.

A commonly used strategy for enhancing membrane permeability of nucleosides has been to increase the lipophilicity by protecting hydroxyls as acetyl, benzoyl, or isobutyryl esters that are cleaved once the compound has crossed the cell membrane.[13] TBS-protection has been shown to enhance the activities of a number of antiproliferative compounds, and activities of several of these compounds have been positively correlated with the increased lipophilicity of the biologically active derivative.[14] TBS-protected cytidine has been shown to facilitate transport of guanosine 5′-monophosphate through a model membrane (in conjunction with a lipophilic phosphonium ion co-carrier),[15] and silylated nucleosides have been shown to penetrate the blood–brain barrier where it is presumed they are desilylated to generate the active species.[16] The lipophilic 2′,3′-bis-*O*-TBS groups could enhance membrane permeability of compound **3a** and serve as a prodrug depot form of the active derivative compound **5**.

Docking studies performed using the Surflex docking program (Sybyl X 1.3) are supportive of such an interpretation.[17] As illustrated in Fig. 2, the highest ranked pose for compound **5** is oriented within the ATP binding cleft of BMPR1b (pdb 3mdy) with the 5′-urea undergoing hydrogen bonding interactions with the highly conserved catalytic triad[18] (Lys 231, Glu 244, Asp 350; Fig. 2). The N^6-phenyl urea moiety in this pose is oriented toward the solvent accessible surface, which is consistent with the relative lack of sensitivity of the antiproliferative activity of **3a–i** to the substitution pattern in the N^6-urea moiety.[1a] In contrast, the top ranked pose for compound **3a** had nearly the opposite orientation to compound **5**, with the N^6-phenyl urea moiety undergoing nonpolar binding interactions with the 'gatekeeper' residue (Leu 277; blue residue; Fig. 2) near the end of the catalytic cleft, in close proximity to the catalytic triad. In this pose, the very hydrophobic 2′,3′-bis-*O*-TBS groups are exposed to the solvent accessible surface. If such a pose were biologically relevant, substitution at the N^6-urea position would be expected to have a much greater effect on the biological activity than the negligible effect that was observed experimentally. (The nature of the R group in **3a–i** had very little impact on their antiproliferative activities).[1a] Furthermore, the hydrophobic effect resulting from protrusion of the very nonpolar TBS groups into the aqueous environment would contribute to an unfavorable entropic term in the overall free energy of binding.

Consistent with these modeling results is the aforementioned observation that compound **5** binds to BMPR1b with $K_d = 11.7 \pm 0.5$ μM), while compound **3a** did not bind at concentrations as high as 30 μM (Fig. 3A and 3B, respectively).[1a] The negative impact of the 2′,3′-*O*-substitution on binding was also illustrated for several representative members of the presently discussed series of 2′,3′-*O*-derivatives of **3a**, none of which showed appreciable binding to BMPR1b in a competitive inhibition of binding experiment[19] at 10 μM concentrations (Fig. 3C). The relative reactivity of silyl protecting groups toward hydrolysis (TES > TBS ≫ TIPS > TBDPS)[20] is in harmony with these results, and is consistent with a mechanism involving cleavage of the silyl moiety before the nucleoside derivative can interact with its primary biological receptor.[21]

In conclusion, we have developed efficient methods for the preparation of a variety of 2′,3′-*O*-substituted derivatives of our



Scheme 1. Reagents: (a) R_3SiCl, imidazole, DMF; (b) PhN=C=O; (c) H_2, Pd–C; (d) p-NO_2-$C_6H_4O_2CNHCH_3$; (e) $(RC{=}O)_2O$; (f) CH_3OH, Δ.

Table 1
Inhibitory effects of the test compounds on the proliferation of murine leukemia cells (L1210), murine mammary carcinoma cells (FM3A), human T-lymphocyte cells (CEM) and human cervix carcinoma cells (HeLa)

Compound	IC_{50}[a] (μg/ml)			
	L1210	FM3A	CEM	HeLa
3a	3.8 ± 0.3	5.9 ± 1.1	8.3 ± 2.9	3.2 ± 0.2
4a	160 ± 56	>200	>200	⩾200
10a	3.8 ± 0.1	3.0 ± 0.3	4.2 ± 0.2	3.7 ± 0.4
10b	>200	>200	⩾200	104 ± 71
10c	>200	>200	142 ± 81	⩾200
13a	97 ± 17	150 ± 39	107 ± 8	>200
13b	154 ± 30	61 ± 2	>200	>200
13c	29 ± 4	44 ± 4	28 ± 0	73 ± 13
13d	20 ± 2	18 ± 1	29	58 ± 25
13e	9.7 ± 3.5	15 ± 1	20	17 ± 1
13f	9.5 ± 0.3	20 ± 1	10 ± 2	15 ± 5
13g	11 ± 0	32 ± 1	12 ± 4	16 ± 9
13h	>100	140 ± 16	>100	>100
13i	>100	>200	>100	>100
14a	112 ± 31	>200	>200	>200
14b	16 ± 1	36 ± 3	19 ± 8	40 ± 7
14c	87 ± 1	107 ± 13	88 ± 33	99 ± 14

[a] 50% Inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%.

recently discovered antiproliferative N^6,5′-bis-ureidoadenosine compounds. Bis-*O*-protection of 5′-azido-5′-deoxyadenosine with either silyl or acyl protecting groups, followed by sequential acylation of the N^6 and 5′-amino groups (with phenylisocyanate or *N*-methyl *p*-nitrophenylcarbamate, respectively) gave 2′,3′-*O*-substituted derivatives of lead compound **3a** (**10a**–**c** and **13a**–**c**) in good to excellent yields. An alternative route from the more advanced intermediate compound **5** gave **13d**–**i** more efficiently than the route applied for **13a**–**c**. Screening of compounds **10a**–**c**, **13a**–**i**, and **14a**–**c** against a panel of murine and human cancer cell lines did not reveal any improved activity relative to lead compound **3a**. Several representative 2′,3′-*O*-substituted derivatives were shown to lack binding affinity for BMPR1b at concentrations near the K_d for desilylated analogue **5**. Taken together, these results suggest that the role of the TBS group in compound **3a** may be to facilitate membrane permeability. Cleavage of the TBS groups within the cytoplasm could give rise to the active derivative (**5**) which previously published screening data[1a] suggest may target BMPR1b as its primary biomolecular target. BMPR1b is part of the BMP-signaling pathway that regulates expression of Id1. Overexpression of Id1 has been reported in numerous cancers.[4–10] Inhibition of the BMP-signaling cascade by desilylated derivative **5** may account for the broad-spectrum activity of compound **3a**.

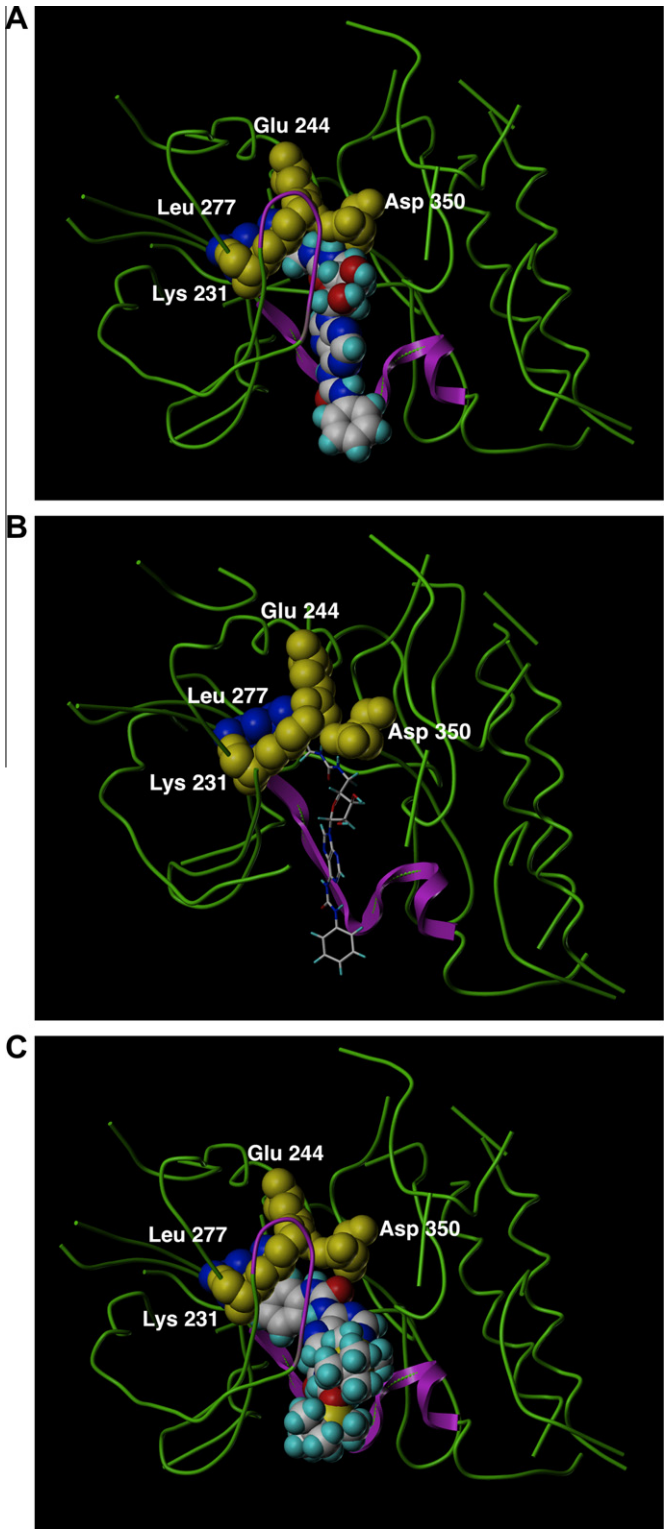


Figure 2. Docking results for **3a** and **5** docked into the active site of BMPR1b (pdb 3mdy). Yellow residues: catalytic triad (K231, E244, D350); blue residue: gatekeeper (L277); magenta tube: G-loop or activation loop (I210, G211, K212, G213, R214, Y215, G216); magenta ribbon: hinge region (I278, T279, D280, Y281, H282, E283, N284, G285, S286).[18] (A) Space-filling model of highest ranked pose of compound **5**. (B) Tube model of highest ranked pose of compound **5** (G-Loop omitted for clarity). (C) Space-filling model of highest ranked pose of compound **3a**.

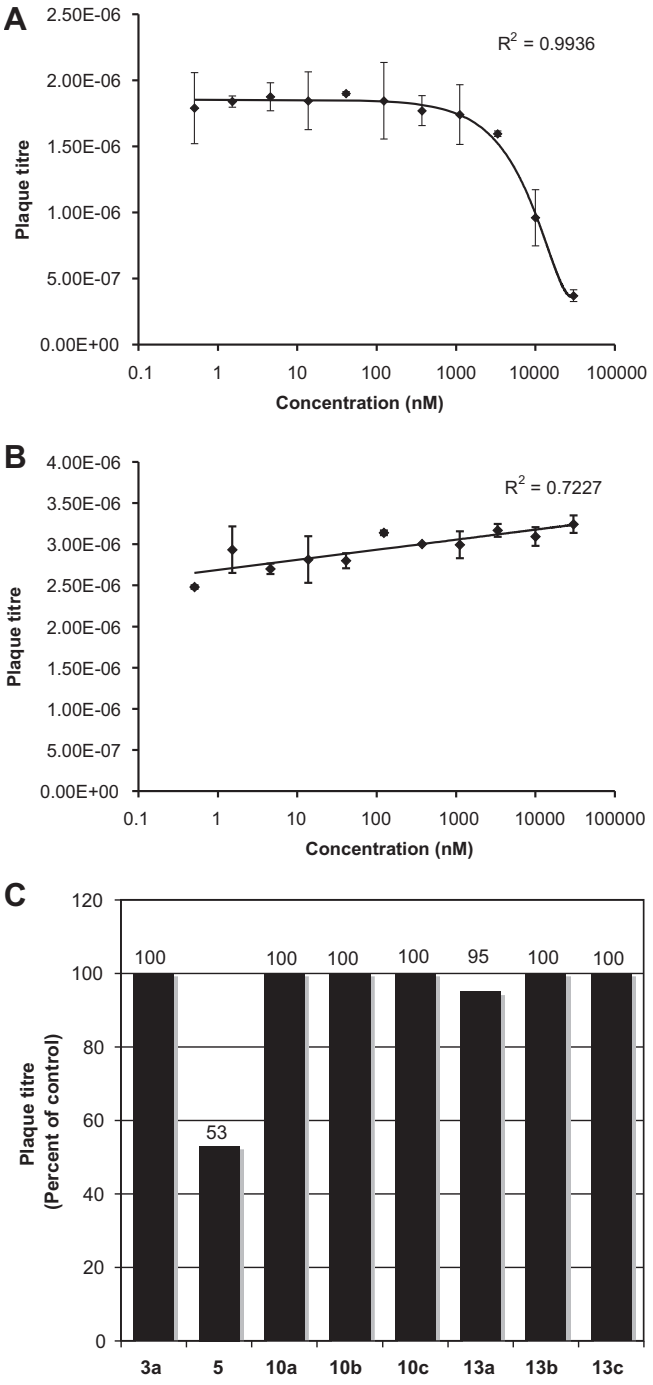


Figure 3. Competitive binding inhibition assays.[19] Effects of compounds on equilibrium competition binding of BMPR1b to immobilized ATP-binding site ligand. (A) Compound **5**. (B) Compound **3a**. (C) Compounds **3a**, **5**, **10a–c**, and **13a–c** at 10 μM (data expressed as percent of control).

We are currently designing 5′-analogues that may more fully exploit interactions with the catalytic triad (Lys 231, Glu 244, Asp 350) and gatekeeper residues (Leu 277), which may lead to enhanced binding, as indicated by the docking study, and thus, increased antiproliferative activity.

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Supplementary data

Supplementary data (experimental procedures and NMR data for all new for compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.050.

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