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Synthesis of C-6 substituted pyrazolo[1,5-*a*]pyridines with potent activity against herpesviruses

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Abstract—A novel series of potent C-6 substituted pyrazolo[1,5-*a*]pyridine inhibitors of herpes simplex viruses has been identified. A synthetic methodology was developed involving functionalization of a C-6 trifluoromethyl pyrazolo[1,5-*a*]pyridine to allow facile access to a diverse set of analogues from common late stage intermediates. The expansion of the SAR of this series at the 6 position allows for modifications to developability parameters such as clog *P*, while maintaining potency comparable to acyclovir.

1. Introduction

Herpesviruses are a large family of viruses that infect most mammalian species.[1] Of particular concern to humans are herpes simplex virus 1 (HSV-1) which causes cold sores and herpes simplex virus 2 (HSV-2) which causes genital infections. The current gold standard therapy based on acyclovir (**1**) and valacyclovir is both safe and efficacious; however, there is significant interest in optimizing treatment for HSV in regard to lesion pain relief, time to healing, viral shedding, and reduction of episodes of reactivation.[2]

Our group recently reported a pyrazolo[1,5-*a*]pyridine scaffold, as exemplified by GW 3733 (**2**), that showed promising antiherpetic activity (Fig. 1).[3,4]

The pyrazolo[1,5-*a*]pyridines of this class were shown not to be DNA synthesis inhibitors and thus have a mechanism of action different from currently marketed polymerase inhibitors. This prompted the directed chemistry effort around this core to identify a potent and safe agent with drug-like properties.

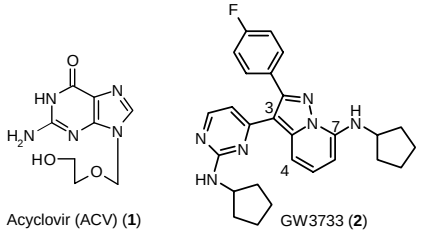

Figure 1.

Although original antiviral activity indicated potency for compounds containing non-polar amines at the C-7 position,[4] it remained unclear whether this was a regiospecific requirement. It was therefore desired to explore the SAR attributes of a series of C-6 substituted pyrazolo[1,5-*a*]pyridines. To accomplish this, a strategy was developed using a previously reported C-6 trifluoromethyl intermediate[5] which would allow synthetic access to both C-6 monosubstituted and C-6/C-7 disubstituted products (Fig. 2).

Keywords: Pyrazolo[1,5-*a*]pyridines; Alkynyl ketone; Pyrimidine synthesis; HSV.
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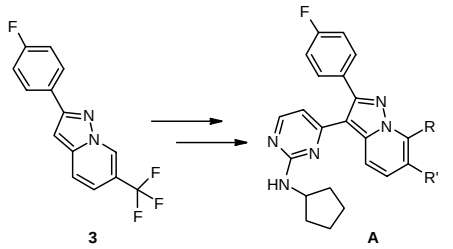


Figure 2. Retrosynthetic scheme.

2. Chemistry

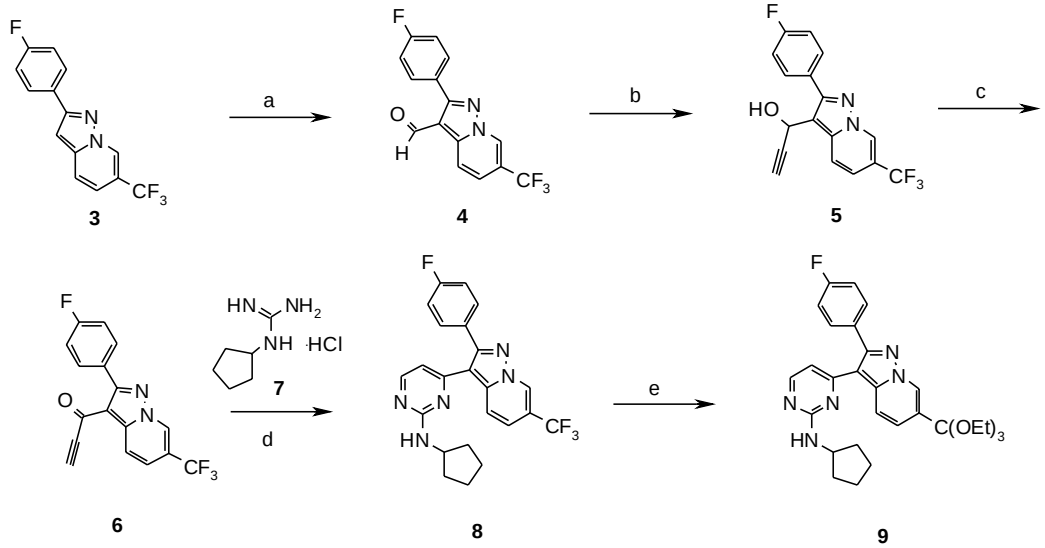
Compounds **4**–**22** were synthesized as is outlined in Schemes 1–3. The previously described pyrazolo[1,5-*a*]pyridine **3**[5] was chosen as a starting material as the 6-trifluoromethyl moiety was known to be labile to alkanolysis and provided a synthetic handle at the 6-position. Vilsmeier–Haack formylation provided the C-3 aldehyde **4** in excellent yield. Grignard addition followed by MnO_2 oxidation of the resulting propargyl alcohol gave the alkynyl ketone **6**. Cyclization with the cyclopentyl guanidine **7**[6] under basic conditions provided clean conversion to the pyrimidine **8**. The choice of the alkynyl ketone as the substrate for cyclization was based on a synthetic methodology developed for similar molecules.[3] Ethanolysis of the triflouromethyl group provided the ortho ester **9** (Scheme 1).[5]

The ortho ester **9** provided a straightforward route to a diverse set of C-6 substituted pyrazolo[1,5-*a*]pyridines. Acid catalyzed hydrolysis provided ethyl ester **10**, which was cleanly saponified to carboxylic acid **11**. Curtius rearrangement followed by deprotection of the resulting *tert*-butylcarbamate provided the amine **13**. Standard reductive amination conditions provided the secondary amines **14** and **15**. The ethyl ester **10** proved to be a versatile intermediate. DIBAL-H reduction of **10** provided 6-hydroxymethyl pyrazolo[1,5-*a*]pyridine **16**, which was converted to the corresponding bromomethyl compound **17**. Tin hydride reduction provided the 6-methyl pyrazolo[1,5-*a*]pyridine **18**. Bromide **17** also provided a route to the *N*-cyclopentylaminomethyl derivative **19** (Scheme 2).

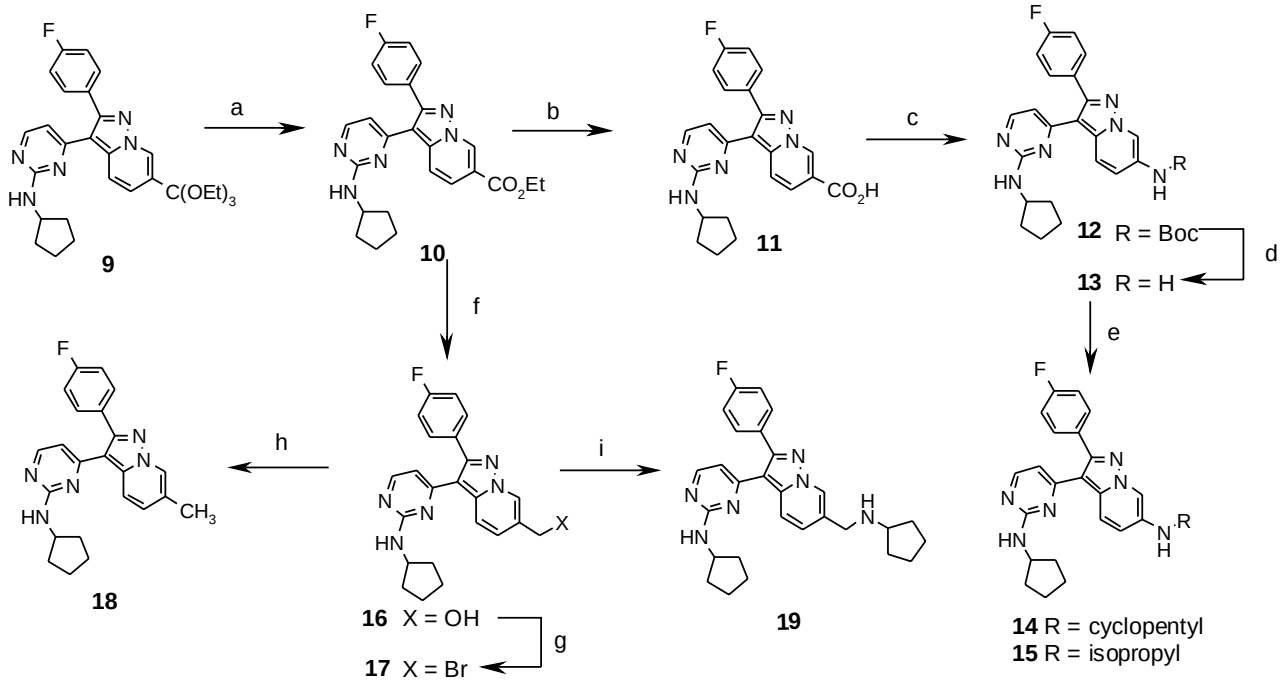
The C-6 orthoester **9** provided a precursor for C-6/C-7 disubstituted products. Lithiation of the 7-position of the pyrazolo[1,5-*a*]pyridine ring system,[7] followed by quenching with CCl_4[4], provided the 7-chloro species **20** (Scheme 3). Thermal displacement of the chloride in neat cyclopentylamine provided **21** in excellent yield. Hydrolysis of the orthoester, followed by saponification, provided carboxylic acid **23**. The C-6 acid allowed for facile access to a series of amido derivatives **24**–**29** via an in situ generated acid chloride intermediate. The ester **22** could also be reduced with DIBAL-H to provide the 7-amino-6-hydroxymethyl derivative **30**. Additionally, the orthoester group in **20** could be hydrolyzed to provide ester **31**, containing the useful 7-chloro substituent. Interestingly, very few examples of Negishi-type dialkylzinc couplings exist in the literature.[8] However, treatment of **31** with Me_2Zn and $Pd(Ph_3P)_4$ provided the desired 7-methyl pyrazolo[1,5-*a*]pyridine **32** in modest yield. Saponification and standard amide formation provided **34** (Scheme 3).

3. Results and discussion

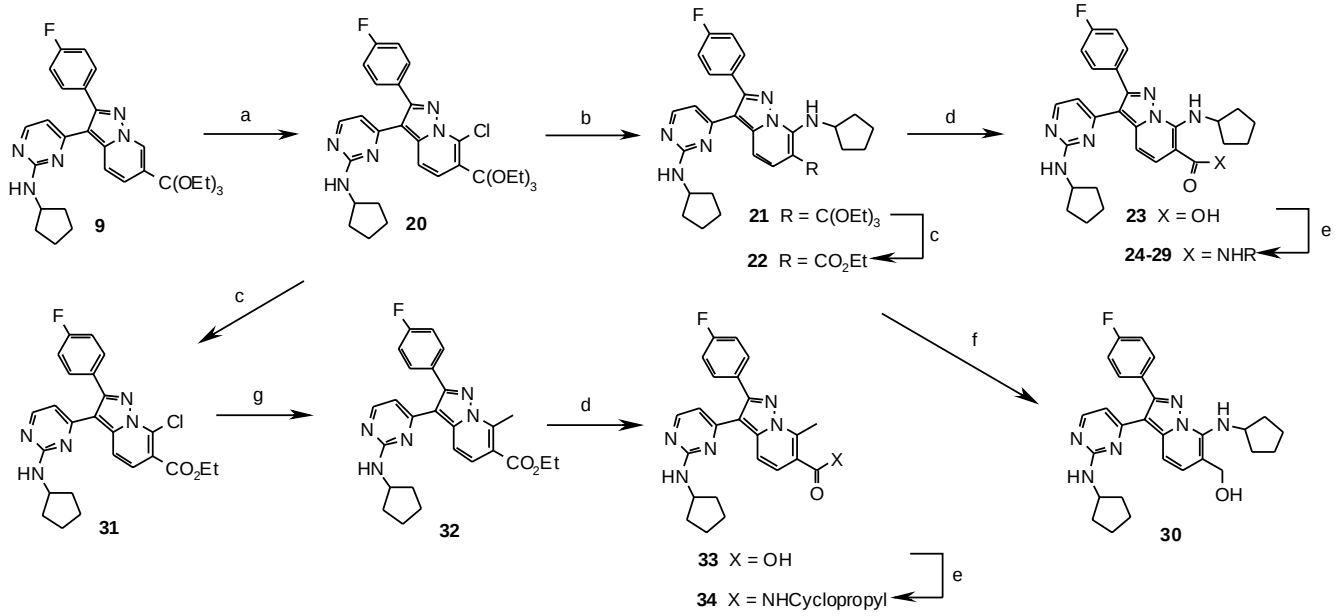
Our initial interest was to gauge the effects of moving the cyclopentylamino group from the 7- to the 6-position. As can be seen in Table 1, the 6-cyclopentylamino analogue **14** exhibited a activity and cytotoxicity profile similar to those of GW 3733 (**2**). Evaluation of additional small polar and non-polar groups indicated that substitution at this position was well tolerated. Interestingly, the methylene spaced cyclopentylamine (entry 6) showed greater cytotoxicity and somewhat less antiviral activity than related compounds in this series. The two possible



Scheme 1. Reagents and conditions: (a) $POCl_3$, DMF, 3 days, 94%; (b) ethynylmagnesium bromide, THF, −78 °C, 96%; (c) MnO_2, $CHCl_3$, 0 °C, 69%; (d) NaOEt, EtOH, 98%; (e) NaOEt, EtOH, 60 °C, 92%.



Scheme 2. Reagents and conditions: (a) pTsOH, H_2O/acetone, 85%; (b) aq LiOH, dioxane, 92%; (c) diphenylphosphoryl azide, Et_3N, *t*-BuOH, 54%; (d) 4 N HCl in dioxane, 27%; (e) alkylamine, HOAc, $NaBH(OAc)_3$, 1,2 DCE; (f) DIBAL-H, CH_2Cl_2, −78 °C, 40%; (g) PBr_3, $CHCl_3$, 43%; (h) Bu_3SnH, AIBN, toluene, 95 °C, 12%; (i) cyclopentylamine, THF, 20%.

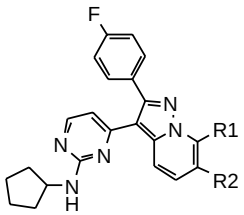


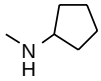
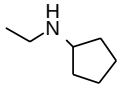
Scheme 3. Reagents and conditions: (a) LDA, CCl_4, THF, −78 °C, 46%; (b) cyclopentylamine, 80 °C, 96%; (c) *p*-TsOH, H_2O/acetone; (d) aq LiOH, dioxane; (e) $SOCl_2$, alkylamine, CH_2Cl_2; (f) DIBAL-H, CH_2Cl_2, −78 °C, 59%; (g) Me_2Zn, $(PPh_3)_4Pd$, THF, 60 °C, 45%.

factors for this cytotoxicity, the increased flexibility and increased basicity of the amine, were not further investigated. As this was the only methylene spaced alkylamino compound synthesized, it remains unclear whether this is a significant SAR trend.

Having established that activity could be retained in 6-substituted analogues, we wished to evaluate C-6/C-7 disubstituted analogues. As can be seen in Table 2, certain polar groups were tolerated at the 6 position (entries 2 and 3). Clearly, there was significant cytotoxicity imparted by the introduction of a 6-amido group (entries 5–10) and in these cases the antiviral activity was not well established from the overlying toxicity. As stated earlier, one of the investigational goals of C-6 substitution was to identify potent compounds with lower clog *P*'s than GW3733 (clog *P* = 6.7), thus imparting a more desirable developability profile. Compounds

Table 1. HSV-1 antiviral activity (Vero cells, SC-16 strain) and cytotoxicity



Entry	Compound	R1	R2	EC_{50} (μM)	CC_{50} (μM)	clog *P*
1	ACV	—	—	0.39	>100	—
2	**2**		H	0.26	>160	6.7
3	**14**	H		0.74 (0.21)	>40	6.7
4	**15**	H		2.09 (0.19)	>40	5.9
5	**13**	H		0.71 (0.08)	>40	4.3
6	**19**	H		4.8	5.8	6.0
7	**18**	H		2.0 (0.51)	>40	5.6
8	**11**	H		0.55 (0.07)	>40	5.1
9	**16**	H		1.8 (0.51)	>40	4.6

Vero cells, SC-16 strain. IC_{50} is the concentration at which 50% efficacy in the antiviral assay is observed.
CC_{50} is the concentration at which 50% cytotoxicity is observed.
Compounds showing overt cytotoxicity were not retested, but all others were retested with $n \geqslant 2$. Standard deviation is shown in brackets. clog *P* values calculated by Daylight© Pomona College and BioByte Inc.

of note include **30** and **34**, which have lower clog *P* values relative to GW3733, while maintaining potency and a large therapeutic index.

4. Conclusions

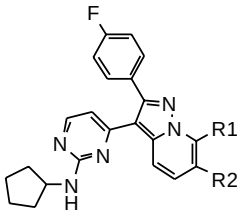
In summary, a series of C-6 substituted and C-6/C-7 disubstituted pyrazolo[1,5-*a*]pyridines with potent antiviral activity has been identified. Synthetic accessibility to the C-6 substituted compounds was provided through a chemically labile C-6 trifluoromethyl group, providing a C-6 ortho ester as a key synthetic intermediate. This intermediate allowed rapid access to a variety of C-6 substituted and C-6/C-7 disubstituted pyrazolo[1,5-*a*]pyridines of varying degrees of size and polarity. Substitution at the 6-position of the monosubstituted compounds was well tolerated with a variety of small functional groups with varying polarity. Disubstitution at the C-6/C-7 positions was also well tolerated, which coupled with the clog *P* lowering effects of the polar substituents, provides a mechanism for improving the drug-like properties of this class of molecules.

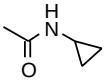
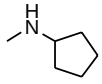
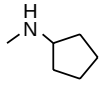
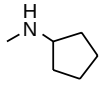
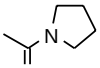
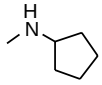
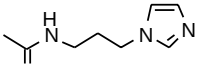
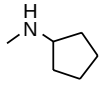
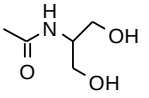
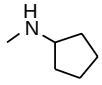
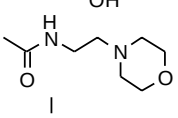
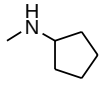
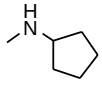
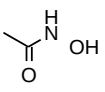
5. Experimental

5.1. General

^{1}H and ^{13}C NMR spectra were obtained on Varian Unity Plus NMR spectrometers at 300 or 400 MHz, and 75 or 100 MHz, respectively. Mass spectra were obtained on Micromass Platform or ZMD mass spectrometers from Micromass Ltd. Altrincham, UK, using either atmospheric chemical ionization (APCI) or electrospray ionization (ESI). Solvents were purchased as anhydrous grade and used without further purification. For TLC, Merck precoated plates (silica gel 60 F_{254}) were used. Unless otherwise stated, column chromatography for the purification of some compounds used Merck Silica gel 60 (230–400 mesh), and the stated solvent system under pressure. All compounds were characterized as their

Table 2. HSV-1 antiviral activity (Vero cells, SC-16 strain) and cytotoxicity



Entry	Compound	R1	R2	EC_{50}(μM)	CC_{50} (μM)	clog *P*
1	**34**			1.77 (0.30)	>40	4.9
2	**23**			0.55 (0.07)	38	7.0
3	**30**			0.75 (0.21)	>40	6.0
4	**22**			6.27 (0.21)	>40	7.8
5	**24**			1.6	11	5.9
6	**25**			0.43	0.63	6.0
7	**26**			4.26	22.4	5.1
8	**27**			5.05	22.1	6.1
9	**28**			2.07	7.2	5.3
10	**29**			1.12	1.1	5.1

Vero cells, SC-16 strain. IC_{50} is the concentration at which 50% efficacy in the antiviral assay is observed.
CC_{50} is the concentration at which 50% cytotoxicity is observed.
Compounds showing overt cytotoxicity were not retested, but all others were retested with $n \geqslant 2$. Standard deviation is shown in brackets. clog *P* values calculated by Daylight © Pomona College and BioByte Inc.

free-base form unless otherwise stated. The corresponding hydrochloride salts were formed to generate solids where noted. In lieu of elemental analysis, compound purity was assessed by high field ^{1}H NMR and HPLC. HPLC was performed using Synergi Max-RP 3.5 μ column, running 15–100% MeOH gradient (0.1% formic acid). Biological results were obtained with compounds of >98% purity as determined by the above methods.

5.2. Synthesis of *N*-cyclopentyl-4-[2-(4-fluorophenyl)-6-(triethoxymethyl)pyrazolo[1,5-*a*]pyridin-3-yl]-2-pyrimidinamine (9)

5.2.1. 2-(4-Fluorophenyl)-6-(trifluoromethyl)pyrazolo[1,5-*a*]pyridine-3-carbaldehyde (4). To a cold (0 °C) solution of phosphorus oxychloride (8.0 mL, 86 mmol) in *N*,*N*-dimethylformamide (160 mL) was added 2-(4-fluorophenyl)-6-(trifluoromethyl)pyrazolo[1,5-*a*]pyridine (**3**) (11.0 g, 39.3 mmol). The reaction mixture was stirred at room temperature for 72 h, and then quenched with ice water. The solid precipitate was collected on a filter to provide **4** (11.4 g, 94%). R_f 0.45 (4:1 hexanes/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.15 (s, 1H), 8.92 (s, 1H), 8.53 (d, J = 9.2 Hz, 1H), 7.80 (m, 2H), 7.70 (d, J = 9.3 Hz, 1H), 7.27 (t, J = 8.5 Hz, 2H).

5.2.2. 1-[2-(4-Fluorophenyl)-6-(trifluoromethyl)pyrazolo[1,5-*a*]pyridin-3-yl]-2-propyn-1-ol (5). To a cold (−78 °C) suspension of 2-(4-fluorophenyl)-6-(trifluoromethyl)pyrazolo[1,5-*a*]pyridine-3-carbaldehyde (**4**) (11.4 g, 37.0 mmol) in tetrahydrofuran (100 mL) was added ethynylmagnesium bromide (111 mL, 0.5 M in tetrahydrofuran, 56 mmol). The reaction mixture was warmed to room temperature and stirred for 14 h. The reaction mixture was

poured into water and adjusted to neutral pH with 1 N aqueous hydrochloric acid. The aqueous mixture was extracted with ethyl acetate. The combined extracts were washed with water and brine. The organic layer was dried over magnesium sulfate. Filtration and concentration provided **5** (11.9 g, 96%) as a tan solid. R_f 0.18 (4:1 hexanes/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.81 (s, 1H), 8.14 (d, J = 9.3 Hz, 1H), 7.76 (m, 2H), 7.34 (dd, J = 9.3, 1.2 Hz, 1 H), 7.19 (app t, J = 8.7 Hz, 2H), 5.76 (m, 1H), 2.70 (d, J = 2.2 Hz, 1H), 2.60 (d, J = 4.0 Hz, 1H); MS m/z 335 (M+1).

5.2.3. 1-[2-(4-Fluorophenyl)-6-(trifluoromethyl)pyrazolo[1,5-*a*]pyridin-3-yl]-2-propyn-1-one (6). To a cold (0 °C) solution of 1-[2-(4-fluorophenyl)-6-(trifluoromethyl)pyrazolo[1,5-*a*]pyridin-3-yl]-2-propyn-1-ol (**5**) (5.00 g, 15.0 mmol) in chloroform (400 mL) was added manganese dioxide (130 g, 1.50 mol). The reaction mixture was stirred at 0 °C for 1.5 h. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated in vacuo to provide **6** (3.44 g, 69%) as a clear oil. R_f 0.39 (4:1 hexanes/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.90 (s, 1H), 8.61 (d, 1H), 7.72–7.69 (m, 3H), 7.17 (m, 2H), 3.06 (s, 1H); MS m/z 333 (M+1).

5.2.4. *N*-Cyclopentyl-4-[2-(4-fluorophenyl)-6-(trifluoromethyl)pyrazolo[1,5-*a*]pyridin-3-yl]-2-pyrimidinamine (8). To a suspension of *N*-cyclopentylguanidine hydrochloride (**7**)[9] (2.20 g, 13.5 mmol) in ethanol (70 mL) was added sodium ethoxide (4.5 mL, 3 M in ethanol, 14 mmol). The mixture was stirred at room temperature for 30 min, and then cooled to 0 °C. To this mixture was added 1-[2-(4-fluorophenyl)-6-(trifluoromethyl)pyrazolo[1,5-*a*]pyridin-3-yl]-2-propyn-1-one (**6**) (3.44 g, 10.4 mmol) portionwise. The reaction mixture was stirred at 0 °C for 30 min, followed by room temperature for 15 h. The reaction mixture was diluted with water (400 mL). The solid precipitate was collected on a filter to provide **8** (4.48 g, 98%) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.83 (s, 1H), 8.50 (d, J = 9.4 Hz, 1H), 8.10 (d, J = 5.1 Hz, 1H), 7.63 (m, 2H), 7.43 (d, J = 9.3 Hz, 1H), 7.16 (app t, J = 8.7 Hz, 2H), 6.33 (d, J = 5.1 Hz, 1H), 5.17 (d, J = 7.2 Hz, 1H), 4.34 (m, 1H), 2.10 (m, 2H), 1.81–1.53 (m, 6H); MS m/z 442 (M+1); mp 155–156 °C.

5.2.5. *N*-Cyclopentyl-4-[2-(4-fluorophenyl)-6-(triethoxymethyl)pyrazolo[1,5-*a*]pyridin-3-yl]-2-pyrimidinamine (9). To a dry round-bottomed flask was added sodium metal (1.9 g, 83 mmol). Ethanol (110 mL) was added and allowed to react with sodium at room temperature until completely dissolved. *N*-Cyclopentyl-4-[2-(4-fluorophenyl)-6-(trifluoromethyl)pyrazolo[1,5-*a*]pyridin-3-yl]-2-pyrimidinamine (**8**) (4.48 g, 10.1 mmol) was added and the reaction mixture was stirred at 60 °C for 18 h. The reaction mixture was cooled and concentrated in vacuo to approximately one-fourth of the original volume. The resulting mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with water and brine, and then dried over magnesium sulfate. Filtration and concentration provided **9** (4.86 g, 92%) as an off-white solid. R_f 0.15 (4:1 hexanes/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.81 (s, 1H), 8.38 (d, J = 9.3 Hz, 1H), 8.06 (d, J = 5.3 Hz, 1H), 7.62 (m, 2H), 7.46 (dd, J = 9.3, 1.1 Hz, 1H), 7.14 (app t, J = 8.7 Hz, 2H), 6.32 (d, J = 5.4 Hz, 1H), 5.14 (d, J = 7.3 Hz, 1H), 4.35 (m, 1H), 3.43 (m, 6H), 2.09 (m, 2H), 1.81–1.51 (m, 6H), 1.21 (m, 9H); MS m/z 520 (M+1).

5.3. 3-[2-(Cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridine-6-carboxylic acid hydrochloride (11)

5.3.1. Ethyl 3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridine-6-carboxylate (10). To a solution of *N*-cyclopentyl-4-[2-(4-fluorophenyl)-6-(triethoxymethyl)pyrazolo[1,5-*a*]pyridin-3-yl]-2-pyrimidinamine (**9**) (1.0 g, 1.9 mmol) in acetone (40 mL) and water (10 mL) was added *p*-toluenesulfonic acid monohydrate (915 mg, 4.81 mmol). The reaction mixture was stirred at room temperature for 2 h. The pH of the reaction mixture was adjusted to slightly basic using saturated aqueous sodium bicarbonate solution. The reaction mixture was concentrated in vacuo to one-third of the original volume and then diluted with water. The precipitate was collected on a filter to provide **10** (722 mg, 85%) as an orange solid. R_f 0.15 (4:1 hexanes/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.22 (s, 1H), 8.39 (d, J = 9.4 Hz, 1H), 8.08 (br, 1H), 7.85 (d, J = 9.4 Hz, 1H), 7.64 (m, 2H), 7.16 (app t, J = 8.6 Hz, 2H), 6.34 (br, 1H), 5.27 (br, 1H), 4.44 (q, J = 7.1 Hz, 2H), 4.35 (br, 1H), 2.10 (m, 2H), 1.82–1.52 (m, 6H), 1.44 (t, J = 7.1 Hz, 3H); MS m/z 446 (M+1).

5.3.2. 3-[2-(Cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridine-6-carboxylic acid hydrochloride (11). To a solution of ethyl 3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridine-6-carboxylate (**10**) (385 mg, 0.864 mmol) in dioxane (9 mL) and water (1 mL) was added lithium hydroxide monohydrate (109 mg, 2.60 mmol). The reaction mixture was heated at 95 °C for 5 h. The reaction mixture was concentrated in vacuo. A suspension of the concentrated residue in water was acidified with 1 N aqueous hydrochloric acid. The solid precipitate was collected on a filter to provide **11** (359 mg, 92%) as an orange solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.27 (s, 1H), 8.72 (br, 1H), 8.51 (br, 1H), 8.12 (br, 1H), 7.96 (br, 1H), 7.66 (m, 2H), 7.36 (app t, J = 8.7 Hz, 2H), 6.40 (br, 1H), 4.18 (br, 1H), 1.95 (br, 2H), 1.71 (br, 2H), 1.56 (br, 4H); MS m/z 418 (M+1).

5.4. 3-[2-(Cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridin-6-amine dihydrochloride (13)

5.4.1. *tert*-Butyl 3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridin-6-ylcarbamate (12). To a suspension of 3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridine-6-carboxylic acid hydrochloride (**11**) (60 mg, 0.13 mmol) in *tert*-butanol were added triethylamine (39 μL, 0.28 mmol) and diphenylphosphoryl azide (34 μL, 0.16 mmol). The reaction mixture was refluxed for 2.5 h. The reaction mixture was cooled to room temperature and diluted with ethyl acetate. The organic layer was washed with 5% aqueous citric acid solution, water, saturated aqueous sodium bicarbonate solution, and brine. The organic

layer was dried over sodium sulfate. Filtration and concentration followed by flash chromatography (39:1 dichloromethane/methanol) provided **12** (35 mg, 54%) as a light green oil. R_f 0.32 (29:1 dichloromethane/methanol); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.94 (br, 1H), 8.34 (d, J = 9.4 Hz, 1H), 8.00 (br, 1H), 7.59 (m, 2H), 7.18 (d, J = 9.4 Hz, 1H), 7.12 (app t, J = 8.7 Hz, 2H), 6.51 (s, 1H), 6.30 (d, J = 5.3 Hz, 1H), 4.33 (m, 1H), 2.07 (m, 2H), 1.79–1.49 (m, 6H), 1.53 (s, 9H); MS m/z 489 (M+1).

5.4.2. 3-[2-(Cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridin-6-amine dihydrochloride (13). To a solution of *tert*-butyl 3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridin-6-ylcarbamate (**12**) (35 mg, 0.072 mmol) in dichloromethane was added hydrogen chloride (144 μL, 4 N in dioxane, 0.58 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with ether and the precipitated solids were collected on a filter to provide **13** (9 mg, 27%) as a brownish yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.23 (br, 1H), 7.96–7.92 (m, 2H), 7.53 (m, 2H), 7.26 (app t, J = 8.9 Hz, 2H), 7.06 (d, J = 9.5 Hz, 1H), 6.15 (br, 1H), 3.97 (br, 1H), 1.85 (m, 2H), 1.66 (m, 2H), 1.48 (m, 4H); MS m/z 389 (M+1).

5.5. *N*-Cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridin-6-amine (14)

To a suspension of 3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridin-6-amine dihydrochloride (**13**) (90 mg, 0.20 mmol) in 1,2 dichloroethane were added cyclopentanone (26 μL, 0.29 mmol), acetic acid (56 μL, 0.98), and sodium triacetoxyborohydride (82 mg, 0.39 mmol). The reaction mixture was stirred at room temperature 16 h and then quenched with water. The resultant mixture was diluted with ethyl acetate and saturated aqueous sodium bicarbonate solution. The organic layer was separated and washed with water and brine. The organic layer was dried over magnesium sulfate. Filtration and concentration followed by flash chromatography (4:1 hexanes/ethyl acetate to 7:3 hexanes/ethyl acetate) provided **14** (40 mg, 45%) as a green oil. R_f 0.25 (2:1 hexanes/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.24 (d, J = 9.4 Hz, 1H), 8.00 (d, J = 5.4 Hz, 1H), 7.79 (d, J = 1.7 Hz, 1H), 7.61 (m, 2H), 7.13 (app t, J = 8.7 Hz, 2H), 6.85 (dd, J = 9.6, 2.1 Hz, 1H), 6.32 (d, J = 5.4 Hz, 1H), 5.23 (br d, J = 6.5 Hz, 1H), 4.35 (m, 1H), 3.71 (m, 1H), 3.54 (d, J = 5.8 Hz, 1H), 2.14–2.03 (m, 4H), 1.80–1.51 (m, 12H); MS m/z 457 (M+1). To a solution of the product in ether was added 1 MHCl in ether. The precipitated solid was isolated to give the corresponding HCl salt.

5.6. 3-[2-(Cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)-*N*-isopropylpyrazolo[1,5-*a*]pyridin-6-amine (15)

In a similar manner as described in for compound **14** from 3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridin-6-amine dihydrochloride (**13**) (40 mg, 0.087 mmol) and acetone (10 μL, 0.13 mmol) was obtained **15** (16 mg, 43%) as a pale green solid. R_f 0.21 (2:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, CD_3OD): δ 8.25 (d, J = 9.5 Hz, 1H), 7.89 (d, J = 5.6 Hz, 1H), 7.72 (d, J = 1.8 Hz, 1H), 7.55 (m, 2H), 7.19 (app t, J = 8.8 Hz, 2H), 7.03 (dd, J = 9.6, 2.0 Hz, 1H), 6.24 (d, J = 5.4 Hz, 1H), 4.22 (m, 1H), 3.52 (m, 1H), 2.02 (m, 2 H), 1.76 (m, 2H), 1.67–1.51 (m, 4H), 1.24 (d, J = 6.2 Hz, 6H); MS m/z 431 (M+1).

5.7. [3-[2-(Cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridin-6-yl]methanol (16)

To a cold (−78 °C) solution of ethyl 3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]-pyridine-6-carboxylate (**10**) (722 mg, 1.62 mmol) in dichloromethane (14 mL) was added diisobutylaluminum hydride (6.5 mL, 1.0 M in hexanes, 6.5 mmol). The reaction mixture was stirred at −78 °C for 1.5 h. The reaction mixture was poured into saturated aqueous Rochelle salt solution and stirred at room temperature for 2 h. The resultant mixture was extracted with ethyl acetate. The organic layer was washed with water and brine, and then dried over magnesium sulfate. Filtration and concentration followed by flash chromatography (4:1 dichloromethane/acetone) provided **16** (261 mg, 40%) as a white solid. R_f 0.41 (4:1 dichloromethane/acetone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.36 (s, 1H), 8.27 (d, J = 9.2 Hz, 1H), 8.00 (d, J = 5.2 Hz, 1H), 7.56 (m, 2H), 7.23 (d, J = 9.1 Hz, 1H), 7.10 (app t, J = 8.6 Hz, 2H), 6.26 (d, J = 5.3 Hz, 1H), 5.23 (d, J = 7.2 Hz, 1H), 4.64 (s, 2H), 4.29 (m, 1H), 2.03 (m, 2H), 1.77–1.45 (m, 6H); MS m/z 404 (M+1).

5.8. *N*-Cyclopentyl-4-[2-(4-fluorophenyl)-6-methylpyrazolo[1,5-*a*]pyridin-3-yl]-2-pyrimidinamine (18)

5.8.1. 4-[6-(Bromomethyl)-2-(4-fluorophenyl)pyrazolo[1,5-*a*]-pyridin-3-yl]-*N*-cyclopentyl-2-pyrimidinamine (17). To a solution of [3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridin-6-yl]methanol (**16**) (65 mg, 0.16 mmol) in chloroform (1 mL) was added phosphorus tribromide (6 μL, 0.06 mmol). The reaction mixture was stirred at room temperature for 2 h, and then quenched with saturated aqueous sodium bicarbonate solution. The resultant mixture was extracted with dichloromethane. The organic layer was washed with water and brine, and then dried over sodium sulfate. Filtration and concentration followed by flash chromatography (19:1 dichloromethane/acetone) provided **17** (32 mg, 43%) as a yellow oil. R_f 0.68 (9:1 dichloromethane/acetone); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.49 (s, 1H), 8.39 (d, J = 9.3 Hz, 1H), 8.05 (d, J = 5.3 Hz, 1H), 7.59 (m, 2H), 7.34 (dd, J = 9.2, 1.5 Hz, 1H), 7.13 (app t, J = 8.7 Hz, 2H), 6.30 (d, J = 5.3 Hz, 1H), 5.21 (br d, J = 7.2 Hz, 1H), 4.52 (s, 2H), 4.33 (m, 1H), 2.07 (m, 2H), 1.78–1.50 (m, 6H); MS m/z 467 (M+1).

5.8.2. *N*-Cyclopentyl-4-[2-(4-fluorophenyl)-6-methylpyrazolo[1,5-*a*]pyridin-3-yl]-2-pyrimidinamine (18). To a solution of 4-[6-(bromomethyl)-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridin-3-yl]-*N*-cyclopentyl-2-pyrimidinamine (**17**) (40 mg, 0.086 mmol) in toluene (5 mL) were added tributyltin hydride (35 μL, 0.13 mmol) and 2,2′-azobisisobutyronitrile (2 mg, 0.009 mmol). The reaction mixture was heated at 95 °C for 3 h. After cooling the reaction mixture to room temperature, Celite was added and the resultant mixture was concentrated in vacuo.

Flash chromatography (19:1 dichloromethane/acetone) provided a crude material which was triturated with ether to provide **18** (4 mg, 12%) as a pale yellow solid. R_f 0.63 (9:1 dichloromethane/acetone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.36–8.29 (m, 2H), 8.00 (br, 1H), 7.60 (m, 2H), 7.23–7.11 (m, 3H), 6.31 (d, J = 5.2 Hz, 1H), 5.37 (br, 1H), 4.35 (m, 1H), 2.39 (s, 3H), 2.08 (m, 2H), 1.81–1.53 (m, 6H); MS *m*/*z* 388 (M+1).

5.9. *N*-Cyclopentyl-4-[6-[(cyclopentylamino)methyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridin-3-yl]-2-pyrimidinamine (19)

To a suspension of 4-[6-(bromomethyl)-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridin-3-yl]-*N*-cyclopentyl-2-pyrimidinamine (**17**) (30 mg, 0.064 mmol) in tetrahydrofuran (2 mL) was added cyclopentylamine (730 μL, 7.4 mmol). The reaction mixture was stirred at room temperature for 3 h, and then diluted with ethyl acetate. The organic layer was washed with saturated aqueous sodium bicarbonate solution, water, and brine. The organic layer was dried over magnesium sulfate. Filtration and concentration followed by flash chromatography (39:1 dichloromethane/methanol to 35:5 dichloromethane/methanol) provided **19** (6 mg, 20%) as a light yellow solid. R_f 0.56 (35:5 dichloromethane/methanol); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (s, 1H), 8.36 (d, J = 9.3 Hz, 1H), 8.03 (d, J = 5.2 Hz, 1H), 7.60 (m, 2H), 7.36 (d, J = 9.4 Hz, 1H), 7.12 (app t, J = 8.7 Hz, 2H), 6.30 (d, J = 5.3 Hz, 1H), 5.10 (d, J = 7.2 Hz, 1H), 4.33 (m, 1H), 3.84 (s, 2H), 3.14 (quint, J = 6.7 Hz, 1H), 2.07 (m, 2H), 1.85 (m, 2H), 1.78–1.38 (m, 12H); MS *m*/*z* 471 (M+1).

5.10. Ethyl 7-(cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl) [1,5-*a*]pyridine-6-carboxylate (22)

5.10.1. 4-[7-Chloro-2-(4-fluorophenyl)-6-(triethoxymethyl) pyrazolo[1,5-*a*]pyridin-3-yl]-*N*-cyclopentyl-2-pyrimidinamine (20). To a cold (0 °C) solution of diisopropylamine (4.1 mL, 29 mmol) in tetrahydrofuran (25 mL) was added butyllithium (17 mL, 1.6 M in hexanes, 28 mmol) dropwise. The reaction mixture was stirred at 0 °C for 10 min and then cooled to −78 °C. The reaction mixture was transferred via syringe to a cold (−78 °C) solution of *N*-cyclopentyl-4-[2-(4-fluorophenyl)-6-(triethoxymethyl)pyrazolo[1,5-*a*]pyridin-3-yl]-2-pyrimidinamine (**9**) (4.86 g, 9.35 mmol) in tetrahydrofuran (25 mL). The reaction mixture was stirred at −78 °C for 30 min. Carbon tetrachloride (3.6 mL, 37 mmol) was added and the resulting mixture was warmed to room temperature and stirred 2 h. The reaction mixture was poured onto ice. After the ice had melted, the aqueous mixture was extracted with ethyl acetate. The organic layer was washed with water and brine, and then dried over sodium sulfate. Filtration and concentration followed by flash chromatography (4:1 hexanes/ethyl acetate) provided **20** (2.37 g, 46%) as a yellow solid. R_f 0.36 (4:1 hexanes/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.36 (d, J = 9.4 Hz, 1H), 8.08 (d, J = 5.2 Hz, 1H), 7.85 (d, J = 9.5 Hz, 1H), 7.66 (m, 2H), 7.15 (app t, J = 8.6 Hz, 2H), 6.32 (d, J = 5.2 Hz, 1H), 5.15 (d, J = 7.2 Hz, 1H), 4.36 (m, 1H), 3.45 (m, 6H), 2.10 (m, 2H), 1.81–1.54 (m, 6H), 1.26 (m, 9H); MS *m*/*z* 554 (M+1).

5.10.2. *N*-Cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)-6-(triethoxymethyl)pyrazolo[1,5-*a*]pyridin-7-amine (21). A mixture of 4-[7-chloro-2-(4-fluorophenyl)-6-(triethoxymethyl)pyrazolo[1,5-*a*]pyridin-3-yl]-*N*-cyclopentyl-2-pyrimidinamine (**20**) (2.37 g, 4.28 mmol) and cyclopentylamine (10 mL, 100 mmol) was heated in a sealed tube at 80 °C for 16 h. The reaction mixture was cooled to room temperature and excess cyclopentylamine was removed in vacuo. The crude solid was triturated in water. The solids were collected on filter to provide **21** (2.48 g, 96%). R_f 0.42 (4:1 hexanes/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03 (d, J = 5.3 Hz, 1H), 7.74 (d, J = 9.2 Hz, 1H), 7.68–7.60 (m, 3H), 7.12 (app t, J = 8.6 Hz, 2H), 6.36–6.31 (m, 2H), 5.32 (m, 1H), 5.06 (d, J = 7.5 Hz, 1H), 4.35 (m, 1H), 3.45 (q, J = 7.2 Hz, 6H), 2.13–1.95 (m, 4H), 1.83–1.47 (m, 12H), 1.25 (t, J = 7.2 Hz, 9H); MS *m*/*z* 603 (M+1).

5.10.3. Ethyl 7-(cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl) [1,5-*a*]pyridine-6-carboxylate (22). To a suspension of *N*-cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)-6-(triethoxymethyl)pyrazolo[1,5-*a*]pyridin-7-amine (**21**) (2.48 g, 4.11 mmol) in acetone (80 mL) and water (20 mL) was added *p*-toluenesulfonic acid monohydrate (1.95 g, 10.2 mmol). The reaction mixture was stirred at room temperature for 3 h, then poured onto ice and neutralized with saturated aqueous sodium bicarbonate solution. The aqueous mixture was concentrated in vacuo to remove acetone. The resulting aqueous mixture was extracted with ethyl acetate. The organic layer was washed with water and brine, and then dried over magnesium sulfate. Filtration and concentration followed by flash chromatography (4:1 hexanes/ethyl acetate to 100% ethyl acetate to 4:1 ethyl acetate/methanol) provided **22** (1.9 g, 88%) as a light yellow solid. R_f 0.20 (4:1 hexanes/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.26 (d, J = 7.5 Hz, 1H), 8.08 (d, J = 5.3 Hz, 1H), 7.77 (d, J = 9.4 Hz, 1H), 7.64 (m, 2H), 7.47 (d, J = 9.5 Hz, 1H), 7.12 (app t, J = 8.7 Hz, 2H), 6.32 (d, J = 5.3 Hz, 1H), 5.51 (m, 1H), 5.10 (d, J = 7.2 Hz, 1H), 4.39–4.28 (m, 3H), 2.12–2.03 (m, 4H), 1.82–1.49 (m, 12H), 1.41 (t, J = 7.2 Hz, 3H); MS *m*/*z* 529 (M+1).

5.11. 7-(Cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridine-6-carboxylic acid (23)

To a solution of ethyl 7-(cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridine-6-carboxylate (**22**) (200 mg, 0.378 mmol) in dioxane (5 mL) and water (700 μL) was added lithium hydroxide monohydrate (48 mg, 1.1 mmol). The reaction mixture was heated for 40 h at 100 °C. The reaction mixture was cooled and concentrated *in vacuo*, and then diluted with water. The aqueous mixture was acidified with 1 N aqueous hydrochloric acid. The solid precipitate was collected on a filter to provide **23** (125 mg, 58%) as an orange solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.06 (br, 1H), 9.46 (d, J = 7.4 Hz, 1H), 8.07 (d, J = 5.6 Hz, 1H), 7.78 (d, J = 9.2 Hz, 1H), 7.65 (m, 2H), 7.34 (app t, J = 8.8 Hz, 2H), 6.28 (br, 1H), 5.37 (m, 1H), 4.10 (m, 1H), 1.99 (m, 2H), 1.87 (m, 2H), 1.74-1.47 (m, 12H); MS *m*/*z* 501 (M+1).

5.12. *N*-Cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)-6-(1-pyrrolidinylcarbonyl)pyrazolo[1,5-*a*]-pyridin-7-amine (24)

7-(Cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridine-6-carboxylic acid dihydrochloride (**23**) (19 mg, 0.033 mmol) was added to a dry flask and cooled to −78 °C. Thionyl chloride (14 μL, 0.19 mmol) was added and the reaction mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated under a stream of nitrogen and then placed under high vacuum (using base trap in vacuum line). To a solution of the crude residue in dichloromethane (600 μL) was added pyrrolidine (150 μL, 1.80 mmol). The reaction mixture was stirred at room temperature for 10 min and then partitioned between ethyl acetate and saturated aqueous sodium bicarbonate solution. The aqueous layer was extracted with ethyl acetate. The combined extracts were washed with water and brine, and then dried over magnesium sulfate. Filtration and concentration followed by flash chromatography (1:1 hexanes/ethyl acetate to 100% ethyl acetate) provided **24** (9 mg, 49%) as a clear oil. R_f 0.13 (1:1 hexanes/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04 (d, J = 5.4 Hz, 1H), 7.72 (d, J = 9.1 Hz, 1H), 7.63 (m, 2H), 7.24 (m, 1H), 7.14 (app t, J = 8.6 Hz, 2H), 6.54 (d, J = 8.7 Hz, 1H), 6.29 (d, J = 5.3 Hz, 1H), 5.11 (d, J = 6.7 Hz, 1H), 4.31 (m, 1H), 4.20 (m, 1H), 3.66 (br, 2H), 3.38 (br, 2H), 2.11–1.90 (m, 8H), 1.80–1.49 (m, 12H); MS *m*/*z* 554 (M+1). To a solution of the product in ether was added 1 M HCl in ether. The precipitated solid was isolated to give the corresponding HCl salt.

5.13. 7-(Cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)-*N*,*N*-dimethylpyrazolo[1,5-*a*]pyridine-6-carboxamide (28)

In a similar manner as described in for compound **24** from 7-(cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridine-6-carboxylic acid dihydrochloride (**23**) (41 mg, 0.071 mmol) and dimethylamine (500 μL, 2.0 M in tetrahydrofuran, 0.25 mmol) was formed **28** (14 mg, 38%) as a clear oil. R_f 0.12 (1:1 hexanes/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04 (d, J = 5.2 Hz, 1H), 7.73 (d, J = 9.0 Hz, 1H), 7.63 (m, 2H), 7.19 (d, J = 8.8 Hz, 1H), 7.15 (app t, J = 8.7 Hz, 2H), 6.51 (d, J = 8.4 Hz, 1H), 6.30 (d, J = 5.3 Hz, 1H), 5.17 (d, J = 6.8 Hz, 1H), 4.32 (m, 1H), 4.13 (m, 1H), 3.09 (br, 6H), 2.11–1.95 (m, 4H), 1.80–1.50 (m, 12H); MS *m*/*z* 528 (M+1). To a solution of the product in ether was added 1 M HCl in ether. The precipitated solid was isolated to give the corresponding HCl salt.

5.14. 7-(Cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)-*N*-hydroxypyrazolo[1,5-*a*]pyridine-6-carboxamide (29)

7-(Cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridine-6-carboxylic acid dihydrochloride (**23**) (52 mg, 0.091 mmol) was added to a dry flask and cooled to −78 °C. Thionyl chloride (38 μL, 0.52 mmol) was added and the reaction mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated under a stream of nitrogen and then placed under high vacuum (using base trap in vacuum line). In a separate flask, a suspension of hydroxylamine hydrochloride (29 mg, 0.42 mmol) and potassium carbonate (29 mg, 0.21 mmol) in tetrahydrofuran (600 μL) was stirred at room temperature for 30 min. To this suspension was added a solution of the crude acid chloride in dichloromethane (600 μL). The resulting mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution, water, and brine. The organic layer was dried over magnesium sulfate. Filtration and concentration followed by chromatography (100% ethyl acetate to 9:1 ethyl acetate/methanol) provided **29** (12 mg, 26%) as a brownish-yellow solid. R_f 0.42 (19:1 ethyl acetate/methanol); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (br, 2H), 7.60 (m, 2H), 7.49 (br, 1H), 7.21 (br, 1H), 7.13 (app t, J = 8.6 Hz, 2H), 6.29 (d, J = 5.2 Hz, 1H), 5.90 (br, 1H), 4.92 (br, 1H), 4.30 (m, 1H), 2.08–1.98 (m, 4H), 1.82–1.54 (m, 12H); MS *m*/*z* 516 (M+1).

5.15. 7-(Cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)-*N*-[2-(4-morpholinyl)ethyl]pyrazolo[1,5-*a*]pyridine-6-carboxamide (27)

In a similar manner as described in for compound **24** from 7-(cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridine-6-carboxylic acid dihydrochloride (**23**) (40 mg, 0.070 mmol) and 4-(2-aminoethyl)morpholine (400 μL, 3.0 mmol) was formed **27** (20 mg, 47%) as an orange solid. R_f 0.27 (27:3 ethyl acetate/methanol); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (br, 1H), 8.06 (d, J = 5.3 Hz, 1H), 7.65–7.61 (m, 3H), 7.40 (d, J = 9.3 Hz, 1H), 7.12 (app t, J = 8.7 Hz, 2H), 6.92 (br, 1H), 6.31 (d, J = 5.2 Hz, 1H), 5.11 (d, J = 7.5 Hz, 1H), 5.00 (br, 1H), 4.30 (m, 1H), 3.74 (t, J = 4.4 Hz, 4H), 3.55 (m, 2H), 2.63 (t, J = 5.9 Hz, 2H), 2.53 (br, 4H), 2.10–1.95 (m, 4H), 1.82–1.48 (m, 12H); MS *m*/*z* 613 (M+1).

5.16. 7-(Cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)-*N*-[3-(1*H*-imidazol-1-yl)propyl]pyrazolo[1,5-*a*]pyridine-6-carboxamide (25)

In a similar manner as described in for compound **24** from 7-(cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridine-6-carboxylic acid dihydrochloride (**23**) (40 mg, 0.070 mmol) and 1-(3-aminopropyl)imidazole (400 μL, 3.4 mmol) was formed **25** (19 mg, 43%) as a yellow solid. R_f 0.09 (27:3 ethyl acetate/methanol); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (d, J = 8.1 Hz, 1H), 8.05 (d, J = 5.2 Hz, 1H), 7.64–7.60 (m, 4H), 7.37 (d, J = 9.4 Hz, 1H), 7.14–7.08 (m, 4H), 6.98 (s, 1H), 6.65 (t, J = 5.8 Hz, 1H), 6.30 (d, J = 5.2 Hz, 1H), 4.94 (m, 1H), 4.29 (m, 1H), 4.08 (m, 2H), 3.46 (m, 2H), 2.14 (m, 2H), 2.07–1.94 (m, 4H), 1.80–1.47 (m, 12H); MS *m*/*z* 608 (M+1).

5.17. 7-(Cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)-*N*-[2-hydroxy-1-(hydroxymethyl)ethyl]pyrazolo[1,5-*a*]pyridine-6-carboxamide (26)

In a similar manner as described in for compound **24** from 7-(cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridine-6-carboxylic acid dihydrochloride (**23**) (40 mg, 0.070 mmol) and serinol (200 mg, 2.2 mmol) was formed **26** (8 mg, 20%) as an orange solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.47 (br d, J = 7.5 Hz, 1H), 7.96 (br, 1H), 7.67–7.55 (m, 4H), 7.43 (d, J = 9.2 Hz, 1H), 7.20–7.09 (m, 3H), 6.24 (d, J = 5.3 Hz, 1H), 4.99 (m, 1H), 4.32 (m, 1H), 4.17 (m, 1H), 3.97 (m, 4H), 2.10–1.94 (m, 4H), 1.83–1.52 (m, 12H); MS m/z 572 (M−1).

5.18. [7-(Cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridin-6-yl]methanol (30)

To a cold (−78 °C) solution of ethyl 7-(cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridine-6-carboxylate (**22**) (50 mg, 0.095 mmol) in dichloromethane (1 mL) was added diisobutylaluminum hydride (490 μL, 1.0 M in hexanes, 0.49 mmol). The reaction mixture was stirred at −78 °C for 2 h then poured into a stirring mixture of ether and aqueous Rochelle salt solution. The resultant mixture was stirred at room temperature for 16 h. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with water and brine. Concentration and flash chromatography (29:1 dichloromethane/methanol) provided **30** (27 mg, 59%) as a green solid. R_f 0.26 (29:1 dichloromethane/methanol); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.99 (d, J = 5.2 Hz, 1H), 7.69 (d, J = 8.9 Hz, 1H), 7.61 (m, 2H), 7.22 (d, J = 8.9 Hz, 1H), 7.13 (app t, J = 8.7 Hz, 2H), 6.28–6.23 (m, 2H), 5.08 (d, J = 7.4 Hz, 1H), 4.79 (s, 2H), 4.46 (br, 1H), 4.29 (m, 1H), 2.10–2.00 (m, 4H), 1.85–1.47 (m, 12H); MS m/z 487 (M+1).

5.19. 3-[2-(Cyclopentylamino)-4-pyrimidinyl]-*N*-cyclopropyl-2-(4-fluorophenyl)-7-methylpyrazolo[1,5-*a*]pyridine-6-carboxamide (34)

5.19.1. Ethyl 7-chloro-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridine-6-carboxylate (31). To a solution of 4-[7-chloro-2-(4-fluorophenyl)-6-(triethoxymethyl)pyrazolo[1,5-*a*]pyridin-3-yl]-*N*-cyclopentyl-2-pyrimidinamine (**20**) (375 mg, 0.677 mmol) in acetone (8 mL) and water (2 mL) was added *p*-toluenesulfonic acid monohydrate (321 mg, 1.69 mmol). The reaction mixture was stirred at room temperature for 2 h and then quenched with ice water. The reaction mixture was neutralized with saturated aqueous sodium bicarbonate solution and then concentrated in vacuo to remove the majority of the acetone. The resultant mixture was extracted with ethyl acetate. The organic layer was washed with water and brine, then dried over magnesium sulfate. Filtration and concentration and followed by flash chromatography (29:1 dichloromethane/methanol) provided **31** (175 mg, 54%) as a brown solid. R_f 0.08 (29:1 dichloromethane/methanol); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.36 (d, J = 9.5 Hz, 1H), 8.08 (d, J = 5.1 Hz, 1H), 7.82 (d, J = 9.2 Hz, 1H), 7.65 (m, 2H), 7.14 (app t, J = 8.7 Hz, 2H), 6.30 (d, J = 5.4 Hz, 1H), 5.19 (d, J = 6.8 Hz, 1H), 4.46 (q, J = 7.1 Hz, 2H), 4.32 (m, 1H), 2.08 (m, 2H), 1.78–1.22 (m, 9H); MS m/z 480 (M+1).

5.19.2. Ethyl 3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)-7-methylpyrazolo[1,5-*a*]pyridine-6-carboxylate (32). To a solution of ethyl 7-chloro-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridine-6-carboxylate (**31**) (90 mg, 0.19 mmol) in tetrahydrofuran (1 mL) were added dimethylzinc (281 μL, 2.0 M in toluene, 0.56 mmol) and tetrakis(triphenylphosphine)palladium (21 mg, 0.018 mmol). The reaction mixture was stirred at 60 °C for 16 h. The reaction mixture was quenched with ice water and then extracted with ethyl acetate. The organic layer was washed with water and brine, and then dried over magnesium sulfate. Filtration and concentration followed by flash chromatography (49:1 dichloromethane/methanol) provided **32** (40 mg, 45%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.32 (d, J = 9.3 Hz, 1H), 8.10 (d, J = 5.0 Hz, 1H), 7.89 (d, J = 9.4 Hz, 1H), 7.70 (m, 2H), 7.19 (app t, J = 8.7 Hz, 2H), 6.36 (d, J = 5.5 Hz, 1H), 5.32 (br, 1H), 4.47 (q, J = 7.1 Hz, 2H), 4.38 (m, 1H), 3.26 (s, 3H), 2.12 (m, 2H), 1.85–1.45 (m, 9H); MS m/z 460 (M+1).

5.19.3. 3-[2-(Cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)-7-methylpyrazolo[1,5-*a*]pyridine-6-carboxylic acid (33). To a solution of ethyl 3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)-7-methylpyrazolo[1,5-*a*]pyridine-6-carboxylate **32** (40 mg, 0.087 mmol) in dioxane (600 μL) was added lithium hydroxide (300 μL, 1 M aqueous, 0.30 mmol). The reaction mixture was stirred at room temperature 16 h. The reaction mixture was concentrated in vacuo to remove dioxane and then diluted with water. The aqueous mixture was acidified with 1 N aqueous hydrochloric acid. Upon standing for 72 h, a solid precipitate had formed which was collected by filtration to provide (**33**) (31 mg, 82%). R_f 0.10 (19:1 dichloromethane/methanol); MS m/z 432 (M+1).

5.19.4. 3-[2-(Cyclopentylamino)-4-pyrimidinyl]-*N*-cyclopropyl-2-(4-fluorophenyl)-methylpyrazolo[1,5-*a*]pyridine-6-carboxamide (34). Thionyl chloride (200 μL, 2.7 mmol) was added to 3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)-7-methylpyrazolo[1,5-*a*]pyridine-6-carboxylic acid **33** (31 mg, 0.072 mmol), pre-cooled to 0 °C. The reaction mixture was stirred at room temperature for 1 h. The excess thionyl chloride was removed in vacuo (using base trap). To a solution of the residue in dichloromethane (300 μL) was added cyclopropylamine (50 μL, 0.72 mmol). The reaction mixture was stirred at room temperature for 30 min. The resultant mixture was quenched with water and diluted with ethyl acetate. Saturated aqueous sodium bicarbonate solution was added to the bi-phasic mixture. The organic layer was washed with water and brine, and then dried over magnesium sulfate. Filtration and concentration, followed by flash chromatography (3:2 hexanes/ethyl acetate to 2:3 hexanes/ethyl acetate) provided (**34**) (15 mg, 44%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (d, J = 9.2 Hz, 1H), 8.05 (d, J = 5.1 Hz, 1H), 7.63 (m, 2H),

7.30 (d, $J = 9.2$ Hz, 1H), 7.13 (app t, $J = 8.6$ Hz, 2H), 6.29 (d, $J = 5.3$ Hz, 1H), 5.10 (d, $J = 7.3$ Hz, 1H), 4.31 (m, 1H), 2.96 (s, 3H), 2.93 (m, 1H), 2.06 (m, 2H), 1.78–1.49 (m, 6H), 0.91 (m, 2H), 0.66 (m, 2H); MS *m/z* 471 (M+1).

5.20. HSV antiviral assay

5.20.1. Cell culture and HSV infection. Vero 76 cells (American Type Culture Collection, Manassas, VA) were grown and passed in MEM with Earle's salts, L-glutamine, penicillin, and streptomycin (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 8% fetal bovine serum (FBS) (Hyclone Laboratories, Logan, UT). The amount of FBS was reduced to 2% for assays.

Vero cells were infected in suspension with either HSV-1 for 45 min at 37 °C at a multiplicity of infection of 0.001. Infected cells were plated at a density of 50,000 cells/well into 96-well tissue culture plates containing antiviral test compounds and incubated at 37 °C for 40–48 h.

5.20.2. HSV DNA hybridization. The effect of compounds on HSV replication was assessed by conventional DNA hybridization. Cell lysates were prepared for hybridization by removing growth medium from HSV-infected Vero cells two days post-infection and adding 150 μL lysis buffer (0.2 N NaOH with 1% NP-40) to each well. The lysates were then incubated at room temperature for five days in a humidified chamber to ensure complete DNA hydrolysis. Samples of the lysates were neutralized in a phosphate-buffered guanidine isothiocyanate (GuSCN) solution and combined with a digoxigenin-labeled 710 bp DNA fragment of the HSV UL15 open reading frame. The hybridization solution was heated to 90 °C for 6 min and incubated at 42 °C overnight. Immobilized hybrids were detected by incubation with anti-digoxigenin HRP-conjugated antibody (Boehringer–Mannheim, Indianapolis, IN) and subsequent addition of SuperSignal? substrate (Pierce, Rockford, MD). The resulting chemiluminescent signal from compound-treated cells was compared to that of compound-free cells to obtain percent inhibitions, which were used to construct dose–response curves to derive 50% inhibitory concentrations (IC_{50}).

5.21. Cytotoxicity assay

Compounds were dissolved in DMSO at a stock concentration of 10 mM and serially diluted 2-fold in DMSO. Dilutions were carried out in columns 1–9 of a 96-well, v-bottomed polypropylene plate. Columns 10, 11, and 12 are control columns that do not contain compound. Plates are then seeded at 10,000 Vero cells/well, columns 1–11, in minimum essential medium containing Earle's salt and L-glutamine and supplemented with 10% heat-inactivated fetal bovine serum, 1% penicillin–streptomycin, and 1% L-glutamine (200 mM) resulting in a final 250-fold drug dilution. Thus, the maximum compound concentration used in the assay is 40 μM, while the final DMSO concentration is 0.4%. Plates are incubated at 37 °C in a humidified, 5% CO_2 atmosphere and growth inhibition is measured after 3 days.

Cytotoxicity is determined in Vero cells using the CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay (Promega Corporation, G1111). Metabolically active cells will convert methylthiazol tetrazolium inner salt (MTS), through the actions of cellular dehydrogenases, into a colorized formazan end product. On day 3, MTS solution is added to the assay plates, incubated for 1.5–2 h at 37 °C in a humidified, 5% CO_2 atmosphere, and absorbance is measured at 490 nm using the Wallac Victor2 1420 multilabel counter (Perkin-Elmer, Wellesley, NA). RoboFit 2000 curve-fitting software is then used to obtain a CC_{50} (50% cytotoxicity concentration) value from the curves generated.

References and notes

1. Roizman, B.; Pellett, P. E.. In Knipe, D. M., Howley, P. M., Eds.; Fields' Virology; Lippincott Williams and Wilkins: Philadelphia, PA, 2001; Vol. 2, p 2381.
2. Corey, L.; Tyring, S.; Sacks, S.; Warren, T.; Beutner, K.; Patel, R.; Wald, A.; Mertz, G.; Paavonen, J. In *Abstract of papers*, 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA; American Society for Microbiology: Washington, DC. 2002; Abstract LB3.
3. Johns, B. A.; Gudmundsson, K. S.; Turner, E. M.; Allen, S. H.; Jung, D. K.; Sexton, C. J.; Boyd, F. L., Jr.; Peel, M. R. *Tetrahedron* **2003**, *59*, 9001.
4. Johns, B. A.; Gudmundsson, K. S.; Turner, E. M.; Allen, S. H.; Samano, V. A.; Ray, J. A.; Freeman, G. A.; Boyd, F. L., Jr.; Sexton, C. J.; Selleseth, D. A.; Creech, K. L.; Moniri, K. R. *Bioorg. Med. Chem.* **2005**, 2397.
5. Alberti, M. J.; Baldwin, I. R.; Cheung, M.; Cockerill, S.; Flack, S.; Harris, P. A.; Jung, D. K.; Peckham, G.; Peel, M. R.; Stanford, J. B.; Stevens, K.; Veal, J. M. WO 02/16359, 2002; *Chem Abstr.* **2002**, 136, 200184.
6. Cyclopentyl guanidine **61** ($NR^3R^4 = c\text{-}C_5H_9$) was made via a modification of methods described in Bannard, R. A. B.; Casselman, A. A.; Cockburn, W. F.; Brown, G. M. *Can. J. Chem.* **1958**, 36.
7. (a) Finkelstein, B. L. *J. Org. Chem.* **1992**, 5538; (b) Aboul-Fadl, T.; Lober, S.; Gmeiner, P. *Synthesis* **2000**, 1727.
8. (a) Herbert, J. M. *Tetrahedron Lett.* **2004**, 87; (b) Erdik, E. *Tetrahedron* **1992**, *44*, 9577.