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Synthesis of novel substituted 2-phenylpyrazolopyridines with potent activity against herpesviruses

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Abstract—Herpesviruses are a significant source of human disease; amongst these herpes simplex virus 1 (HSV-1) and HSV-2 are very prevalent and cause recurrent infections. We recently identified a pyrazolo[1,5-*a*]pyridine scaffold that showed promising activity against HSV-1 and HSV-2 in Vero cell antiviral assays. Here, we describe the synthesis and anti-herpetic activity of several 3-pyrimidinyl-2-phenylpyrazolo[1,5-*a*]pyridines with differing 2-phenyl substitution patterns. Approaches to rapidly access a number of analogs with different 2-phenyl substitution patterns are outlined. Several of the compounds described have comparable activity to acyclovir against HSV-1 and HSV-2.

1. Introduction

The herpesvirus family (Herpesviridae) is highly disseminated in nature and most animal species host at least one herpesvirus. This family contains eight known human viruses, herpes simplex virus 1 (HSV-1), HSV-2, varicella zoster virus (VZV), human cytomegalovirus (HCMV), Epstein–Barr virus (EBV), and human herpesviruses 6–8 (HHV-6, HHV-7, and HHV-8).[1] These herpesviruses are associated with a diverse set of diseases, ranging in severity from asymptomatic to life-threatening illness.

Much research has been focused on HSV-1 and HSV-2 as these viruses have a high incidence rate (~1.6 million new cases of HSV-2 predicted per year in the United States) and a high prevalence of 50–95% (HSV-1) and 6–50% (HSV-2) depending on other factors such as sex, age, and marital status.[2] HSV-1 and HSV-2 cause mucocutaneous infections, such as cold sores (HSV-1) and genital infection (HSV-2). A key feature of these viruses is their ability to cause latent infection of neurons, which upon reactivation leads to recurrent lesions at the innervated dermatome, the mucocutaneous area of initial infection. Frequency of recurrence is higher for HSV-2 than HSV-1, occurring about 2–3 times every 100 days. Thus, once latency is established, the primary infection of HSV is not completely cleared but persists chronically throughout life.[3]

Previous antiviral research on HSVs has primarily focused on the development of nucleoside analogs that target the viral polymerase.[4] Early nucleosides included idoxuridine, vidarabine, and trifluridine. These are still used topically, but are too toxic for use as first-line treatment.[5] Development of acyclovir (**1**, Zovirax),[6] a potent, specific, and well-tolerated nucleoside inhibitor of herpes DNA polymerase, was a milestone in the development of antiviral drugs in the late 1970s and spurred development of a number of other nucleoside analogs. Currently, only the nucleosides acyclovir, valacyclovir (**2**),[7] famciclovir (**3**),[8] and penciclovir (**4**) are recommended for the treatment of herpes simplex disease (Fig. 1).

Though numerous strategies and considerable effort have been expended in the search for the next generation

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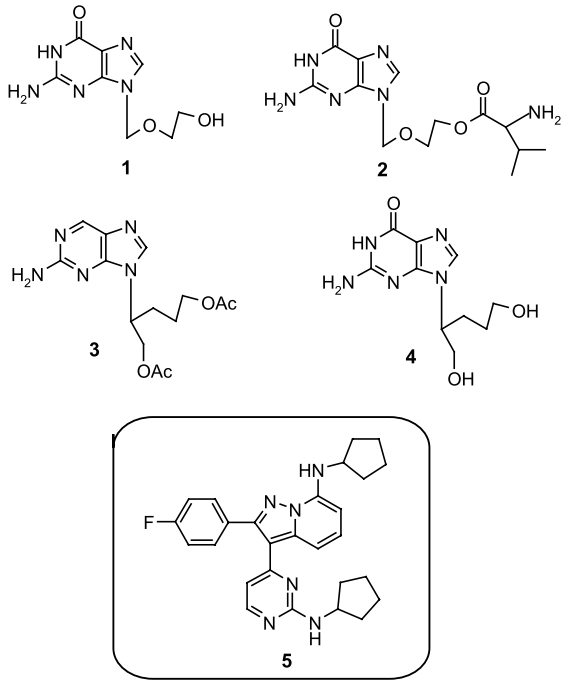


Figure 1. Drugs approved for treatment of herpes simplex infections (**1**–**4**) and recently described pyrazolo[1,5-*a*]pyridine (**5**).

anti-herpetic therapy, it has been proved difficult to outperform acyclovir.[9] Vaccines,[10] interleukins,[11] interferons,[12] therapeutic proteins, and antibodies with specific or non-specific mode of action have lacked either the specificity or the safety to replace the currently available nucleosides as first-line treatment. Small molecule drugs have recently received considerable attention as potential next generation anti-herpetics. Immunomodulators (imiquimod and resiquimod),[13] non-nucleoside viral polymerase inhibitors (4-hydroxyquinoline-3-carboxamides),[14] and viral helicase inhibitors (thiazolylphenyl and thiazolylamide)[15] appear to be among the most promising investigational drugs.

We recently identified pyrazolo[1,5-*a*]pyridine scaffold **5** that showed activity similar to acyclovir against HSV-1 and HSV-2 in our cellular assay.[16] Interestingly, few reports exist in the literature regarding the chemistry of pyrazolopyridines. We have previously described the methodology developed for the synthesis of **5**: here, we report structure–activity trends (SAR) observed when the substituents on the 2-phenyl portion of the pyrazolopyridine are varied (Fig. 2).

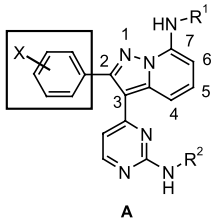


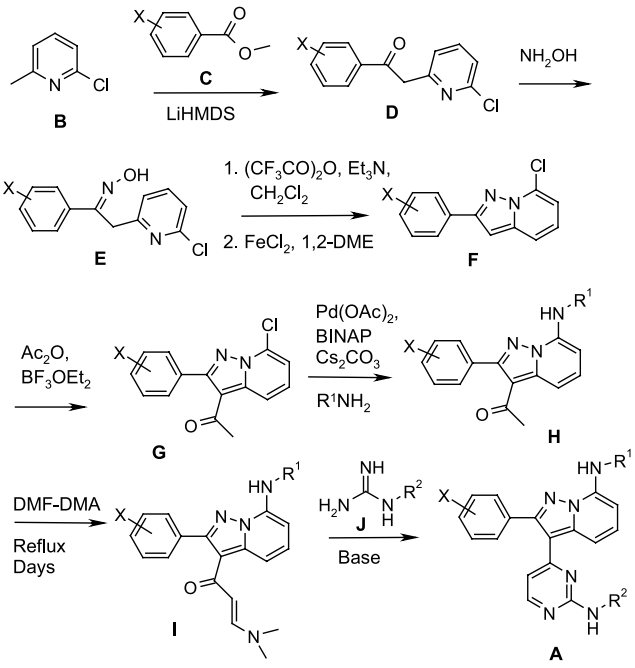
Figure 2. Pyrazolopyridines, SAR focused on varying substituents on the 2-phenyl moiety (R^1 and R^2 = cyclopentyl or *n*-butyl).

2. Chemistry

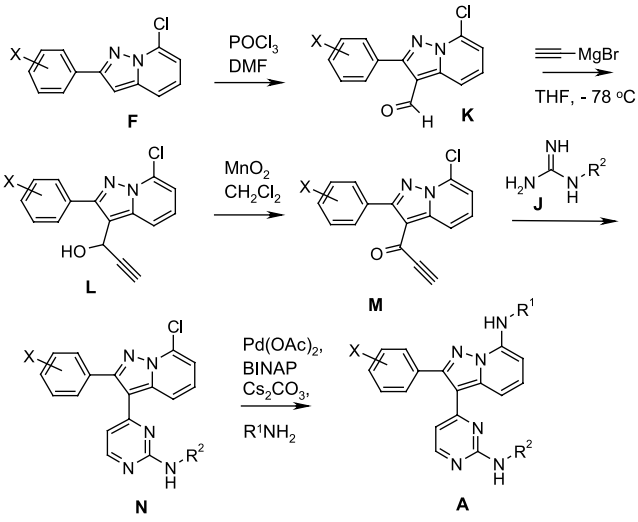
For this SAR study, we chose to keep the C-7 position substituted with either a cyclopentylamino or a *n*-butylamino group.[17] The C-3 pyrimidine moiety was also kept unchanged so we could focus our attention on identifying optimal substitution on the 2-phenyl moiety.

Key intermediates in the synthesis of the desired pyrazolopyridines were 7-chloro-2-phenylpyrazolopyridines (**F**). These were synthesized from 6-chloropicoline (**B**) and appropriately substituted benzoate esters (**C**) as outlined in Scheme 1 using previously described methodology.[16] The 3-pyrimidinyl-2-phenylpyrazolopyridines **A** were subsequently synthesized from **F** using either of two methods for construction of the 3-pyrimidinyl moiety as outlined in Schemes 1 and 2, respectively.

Briefly, treatment of **F** with Ac_2O and BF_3OEt_2 in toluene gave the 3-acetyl derivatives **G**. The desired C-7 amine was installed using the Pd(O)-mediated Buchwald–Hartwig[18] amination, using $Pd(OAc)_2$, BINAP, Cs_2CO_3 in toluene to give **H**. Refluxing in dimethylformamide dimethylacetal (DMF-DMA) or dimethylformamide di-*tert*-butyl acetal (DMF-DTBA) gave the vinylogous amide **I**. Treatment of this vinylogous amide with guanidines (**J**) gave the desired compound **A**. Alternatively, **F** could be formylated under Vilsmeier Haack conditions as outlined in Scheme 2. The resulting 3-formyl derivatives (**K**) were treated with an ethynyl Grignard reagent to give alcohols (**L**). Oxidation of the alcohols (**L**) by MnO_2 gave the alkynyl ketones (**M**) that were treated with guanidines (**J**) to give the 7-chloro derivatives (**N**). Buchwald–Hartwig methodology or thermal displacement was then used to install the C-7 amine to give the desired product **A**.

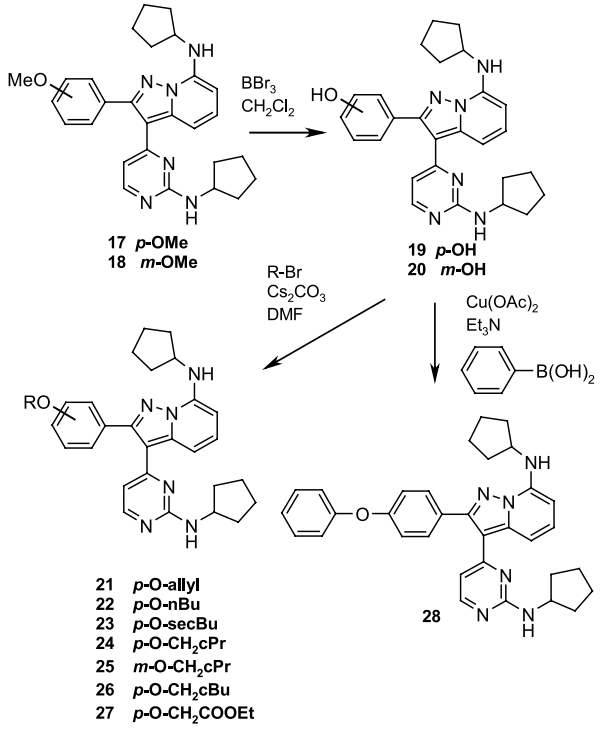


Scheme 1. Synthetic route for general procedure I.

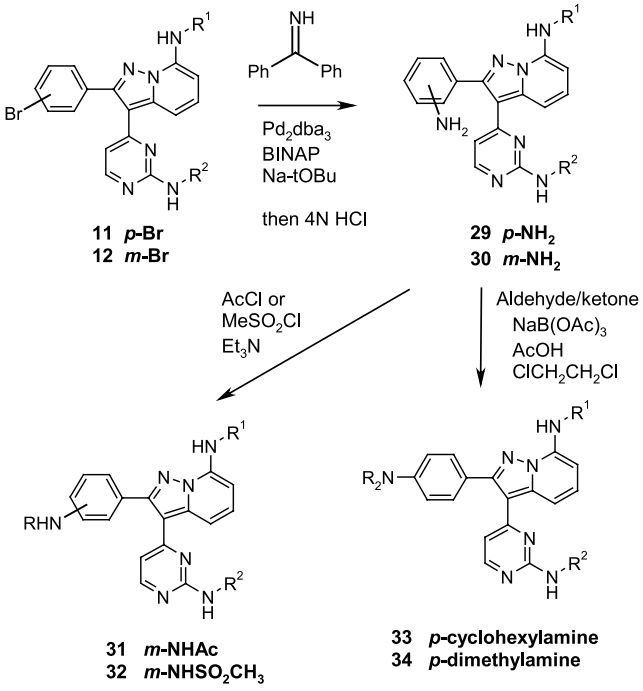


Scheme 2. Synthetic route for general procedure II.

Yield, scaleability, and ease of purification dictated whether general procedure I or II was used for the synthesis of individual derivatives. Initially, general procedure I was used to synthesize a set of *para*-F analogs, where R^1 and R^2 were either cyclopentyl or *n*-butyl (compounds **5**–**7**; Table 1). Then, a set of halogenated derivatives was synthesized to investigate how *para* vs. *meta* or *ortho* halogen (F, Cl, Br) substituent would affect activity (compounds **8**–**13**, synthesized according to procedure I from appropriate halogenated benzoic acid derivative). The unsubstituted 2-phenyl derivative **14** (X = H), the *m*-CH_3 derivative (**15**), and the *m*-CF_3 derivative (**16**) were also synthesized using the methodologies outlined in Schemes 1 and 2.



Scheme 3. Synthesis of alkoxy derivatives **17**–**28**.



Scheme 4. Synthesis of various amine derivatives.

Because of the promising activity of the *p*- and *m*-chloro-substituted compounds, we chose to synthesize the isosteric methoxy derivatives. These methoxy derivatives **17** and **18** showed very promising anti-HSV activity and we became interested in synthesizing additional alkoxy analogs (Scheme 3). For the synthesis of these, the methoxy derivatives **17** and **18** were demethylated using BBr_3 in dichloromethane to give the corresponding phenols **19** and **20**. The phenols were alkylated with a variety of alkyl bromides in the presence of Cs_2CO_3 or K_2CO_3 to give alkoxy derivatives **21**–**27**. The bipenyl ether **28** was synthesized by condensing the *p*-phenol derivative **19** with phenylboronic acid in the presence of $Cu(OAc)_2$ and Et_3N using conditions similar to those described by Evans et al.[19]

To further explore electron-donating substituents on the 2-phenyl portion of the pyrazolopyridines, the bromo derivatives **11** and **12** were used to access amine derivatives **29** and **30** (Scheme 4). These were synthesized via Buchwald amination, where the bromo derivatives **11** and **12**, respectively, were treated with benzophenone imine, Pd_2dba_3, and BINAP in the presence of base to give imines that were subsequently hydrolyzed in aqueous acid to give the desired amines **29** and **30**. To further investigate how changes in the basicity and electron-donating effect of the amine would affect anti-viral activity, the acetylated and sulfonylated derivatives **31** and **32** were synthesized. In addition, prepared from **29** were alkylamine derivatives **33** and **34**.

To explore the steric tolerance around the 2-phenyl moiety, biphenyl derivatives **35** and **36** were prepared from bromo derivatives **11** and **12** and phenyl boronic acids using Suzuki coupling methodology (Scheme 5).[20]

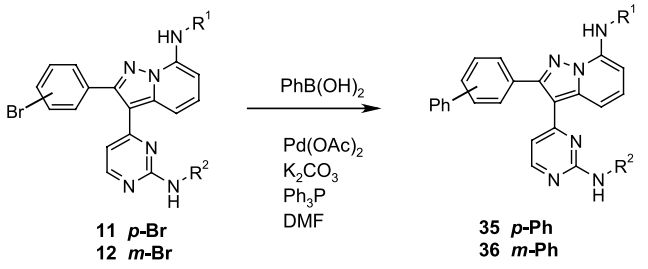
Finally, we were interested in the synthesis of a carboxylic acid derivative, to investigate how a negative charge at physiological pH would affect the anti-viral activity. We opted to synthesize the carboxylic acid via nitrile derivative **37** (Scheme 6). The nitrile derivative **37** was synthesized by palladium (Pd_2dba_3)-mediated coupling of **12** and $Zn(CN)_2$ using conditions similar to those previously described in the literature.[21] Treatment of **37** with KOH at elevated temperature gave the desired acid **38**. Additionally, treatment of **37** with ammonium hydroxide and hydrogen peroxide gave the amide **39**.[22]

3. Results and discussion

The new pyrazolopyridine derivatives were evaluated for activity against HSV-1 (SC-16) and for cytotoxicity in Vero cells (Table 1). Several of the pyrazolopyridine analogs in Table 1 show similar or better anti-HSV activity than acyclovir.

The choice of R^1 and R^2 as cyclopentyl or *n*-butyl does not significantly affect the antiviral activity (compounds **5**, **6**, and **7** in Table 1). On the other hand, the choice of substituent on the 2-phenyl can significantly change the antiviral activity as well as the toxicity profile of the pyrazolopyridines. In general, small substituents appear to be preferred over large substituents, thus halogens, alkyl, alkoxy, and cyano substituents gave compounds with better anti-viral activity than bulky phenyl (**35** and **36**) or amine (**31** and **32**) substituents. Hydrophobic substituents were also generally preferred over hydrophilic substituents. Thus, halogen (**5–13**) and alkoxy (**17–26**) substituted derivatives showed better activity than amino (**29–34**) or carboxyl (**38–39**) substituted derivatives. Electronic nature of the substituent did not affect the antiviral activity to a significant extent. Strongly donating groups such as methoxy did not give compounds with better antiviral activity than strongly withdrawing substituents such as fluorine. In addition, for smaller substituents whether the substituent was at the *para* or *meta* position did not make significant difference (e.g., methoxy derivatives **17** and **18** or fluoro derivatives **5** and **8** showed similar activity) (Table 1).

A selected set of halogenated and alkoxy derivatives that showed good activity against HSV-1 and good separation between anti-HSV activity and toxicity along with promising developability profile were tested for activity against HSV-2 (Table 2).



Scheme 5. Synthesis of biphenyl derivatives.

The pyrazolopyridines showed similar activity against HSV-1 and HSV-2 in Vero cells. Several of the compounds showed activity similar to acyclovir against HSV-1 and HSV-2. Several representative compounds have also been tested for the activity against other viruses (e.g., HIV and HBV) and have not shown significant activity. We are continuing to investigate the SAR of these novel pyrazolopyridines and these studies will be the subject of future publications.

4. Conclusions

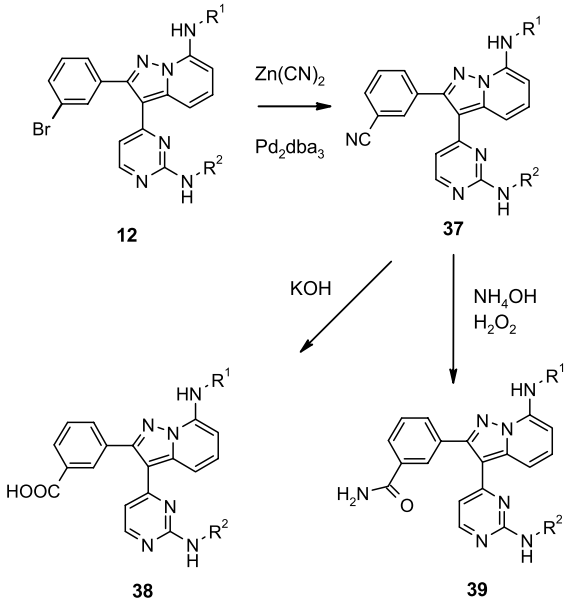
In this study, we have described the synthesis of a diverse set of substituted 2-phenylpyrazolopyridines. Halogen- and alkoxy-substituted derivatives were identified as the most promising leads.

Several of these novel compounds show similar activity against HSV-1 and HSV-2 to that of the gold standard acyclovir. Furthermore, this new series of inhibitors are not DNA synthesis inhibitors, like the currently marketed nucleoside analogs.[23] This new class of inhibitors shows potent antiviral activity separated from cytotoxicity that suggest its potential use as a therapeutic intervention in HSV-infected patients.

5. Experimental

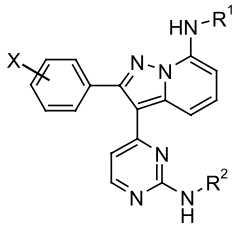
5.1. General

^1H and ^{13}C NMR spectra were obtained on Varian Unity Plus NMR spectrometers at 300 or 400 MHz and 75 or 100 MHz, respectively. ^{19}F NMR spectra were recorded at 282 MHz. Mass spectra were obtained on Micromass Platform or ZMD mass spectrometers from Micromass (Altrincham, UK), using either atmospheric



Scheme 6. Synthesis of nitriles, acids, and amides.

Table 1. Activity against HSV-1 (SC-16 strain) and cytotoxicity in Vero cells



Compound	X	R^1	R^2	IC_{50} (μM)	CC_{50} (μM)
5	*p*-F	c-Pent	c-Pent	0.26 (0.01)	>160
6	*p*-F	n-Bu	n-Bu	0.51 (0.05)	>250
7	*p*-F	n-Bu	c-Pent	0.41 (0.06)	>40
8	*m*-F	c-Pent	c-Pent	0.23 (0.01)	>40
9	*p*-Cl	c-Pent	c-Pent	0.86 (0.25)	>40
10	*m*-Cl	c-Pent	c-Pent	0.17 (0.03)	>40
11	*p*-Br	n-Bu	n-Bu	2.82 (1.14)	>40
12	*m*-Br	c-Pent	c-Pent	0.31 (0.06)	>100
13	*o*-Br	c-Pent	c-Pent	0.85 (0.06)	>40
14	H	c-Pent	c-Pent	0.62 (0.11)	>40
15	*m*-CH_3	c-Pent	c-Pent	0.22 (0.03)	>40
16	*m*-CF_3	c-Pent	c-Pent	0.27 (0. 05)	>40
17	*p*-OCH_3	c-Pent	c-Pent	0.22 (0.02)	>100
18	*m*-OCH_3	c-Pent	c-Pent	0.20 (0.02)	>40
19	*p*-OH	c-Pent	c-Pent	10.1 (4.6)	>50
20	*m*-OH	c-Pent	c-Pent	0.66 (0.02)	>40
21	*p*-$OCH_2CH{=}CH_2$	c-Pent	c-Pent	0.20 (0.03)	>100
22	*p*-O-n-Bu	c-Pent	c-Pent	3.13 (0.40)	>100
23	*p*-O-sec-Bu	c-Pent	c-Pent	0.58 (0.05)	>100
24	*p*-OCH_2c-Pr	c-Pent	c-Pent	0.17 (0.02)	>100
25	*m*-OCH_2c-Pr	c-Pent	c-Pent	0.17 (0.04)	>40
26	*p*-OCH_2c-Bu	c-Pent	c-Pent	0.35	>100
27	*p*-OCH_2CO_2Et	c-Pent	c-Pent	3.29 (0.21)	>100
28	*p*-OPh	c-Pent	c-Pent	0.90 (0.39)	>40
29	*p*-NH_2	n-Bu	n-Bu	1.07	19
30	*m*-NH_2	c-Pent	c-Pent	0.64 (0.02)	
31	*m*-$NHCOCH_3$	c-Pent	c-Pent	2.26 (0.13)	>40
32	*m*-$NHSO_2CH_3$	c-Pent	c-Pent	2.09 (0.45)	>40
33	p-NH-c-Hexyl	n-Bu	n-Bu	3.24 (0.81)	>40
34	*p*-$N(CH_3)_2$	n-Bu	n-Bu	1.70 (0.46)	
35	*p*-Ph	n-Bu	n-Bu	2.79 (0.47)	
36	*m*-Ph	c-Pent	c-Pent	2.18 (0.64)	>40
37	*m*-CN	c-Pent	c-Pent	0.28 (0.04)	>20
38	*m*-COOH	c-Pent	c-Pent	3.08 (0.37)	>40
39	*m*-$CONH_2$	c-Pent	c-Pent	1.67 (0.32)	>30
1	ACV			0.39 (0.01)	>200

Vero cells, SC-16 strain. IC_{50} is the concentration at which 50% efficacy in the anti-viral assay is observed, standard error is shown in brackets. CC_{50} is the concentration at which 50% cytotoxicity is observed. c-Pent is cyclopentyl; n-Bu is *n*-butyl.

chemical ionization (APCI) or electrospray ionization (ESI). Solvents were purchased as anhydrous grade and used without further purification. 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 free-base form unless otherwise stated. On occasion, the corresponding hydrochloride salts were formed to generate solids as noted. Combustion analyses were performed by Atlantic Microlabs (Norcross, GA, USA). In general, compound purity was assessed by high field ^1H NMR and HPLC. Biological results were obtained with compounds of >98% purity as determined by the above methods.

Table 2. Activity of selected compounds against HSV-1 (SC-16 strain in vero cells) and HSV-2

Compound	IC_{50} (HSV-1) (μM)	IC_{50} (HSV-2) (μM)	CC_{50} (μM)
5	0.26	0.2	>160
12	0.31	0.25	>100
17	0.22	0.20	>100
18	0.20	0.12	>40
23	0.58	0.50	>100
24	0.17	0.1	>100
26	0.35	0.21	>100
1 (ACV)	0.39	0.23	>200

IC_{50} is the concentration at which 50% efficacy in the anti-viral assay is observed. CC_{50} is the concentration at which 50% cytotoxicity is observed.

5.2. General procedure I (Scheme 1)

5.2.1. Synthesis of 2-(6-chloro-2-pyridinyl)-1-phenylethanone derivatives (D). To a cold (0 °C) solution of 6-chloro-2-picoline (**B**, 1 equiv.) and ethyl benzoate (**C**, 2 equiv.) in THF, lithium bis(trimethylsilyl)amide (2 equiv. in THF) was added dropwise *via* a pressure equalizing funnel over 1 h. Upon complete addition, the cold bath was removed and the resultant solution was heated at 45 °C until the reaction was complete as judged by TLC. The mixture was cooled to room temperature and quenched by the addition of water. Ether was added and the organic layer was washed with brine. The aqueous layer was extracted with ether, and the combined organics were dried over magnesium sulfate. Filtration and concentration gave a product **D** that was purified by recrystallization or silica gel chromatography.

5.2.2. Synthesis of 2-(6-chloro-2-pyridinyl)-1-phenylethanone oximes (E). To a solution of ketone **D** (1 equiv.) in MeOH (~10 mL/g), hydroxylamine hydrochloride (5 equiv.) was added followed by NaOH (5 equiv., 10% aqueous). The resultant suspension was heated to reflux until complete by TLC and then cooled to room temperature. The mixture was concentrated in vacuo and the residue taken up in ether and water. The organic layer was washed with brine. The aqueous layer was extracted with ether, and the combined organics were dried over magnesium sulfate. Filtration and concentration gave a residue that was purified by recrystallization or silica gel chromatography to give **E**.

5.2.3. Formation of 7-chloro-2-(phenyl)pyrazolo[1,5-*a*]-pyridines (F). To a solution of **E** (1 equiv.) in 1,2-dimethoxyethane (1 mL/g) at 0 °C was added trifluoroacetic anhydride (1 equiv.), keeping the temperature below 10 °C. After the addition was complete, the reaction was allowed to warm to room temperature. The solution was then cooled to 4 °C and a solution of triethylamine (2 equiv.) in 1,2-dimethoxyethane was added over 0.5 h. After warming to room temperature, the mixture was stirred until the starting material was consumed. To this, iron(II)chloride (0.1 equiv.) was added, and the reaction was heated to reflux for 8 h. The reaction was concentrated, and the resulting solid was purified by recrystallization or silica gel chromatography to give **F**.

5.2.4. 1-[7-Chloro-2-phenylpyrazolo[1,5-*a*]pyridin-3-yl]-ethanone (G). To a solution of **F** (1 equiv.) in toluene (22 mL/g) at room temperature, acetic anhydride (1.25 equiv.) was added. Boron trifluoride diethyletherate (1.1 equiv.) was then added dropwise, and the resultant solution was heated to reflux until complete as determined by TLC. The reaction mixture was cooled to room temperature and quenched by the dropwise addition of aqueous sodium bicarbonate. Ether was added and the organic layer was washed with brine. The aqueous layer was extracted with ether, and the combined organics were dried over magnesium sulfate, filtered, and concentrated. The residue was purified by recrystallization or silica gel chromatography to give **G**.

5.2.5. 1-[7-Cyclopentylamino-2-phenylpyrazolo[1,5-*a*]-pyridin-3-yl]ethanone (H). To a solution of ketone **G** (1 equiv.) in toluene (10 mL/g) was added successively *racemic*-BINAP (0.06 equiv.), cesium carbonate (1.5 equiv.), cyclopentylamine (5 equiv.), and palladium (II) acetate (0.04 equiv.). The resultant mixture was heated at 100 °C until the reaction was as complete judged by TLC. The solution was cooled to room temperature and ether was added. The organic layer was washed with water and brine. The aqueous layer was extracted with ether, and the combined organics dried over magnesium sulfate. Filtration and concentration followed by silica gel chromatography gave the desired compound **H** (where R^1 is cyclopentyl).

5.2.6. 1-[7-Cyclopentylamino-2-phenylpyrazolo[1,5-*a*]-pyridin-3-yl]-3-(dimethylamino)-2-propen-1-one (I). A solution of **H** (1 equiv.) in *N*,*N*-dimethylformamide dimethyl acetal (8 mL/g) was heated to reflux for 6 days. The mixture was cooled to room temperature, and EtOAc was added followed by water. The organic layer was washed with brine. The aqueous layer was extracted with EtOAc, and the combined organics were dried over magnesium sulfate. Filtration and concentration followed by silica gel chromatography (EtOAc) provided the desired vinylogous amide **I**.

5.2.7. Synthesis of guanidines (J). *N*-cyclopentylguanidine hydrochloride[16] and *N*-butylguanidine sulfate[24] were synthesized as described in the literature.

5.2.8. *N*-Cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-phenylpyrazolo[1,5-*a*]pyridin-7-amine (A). To a solution of **I** (1 equiv.) in EtOH (10 mL/g) the desired guanidine (**J**) (1.2 equiv.) was added, followed by anhydrous K_2CO_3 (2.5 equiv.). The resultant solution was heated at reflux until reaction was complete. Upon cooling to room temperature, ether was added followed by water. The organics were washed with brine, and the aqueous layer was extracted with ether. The combined organics were dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography to give the desired product **A**.

5.3. General procedure II (Scheme 2)

5.3.1. 7-Chloro-2-phenylpyrazolo[1,5-*a*]pyridine-3-carbaldehydes (K). DMF (5–10 mL/g) was cooled to 0 °C and treated with phosphorous oxychloride (1.5–2 equiv.). After the addition was complete, the mixture was warmed to room temperature and stirred for 1 h. To this, the desired **F** (1 equiv.) was added and the resultant solution was stirred overnight. Water was added, followed by dichloromethane. The aqueous layer was extracted with dichloromethane. The combined organics were washed with brine, dried over magnesium sulfate, filtered, and concentrated. The residue was recrystallized to give the desired aldehyde **K**.

5.3.2. 1-[7-Chloro-2-phenylpyrazolo[1,5-*a*]pyridin-3-yl]-2-propyn-1-ol (L). To a cold (−78 °C) solution of aldehyde

K (1 equiv.) in THF (0.15 M) was added alkynyl magnesium bromide (2.5 equiv.) in THF (0.5–1.0 M) dropwise. The resulting mixture was allowed to warm to 0 °C and stirred at that temperature until the reaction was complete as judged by TLC or LC/MS methods. The resultant solution was poured into water and extracted with ether. The organic layer was washed with water and brine, and the combined organics were dried over sodium sulfate. Filtration and concentration followed by flash chromatography or recrystallization provide the desired alcohol **L**.

5.3.3. 1-[7-Chloro-2-phenylpyrazolo[1,5-*a*]pyridin-3-yl]-2-propyn-1-one (M). To a solution of alcohol **L** (1 equiv.) in CH_2Cl_2 (0.05 M), manganese dioxide (40 equiv.) was added. The reaction mixture was stirred at room temperature until complete as judged by TLC or LC/MS methods. The suspension was filtered through a pad of Celite, and the filtrate was concentrated in vacuo to give ketone **M**. This material is used without further purification.

5.3.4. 4-[7-Chloro-2-phenylpyrazolo[1,5-*a*]pyridin-3-yl]-*N*-cyclopentyl-2- pyridinamine (N). To a dry round bottom flask added sodium metal (1.3 equiv.). Ethanol (0.15 M) was added and allowed to react with sodium at room temperature until completely dissolved. Guanidinium salt **J** (1.3 equiv.) was added, and the mixture was allowed to stir at room temperature for 10 min. To the resultant mixture, ketone **M** (1 equiv.) was added, and the reaction mixture was stirred at room temperature or heated to 70 °C until complete as judged by TLC or LC/MS. The reaction mixture was cooled to room temperature and diluted with water. The mixture was extracted with ethyl acetate, and the combined extracts were washed with water and brine. The organic layer was dried over sodium sulfate. Filtration and concentration followed by flash chromatography or recrystallization provided pyrimidine **N**. An alternative method involved heating a solution of alkynyl ketone **M** (1 equiv.) guanidine **J** (1.3 equiv.), and K_2CO_3 (1.3 equiv.) in NMP or DMF (0.15 M) at 120 °C to give **N**.

5.3.5. *N*-Cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-phenylpyrazolo[1,5-*a*]pyridin-7-amine (A). To a solution of chloride **N** (1 equiv.) in toluene (0.2 M), successively racemic-BINAP (6 mol%), cesium carbonate (1.5 equiv.), cyclopentylamine (5 equiv.), and palladium (II) acetate (4 mol%) were added. The resultant mixture was heated to 95 °C until the reaction was complete as judged by TLC or LC/MS. The solution was cooled to room temperature and ether was added. The organic layer was washed with water and brine. The aqueous layer was extracted with ether and the combined organics dried over sodium sulfate. Purification by flash chromatography or recrystallization provided 7-amino derivative **A** (where R^1 is cyclopentyl).

Alternatively, chloride **N** was heated in either neat amine at 130–150 °C or a solution of excess amine in ethanol at reflux followed by similar workup to provide the desired 7-amino derivative **A**.

5.4. *N*-Butyl-3-[2-(butylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridin-7-amine (6)

5.4.1. 1-[7-(Butylamino)-2-(4-fluorophenyl)pyrazolo-[1,5-*a*]pyridin-3-yl]ethanone (40). 1-[7-Chloro-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridin-3-yl]ethanone (969 mg, 3.4 mmol) was treated according to the procedure I to give after flash chromatography (2:1, hexanes/ethyl acetate) **40** (921 mg, 84%) as an oil. ^{1}H NMR ($CDCl_3$): δ 7.71 (d, 1H), 7.63 (dd, 2H), 7.49 (t, 1H), 7.23 (t, 2H), 6.16 (d, 1H), 6.10 (m, 1H), 3.40 (m, 2H), 2.18 (s, 3H), 1.78 (m, 2H), 1.52 (m, 2H), 1.02 (t, 3H); MS *m*/*z* 326 (M + 1); R_f 0.6 (1:1 hexanes/ethyl acetate).

5.4.2. (2*E*)-1-[7-(Butylamino)-2-(4-fluorophenyl)-pyrazolo-[1,5-*a*]pyridin-3-yl]-3-(dimethylamino)-2-propen-1-one (41). 1-[7-(Butylamino)-2-(4-fluorophenyl)-pyrazolo[1,5-*a*]pyridin-3-yl]ethanone (**40**, 880 mg, 2.7 mmol) was treated as described in the general procedure I to give **41** (971 mg, 95%) as yellow foam. ^{1}H NMR ($CDCl_3$): δ 7.67 (dd, 2H), 7.60 (d, 1H), 7.53 (d, 1H), 7.27 (t, 1H), 7.09 (t, 2H), 5.96–5.93 (m 2H), 5.01 (d, 1H), 3.30 (m, 2H), 3.10–2.30 (br, 6H), 1.69 (m, 2H), 1.43 (m, 2H), 0.93 (t, 3H); MS *m*/*z* 381 (M + 1).

5.4.3. *N*-Butyl-3-[2-(butylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridin-7-amine (6). In a similar manner as described in the general procedure I, from **41** (34 mg, 0.09 mmol) and *N*-butylguanidine sulfate, **6** (33 mg, 85%) was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.98 (d, 1H), 7.68 (d, 1H), 7.59 (m, 2H), 7.27 (t, 1H), 7.10 (t, 2H), 6.25 (d, 1H), 6.00–5.96 (m, 2H), 5.05 (m, 1H), 3.41 (m, 2H), 3.33 (m, 2H), 1.71 (m, 2H), 1.60 (m, 2H), 1.50–1.36 (m, 4H), 0.97–0.91 (m, 6H); MS *m*/*z* 433 (M + 1); R_f 0.67 (1:1, hexanes/ethyl acetate).

5.5. *N*-Butyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-fluorophenyl)pyrazolo[1,5-*a*]pyridin-7-amine (7)

In a similar manner as described in the general procedure I, from **41** (258 mg, 0.68 mmol) and *N*-cyclopentylguanidine, **7** (260 mg, 87%) was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 8.01 (d, 1H), 7.73 (d, 1H), 7.62 (m, 2H), 7.29 (t, 1H), 7.12 (t, 2H), 6.28 (d, 1H), 6.05 (m, 1H), 5.98 (d, 1H), 5.27 (br, 1H), 4.32 (m, 1H), 3.34 (m, 2H), 2.10–2.00 (m, 2H), 1.77–1.41 (m, 10H), 0.97 (t, 3H); ^{13}C NMR ($CDCl_3$) δ 163.03 (d, J_{CF} = 244.5 Hz), 162.14, 161.41, 156.74, 152.10, 142.99, 141.02, 131.36 (d, J_{CF} = 8.0 Hz) 129.92 (d, J_{CF} = 3.0 Hz), 128.31, 115.36 (d, J_{CF} = 21.0 Hz), 108.58, 107.9, 105.17, 89.21, 52.79, 42.27, 33.40, 31.06, 23.65, 20.13, 13.70; ^{19}F NMR ($CDCl_3$) δ −113.42; MS *m*/*z* 445 (M + 1).

5.6. *N*-Cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(3-fluorophenyl)pyrazolo[1,5-*a*]pyridin-7-amine (8)

Compound **8** was obtained according to the general procedure I. R_f 0.43 (99:1, dichloromethane/methanol); ^{1}H NMR ($CDCl_3$): δ 8.06 (d, 1H), 7.76 (d, 1H), 7.47–7.30 (m, 4H), 7.17 (m, 1H), 6.35 (d, 1H), 6.10–6.04 (m, 2H), 5.15 (d, 1H), 4.36 (m, 1H), 4.05 (m, 1H), 2.21–2.07 (m, 4H), 1.88–1.54 (m, 12H); MS *m*/*z* 457 (M + 1).

5.7. 2-(4-Chlorophenyl)-*N*-cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (9)

Compound **9** was obtained according to procedure I. ^{1}H NMR ($CDCl_3$): δ 8.02 (d, 1H), 7.72 (m, 1H), 7.60 (d, 2H), 7.41 (d, 2H), 7.35 (m, 1H), 6.31 (d, 1H), 6.03 (m, 2H), 5.17 (br, 1H), 4.32 (m, 1H), 4.00 (m, 1H), 2.18–2.05 (m, 4H), 1.82–1.50 (m, 12H). MS *m*/*z* 474 (M + 1).

5.8. 2-(3-Chlorophenyl)-*N*-cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (10)

Compound **10** was prepared according to procedure I as a yellow solid. R_f 0.48 (49:1 dichloromethane/methanol); ^{1}H NMR (DMSO-d_6): δ 8.02 (d, 1H), 7.73–7.67 (br, 2H), 7.56–7.44 (m, 2H), 7.36 (t, 1H), 7.01 (d, 1H), 6.61 (d, 1H), 6.22–6.17 (m, 2H), 4.09 (br, 1H), 3.98 (m, 1H), 2.04 (m, 2H), 1.84 (m, 2H), 1.72–1.48 (m, 12H); MS *m*/*z* 473 (M + 1). Anal. Calcd for $C_{27}H_{29}ClN_6$: C, 68.56; H, 6.18; N, 17.77. Found: C, 68.55; H, 6.20; N, 17.64.

5.9. 2-(4-Bromophenyl)-*N*-butyl-3-[2-(butylamino)-4-pyrimidinyl]pyrazolo-[1,5-*a*]pyridin-7-amine (11)

5.9.1. 1-(4-Bromophenyl)-2-(6-chloro-2-pyridinyl)ethanone (42). In a similar manner as described in the general procedure I, from ethyl 4-bromobenzoate (12.8 mL, 78.3 mmol) and 6-chloro-2-picoline (4.3 mL, 39.2 mmol), **42** (9.6 g, 82%) was obtained as a crystalline solid existing as a keto–enol tautomeric mixture. ^{1}H NMR ($CDCl_3$): for the keto tautomer δ 7.95 (d, 2H), 7.74–7.56 (m, 3H), 7.27 (m, 2H), 4.47 (s, 2H); MS *m*/*z* 310 (M + 1).

5.9.2. 1-(4-Bromophenyl)-2-(6-chloro-2-pyridinyl)-ethanone oxime (43). In a similar manner as described in the procedure I, from **42** (9.5 g, 30.6 mmol), **43** (10.0 g, 99%) was obtained as a white solid. ^{1}H NMR ($CDCl_3$): δ 9.78 (br, 1H), 7.74–7.47 (m, 5H), 7.21–7.17 (m, 2H), 4.39 (s, 2H); MS *m*/*z* 325 (M + 1).

5.9.3. 2-(4-Bromophenyl)-7-chloropyrazolo[1,5-*a*]pyridine (44). In a similar manner as described in the procedure I, from **43** (45.2 g, 139 mmol), **44** (30.5 g, 72%) was obtained as a pale yellow crystalline solid. ^{1}H NMR ($CDCl_3$): δ 7.85 (dd, 2H), 7.54 (dd, 2H), 7.46 (d, 1H), 7.04 (m, 1H), 6.87 (m, 2H); MS *m*/*z* 307 (M + 1).

5.9.4. 1-[2-(4-Bromophenyl)-7-chloropyrazolo[1,5-*a*]-pyridin-3-yl]ethanone (45). In a similar manner as described in the general procedure I, from **44** (10.0 g, 32.5 mmol), **45** (7.63 g, 67%) was obtained as pink needles. ^{1}H NMR ($CDCl_3$): δ 8.37 (d, 1H), 7.62 (d, 2H), 7.43 (m, 3H), 7.14 (d, 1H), 2.13 (s, 3H); MS *m*/*z* 349 (M + 1).

5.9.5. 1-[2-(4-Bromophenyl)-7-(butylamino)pyrazolo[1,5-*a*]pyridin-3-yl]ethanone (46). In a similar manner as described in the general procedure I, from **45** (2.55 g, 7.3 mmol), **46** (2.15 g, 76%) was obtained as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.61 (m, 3H), 7.43 (m, 3H), 6.08 (d, 1H), 6.02 (bs, 1H), 3.33 (q, 2H), 2.12 (s, 3H), 1.70 (m, 2H), 1.44 (m, 2H), 0.94 (t, 3H); MS *m*/*z* 386 (M + 1).

5.9.6. (2*E*)-1-[2-(4-Bromophenyl)-7-(butylamino)-pyrazolo[1,5-*a*]pyridin-3-yl]-3-(dimethylamino)-2-propen-1-one (47). In a similar manner as described in the general procedure I, from **46** (5.5 g, 14.2 mmol), **47** (5.78 g, 92%) was obtained as a brown oil. ^{1}H NMR ($CDCl_3$): δ 7.57 (m, 6H), 7.28 (t, 1H), 5.95 (m, 2H), 5.03 (d, 1H), 3.32 (q, 2H), 2.92 (bs, 3H), 2.52 (bs, 3H), 1.71 (m, 2H), 1.44 (m, 2H), 0.94 (t, 3H); MS *m*/*z* 441 (M + 1).

5.9.7. 2-(4-Bromophenyl)-*N*-butyl-3-[2-(butylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (11). In a similar manner as described in the general procedure I, from **47** (5.78 g, 13.1 mmol) and *N*-butylguanidine sulfate, **11** (5.18 g, 80%) was obtained as a pale yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.98 (d, 1H), 7.71 (d, 1H), 7.56 (m, 4H), 7.33 (t, 1H), 6.35 (d, 1H), 6.03 (m, 2H), 3.46 (q, 2H), 3.38 (q, 2H), 1.81–1.40 (m, 8H), 0.98 (m, 6H); MS *m*/*z* 493 (M + 1).

5.10. 2-(3-Bromophenyl)-*N*-cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (12)

5.10.1. 1-(3-Bromophenyl)-2-(6-chloro-2-pyridinyl)ethanone (48). In a similar manner as described in the procedure I, from ethyl 3-bromobenzoate (50.6 g, 220 mmol) and 6-chloro-2-picoline (24 mL, 220 mmol), **48** (59.4 g, 87%) was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.96 (br s, 1H), 7.71 (d, 1H), 7.35–7.25 (m, 3H), 6.98 (t, 1H), 6.81 (d, 1H), 6.58 (d, 1H), 5.84 (s, 1H); MS *m*/*z* 310 (M + 1).

5.10.2. 1-(3-Bromophenyl)-2-(6-chloro-2-pyridinyl)ethanone oxime (49). In a similar manner as described in the procedure I, from **48** (59.1 g, 190 mmol), **49** (58.3 g, 94%) was obtained as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.96 (s, 1H), 7.67–7.50 (m, 3H), 7.28–7.18 (m, 3H), 4.80 (br, 1H), 4.39 (s, 2H); MS *m*/*z* 325 (M + 1).

5.10.3. 2-(3-Bromophenyl)-7-chloropyrazolo[1,5-*a*]pyridine (50). In a similar manner as described in the procedure I, from **49** (59.1 g, 181.5 mmol), **50** (24.5 g, 44%) was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 8.13 (dd, 1H), 7.90 (d, 1H), 7.47 (m, 2H), 7.29 (t, 1H), 7.05 (t, 1H), 6.88 (m, 2H); MS *m*/*z* 307 (M + 1).

5.10.4. 1-[2-(3-Bromophenyl)-7-chloropyrazolo[1,5-*a*]-pyridin-3-yl]ethanone (51). In a similar manner as described in the procedure I, from **50** (16.5 g, 53.6 mmol), **51** (8.6 g, 46%) was obtained as pinkish needles. ^{1}H NMR ($CDCl_3$): δ 8.46 (d, 1H), 7.83 (s, 1H), 7.69 (d, 1H), 7.59–7.40 (m, 3H), 7.22 (d, 1H), 2.21 (s, 3H); MS *m*/*z* 349 (M + 1).

5.10.5. 1-[2-(3-Bromophenyl)-7-(cyclopentylamino)-pyrazolo[1,5-*a*]pyridin-3-yl]ethanone (52). In a similar manner as described in the procedure I, from **51** (3.00 g, 8.6 mmol), **52** (1.90 g, 56%) was obtained as a yellow syrup. ^{1}H NMR ($CDCl_3$): δ 7.74 (dd, 1H), 7.61–7.57 (m, 2H), 7.50 (d, 1H), 7.41 (t, 1H), 7.32 (t, 1H), 6.10 (dd, 1H), 5.99 (d, 1H), 3.95 (m, 1H), 2.12–2.05 (m, 5H), 1.78–1.63 (m, 6H); MS *m*/*z* 398 (M + 1).

5.10.6. (2*E*)-1-[2-(3-Bromophenyl)-7-(cyclopentylamino)-pyrazolo[1,5-*a*]pyridin-3-yl]-3-(dimethylamino)-2-propen-1-one (53). In a similar manner as described in the procedure I, from **52** (1.90 g, 4.8 mmol), **53** (1.87 g, 86%) was obtained as a brown syrup. ^{1}H NMR ($CDCl_3$): δ 7.88 (s, 1H) 7.64–7.49 (m, 4H), 7.30–7.24 (m, 2H), 6.00 (d, 1H), 5.93 (d, 1H), 5.03 (d, 1H), 3.95 (m, 1H), 3.10–2.35 (br, 6H), 2.10–2.06 (m, 2H), 1.77–1.62 (m, 6H); MS *m*/*z* 455 (M + 1).

5.10.7. 2-(3-Bromophenyl)-*N*-cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (12). In a similar manner as described in the procedure I, from **53** (500 mg, 1.1 mmol) and *N*-cyclopentylguanidine, the title compound **12** (500 mg, 88%) was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 8.00 (d, 1H), 7.83 (s, 1H), 7.65 (d, 1H), 7.51 (m, 2H), 7.29–7.22 (m, 2H), 6.28 (d, 1H), 6.00 (d, 1H), 5.96 (d, 1H), 5.00 (d, 1H), 4.27 (m, 1H), 3.97 (m, 1H), 2.11–2.00 (m, 4H), 1.79–1.46 (m, 12H); MS *m*/*z* 517 (M + 1).

5.11. 2-(2-Bromophenyl)-*N*-cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (13)

5.11.1. 1-(2-Bromophenyl)-2-(6-chloro-2-pyridinyl)-ethanone (54). In a similar manner as described in the procedure I, from ethyl 2-bromobenzoate (50.0 g, 218 mmol) and 6-chloro-2-picoline (24 mL, 218 mmol), **54** (52.4 g, 77%) was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.53 (d, 1H), 7.45 (d, 1H), 7.25 (d, 1H), 7.05 (d, 1H), 6.97 (d, 1H), 6.67 (d, 1H), 6.53 (d, 1H), 5.28 (s, 1H); MS *m*/*z* 310 (M + 1).

5.11.2. 1-(2-Bromophenyl)-2-(6-chloro-2-pyridinyl)-ethanone oxime (55). In a similar manner as described in the procedure I, from **54** (52.4 g, 169 mmol) **55** (36.5 g, 66%) was obtained as a pale yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.50–7.45 (m, 2H), 7.23–7.07 (m, 6H), 4.29 (s, 2H); MS *m*/*z* 325 (M + 1).

5.11.3. 2-(2-Bromophenyl)-7-chloropyrazolo[1,5-*a*]pyridine (56). In a similar manner as described in the procedure I, from **55** (36.5 g, 112 mmol), **56** (21.0 g, 61%) was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.83 (dd, 1H), 7.65 (d, 1H), 7.51 (d, 1H), 7.37 (t, 1H), 7.21 (m, 1H), 7.07 (m, 2H), 6.89 (d, 1H); MS *m*/*z* 307 (M + 1).

5.11.4. 1-[2-(2-Bromophenyl)-7-chloropyrazolo[1,5-*a*]-pyridin-3-yl]ethanone (57). In a similar manner as described in the procedure I, from **56** (21.0 g, 68.3 mmol), **57** (15.7 g, 66%) was obtained as orange needles. ^{1}H NMR ($CDCl_3$): δ 8.48 (dd, 1H), 7.72 (d, 1H), 7.49–7.36 (m, 4H), 7.18 (dd, 1H), 2.06 (s, 3H); MS *m*/*z* 349 (M + 1).

5.11.5. 1-[2-(2-Bromophenyl)-7-(cyclopentylamino)pyrazolo[1,5-*a*]pyridin-3-yl]ethanone (58). In a similar manner as described in the procedure I, from **57** (3.00 g, 8.6 mmol), **58** (0.93 g, 27%) was obtained as a yellow syrup. ^{1}H NMR ($CDCl_3$): δ 7.73–7.68 (m, 2H), 7.43 (m, 3H), 7.35–7.31 (m, 1H), 6.12 (d, 1H), 5.98 (d, 1H), 3.95 (m, 1H), 2.08 (m, 2H), 1.98 (s, 3H), 1.76–1.53 (m, 6H); MS *m*/*z* 398 (M + 1).

5.11.6. (2*E*)-1-[2-(2-Bromophenyl)-7-(cyclopentylamino)pyrazolo[1,5-*a*]pyridin-3-yl]-3-(dimethylamino)-2-propen-1-one (59). In a similar manner as described in the procedure I, from **58** (0.93 g, 2.3 mmol), **59** (0.57 g, 54%) was obtained as a brown syrup. ^{1}H NMR ($CDCl_3$): δ 7.78 (d, 1H), 7.66 (d, 1H), 7.53 (d, 1H), 7.46 (m, 1H), 7.39 (t, 1H), 7.31–7.22 (m, 2H), 6.02 (d, 1H), 5.85 (d, 1H), 4.80 (d, 1H), 3.95 (m, 1H), 2.90 (br s, 3H), 2.30 (br s, 3H), 2.08 (m, 2H), 1.77–1.63 (m, 6H); MS *m*/*z* 455 (M + 1).

5.11.7. 2-(2-Bromophenyl)-*N*-cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (13). In a similar manner as described in the procedure I, from **59** (200 mg, 0.46 mmol), **13** (110 mg, 48%) was obtained as a yellow foam. ^{1}H NMR ($CDCl_3$): δ 7.90 (m, 2H), 7.66 (d, 1H), 7.44 (m, 1H), 7.39 (t, 1H), 7.32–7.27 (m, 2H), 6.04–5.96 (m, 3H), 5.02 (m, 1H), 4.21 (m, 1H), 3.95 (m, 1H), 2.10–1.96 (m, 4H), 1.75–1.43 (m, 12H); MS *m*/*z* 517 (M + 1).

5.12. *N*-Cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-phenylpyrazolo[1,5-*a*]pyridin-7-amine (14)

To a solution of **13** (100 mg, 0.19 mmol) in toluene (10 mL) tributyltin hydride (112 mg, 0.39 mmol) and 2,2′-azobisisobutyronitrile (9.4 mg, 0.057 mmol) were added. After heating at reflux for 4 h, the reaction mixture was allowed to cool to room temperature. Concentration followed by purification with flash chromatography (40:60, ethyl acetate/hexanes) gave **14** as a yellow foam (39 mg, 46%). ^{1}H NMR ($CDCl_3$): δ 7.98 (d, 1H), 7.78 (d, 1H), 7.64 (m, 2H), 7.45 (m, 2H), 7.33–7.26 (m, 2H), 6.27 (d, 1H), 6.04 (m, 2H), 5.12 (d, 1H), 4.35 (m, 1H), 4.00 (m, 1H), 2.09–2.03 (m, 4H), 1.81–1.59 (m, 12H); MS *m*/*z* 439 (M + 1).

Alternatively, **14** could be prepared from ethyl benzoate using general procedure I.

5.13. *N*-Cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(3-methylphenyl)-pyrazolo[1,5-*a*]pyridin-7-amine (15)

5.13.1. 2-(6-Chloro-2-pyridinyl)-1-(3-methylphenyl)-ethanone (60). In a similar manner as described in the procedure I, from ethyl 3-methylbenzoate (30 g, 183 mmol), **60** (33.6 g, 75% yield) was obtained as a mixture of ketone and enol tautomers. This mixture was used directly in the next step.

5.13.2. 2-(6-Chloro-2-pyridinyl)-1-(3-methylphenyl)-ethanone oxime (61). In a similar manner as described in the procedure I, from **60** (33.6 g, 137 mmol), **61** (26.1 g, 73% yield) was obtained as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.54–7.47 (m, 3H), 7.26–7.15 (m, 4H), 4.40 (s, 2H), 2.34 (s, 3H). MS *m*/*z* 243 (M + 1).

5.13.3. 7-Chloro-2-(3-methylphenyl)pyrazolo[1,5-*a*]-pyridin (62). In a similar manner as described in the procedure I, from **61** (13 g, 50 mmol), **62** (11.5 g, 99% yield) was obtained as a yellow crystalline solid. ^{1}H NMR

($CDCl_3$): δ 7.86 (s, 1H), 7.79 (d, 1H), 7.48 (d, 1H), 7.34 (t, 1H), 7.20 (d, 1H), 7.05 (dd, 1H), 6.91 (s, 1H), 6.88 (d, 1H), 2.44 (s, 3H). MS *m*/*z* 243 (M + 1).

5.13.4. 7-Chloro-2-(3-methylphenyl)pyrazolo[1,5-*a*]pyridine-3-carbaldehyde (63). 7-Chloro-2-(3-methylphenyl)pyrazolo[1,5-*a*]pyridine (**62**, 16.3 g, 67 mmol) was treated according to procedure II to give after recrystallization from diethyl ether and hexanes **63** (14.3 g, 79%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$): δ 10.11 (s, 1H), 8.41 (dd, 1H), 7.63 (s, 1H), 7.58 (d, 1H), 7.49 (dd, 1H), 7.42 (t, 1H), 7.33 (d, 1H), 7.20 (dd, 1H), 2.45 (s, 3H); MS *m*/*z* 271 (M + 1).

5.13.5. 1-[7-Chloro-2-(3-methylphenyl)pyrazolo[1,5-*a*]-pyridin-3-yl]-2-propyn-1-ol (64). 7-Chloro-2-(3-methylphenyl)pyrazolo[1,5-*a*]pyridine-3-carbaldehyde (**63**, 10.38 g, 36.2 mmol) was treated according to procedure II to give after flash chromatography (4:1, hexanes/ethyl acetate to 7:3, hexanes/ethyl acetate) **64** (11.3 g, 77%) as a yellow foam. ^{1}H NMR ($CDCl_3$) δ 8.03 (d, 1H), 7.60 (s, 1H), 7.54 (d, 1H), 7.37 (t, 1H), 7.26 (d, 1H), 7.17 (dd, 1H), 6.98 (d, 1H) , 2.67 (s, 1H), 2.43 (s, 3H), 2.38 (s, 1H); MS *m*/*z* 297 (M + 1).

5.13.6. 1-[7-Chloro-2-(3-methylphenyl)pyrazolo[1,5-*a*]pyridin-3-yl]-2-propyn-1-one (65). 1-[7-Chloro-2-(3-methylphenyl)pyrazolo[1,5-*a*]pyridin-3-yl]-2-propyn-1-ol (**64**, 11.3 g, 36.2 mmol) in chloroform (300 mL) was treated according to procedure II to give after purification by flash chromatography (7:3, hexanes/ethyl acetate) **65** (4.57 g, 41%) as a pale yellow crystalline. ^{1}H NMR ($CDCl_3$): δ 8.48 (d, 1H), 7.51 (m, 3H), 7.33 (t, 1H), 7.29 (d, 1H), 8.21 (dd, 1H), 2.89 (s, 1H), 2.42 (s, 3H); MS *m*/*z* 295 (M + 1).

5.13.7. 4-[7-Chloro-2-(3-methylphenyl)pyrazolo[1,5-*a*]-pyridin-3-yl]-*N*-cyclopentyl- 2-pyrimidinamine (66). 1-[7-Chloro-2-(3-methylphenyl)pyrazolo[1,5-*a*]pyridin-3-yl]-2-propyn-1-one (**65**, 4.04 g, 13.0 mmol) and cyclopentyl guanidine hydrochloride (6.36 g, 39 mmol) were treated according to procedure II to give after purification by silica gel flash chromatography (3:10, ethyl acetate/hexanes) **66** (2.39 g, 43%) as a yellow foam. ^{1}H NMR ($CDCl_3$): δ 8.47 (d, 1H), 7.99 (d, 1H), 7.48 (s, 1H), 7.39 (d, 1H), 7.33–7.24 (m, 3H), 7.05 (d, 1H), 6.33 (d, 1H), 5.38 (br s, 1H), 4.36 (m, 1H), 2.40 (s, 3H), 2.10 (m, 2H), 1.78 (m, 2H), 1.67 (m, 2H), 1.58 (m, 2H); MS *m*/*z* 404 (M + 1).

5.13.8. *N*-Cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(3-methylphenyl)pyrazolo[1,5-*a*]pyridin-7-amine (15). 4-[7-Chloro-2-(3-methylphenyl)pyrazolo[1,5-*a*]pyridin-3-yl]-*N*-cyclopentyl-2-pyrimidinamine (**66**, 500 mg, 1.24 mmol) in cyclopentylamine (50 mL) was treated according to procedure II to give after purification by flash chromatography (4:1, hexanes/ethyl acetate) **15** (360 mg, 64%) as a yellow foam. ^{1}H NMR ($CDCl_3$): δ 7.94 (d, 1H), 7.79 (d, 1H), 7.46 (s, 1H), 7.40 (d, 1H), 7.34–7.29 (m, 2H), 7.24 (d, 1H), 6.27 (d, 1H), 6.03 (m, 2H), 5.12 (br s, 1H), 4.35 (m, 1H), 3.99 (m, 1H), 2.40 (s, 3H), 2.14–2.04 (m, 4H), 1.81–1.52 (m, 12H); MS *m*/*z* 453 (M + 1).

5.14. *N*-Cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-[3- (trifluoromethyl)phenyl]pyrazolo[1,5-*a*]pyridin-7-amine (16)

The title compound was prepared using procedure II in a similar fashion as outlined for **15**. R_f 0.22 (4:1, hexanes/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06–8.02 (m, 2H), 7.84 (d, 1H), 7.71–7.67 (m, 2H), 7.55 (t, 1H), 7.34 (t, 1H), 6.32 (d, 1H), 6.07 (d, 1H), 6.03 (d, 1H), 5.08 (d, 1H), 4.28 (m, 1H), 4.03 (m, 1H), 2.17 (m, 2H), 2.04 (m, 2H), 1.86–1.51 (m, 12H); MS *m*/*z* 507 (M + 1).

5.15. *N*-Cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-methoxyphenyl)pyrazolo[1,5-*a*]pyridin-7-amine (17)

5.15.1. 2-(6-Chloro-2-pyridinyl)-1-(4-methoxyphenyl)-ethanone (67). 6-Chloro-2-picoline (18.3 mL, 166.5 mmol) and ethyl 4-methoxybenzoate (30.0 g, 166.5 mmol) were treated as outlined in the general procedure I to give **67** (37.4 g, 86%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.99 (d, 2H), 7.57 (t, 1H), 7.22–7.19 (m, 2H), 6.90 (d, 2H), 4.39 (s, 2H), 3.83 (s, 3H); MS m/z 262 (M + 1).

5.15.2. 2-(6-Chloro-2-pyridinyl)-1-(4-methoxyphenyl)-ethanone oxime (68). 2-(6-Chloro-2-pyridinyl)-1-(4-methoxyphenyl)ethanone (**67**, 37.4 g, 142.9 mmol) was treated as outlined in the general procedure I to give **68** (38.7 g, 97%) as a white solid. ^{1}H NMR ($CDCl_3$): δ 8.23 (br, 1H), 7.63 (d, 2H), 7.48 (d, 1H), 7.12 (m, 2H), 6.83 (dd, 2H), 4.33 (s, 2H), 3.76 (s, 3H); MS *m*/*z* 277 (M + 1).

5.15.3. 7-Chloro-2-(4-methoxyphenyl)pyrazolo[1,5-*a*]pyridine (69). 2-(6-Chloro-2-pyridinyl)-1-(4-methoxyphenyl)ethanone oxime (**68**, 38.7 g, 140 mmol) was treated as outlined in the general procedure I to give **69** (18.7 g, 52%) as pale yellow needles. ^{1}H NMR ($CDCl_3$): δ 7.91 (d, 2H), 7.43 (d, 1H), 7.01 (t, 1H), 6.95 (d, 2H), 6.81 (d, 1H), 6.80 (s, 1H), 3.83 (s, 3H); MS *m*/*z* 259 (M + 1).

5.15.4. 1-[7-(Chloro)-2-(4-methoxyphenyl)pyrazolo[1,5-*a*]pyridin-3-yl]ethanone (70). 7-Chloro-2-(4-methoxyphenyl)pyrazolo[1,5-*a*]pyridine (**69**, 18.7 g, 72.4 mmol) was treated as outlined in the general procedure I to give after recrystallization from ethyl acetate hexanes **70** (14.2 g, 65%) as reddish needles.^{1}H NMR ($CDCl_3$): δ 8.37 (dd, 1H), 7.49 (dd, 2H), 7.39 (dd, 1H), 7.10 (dd, 1H), 6.98 (dd, 2H), 3.84 (s, 3H), 2.13 (s, 3H); MS *m*/*z* 301 (M + 1).

5.15.5. 1-[7-(Cyclopentylamino)-2-(4-methoxyphenyl)-pyrazolo[1,5-*a*]pyridin-3-yl]ethanone (71). 1-[7-(Chloro)-2-(4-methoxyphenyl)pyrazolo[1,5-*a*]pyridin-3-yl]ethanone (**70**, 5.0 g, 16.6 mmol) was treated as outlined in the general procedure I to give after purification by flash chromatography (4:1, hexanes/ethyl acetate) **71** (5.66 g, 97%) as a yellow foam. ^{1}H NMR ($CDCl_3$): δ 7.65 (d, 1H), 7.48 (d, 2H), 7.39 (t, 1H), 6.99 (d, 2H), 6.09 (d, 1H), 6.01 (d, 1H), 3.95 (m, 1H), 3.84 (s, 3H), 2.09 (s, 3H), 2.09–2.00 (m, 2H) 1.76–1.22 (m, 6H); MS *m*/*z* 350 (M + 1).

5.15.6. (2*E*)-1-[7-(Cyclopentylamino)-2-(4-methoxyphenyl)pyrazolo[1,5-*a*]pyridin-3-yl]-3-(dimethylamino)-2-propen-1-one (72). 1-[7-(Cyclopentylamino)-2-(4-methoxyphenyl)-pyrazolo[1,5-*a*]pyridin-3-yl]ethanone (**71**, 5.56 g, 15.9 mmol) in *N*,*N*-dimethylformamide dimethyl acetal (25 mL) was heated at reflux for 5 days as outlined in the general procedure I to give after flash chromatography (7:3, ethyl acetate/acetone) **72** (5.97 g, 93%) as a colored syrup. ^{1}H NMR ($CDCl_3$): δ 7.96–7.59 (m, 3H), 7.53 (d, 1H), 7.23 (dd, 1H), 6.93 (d, 2H), 5.97–5.94 (m, 2H), 5.07 (d, 1H), 3.95 (m, 1H), 3.81 (s, 3H), 3.0–2.3 (br, 6H), 2.07 (m, 2H), 1.76–1.60 (m, 6H); MS *m*/*z* 405 (M + 1).

5.15.7. *N*-Cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-methoxyphenyl)pyrazolo[1,5-*a*]pyridin-7-amine (17). (2*E*)-1-[7-(Cyclopentylamino)-2-(4-methoxyphenyl)-pyrazolo[1,5-*a*]pyridin-3-yl]-3-(dimethylamino)-2-propen-1-one (**72**, 5.97 g, 14.7 mmol) was treated as outlined in the general procedure I to give after purification by flash chromatography (4:6, ethyl acetate/hexane) **17** (5.02 g, 73%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.95 (d, 1H), 7.72 (d, 1H), 7.54 (dd, 2H), 7.25 (t, 1H), 6.94 (dd, 2H), 6.28 (d, 1H), 6.00–5.97 (m, 2H), 5.03 (d, 1H), 4.33–4.31 (m, 1H), 3.96 (m, 1H), 3.83 (s, 3H), 2.10–2.01 (m, 4H), 1.77–1.50 (m, 12H); MS *m*/*z* 469 (M + 1); Anal. Calcd for $C_{28}H_{32}N_6O$: C, 71.77; H, 6.88; N, 17.93. Found: C, 71.41; H, 7.02; N, 17.89.

5.16. *N*-Cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(3-methoxyphenyl)pyrazolo[1,5-*a*]pyridin-7-amine (18)

5.16.1. 2-(6-Chloro-2-pyridinyl)-1-(3-methoxyphenyl)-ethanone (73). In a similar manner as described in the procedure I, from ethyl 3-methoxybenzoate (30 g, 166 mmol) and 6-chloropicoline (21.2 g, 166 mmol), **73** was obtained as a mixture of ketone and enol tautomers. This product was used directly in the next step.

5.16.2. 2-(6-Chloro-2-pyridinyl)-1-(3-methoxyphenyl)-ethanone oxime (74). In a similar manner as described in the procedure I, from **73** **74** (33.1 g, yield for the two steps 72%) was obtained as a white solid. ^{1}H NMR ($CDCl_3$): δ 8.22 (br s, 1H), 7.52 (t, 1H), 7.34 (m, 1H), 7.26–7.25 (m, 2H), 7.17–7.05 (d, 2H), 6.90 (m, 1H), 4.36 (s, 2H), 3.81 (s, 3H); MS *m*/*z* 277.

5.16.3. 7-Chloro-2-(3-methoxyphenyl)pyrazolo[1,5-*a*]pyridine (75). In a similar manner as described in the procedure I, from **74** (33.0 g, 119 mmol) **75** (23.1 g, 75% yield) was obtained as a pale yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.59 (m, 2H), 7.49 (d, 1H), 7.37 (t, 1H), 7.07 (t, 1H), 6.95–6.94 (dd, 1H), 6.91 (s, 1H), 6.88 (d, 1H), 3.90 (s, 3H); MS *m*/*z* 259.

5.16.4. 7-Chloro-2-(3-methoxyphenyl)pyrazolo[1,5-*a*]pyridine-3-carbaldehyde (76). In a similar manner as described in the procedure II, from **75** (23 g, 88.9 mmol) **76** (21.6 g, 84%) was obtained as a white solid. ^{1}H NMR ($CDCl_3$): δ 10.13 (s, 1H), 8.40 (d, 1H), 7.52–7.42 (m, 2H), 7.35 (m, 2H), 7.21 (d, 1H), 7.06 (d, 1H), 3.89 (s, 3H); MS *m*/*z* 287.

5.16.5. 1-[7-Chloro-2-(3-methoxyphenyl)pyrazolo[1,5-*a*]-pyridin-3-yl]-2-propyn-1-ol (77). In a similar manner as described in the procedure II, from **76** (17.25 g, 60.1 mmol) and ethynylmagnesium bromide, **77** (19.0 g, 100%) was obtained. ^{1}H NMR ($CDCl_3$): δ 8.02 (d, 1H), 7.38–7.32 (m, 3H), 7.17 (t, 1H), 6.98–6.97 (d, 2H), 5.82 (m, 1H), 3.86 (s, 3H), 2.67 (s, 1H), 2.53 (d, 1H); MS *m*/*z* 313.

5.16.6. 1-[7-Chloro-2-(3-methoxyphenyl)pyrazolo[1,5-*a*]-pyridin-3-yl]-2-propyn-1-one (78). In a similar manner as described in the procedure II, from **77** (19 g, 60.8 mmol) **78** (14.4 g, yield 76%) was obtained as an orange colored solid. ^{1}H NMR ($CDCl_3$): δ 8.47 (d, 1H), 7.52 (t, 1H), 7.36 (t, 1H), 7.29–7.21 (m, 3H), 7.02 (dd, 1H), 3.86 (s, 3H), 2.92 (s, 1H); MS *m*/*z* 311.

5.16.7. 4-[7-Chloro-2-(3-methoxyphenyl)pyrazolo[1,5-*a*]-pyridin-3-yl]-*N*-cyclopentyl-2-pyrimidinamine (79). In a similar manner as described in the procedure II, from **78** (1.0 g, 3.2 mmol) and cyclopentyl guanidine hydrochloride **79** (0.34 g, yield 25%) was obtained as a pale yellow foam. ^{1}H NMR ($CDCl_3$): δ 8.52 (d, 1H), 8.05 (d, 1H), 7.43–7.23 (m, 4H), 7.11 (d, 1H), 7.03 (dd, 1H), 6.40 (d, 1H), 5.45 (br s, 1H), 4.40 (m, 1H), 3.86 (s, 3H), 2.10 (m, 2H), 1.85–1.59 (m, 6H); MS *m*/*z* 420.

5.16.8. *N*-Cyclopentyl-3-[2-(cyclopenylamino)-4-pyrimidinyl]-2-(3-methoxyphenyl)pyrazolo[1,5-*a*]pyridin-7-amine (18). In a similar manner as described in the procedure II, from **79** (500 mg, 1.19 mmol) and cyclopentylamine, **18** (389 mg, yield 70%) was obtained as a yellow foam. ^{1}H NMR ($CDCl_3$): δ 7.92 (d, 1H), 7.79 (d, 1H), 7.38–7.30 (m, 2H), 7.21–7.18 (m, 2H), 6.99 (d, 1H), 6.30 (d, 1H), 6.04 (m, 2H), 5.38 (br s, 1H), 4.36 (m, 1H), 4.00 (m, 1H), 3.82 (s, 3H), 2.14–2.06 (m, 4H), 1.82–1.57 (m, 12H); MS *m*/*z* 469.

5.17. 4-{7-(Cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-2-yl}phenol (19)

To a solution of **17** (1.16 g, 2.48 mmol) in dichloromethane (50 mL) at –78 °C was added boron tribromide (9.92 mL, 1.0 M in dichloromethane, 9.92 mmol) dropwise. The resulting solution was allowed to warm to room temperature. After stirring for 15 h at room temperature, the mixture was cooled to 0 °C and quenched by the addition of water. The mixture was extracted with ethyl acetate. The organic phase was dried (magnesium sulfate), filtered, and concentrated to give a solid residue which was purified by flash chromatography (95:5, chloroform/methanol). **19** was obtained as a yellow solid (0.80 g, 71%). ^{1}H NMR ($CDCl_3$): δ 7.91 (d, 1H), 7.70 (d, 1H), 7.46 (d, 2H), 7.26 (t, 1H), 6.86 (d, 2H), 6.27 (d, 1H), 6.00–5.97 (m, 2H), 5.06 (d, 1H), 4.33 (m, 1H), 3.96 (m, 1H), 2.10–2.03 (m, 4H), 1.78–1.47 (m, 12H); MS *m*/*z* 455 (M + 1).

5.18. 3-{7-(Cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-2-yl}phenol (20)

In a similar manner as described above, from **18** (150 mg, 0.32 mmol) **20** (126 mg, yield 87%) was obtained as a yellow foam. ^{1}H NMR ($CDCl_3$): δ 7.83 (d, 1H), 7.77 (d, 1H),

7.35–7.28 (m, 2H), 7.21 (d, 1H), 7.06 (s, 1H), 6.95 (dd, 1H), 6.39 (d, 1H), 6.05–6.02 (m, 2H), 5.29 (s, 1H), 5.22 (m, 1H), 4.31 (m, 1H), 3.99 (m, 1H), 2.12–1.98 (m, 4H), 1.80–1.46 (m, 12H); MS *m/z* 455.

5.19. 2-[4-(Allyloxy)phenyl]-*N*-cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (21)

To a solution of **19** (100 mg, 0.22 mmol) in *N*,*N*-dimethylformamide (5 mL), allyl bromide (21 μL, 0.24 mmol) and potassium carbonate (122 mg, 0.88 mmol) were added. The mixture was heated at reflux for 3 h. The mixture was allowed to cool to room temperature and water was added. The mixture was extracted with ethyl acetate. The ethyl acetate phase was dried (magnesium sulfate), filtered, and concentrated to a solid. The residue was purified by flash chromatography (1:1, ethyl acetate/hexanes) to give **21** (67 mg, 61%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.94 (d, 1H), 7.73 (d, 1H), 7.53 (d, 2H), 7.24 (t, 1H), 6.95 (d, 2H), 6.28 (d, 1H), 6.08–5.96 (m, 3H), 5.41 (dd, 1H), 5.27 (dd, 1H), 5.10 (d, 1H), 4.56 (d, 2H), 4.32 (m, 1H), 3.95 (m, 1H), 2.10–2.00 (m, 4H), 1.76–1.45 (m, 12H); MS *m/z* 495 (M + 1); Anal. Calcd for $C_{30}H_{34}N_6O$: C, 72.86; H, 6.93; N,16.99. Found: C, 72.47; H, 7.05; N, 16.75.

5.20. 2-(4-Butoxyphenyl)-*N*-cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (22)

In a similar manner as described above, from **19** (100 mg, 0.22 mmol) and butyl bromide, **22** (80 mg, 71%) was formed as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 8.00 (d, 1H), 7.78 (d, 1H), 7.57 (d, 2H), 7.28 (t, 1H), 6.97 (d, 2H), 6.34 (d, 1H), 6.05 (d, 1H), 6.00 (d, 1H), 5.22 (d, 1H), 4.37 (m, 1H), 4.02 (m, 3H), 2.12–2.05 (m, 4H), 1.82–1.49 (m, 16H), 1.00 (t, 3H); MS *m/z* 511 (M + 1).

5.21. *N*-Cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4-isobutoxyphenyl)pyrazolo[1,5-*a*]pyridin-7-amine (23)

In a similar manner as described above, from **19** (100 mg, 0.22 mmol) and isobutyl bromide, **23** (76 mg, 68%) was formed as a yellow foam. ^{1}H NMR ($CDCl_3$): δ 8.00 (d, 1H), 7.78 (d, 1H), 7.57 (d, 2H), 7.29 (t, 1H), 6.98 (d, 2H), 6.35 (d, 1H), 6.05 (d, 1H), 6.01 (d, 1H), 5.16 (d, 1H), 4.37 (m, 1H), 4.00 (m, 1H), 3.79 (d, 2H), 2.16–2.05 (m, 5H), 1.81–1.52 (m, 12H), 1.06 (d, 6H); MS *m/z* 511 (M + 1).

5.22. *N*-Cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-[4-(cyclopropyl- methoxy)phenyl]pyrazolo[1,5-*a*]-pyridin-7-amine (24)

In a similar manner as described above, from **19** (100 mg, 0.22 mmol), Cs_2CO_3, (bromomethyl)cyclopropane, **24** (48 mg, 44%) was formed as a yellow foam. ^{1}H NMR ($CDCl_3$): δ 7.99 (d, 1H), 7.77 (d, 1H), 7.57 (dd, 2H), 7.30 (t, 1H), 6.97 (d, 2H), 6.33 (d, 1H), 6.04–6.00 (m, 2H), 5.08 (d, 1H), 4.37 (m, 1H), 4.00 (m, 1H), 3.87 (d, 2H), 2.14–2.07 (m, 4H), 1.82–1.52 (m, 12H), 1.32 (m, 1H), 0.65 (m, 2H), 0.38 (m, 2H); MS *m/z* 509 (M + 1).

5.23. *N*-Cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-[3- (cyclopropylmethoxy)phenyl]pyrazolo[1,5-*a*]pyridin-7-amine (25)

To a solution of **20** (400 mg, 0.88 mmol) in acetonitrile (80 mL) cesium carbonate (315 mg, 0.97 mmol) and (bromomethyl)cyclopropane (0.26 mL, 2.64 mmol) were added. The reaction mixture was heated at reflux for 6 h. After the reaction was cooled to room temperature, ethyl acetate was added and the organic phase was washed with water, brine and dried over magnesium sulfate. Filtration and concentration followed by purification with silica gel chromatography (3:2, hexanes/ethyl acetate) gave **25** (320 mg, 71%) as yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.97 (d, 1H), 7.78 (d, 1H), 7.33 (m, 2H), 7.18 (m, 2H), 6.98 (m, 1H), 6.30 (d, 1H), 6.02 (m, 2H), 5.08 (d, 1H), 4.35 (m, 1H), 3.99 (m, 1H), 3.80 (d, 2H), 2.014 (m, 4H), 1.83–1.55 (m, 12H), 1.22 (m, 1H), 0.61 (m, 2H), 0.35 (m, 2H). MS m/z 509 (M + 1).

5.24. 2-[4-(Cyclobutylmethoxy)phenyl]-*N*-cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (26)

In a similar manner as described above, from **19** (67 mg, 0.15 mmol) and (bromomethyl)cyclobutane, **26** (50 mg, 65%) was formed as a yellow foam. ^{1}H NMR ($CDCl_3$): δ 7.99 (d, 1H), 7.78 (d, 1H), 7.57 (d, 2H), 7.30 (t, 1H), 6.98 (d, 2H), 6.34 (d, 1H), 6.05–6.00 (m, 2H), 5.13 (d, 1H), 4.37 (m, 1H), 3.99 (m, 3H), 2.82 (m, 1H), 2.20–1.50 (m, 22H); MS *m/z* 523 (M + 1).

5.25. Ethyl (4-{7-(cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]-pyrazolo[1,5-*a*]pyridin-2-yl}phenoxy)acetate (27)

To a solution of **19** (100 mg, 0.22 mmol) in acetone (10 mL) ethyl α-bromoacetate (49 μL, 0.44 mmol) and potassium carbonate (304 mg, 2.2 mmol) were added. The mixture was heated to reflux for 3 h. The reaction mixture was cooled to room temperature and water was added. The solution was extracted with ethyl acetate. The organics were dried (magnesium sulfate), filtered, and concentrated, followed by purification with flash chromatography (1:1, ethyl acetate/hexanes) to give **27** (85 mg, 71%) as a brown oil. ^{1}H NMR ($CDCl_3$): δ 7.94 (d, 1H), 7.70 (d, 1H), 7.55 (d, 2H), 7.23 (t, 1H), 6.95 (d, 2H), 6.25 (d, 1H), 5.98–5.95 (m, 2H), 5.13 (d, 1H), 4.62 (s, 2H), 4.31–4.21 (m, 3H), 3.94 (m, 1H), 2.07–1.99 (m, 4H), 1.75–1.46 (m, 12H), 1.28 (t, 3H); MS *m/z* 541 (M + 1).

5.26. *N*-Cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-(4- phenoxyphenyl)pyrazolo[1,5-*a*]pyridin-7-amine (28)

To a solution of **19** (100 mg, 0.22 mmol) in dichloromethane (5 mL), copper (II) acetate (40 mg, 0.22 mmol), phenylboronic acid (80 mg, 0.66 mmol), triethylamine (92 μL, 0.66 mmol) and molecular sieves were added. The mixture was stirred at room temperature for 24 h. Solids were removed by filtration and the filtrate was concentrated, followed by purification by flash chromatography (2:3, ethyl acetate/hexanes) to give **28** (14 mg,

12%) as a yellow foam. ^{1}H NMR ($CDCl_3$): δ 8.05–6.85 (m, 12H), 6.40 (d, 1H), 6.06 (m, 2H), 5.12 (d, 1H), 4.40 (m, 1H) 4.01 (m, 1H), 2.20–2.09 (m, 4H), 1.82–1.59 (m, 12H); MS *m*/*z* 531 (M + 1).

5.27. *N*-{4-[2-(4-Aminophenyl)-7-(butylamino)pyrazolo-[1,5-*a*]pyridin-3-yl]-2-pyrimidinyl}-*N*-butylamine (29)

To a solution of **11** (1.0 g, 2.0 mmol) in toluene (20 mL), *racemic*-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (200 mg, 0.30 mmol), tris(dibenzylideneacetone)dipalladium(0) (92 mg, 0.10 mmol), diphenylimine (1.02 mL, 6.1 mmol), and sodium *tert*-butoxide (582 mg, 6.1 mmol) were added. The mixture was heated to 100 °C for 50 min. The resultant solution was cooled to room temperature and diluted with ether and water was added. The organic layer was washed with brine. The aqueous layer was extracted with ether, and the combined organics dried over magnesium sulfate. Filtration and concentration followed by flash chromatography (4:1, hexanes/ethyl acetate with 1% triethylamine) provided *N*-butyl-3-[2-(butylamino)-4-pyrimidinyl]-2-{4-[(diphenylmethylene)-amino]phenyl}-pyrazolo[1,5-*a*]pyridin-7-amine (1.0 g, 84%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.90 (d, 1H), 7.80–7.28 (m, 12H), 7.19–7.16 (m, 2H), 6.80 (d, 2H), 6.07 (d, 1H), 6.02 (t, 1H), 5.97 (d, 1H), 4.99 (t, 1H), 3.48 (m, 2H), 3.36 (m, 2H), 1.80–1.43 (m, 8H), 1.00–0.96 (m, 6H); MS *m*/*z* 594 (M + 1).

To the solution of *N*-butyl-3-[2-(butylamino)-4-pyrimidinyl]-2-{4-[(diphenylmethylene)- amino]phenyl}pyrazolo[1,5-*a*]pyridin-7-amine (1.0 g, 1.7 mmol) in tetrahydrofuran (50 mL) was added hydrochloric acid (10 mL, 4 N aqueous). The resultant solution was stirred at room temperature for 30 min. Ether was added and the solution was made basic by the slow addition of saturated aqueous sodium bicarbonate. The organic layer was washed with brine. The aqueous layer was extracted with ether and the combined organics dried over magnesium sulfate. Filtration and concentration followed by flash chromatography (1:1 hexanes/ethyl acetate) provided **29** as an orange oil. ^{1}H NMR ($CDCl_3$): δ 8.02 (d, 1H), 7.81 (d, 1H), 7.47 (d, 2H), 7.31 (m, 1H), 6.77 (d, 2H), 6.42 (d, 1H), 6.08 (m, 1H), 6.00 (d, 1H), 5.18 (br, 1H), 3.86 (br, 2H), 3.53 (m, 2H), 3.39 (m, 2H), 1.82-1.64 (m, 4H), 1.58-1.46 (m, 4H), 1.04-0.99 (m, 6H); MS *m*/*z* 430 (M + 1).

5.28. 2-(3-Aminophenyl)-*N*-cyclopentyl-3-[2-(cyclopentyl-amino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (30)

2-(3-Bromophenyl)-*N*-cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (**12**, 3.00 g, 5.8 mmol) in toluene (60 mL) was treated with benzophenone imine (3.15 g, 17.4 mmol), tris(dibenzylideneacetone)dipalladium (0.26 g, 0.3 mmol), *rac*-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (0.54 g, 0.15 mmol) and sodium *tert*-butoxide (1.67 g, 17.4 mmol) as described above to give after flash chromatography (4:6 ethyl acetate/hexanes) *N*-cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-{3-[(diphenyl-methylene) amino]phenyl}-pyrazolo[1,5-*a*]pyridin-7-amine (2.61 g, 73%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.89 (d, 1H), 7.80–7.75 (m, 3H), 7.48 (m, 1H), 7.41 (m, 2H), 7.27 (m, 5H), 7.23–7.12 (m, 4H), 7.04 (s, 1H), 6.80 (d, 1H), 6.03-5.97 (m, 3H), 4.39 (m, 1H), 4.00 (m, 1H), 2.15–2.05 (m, 4H), 1.83–1.56 (m, 12H); MS *m*/*z* 618 (M + 1). The *N*-cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-{3-[(diphenylmethylene)amino]phenyl}-pyrazolo[1,5-*a*]pyridin-7-amine (2.61 g, 4.22 mmol) in tetrahydrofuran (30 mL) at 0 °C was treated with 4 N hydrochloric acid (20 mL) as described above to give after recrystallization from ethyl acetate–hexanes, **30** (1.78 g, 93%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.92 (d, 1H), 7.82 (d, 1H), 7.32 (t, 1H), 7.22 (m, 2H), 7.00-6.94 (m, 2H), 6.78 (m, 1H), 6.34 (d, 1H), 6.04 (m, 2H), 4.38 (m, 1H), 4.00 (m, 1H), 3.75 (br, 2H), 2.14-2.05 (m, 4H), 1.83–1.54 (m, 12H); MS *m*/*z* 454 (M + 1).

5.29. *N*-(3-{7-(cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-2-yl}phenyl)acetamide (31)

To a suspension of 2-(3-aminophenyl)-*N*-cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (**30**, 150 mg, 0.33 mmol) in dimethylformamide (10 mL) was added triethylamine (51 μL, 0.36 mmol). The reaction mixture was cooled to 0 °C and flushed with nitrogen, then acetyl chloride (26 μL, 0.36 mmol) was added dropwise. The reaction mixture was stirred at room temperature overnight. Water was added and the resulting mixture was extracted with ethyl acetate. The ethyl acetate phase was dried (magnesium sulfate), filtered and concentrated to a solid. This solid was purified by flash chromatography (95:5 chloroform/methanol) to give **31** (151 mg, 92%) as a tan solid. ^{1}H NMR ($CDCl_3$): δ 8.19 (s, 1H), 7.95 (d, 1H), 7.82 (d, 1H), 7.75 (d, 1H), 7.63 (s, 1H), 7.36–7.25 (m, 3H), 6.28 (d, 1H), 6.00 (m, 2H), 5.17 (d, 1H), 4.32 (m, 1H), 3.96 (m, 1H), 2.11 (s, 3H), 2.11–2.02 (m, 4H), 1.76–1.48 (m, 12H); MS *m*/*z* 496 (M + 1).

5.30. *N*-(3-{7-(cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-2-yl}phenyl)methanesulfonamide (32)

To a suspension of 2-(3-aminophenyl)-*N*-cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (**30**, 150 mg, 0.33 mmol) in *N*,*N*-dimethylformamide (5 mL) was added pyridine (40 μL, 0.49 mmol). The reaction mixture was cooled to 0 °C under nitrogen, then methanesulfonyl chloride (28 μL, 0.36 mmol) was added dropwise. After stirring at room temperature for 18 h, the reaction mixture turned clear. Ethyl acetate and water were added and the phases separated. The organic phase was washed with water and brine, then dried over magnesium sulfate. Filtration and concentration, followed by flash chromatography (95:5 chloroform/methanol), gave **32** (170 mg, 96%) as a yellow syrup. ^{1}H NMR ($CDCl_3$): δ 7.93 (d, 1H), 7.66 (d, 1H), 7.42 (m, 1H), 7.35–7.25 (m, 5H), 6.25 (m, 1H), 5.99 (m, 2H), 5.62 (br, 1H), 4.29 (m, 1H), 3.96 (m, 1H), 2.94 (s, 3H), 2.10–1.99 (m, 4H), 1.77–1.49 (m, 12H). MS *m*/*z* 532 (M + 1).

5.31. *N*-Butyl-3-[2-(butylamino)-4-pyrimidinyl]-2-[4-(cyclohexylamino)phenyl]pyrazolo[1,5-*a*]pyridin-7-amine (33)

A solution of *N*-{4-[2-(4-aminophenyl)-7-(butylamino)pyrazolo[1,5-*a*]pyridin-3-yl]-2-pyrimidinyl}-*N*-butylamine (**29**, 62 mg, 0.15 mmol) in 1,2-dichloroethane (2 mL) was treated with cyclohexanone (0.02 mL, 0.22 mmol), acetic acid (0.04 mL, 0.72 mmol), and sodium triacetoxyborohydride (61 mg, 0.29 mmol). The resultant solution was stirred at room temperature for 18 h. Saturated aqueous sodium bicarbonate was added dropwise followed by ether. The organic layer was washed with brine. The aqueous layer was extracted with ether and the combined organics dried over magnesium sulfate. Filtration and concentration followed by flash chromatography (4:1 to 2:1 hexanes–ethyl acetate) provided **33** (54 mg, 73%) as an orange oil. ^{1}H NMR ($CDCl_3$): δ 7.94 (d, 1H), 7.73 (d, 1H), 7.39 (d, 2H), 7.23 (m, 1H), 6.59 (d, 2H), 6.41 (d, 1H), 6.03 (m, 1H), 5.91 (d, 1H), 5.11 (br, 1H), 3.66 (br, 1H), 3.44 (m, 2H), 3.30 (m, 3H), 2.05 (m, 2H), 1.75–1.12 (m, 16H), 0.95–0.92 (m, 6H); MS *m*/*z* 512 (M + 1). This material was treated with anhydrous hydrochloric acid in ether to provide the corresponding hydrochloride salt.

5.32. *N*-Butyl-3-[2-(butylamino)-4-pyrimidinyl]-2-[4-(dimethylamino)phenyl]pyrazolo[1,5-*a*]pyridin-7-amine (34)

In a similar manner as described above from *N*-{4-[2-(4-aminophenyl)-7-(butylamino)pyrazolo[1,5-*a*]pyridin-3-yl]-2-pyrimidinyl}-*N*-butylamine (50 mg, 0.12 mmol), formaldehyde (13 μL, 37% aqueous solution, 0.17 mmol) and sodium triacetoxyborohydride (99 mg, 0.47 mmol) was prepared **34** (40.5 mg, 76%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.80 (m, 2H), 7.50 (d, 2H), 7.33 (t, 1H), 6.79 (d, 2H), 6.44 (d, 1H), 6.10 (t, 1H), 6.03 (d, 1H), 3.54 (q, 2H), 3.38 (q, 2H), 3.03 (s, 6H), 1.80–1.43 (m, 8H), 0.99 (t, 6H); MS *m*/*z* 458 (M + 1).

5.33. 2-[1,1′-Biphenyl]-4-yl-*N*-butyl-3-[2-(butylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (35)

To a solution of 2-(4-Bromophenyl)-*N*-butyl-3-[2-(butylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (**11**, 52 mg, 0.11 mol) in tetrahydrofuran was added phenylboronic acid (26 mg, 0.21 mmol), sodium carbonate (0.21 mL, 2 M aqueous, 0.42 mmol), and dichlorobistriphenylphosphine palladium (II) (7.5 mg, 0.01 mmol). The mixture was heated at reflux for 3.5 h. The resultant solution was cooled to room temperature and diluted with ether and water was added. The organic layer was washed with brine. The aqueous layer was extracted with ether and the combined organics dried over magnesium sulfate. Filtration and concentration followed by flash chromatography provided **35** (37 mg, 73%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 8.05 (d, 1H), 7.83–7.69 (m, 7H), 7.51 (m, 2H), 7.43–7.33 (m, 2H), 6.44 (d, 1H), 6.12 (t, 1H), 6.05 (d, 1H), 5.16 (br, 1H), 3.52 (m, 2H), 3.42 (m, 2H), 1.84–1.43 (m, 8H), 1.05–0.99 (m, 6H); MS *m*/*z* 491 (M + 1).

5.34. 2-[1,1′-Biphenyl]-3-yl-*N*-cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (36)

To a solution of 2-(3-bromophenyl)-*N*-cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (**12**, 100 mg, 0.19 mmol) in dimethylformamide (6 mL) was added phenylboronic acid (47 mg, 0.39 mmol), palladium (II) acetate (4.3 mg, 0.02 mmol), potassium carbonate (54 mg, 0.39 mmol) and triphenyl phosphine (30 mg, 0.08 mmol). The reaction was heated at 100 °C for 24 h. After allowing the reaction mixture to cool to room temperature, ethyl acetate and water were added. The organic layer was separated and washed with water, then brine and dried (magnesium sulfate). Filtration and concentration, followed by purification with flash chromatography (4:6 ethyl acetate/hexanes) gave **36** as a yellow foam (87 mg, 89%). ^{1}H NMR ($CDCl_3$): δ 8.01 (d, 1H), 7.92 (s, 1H), 7.81 (d, 1H), 7.69–7.62 (m, 4H), 7.53 (t, 1H), 7.47–7.43 (m, 2H), 7.38–7.32 (m, 2H), 6.40 (d, 1H), 6.09–6.05 (m, 2H), 5.16 (m, 1H), 4.37 (m, 1H), 4.03 (m, 1H), 2.15–2.06 (m, 4H), 1.82–1.64 (m, 12H); MS *m*/*z* 515 (M + 1).

5.35. 3-{7-(Cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-2-yl}benzonitrile (37)

To a solution of 2-(3-bromophenyl)-*N*-cyclopentyl-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-7-amine (**12**, 500 mg, 0.97 mmol) in *N*,*N*-dimethylformamide (25 mL) was added zinc cyanide (68 mg, 0.58 mmol), tris(dibenzylidineacetone)bipalladium(0) (888 mg, 0.97 mmol) and 1,1′-bis(diphenylphosphino) ferrocene (1.29 g, 2.3 mmol). The resultant mixture was heated at 120 °C for 20 h. After cooling to room temperature, ethyl acetate was added to the reaction mixture. The organic phase was washed with water, brine and dried over magnesium sulfate. Filtration and concentration, followed by purification with flash chromatography (40:60 ethyl acetate/hexanes) gave **37** (0.19 g, yield 42%) as a yellow foam. ^{1}H NMR ($CDCl_3$): δ 8.07 (m, 2H), 7.89 (d, 1H), 7.70 (d, 1H), 7.62 (d, 1H), 7.52 (t, 1H), 7.33 (t, 1H), 6.33 (d, 1H), 6.06 (d, 1H), 6.00 (d, 1H), 5.16 (d, 1H), 4.25 (m, 1H), 4.02 (m, 1H), 2.15 (m, 2H), 2.03 (m, 2H), 1.85–1.51 (m, 12H). MS *m*/*z* 464 (M + 1).

5.36. 3-{7-(Cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo-[1,5-*a*]pyridin-2-yl}benzoic acid (38)

To a solution of 3-{7-(cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-2-yl}-benzonitrile (**37**, 50 mg, 0.1 mmol) in methanol was added 4 N potassium hydroxide. After heating at 85 °C for 2 days, the reaction mixture was cooled to room temperature and acidified with 2 N hydrochloric acid. The solution was extracted with ethyl acetate. The organic phases were combined and dried over magnesium sulfate. Filtration and concentration, followed by purification by flash chromatography (90:10 ethyl acetate/methanol) gave **38** (10 mg, 19%) as a brown foam. ^{1}H NMR ($CDCl_3$): δ 8.11 (s, 1H), 8.00 (d, 1H),

7.96 (d, 1H), 7.80 (br s, 1H), 7.64 (d, 1H), 7.46 (t, 1H), 7.37 (t, 1H), 6.98 (d, 1H), 6.57 (d, 1H), 6.21 (d, 1H), 6.11 (br, 1H), 4.12 (m, 1H), 4.01 (m, 1H), 2.06 (m, 2H), 1.88 (m, 2H), 1.69–1.49 (m, 12H). MS *m*/*z* 483 (M + 1); 481 (M − 1).

5.37. 3-{7-(Cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-2-yl }benzamide (39)

3-{7-(Cyclopentylamino)-3-[2-(cyclopentylamino)-4-pyrimidinyl]pyrazolo[1,5-*a*]pyridin-2-yl}benzonitrile (**37**, 45 mg, 0.097 mmol) was dissolved in hot methanol (2 mL). Subsequently, the solution was cooled down to room temperature and 30% ammonium hydroxide (2 mL) was added. The reaction mixture was then cooled to 0 °C, and 30% hydrogen peroxide was added. After stirring at room temperature for 8 h, water was added and the resulting mixture extracted with ethyl acetate. The organics were dried over magnesium sulfate. Filtration and concentration, followed by purification with flash chromatography (95:5 dichloromethane/methanol), gave **39** (18 mg, 39% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 8.12 (s, 1H), 7.99 (d, 1H), 7.92 (d, 1H), 7.79 (d, 1H), 7.70 (d, 1H), 7.51 (t, 1H), 7.32 (t, 1H), 6.28 (d, 1H), 6.18 (br, 1H), 6.05 (d, 1H), 6.02 (d, 1H), 5.82 (br, 1H), 5.25 (d, 1H), 4.30 (m, 1H), 4.00 (m, 1H), 2.15 (m, 2H), 2.03 (m, 2H), 1.81–1.50 (m, 12H). MS *m*/*z* 482 (M + 1).

5.38. HSV anti-viral assay

5.38.1. 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, 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 or HSV-2 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 anti-viral test compounds and incubated at 37 °C for 40–48 h.

5.38.2. 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 2 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 5 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-labelled 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}).

5.39. Cytotoxicity assay

Compounds were dissolved in DMSO at a stock concentration of 10 mM and serially diluted twofold in DMSO. Dilutions were carried out in columns 1–9 of a 96-well, v-bottom polypropylene plate. Columns 10, 11, and 12 were control columns that did not contain compound. Plates were then seeded at 10,000 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, penicillin–streptomycin, and 1% L-glutamine 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 were incubated at 37 °C in a humidified, 5% CO_2 atmosphere and growth inhibition was measured after 3 days.

Cytotoxicity was 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 was added to the assay plates, incubated for 1.5 to 2 h at 37 °C in a humidified, 5% CO_2 atmosphere, and absorbance was measured at 490 nm using the Wallac Victor^2 1420 multilabel counter (Perkin-Elmer, Wellesley, MA). RoboFit 2000 curve-fitting software was then used to obtain a CC_{50} (50% cytotoxicity concentration) value from the curves generated.

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