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Pyrazolopyridines with potent activity against herpesviruses: Effects of C5 substituents on antiviral activity

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Abstract—Synthesis of a series of 5-substituted as well as 5,7-disubstituted 3-[2-(cyclopentylamino)-4-pyrimidinyl]-2-phenylpyrazolo [1,5-*a*]pyridin-7-amines with potent activity against herpes simplex viruses is described. Synthetic approaches allowing for variation of the substitution pattern are outlined and resulting changes in antiviral activity are highlighted. Several compounds with in vitro antiviral activity similar to or better than acyclovir are described.

The herpesvirus family contains eight known human viruses, amongst them herpes simplex virus 1 (HSV-1) and herpes simplex virus 2 (HSV-2).[1] HSV-1 and HSV-2 cause mucocutaneous infections, resulting in cold sores (HSV-1) and genital lesions (HSV-2), respectively. 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 US) and a high prevalence.[2]

Previous antiviral research on herpes simplex viruses has primarily focused on the development of nucleoside analogs,[3] such as acyclovir (Zovirax),[4] valacyclovir,[5] famciclovir,[6] and penciclovir. Recently, immunomodulators (imiquimod and resiquimod),[7] nonnucleoside viral polymerase inhibitors (4-hydroxyquinoline-3-carboxamides),[8] and viral helicase inhibitors (thiazolylphenyl and thiazolylamide)[9] have received considerable attention. Though numerous strategies and considerable effort have been spent in the search for the next generation antiherpetic therapy, it has proved difficult to outperform acyclovir.[10]

We have recently described a series of pyrazolo[1,5-*a*]pyridines, such as **1**, which show potent and selective inhibition of HSV-1 and HSV-2 (Fig. 1).[11] The medical significance of herpesvirus infection combined with the limited number of potential new therapies compelled our efforts to fully explore the pyrazolopyridine scaffold. In previous reports, we have disclosed optimization of substituents on the 2-phenyl moiety,[11b] optimization of the C7 pyrazolopyridine substituent[11d] and 6,7-disusbstituted pyrazolopyridines[11c] as well as optimization of the C3 heterocycle.[11e] Substitution of the remaining 4 and 5 positions on the pyrazolopyridine wase the next area of our focus. Due to the tight SAR requirements of the C3 pyrimidine[11e] and the likelihood of significant interaction with substituents at the 4 position, we elected to focus our efforts on C5 and combinations with the C7 position. Additionally, the resonance electronic similarity of the 5 position to that of the 7 position with respect to the core heterocycle electron density made examination of the 5 position of significant interest. Herein, we describe the synthesis and antiviral activity of 5-substituted pyrazolopyridines along with 5,7-disubstituted pyrazolopyridines.

The 5-chloropyrazolopyridine **6** was prepared in three steps from commercially available 4-chloro-2-picoline

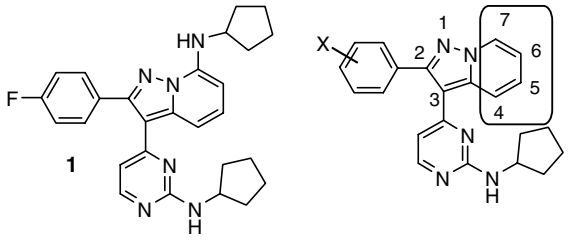

Fig. 1. Pyrazolopyridines.

Keywords: Pyrazolopyridine; HSV; Antiviral; Herpes viruses; SAR.
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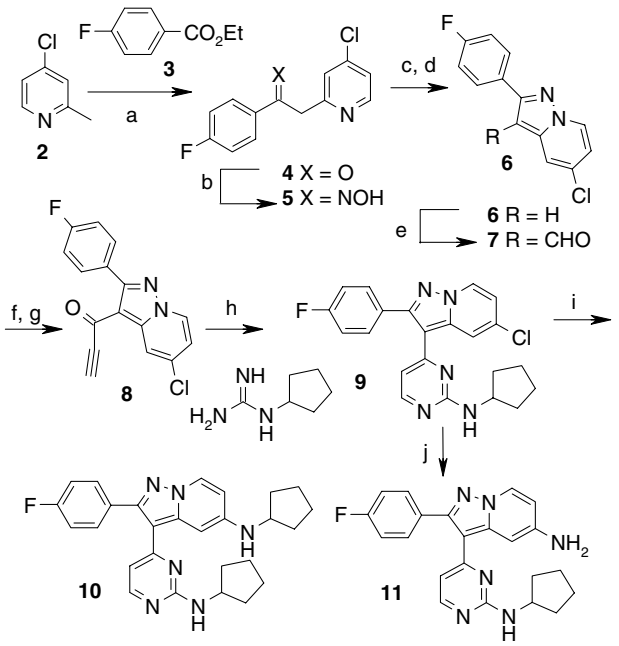


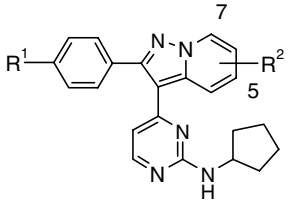
Scheme 1. Reagents and conditions: (a) LiN $(TMS)_2$, THF, 0–20 °C, 15 h, 99%; (b) hydroxylamine HCl, NaOH, MeOH, reflux, 2 h, 84%; (c) $(CF_3CO)_2O$, Et_3N, DME, 0 °C–rt; (d) $FeCl_2$, 1,2-dimethoxyethane, 75 °C, 15 h, 57% for 2 steps; (e) $POCl_3$ (1.5–2 equiv), DMF, rt, 12 h (85%), (f) ethynyl magnesium bromide (2.4 equiv), THF, −78 °C to 0 °C, 2 h; (g) MnO_2 (excess), CH_2Cl_2, 1 h (62%, 2 steps); (h) cyclopentyl guanidine HCl (2 equiv), K_2CO_3 (2 equiv), DMF, 80 °C, 12 h (74%); (i) $Pd(OAc)_2$ (0.2 equiv), BINAP (0.3 equiv), Cs_2CO_3 (1.5 equiv), cyclopentylamine (neat), 80 °C, 24 h (70%); (j) benzophenone imine (3 equiv), Pd_2dba_3 (0.1 equiv), BINAP (0.3 equiv), NaOtBu (3 equiv), toluene, 100 °C, 3 h; 4 N HCl, THF, 0 °C, 30 min (57% for 2 steps).

(**2**) and ethyl 4-fluorobenzoate (**3**) as outlined in Scheme 1, using the same approach as we previously reported for preparation of 7-chloropyrazolopyridine.[11] Formylation of **6** under Vilsmeier–Haack conditions gave an excellent yield of aldehyde **7**. This aldehyde was treated with the commercially available ethynyl Grignard reagent at low temperature to give the propargyl alcohol. This alcohol was easily oxidized to the ketone **8** using MnO_2. Treatment of the alkynyl ketone **8** with cyclopentylguanidine resulted in formation of the desired 5-chloro substituted pyrazolopyridine **9**. Previously, we have shown that a 7-chloro substituent on pyrazolopyridine can be nucleophilically displaced by amines (e.g., formation of **1** from the corresponding 7-chloropyrazolopyridine). In the case of the 5-chloropyrazolopyridine **9** this was not the case. Several attempts at thermally displacing the 5-chloro substituent of **9** with cyclopentylamine at elevated temperature failed, but the 5 position could be coupled with cyclopentylamine using Buchwald amination conditions ($Pd(OAc)_2$, *rac*-BINAP, Cs_2CO_3)[12] to give **10**. Thus, the 5-chloropyrazolopyridine **9** behaves quite differently toward nucleophilic substitution than the analogous 7-chloropyrazolopyridine.

The 5-amine derivative **11** was prepared from **9** by treatment with benzophenone imine, Pd_2dba_3, and BINAP in the presence of base to give the imine that was subsequently hydrolyzed in aqueous acid to give **11**.

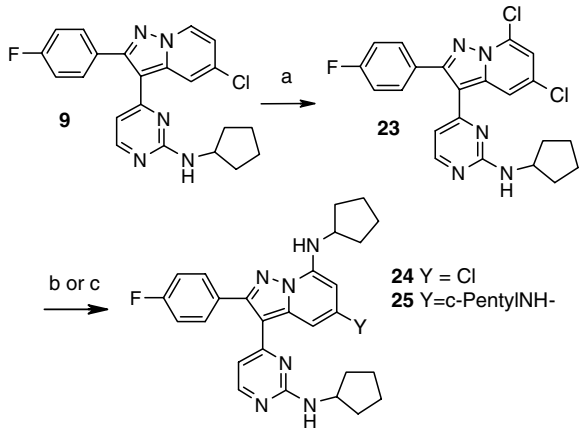
The 5-cyclopentylamine derivative **10** showed similar anti-HSV activity as the corresponding 7-cyclopentylamino derivative **1**, while the less lipophilic amine **11**

Table 1. HSV-1 antiviral activity and cytotoxicity of C5-substituted pyrazolopyridine analogs

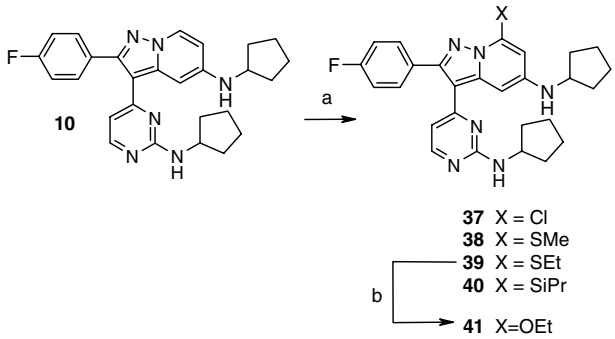


Compound	Subst. R^1	Subst. R^2	IC_{50}[a] (μM)	CC_{50}[b] (μM)
9	F	5-Cl	>5	>40
10	F	5-*c*-PentylNH	0.53	18
11	F	5-NH_2	5.07	20
12	F	5-(1-Pyrrolidinyl)	1.35	>40
13	F	5-$CH_3O(CH_2)_2NH$	0.83	>40
14	F	5-*i*-PropylNH	2.73	>40
15	F	5-Morpholino	3.63	34
16	CH_3O	5-Cl	4.27	>40
17	CH_3O	5-*c*-PentylNH	0.21	12.3
18	CH_3O	5-*i*-PropylNH	0.92	>40
19	*c*-PropylCH_2O	5-*c*-PentylNH	0.33	>40
20	*i*-PropylCH_2O	5-*c*-PentylNH	0.56	27
1[c]	F	7-*c*-PentylNH	0.26	>160
21[c]	CH_3O	7-*c*-PentylNH	0.22	>100
22[c]	*c*-PropylCH_2O	7-*c*-PentylNH	0.17	>40
ACV	Acyclovir		0.39	>200

[a] Vero cells, HSV-1, SC-16 strain. IC_{50} is the concentration at which 50% efficacy in the antiviral assay is observed using a capture hydrid method.[11b]
[b] CC_{50} is the concentration at which 50% cytotoxicity is observed.
[c] C-7 analogs included for comparison were prepared as described in Ref. 11b.



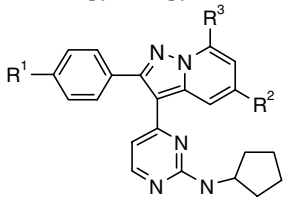
Scheme 2. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, then CCl_4, (45%); (b) cyclopentylamine (neat), $Pd(OAc)_2$, *rac*-BINAP, Cs_2CO_3, 80 °C, 24 h (68% of **24**); (c) cyclopentylamine (neat), $Pd(OAc)_2$, *rac*-BINAP, Cs_2CO_3, 130 °C, sealed tube, 24 h (37% of **25**).



Scheme 3. Reagents and conditions: (a) for **37**: *n*-BuLi (5 equiv), THF, −78 °C, then CCl_4, (25%); for **38**: *n*-BuLi (5 equiv), THF, −78 °C, then MeSSMe (40%); (c) for **39**: *n*-BuLi (5 equiv), THF, −78 °C, then EtSSEt (70%); for **40**: *n*-BuLi (5 equiv), THF, −78 °C, then *i*-PrSSiPr (72%); (b) compound **39**, *m*-CPBA (4 equiv), CH_2Cl_2, rt, 30 min. Then NaOEt, EtOH, reflux, 2 h (40% for 2 steps).

was less potent (Table 1). The potent anti-HSV activity of **10** prompted us to synthesize a number of additional 5-substituted pyrazolopyridines (**12**–**20**) containing either fluorine (**12**–**15**) or alkoxysubstituted (**16**–**20**) 2-phenyl moiety using the synthesis outlined in Scheme 1. Anti-HSV activity for **12**–**20**, along with activity for

Table 2. HSV-1 antiviral activity and cytotoxicity of disubstituted pyrazolopyridine analogs



Compound	Subst. R^1	Subst. (C5) R^2	Subst. (C7) R^3	IC_{50}[a] (μM)	CC_{50}[b] (μM)
23	F	Cl	Cl	7	>40
24	F	Cl	*c*-PentylNH	0.33	>40
25	F	*c*-PentylNH	*c*-PentylNH	0.24	>40
26	F	Cl	*c*-PropylNH	0.33	>40
27	F	Cl	*i*-PropylNH	1.5	>40
28	F	*i*-PropylNH	*i*-PropylNH	0.55	>40
29	F	Cl	Morpholine	0.18	>40
30	F	Cl	Piperidine	0.15	>40
31	F	Cl	Me_2NH	0.38	>40
32	OMe	Cl	Cl	5.3	>40
33	OMe	Cl	*c*-PentylNH	0.22	>40
34	OMe	Cl	*c*-PropylNH	0.34	>40
35	OR^4	Cl	*c*-PentylNH	0.43	>40
36	OR^4	Cl	*c*-PropylNH	0.42	>40
37	F	*c*-PentylNH	Cl	1.36	>40
38	F	*c*-PentylNH	MeS	0.17	>40
39	F	*c*-PentylNH	EtS	0.07	>40
40	F	*c*-PentylNH	*i*-PrS	0.37	>40
41	F	*c*-PentylNH	EtO	0.16	20.6
42	F	*c*-PentylNH	$MeO(CH_2)_2O$	0.08	32.5
43	OMe	*c*-PentylNH	MeS	0.22	>40
44	OMe	*c*-PentylNH	EtS	0.04	>40
45	OMe	*c*-PentylNH	*i*-PrS	0.21	>40
46	OMe	*c*-PentylNH	PhS	1.8	>40
50	F	MeO	H	>36	>36
52	F	MeO	*c*-PentylNH	0.13	>40
54	F	$MeO(CH_2)_2O$	*c*-PentylNH	0.32	>40
55	F	*i*-PrO	*c*-PentylNH	0.07	>40
56	F	*c*-PropylCH_2O	*c*-PentylNH	0.17	>40
ACV		Acyclovir		0.39	>200

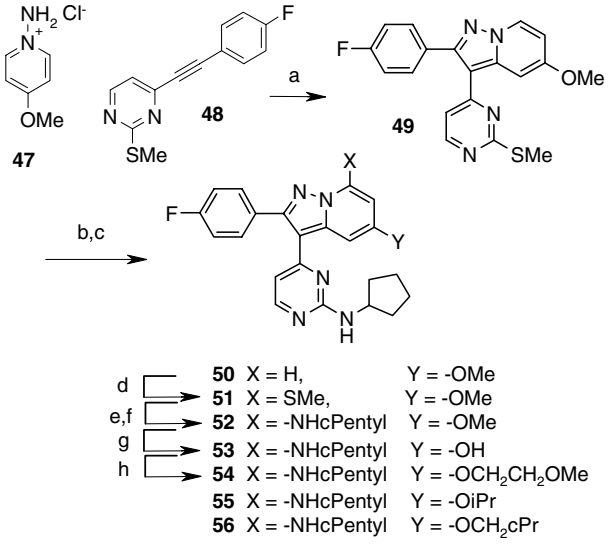
[a] Vero cells, HSV-1, SC-16 strain. IC_{50} is the concentration at which 50% efficacy in the antiviral assay is observed using a capture hydrid method.[11b]
[b] CC_{50} is the concentration at which 50% cytotoxicity is observed.

selected 7-substituted pyrazolopyridines[11b] (**1**, **21**–**22**) for comparison, is shown in Table 1. As can be determined from Table 1 the 5- or 7-substituted pyrazolopyridines show similar potency. In general the C5 amines are significantly more potent than the C5 chloro compounds. Also the more lipophilic amines (e.g., cyclopentylamine) are in general more potent than the smaller, less lipophilic amines.

Potent activity of both 7-substituted as well as 5-substituted pyrazolopyridines prompted us to consider making 5,7-disubstituted pyrazolopyridines. As outlined in Scheme 2 the C7 protio derivative **9** could be treated with a strong base to selectively deprotonate the 7 position.[13] This could be further functionalized by trapping the resulting anion with an electrophile, such as CCl_4, to give the 5,7-dichloropyrazolopyridine **23**.

Use of *n*-BuLi for deprotonation did not seem to result in significant C5 chloro exchange (the C5 protio derivative was not isolated from the reaction mixture) nor did it appear to direct the deprotonation to the 4 or 6 positions. Buchwald coupling conditions were then used to cleanly replace the C7 chlorine (to give **24**) without significant reaction of the C5 chlorine as long as the reaction was carried out under 100 °C. At higher temperatures both the C5 and C7 chlorines could be replaced with alkylamines to give **25**. Compounds **26**–**36** were made in a similar fashion as outlined in Scheme 2.

Additional 5,7-disubstituted derivatives were synthesized from **10** as outlined in Scheme 3. Compound **10** was treated with excess *n*-BuLi followed by quenching with a suitable electrophile. Especially potent were compounds **38**–**40**, obtained by quenching lithiated **10** with alkyl disulfides. 7-Alkoxy derivatives **41** and **42** were obtained by *m*-CPBA oxidation of **39** to the sulfoxide, followed by treatment with an alkoxide. Compounds **41** and **42** are better suited for development than the thiol derivatives, which are potentially liable to in vivo oxidation and thus risk of formation of a reactive metabolite. Compounds **43**–**46** (Table 2) were prepared similarly.



Scheme 4. Reagents and conditions: (a) DBU, MeCN (55%); (b) *m*-CPBA, CH_2Cl_2 0 °C; (c) cyclopentylamine (neat), 85 °C, 1 h (69%); (d) LDA, THF −78 °C, then MeSSMe (91%); (e) *m*-CPBA, CH_2Cl_2 0 °C (99%); (f) cyclopentylamine (neat), 130–145 °C, 24 h (65%); (g) BBr_3, CH_2Cl_2 −78 °C to rt, 3 d (66%); (h) Cs_2CO_3, MeCN, alkyl halide; for **54** $MeOCH_2CH_2Br$ (47%), for **55** 2 bromopropane (35%), for **56** *c*-$PrCH_2Br$ (26%).

Since the 5-amino derivatives (like **10**) were displaying increased activity over the chloro analogs (like **9**), it became of interest to look into ether substituents at the C5 position. Since transition metal catalyzed couplings of alcohols with aryl halides are significantly less developed than the amine coupling reaction, we sought an alternative synthetic route to access the 5-alkoxy derivatives. Toward that end, the *N*-amino-4-methoxypyridine **47** was treated with diaryl acetylene **48** to give the 5-methoxypyrazolopyridine **49** as outlined in Scheme 4. The 2-cyclopentyl amine substituent on the pyrimidine was installed through oxidation of **49** to the sulfoxide and subjected to thermal displacement to provide the desired scaffold **50** as the 7-protio derivative. Lithiation of the 7 position as described above and sulfenylation proceeded smoothly to provide a handle for further functionalization. Oxidation and amine displacement again served to provide the 5,7-disubstituted analog **52**. Standard demethylation with BBr_3 gave the phenol and realkylation with assorted alkyl halides resulted in the 5-alkoxy analogs **54**–**56**. The above SAR study further delineates the SAR of the pyrazolopyridine scaffold. Several molecules with significantly (5- to 10-fold) better potency than the current gold standard acyclovir were identified, thus establishing the disubstituted pyrazolopyridines as an exciting template for anti-HSV drug discovery.

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