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Pyrazolo[1,5-*a*]pyridine antiherpetics: Effects of the C3 substituent on antiviral activity

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Abstract—A recently disclosed series of pyrazolo[1,5-*a*]pyridine inhibitors of herpes virus replication has been closely examined herein for effects of the C3 substituent on antiviral activity. Significant changes in activity are observed by alterations of the heteroatom basicity and orientation of the group at C3. These results in combination with previous studies have served to further elaborate the minimal pharmacophore required for potency of this novel series of antiviral agents. During the course of these studies, several novel synthetic approaches were developed and are described.

Herpesviruses are among the most prevalent infectious agents known and have the ability to infect nearly every animal species.[1] There are currently eight herpesviruses that infect humans including herpes simplex 1 and 2 (HSV-1, HSV-2). The severity of the viral infections can range from the host being asymptomatic due to adequate control of the virus by the immune system to discomfort caused by oral fever blisters to death in certain immunocompromised individuals or neonates.[2] The current gold standard therapy, valacyclovir, is well known, widely used, and exceptionally well tolerated. While this has been an extremely successful therapy for patients, there is still a goal to improve treatment in the areas of time to healing of lesions, attenuation of pain, and reduction in the frequency of reactivations. A major goal of our current efforts has been to find novel therapeutic agents to control viral replication through alternative mechanisms of action (MOA) such that potential synergy and complimentarity with valacyclovir type of treatments may exist.

We have recently disclosed a series of heterocyclic molecules containing the pyrazolo[1,5-*a*]pyridine core which show potent and selective inhibition of HSV-1 and -2, and do not act via the traditional herpes polymerase MOA (Fig. 1).[3] Previous reports from our laboratories

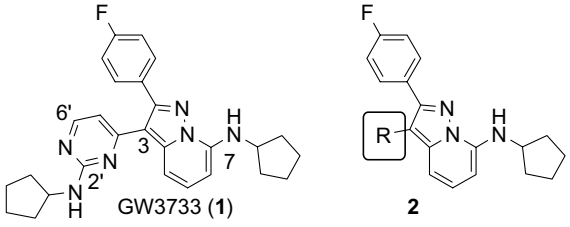

Figure 1.

described the synthesis and SAR for the C2, C6 and the effects resulting from combinations of C2′ and C7 substituent alterations.[4] While those initial reports kept the C3 pyrimidine substituent constant, the current investigation was aimed to examine the importance of the pyrimidine ring appended at C3 with respect to the heteroatom requirements within the pyrimidine ring along with their basicity and orientation for optimal activity.

Our initial strategy to examine the C3 substituent effects was to gradually tear down the 2′-aminopyrimidine group of the lead structure GW3733 beginning with the removal of the individual ring heteroatoms through a series of pyridine derivatives. This series of analogs were made starting from the previously reported 3-bromo derivative **3**[3] through a standard Suzuki cross-coupling sequence as depicted in Scheme 1. The 4-pyridyl (**6**) and 2-fluoro-4-pyridyl derivatives (**7**) were

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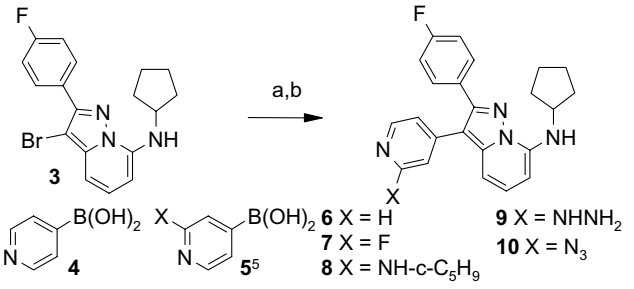
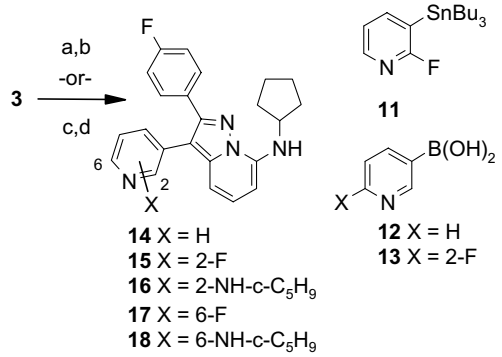


Scheme 1. Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, 2 M (aq) Na_2CO_3, DMF 100 °C, **4** or **5**, 18 h (for **6**, 46%, for **7**, 77%); (b) starting from **7**: for **8**, cyclopentylamine, 150 °C neat, 12 h (72%), for **9**, hydrazine (anhyd), EtOH, 100 °C, 13 h (65%); for **10**, NaN_3, NMP, 129 °C, 4 d (52%). See above-mentioned references for further information.

constructed using this method. The 2-fluoro-4-pyridyl derivative proved to be not only interesting for SAR purposes but also was a useful intermediate for further displacements with various nucleophiles resulting in the cyclopentylamino (**8**), hydrazino (**9**), and azido (**10**) substituted variants.

The unsubstituted 3-pyridyl derivative **14** was made using analogous Suzuki chemistry using the boronic acid **12** (Scheme 2). In addition, it was desired to construct the corresponding cyclopentylamine substituted 3-pyridyl derivatives having the amine flank either side of the pyridine nitrogen. These analogs were made using a related strategy from the appropriate fluoropyridine coupling partners through Stille or Suzuki methodology to couple bromide **3** with stannane **11**[6] and boronic acid **13**[7] to give the fluoro derivatives **15** and **17**, respectively. To complete the synthesis, nucleophilic displacement of the fluorine was cleanly accomplished in each case by heating in neat cyclopentylamine in a sealed tube for 5–7 days resulting in the aminopyridine analogs **16** and **18**.

In addition to the pyridine replacements at C3, we also desired to look into additional analogs containing multiple nitrogens in the 6-membered heterocycle.

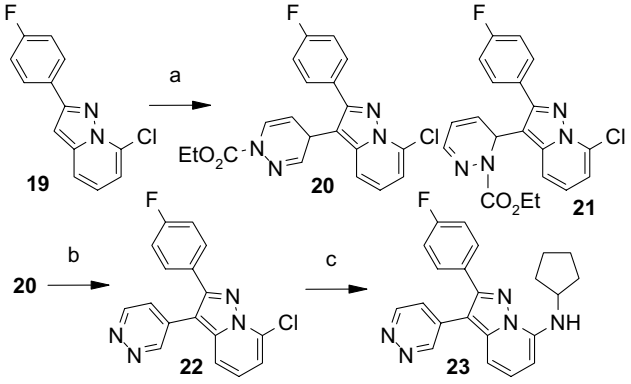


Scheme 2. Reagents and conditions for **15**: (a) $Pd(PPh_3)_4$, **11**, DMF 100 °C, 18 h (28%), for **16**; (b) cyclopentylamine, 140 °C neat, 7 d (87%), for **14** and **16**; (c) $PdCl_2(PPh_3)_2$, **12** or **13**, 2 M (aq) Na_2CO_3, DMF 100 °C, 18 h (for **14**, 46%, for **16**, 27%), for **18**; (d) cyclopentylamine, 140 °C neat, 5 d (99%).

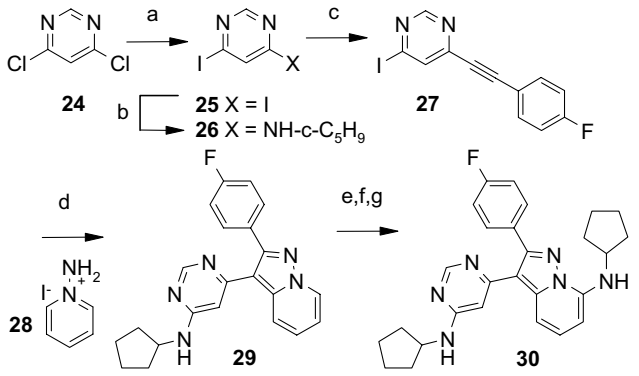
Toward this goal, the 4-pyridazine derivative **23** was made by exploiting the nucleophilicity of the 3-position of the pyrazolopyridine ring system (Scheme 3). From previous work, it was determined that the 7-chloro derivative **19** had the optimal balance of C3 reactivity and did not undergo side reactions at the 4- and 6-positions as is the case if the 7-chloro is replaced at this stage with an amino substituent.[3] Treatment of **19** with pyridazine and ethyl chloroformate at 0 °C resulted in a separable 2:1 mixture of the 4- and 2-substituted dihydropyridazine carbamates **20** and **21**.[8] The desired 4-substituted intermediate **20** was re-aromatized to the 4-pyridazinyl analog **22** by treatment with LiOH in the presence of air. The 7-chloro substituent was displaced under Buchwald type coupling conditions in the presence of cyclopentylamine resulting in the pyridazine containing analog **23**.[9]

The isomeric pyrimidine **30** was the next target examined. This analog effectively moves the 3-position nitrogen in the parent GW3733 structure to the 5-position. The seemingly simple change however required a vastly different synthetic approach. Our strategy to construct this analog was to make use of the [3+2] cyclization of *N*-aminopyridinium iodide with an alkyne which has been shown in the past to be a powerful method of constructing the pyrazolopyridine ring system (Scheme 4).[4c] It was found that conversion of commercially available 4,6-dichloropyrimidine (**24**) to its corresponding diiodide **25** resulted in a substrate that could be selectively converted to the 4-amino-6-iodopyrimidine **26**. The amine displacement chemistry works as well with the dichloro starting material but we were unable to convert the remaining chlorine into iodide **26** once the amine was introduced. The mono-chloro derivative was also much slower to undergo the subsequent Sonogashira coupling thus we used the sequence of steps shown in Scheme 4.

With the iodo-pyrimidine **26** in hand, a Sonogashira coupling with the commercially available 4-fluorophenyl acetylene proceeded smoothly to give the alkyne **27**. This material served as the key intermediate for [3+2] cyclization with the aminopyridinium salt **28** to give



Scheme 3. Reagents and conditions: (a) pyridazine, $ClCO_2Et$, CH_2Cl_2, 0 °C 19:20 2:1 (99%); (b) 1 M LiOH (aq), $MeOH/H_2O$, air (86%); (c) $Pd(OAc)_2$, BINAP, Cs_2CO_3, cyclopentylamine, PhMe, 105 °C (93%).



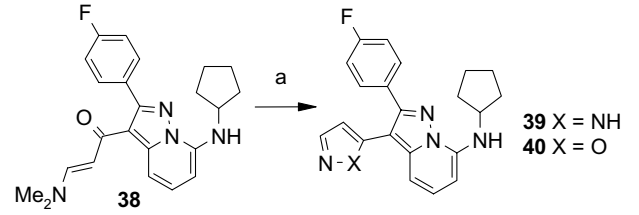
Scheme 4. Reagents and conditions: (a) NaI in 57% HI (58%); (b) cyclopentylamine, THF 0 °C to rt (91%); (c) $PdCl_2(PPh_3)_2$, 4-FC_6H_4CCH, CuI, Et_3N, THF, rt (97%); (d) DBU, MeCN (31%); (e) LDA, MeSSMe, THF −78 °C (85%); (f) *m*-CPBA, CH_2Cl_2; (g) cyclopentylamine 145 °C 6 h (20%).

the 3 substituted pyrazolopyridine **29** as a single regioisomer. All that remained was to deprotonate the 7-position and functionalize by treatment of the anion at low temperature with dimethyl disulfide. The 7-methylthio intermediate was smoothly oxidized to the sulfoxide which was displaced under thermal conditions in cyclopentylamine/THF in a sealed tube to give amine **30**.

The final synthetic goal in the C3 6-membered ring series of analogs was to remove the ring heteroatoms and maintain the amine substituent. The simple aniline derivative **31** seemed to be an appropriate derivative to answer the question of the importance of removing ring nitrogens while keeping the exocyclic amine. This was made via standard Suzuki coupling of bromide **3** and 3-aminophenyl boronic acid. The corresponding pyrmidine containing compound **32** (2′-NH_2) was also included for activity comparison and was made as previously reported.[4] In addition, several 5-membered ring heterocycles which were devoid of basic nitrogens (examples **33**–**37**) were also appended at C3 via the Suzuki or Stille methodology as described above.

Pyrazole and isoxazole derivatives **39** and **40** were made by treating vinylogous amide **38**[3] with hydrazine or hydroxylamine in ethanol, respectively (Scheme 5).

Substituted pyrazoles were not accessible through the above methods of simple condensation with earlier intermediates or via cross-coupling strategies so an alternate route was developed. Treatment of **19** with

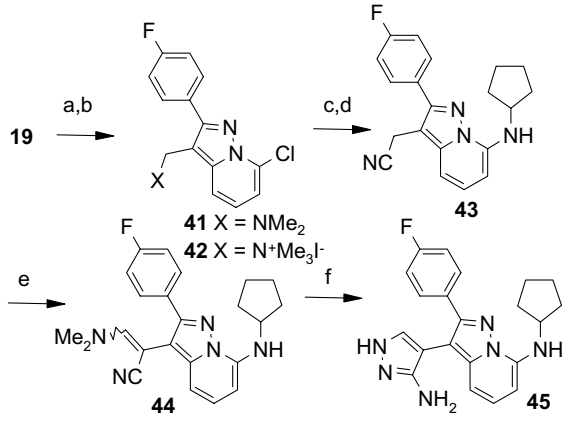


Scheme 5. Reagents and conditions: (a) for **39** anhyd H_2NNH_2, EtOH, reflux, 2 h (86%), for **40**, H_2NOH HCl, K_2CO_3, EtOH (93%).

Eschenmoser's salt in the presence of trifluoroacetic acid results in complete regioselective formation of the *N*,*N*-dimethyl-aminomethyl substituted intermediate **41** (Scheme 6). This benzylic amine could be activated as its quaternary ammonium salt by treatment with methyl iodide to give **42** which was smoothly converted to the benzylic nitrile **43** upon heating with KCN in NMP. The activated methylene of the nitrile was homologated to enamine **44** with dimethylformamide dimethylacetal and DBU, and finally condensed with hydrazine to give the desired amino-substituted pyrazole **45**.

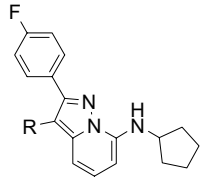
A final series of analogs were made to examine the effects of an imidazole, thiazole, and oxazole at the C3 position. Previously known methyl ketone **46**[3] was converted to its silyl enol ether and brominated with NBS in a one-pot method to give the α-bromoketone **47**. This material was condensed with thiourea to give the corresponding 4-(2-aminothiazole) substituted pyrazolopyridine. A similar condensation was accomplished using guanidine and amide nucleophiles to give the desired imidazole and oxazole derivatives **49**–**51** albeit not unexpectedly in significantly lower yields than the thiazole counterpart.

The objective of the current study was to investigate potential replacements for the C3 pyrimidine in GW3733. The goal was to look for alternative C3 groups that reduce MW and lipophilicity. Toward that end the closest analogs were envisioned to be a set of pyridines that simply removed one of the pyrimidine ring heteroatoms. While a pyridine replacement might add increased solubility, it could be imagined that replacement of the pyrimidine N-3 with a CH might cause unfavorable steric interactions with the neighboring pyrazolopyridine ring. Compound **8** clearly shows that the pyridine ring is tolerated with little to no effect on antiviral activity resulting from removal of the 'inner' pyrimidine nitrogen (Table 1) (Scheme 7).



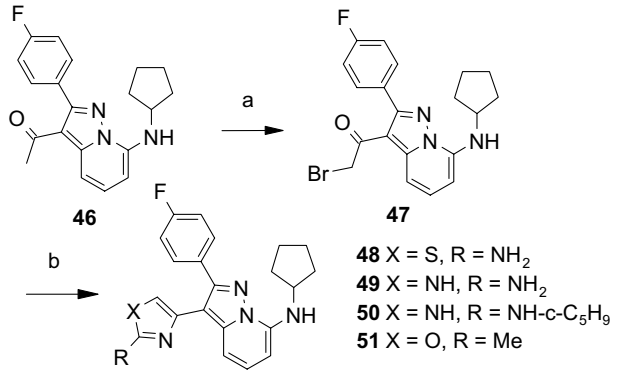
Scheme 6. Reagents and conditions: (a) Eschenmosher's salt, TFA, CH_2Cl_2 (99%); (b) MeI, MeOH (96%); (c) KCN, NMP, 100 °C (68%); (d) $Pd(OAc)_2$, BINAP, Cs_2CO_3, cyclopentylamine, PhMe, 105 °C (89%); (e) DMF–DMA, DBU, 115 °C (35%); (f) anhyd H_2NNH_2, EtOH, AcOH (90%).

Table 1. HSV-1 antiviral activity and cytotoxicity of C3 analogs



Compound	R	EC_{50}[a] (μM)	CC_{50}[b] (μM)	TI[c]
1	4-(2-*c*-C_5H_9NH-pyrimidyl)–	0.26	>160	>615
6	4-Pyridyl–	2.5	~20	80
7	4-(2-F-pyridyl)–	>40	n.d.	n.d.
8	4-(2-*c*-C_5H_9NH-pyridyl)–	0.37	>40	>108
9	4-(2-Hydrazino-pyridyl)–	5.7	40	7
10	4-(2-N_3-pyridyl)–	>40	n.d.	n.d.
14	3-Pyridyl–	3.4	>40	>11
15	3-(2-F-pyridyl)–	n.d.	n.d.	n.d.
16	3-(2-*c*-C_5H_9NH-pyridyl)–	>40	n.d.	n.d.
17	3-(6-F-pyridyl)–	>40	n.d.	n.d.
18	3-(6-*c*-C_5H_9NH-pyridyl)–	>40	n.d.	n.d.
23	4-Pyridazinyl–	>10	n.d.	n.d.
30	4-(6-*c*-C_5H_9NH-pyrimidyl)–	0.85	>40	>46
31	3-NH_2–C_6H_4–	>10	n.d.	n.d.
32	4-(2-NH_2-pyrimidyl)–	4.1	46	11
33	5-Indolyl–	3.9	20	5
34	3-Thiopheneyl–	>10	n.d.	n.d.
35	3-Furanyl–	>10	n.d.	n.d.
36	2-Furanyl–	>10	n.d.	n.d.
37	4-Pyrazolo–	4.7	10	2
39	3-Pyrazolo–	2.5	14	6
40	5-Isoxazolo–	>10	n.d.	n.d.
45	4-(3-NH_2-pyrazolo)–	4.9	20	4
48	4-(2-NH_2-thiazolo)–	8.6	n.d	n.d.
49	4-(2-NH_2-imidazolo)–	>10	n.d.	n.d.
50	4-(2-*c*-C_5H_9NH-imidazolo)–	7.9	40	5
51	4-(2-CH_3-oxazolo)–	>40	n.d.	n.d.

n.d., not determined.
[a] Vero cells, SC-16 strain. EC_{50} is the concentration at which 50% efficacy in the antiviral assay is observed using a capture hydrid method.
[b] CC_{50} is the concentration at which 50% cytotoxicity is observed.
[c] Therapeutic index (CC_{50}/EC_{50}).



Scheme 7. Reagents and conditions: (a) TBSOTf, *i*-PrNEt, CH_2Cl_2, 0 °C 30 min, then add NBS in THF (75%); (b) for **48**, $H_2NC(=S)NH_2$, DMF, 1 h, 90 °C (56%); for **49**, $H_2NC(=NH)NH_2$, K_2CO_3, DMF, 80 °C (18%); for **50**, $H_2NC(=NH)NH$–*c*-C_5H_9, K_2CO_3, DMF, 80 °C (17%); for **51**, H_2NCOMe, DMF, 90 °C (20%).

It is also consistent with our previous work that removal of the cyclopentylamine group as is the case for analog **6** has a deleterious effect on potency. Decreasing the basicity of the pyridine nitrogen as is the case in **7** resulted in a dramatic loss in activity. The 3-pyridyl series (**14**) showed comparable activity to the 4-pyridyl derivative **6**. However in this case, the potency could not be improved by the addition of hydrophobic amines at either the 2- or 6-positions (**16** and **18**). The pyrimidine nitrogen in the 3-position could also be 'moved' across the ring to give formally the 6-cyclopentylamine substituted pyrimidine **30** with little loss in activity. As expected, the complete removal of the pyridine or pyrimidine nitrogen as in examples **31** and **33**–**36** results in no appreciable antiviral activity.

The remaining question was to determine if any 5-membered ring heterocycles could retain the activity of the pyrimidine/pyridine series. Interestingly, after a fairly extensive amount of work to make imidazole, thiazole, oxazole, and pyrazole examples only modest activity was observed and typically was not well separated from cytotoxicity.

The above SAR study has served to map out the C3 pharmacophore requirements in detail. A clear

understanding that a 4-substituted, Lewis basic 6-membered ring heterocycle was essential to the potent antiviral activity of the series was a key outcome of these investigations. In addition several new strategies for the construction of novel 3-substituted pyrazolopyridine ring systems have been developed.

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6. Stannane **11** was prepared from 2-F-pyridine and LDA in THF followed by quenching with Bu_3SnCl.
7. Boronic acid **13** was prepared from 2-F,5-Br-pyridine and BuLi in THF followed by treatment with $B(OMe)_3$ and acidic workup.
8. The structure of **21** was confirmed by X-ray.
9. Interestingly, 2-substituted dihydro intermediate **21** did not undergo the aromatization step when subjected to the same conditions as its 4-substituted counterpart, **20**.