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Pyrazolopyrimidines and pyrazolotriazines with potent activity against herpesviruses

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ABSTRACT

Synthesis of several pyrazolo[1,5-*c*]pyrimidines, pyrazolo[1,5-*a*]pyrimidines and pyrazolo[1,5-*a*][1,3,5]triazines 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 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] non-nucleoside 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 has been spent in the search for the next generation antiherpetic therapy, it has proved difficult to outperform valacyclovir.[10]

We have recently described SAR for 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] We have also described SAR for a closely related series of imidazopyridines.[12] With substitution of the pyrazolo[1,5-*a*]pyridine extensively optimized[11] we became interested in preparing other heterocyclic cores, such as pyrazolopyrimidines and pyrazolotriazines. These alternative cores would allow us to maintain optimized substitution profile, while potentially improving developability characteristics, by changing lipophilicity of the core scaffold. Herein, we describe the synthesis and antiviral activity of certain pyrazolo[1,5-*c*]pyrimidines, pyrazolo[1,5-*a*]pyrimidines and pyrazolo[1,5-*a*][1,3,5]triazines.

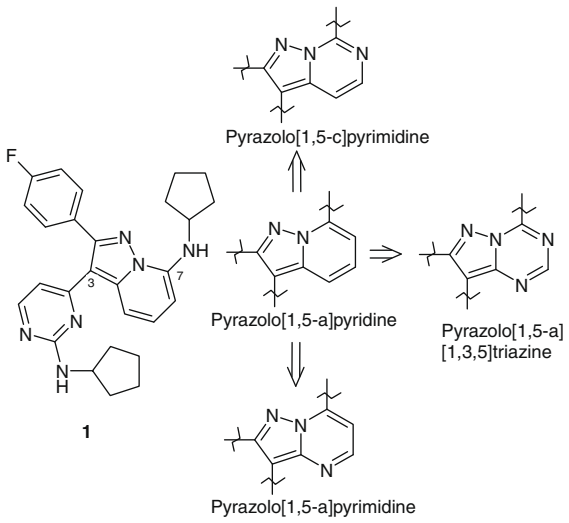

Figure 1. Pyrazolopyridine and related heterocyclic scaffolds.

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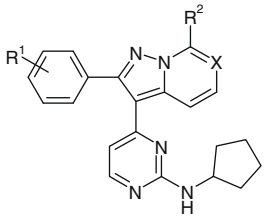
For the synthesis of the desired pyrazolo[1,5-*c*]pyrimidine the first step involved benzoylation of 4-methyl-2-(methylsulfanyl)pyrimidine (**2**) in the presence of lithium bistrimethylsilylamide to give ketone **4** (Scheme 1). Subsequent treatment of **4** with hydroxylamine gave the oxime **5**. Treatment of **5** with trifluoroacetic anhydride and warming to room temperature in the presence of base facilitated formation of an azirine intermediate. This intermediate was not isolated, but heated in the presence of $FeCl_2$ in DME, resulting in a clean conversion to the desired pyrazolopyrimidine **6.** Friedel–Crafts acetylation was used to functionalize the C3 position to form methylketone **7**. Oxidation of the thiomethyl group to the corresponding sulfoxide followed by displacement with cyclopentylamine gave **8**. Heating methyl ketone **8** in di-*tert*-butyl acetal of dimethylformamide (DMF-DTBA) in DMF resulted in complete conversion to the vinylogous amide **9**. Treatment of **9** with the cyclopentylamine derived guanidine **10**[11a], under basic conditions, gave the desired pyrazolopyrimidine **11**.

Pyrazolopyrimidines **12–18** (Table 1) were made in a similar fashion as outlined in Scheme 1. Alkoxyderivatives **19–24** were prepared from **18**, by demethylation with BBr_3 followed by alkylation with desired alkyl halides.

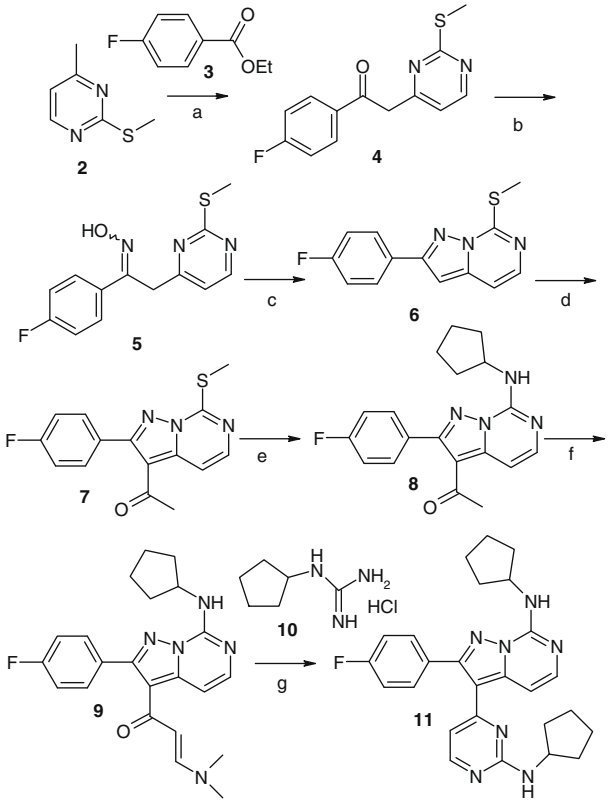
Table 1 shows anti-HSV-1 activity for several pyrazolo[1,5-*c*]pyrimidines. In general these showed comparable activity to the pyrazolopyridines (e.g., pyrazolopyrimidines **11**, **18** and **23** have similar anti-HSV activity as the corresponding pyrazolopyridines **1**, **25** and **26**). This demonstrated the viability of the pyrazolo[1,5-*c*]pyrimidines as an alternative to the pyrazolo[1,5-*a*]pyridine scaffold. Given the promising activity of the pyrazolo[1,5-*c*]pyrimidines we then became interested in synthesizing the pyrazolo[1,5-*a*]pyrimidine scaffold (Scheme 2). Reaction of aminopyrazole **28**

Table 1
HSV-1 antiviral activity and cytotoxicity of pyrazolo[1,5-*c*]pyrimidine analogs

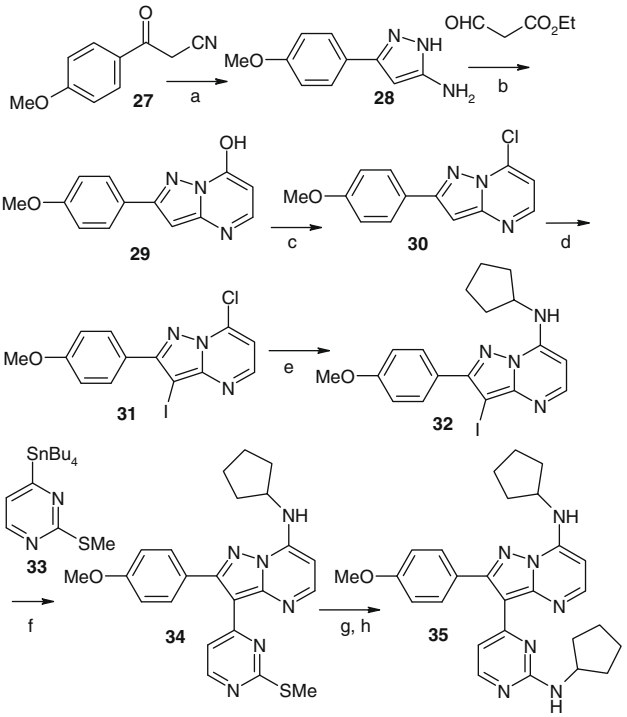


Compd	R^1	R^2	X	IC_{50} (μM)	CC_{50} (μM)
11	*p*-F	*c*-PentylNH	N	0.17	>40
12	*p*-F	1-Piperidinyl	N	0.98	>40
13	*p*-F	1-Pyrrolidinyl	N	1.90	>40
14	*p*-F	*t*-BuNH	N	6.61	>40
15	*m*-Cl	*c*-PentylNH	N	0.46	>40
16	*m*-Cl	1-Morpholine	N	0.20	>40
17	*m*-Cl	$CH_3O(CH_2)_2N$	N	0.30	>40
18	*p*-CH_3O	*c*-PentylNH	N	0.28	>40
19	*p*-HO	*c*-PentylNH	N	4.0	27
20	*p*-(*n*-PrO)	*c*-PentylNH	N	0.54	>20
21	*p*-(*n*-BuO)	*c*-PentylNH	N	0.47	>40
22	*p*-(*i*-PropylCH_2O)	*c*-PentylNH	N	0.61	>40
23	*p*-(*c*-PropylCH_2O)	*c*-PentylNH	N	0.69	>40
24	*p*-($CH_3OCH_2CH_2O$)	*c*-PentylNH	N	1.12	>40
1[c]	*p*-F	*c*-PentylNH	CH	0.26	>160
25[c]	*p*-CH_3O	*c*-PentylNH	CH	0.22	>100
26[c]	*p*-(*c*-PropylCH_2O)	*c*-PentylNH	CH	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 hybrid capture method.[11b]
[b] CC_{50} is the concentration at which 50% cytotoxicity is observed in vero cells.
[c] Pyrazolopyridine analogs included for comparison, prepared as described in Ref. 11b.



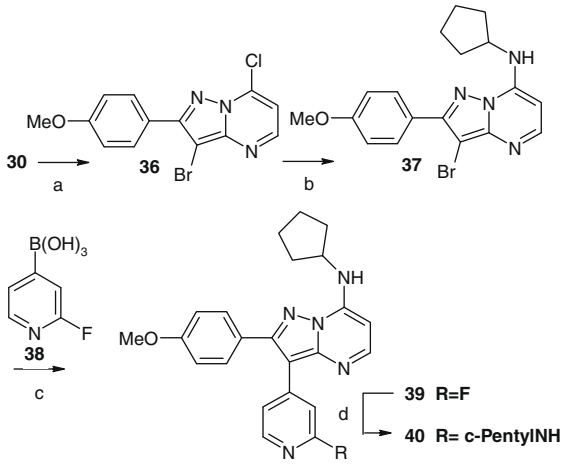
Scheme 1. Reagents and conditions: (a) $LiN(TMS)_2$, THF, 0 to 20 °C, 1.5 h (75%); (b) hydroxylamine HCl, NaOAc, CH_3CN, rt, 24 h (69%); (c) $(CF_3CO)_2O$, Et_3N, DME, 0 °C to rt; then $FeCl_2$ (0.3 equiv added in 3 aliquots), DME, 80 °C, 8 h (68%); (d) Ac_2O (neat), H_2SO_4 (cat), reflux, 2 h (64%); (e) *m*-CPBA, $NaHCO_3$, CH_2Cl_2, rt, 2 h; then cyclopentylamine (neat), 2 h, rt (44% for two steps); (f) DMF-DTBA, DMF, 80 °C, 30 min (69%); (g) **10** (2 equiv), K_2CO_3 (2 equiv), DMF, 140 °C, 4 h (75%).



Scheme 2. Reagents and conditions: (a) NH_2NH_2, EtOH, reflux, 12 h (95%); (b) ethylformyl acetate, Na/EtOH, rt, 8 h (66%); (c) $POCl_3$ (neat), 100 °C, 8 h (90%); (d) NIS, CH_2Cl_2, rt, 2 h; (e) cyclopentylamine (neat), rt, 12 h (45% for steps d and e); (f) $PdCl_2(PPh_3)_2$, toluene, 110 °C, 16 h; (g) *m*-CPBA, CH_2Cl_2, 0 °C to rt, 2 h; (h) cyclopentylamine (neat), rt, 2.5 h (11% from **32**).

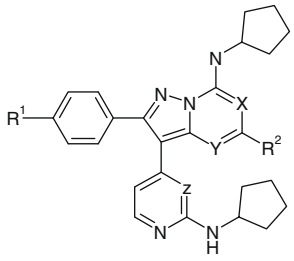
with the sodium salt of ethylformylacetate[13] gave **29**. Treatment of **29** with $POCl_3$ gave the 7-chloro derivative **30**. Iodination with NIS in CH_2Cl_2 gave the 3-iodo derivative **31**. Displacement of the chlorine with cyclopentylamine gave **32.** Stille coupling of **32** and tributylstannylpyrimidine **33**[14] gave the C3-pyrimidine substituted derivative **34**. Oxidation and displacement of the thiomethyl group gave the desired fully elaborated scaffold **35**.

Alternatively, compound **30** could be used for the synthesis of a C3 substituted pyridine, rather than C-3 substituted pyrimidine derivatives (Scheme 3). This was accomplished via bromination of **30** by NBS to give **36**, followed by displacement of the chlorine with cyclopentylamine to give cross-coupling precursor **37**. Suzuki coupling of **37** with 2-fluoropyridin-4-ylboronic acid **38**[15] gave the C3-pyridine substituted derivative **39**. Subsequent displacement of the fluorine with cyclopentylamine at elevated temperature gave **40**. Other C3-substituted pyridine derivatives (**41** and **42**) were synthesized in an analogous manner (Table 2).



Scheme 3. Reagents and conditions: (a) NBS, CH_2Cl_2, rt, 30 min (82%); (b) cyclopentylamine, EtOH, reflux, 10 min (77%); (c) $PdCl_2(PPh_3)_2$, Na_2CO_3, DMF, 110 °C, 24 h (32%); (d) cyclopentylamine (neat) in pressure tube, 165 °C, 2 days (27%).

Table 2
HSV-1 antiviral activity and cytotoxicity of pyrazolopyrimidines and pyrazolotriazine analogs



Compd	R^1	R^2	X	Y	Z	IC_{50} (μM)	CC_{50} (μM)
35	OMe	H	CH	N	N	2.4	32
40	OMe	H	CH	N	CH	0.6	10
41	F	H	CH	N	CH	1.2	16
42	H	H	CH	N	CH	0.3	14
51	H	H	N	N	N	6.4	>40
54	H	*c*-PentNH	N	N	CH	0.12	>40
1[c]	F	H	CH	CH	N	0.26	>40
55[c]	H	H	CH	CH	N	0.62	>40
56[c]	F	*c*-PentNH	CH	CH	N	0.24	>40
ACV	Acyclovir					0.39	>200

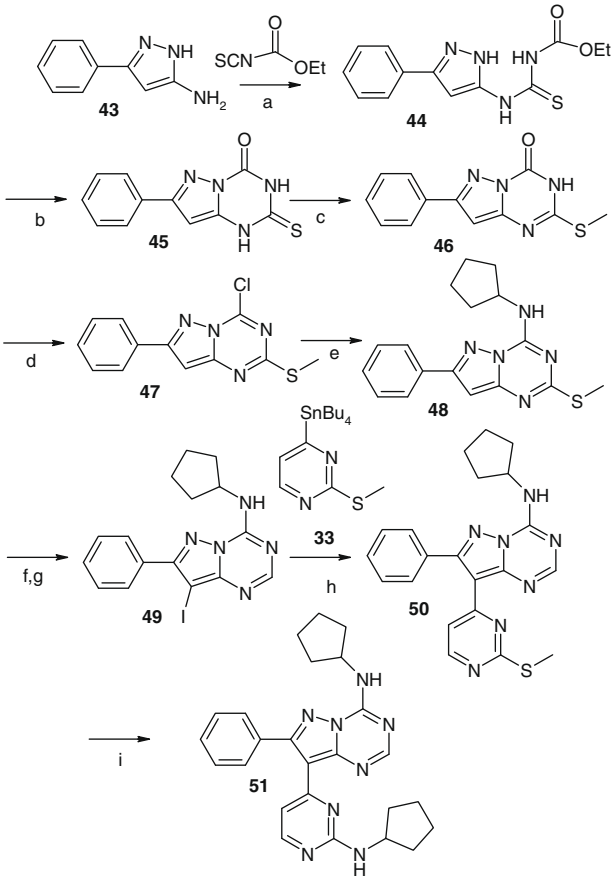
[a] Vero cells, HSV-1, SC-16 strain. IC_{50} is the concentration at which 50% efficacy in the antiviral assay is observed using a hybrid capture method.[11b]
[b] CC_{50} is the concentration at which 50% cytotoxicity is observed in Vero cells.
[c] Pyrazolopyridine analogs included for comparison from Ref. 11b (**1** and **55**) and 11f (**56**).

Anti-HSV activity for pyrazolo[1,5-*a*]pyrimidines is shown in Table 2. In general the pyrazolo[1,5-*a*]pyrimidine derivatives (**35**, **40**, **41** and **42**) had diminished anti-HSV activity when compared to the corresponding pyrazolo[1,5-*a*]pyridines, furthermore the pyrazolo[1,5-*a*]pyrimidines showed significantly more toxicity in Vero cells than previously observed for the pyrazolo[1,5-*a*]pyridines. This loss of selective antiviral activity over toxicity for compounds **35**, **40**, **41** and **42** prompted us not to pursue this series further.

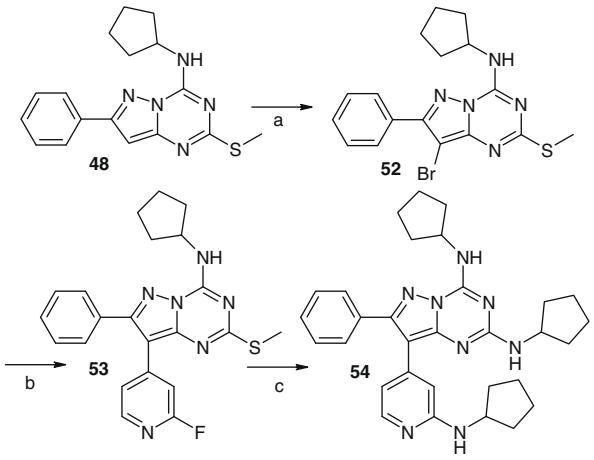
Finally we turned our attention to synthesis of the pyrazolotriazine derivatives (Scheme 4).

Treatment of 3-amino-5-phenylpyrazole **43** with ethoxycarbonyl isothiocyanate gave **44**. Compound **44** was treated with sodium hydroxide and underwent cyclization to give **45**. Methylation of the sulfur with methyliodide gave **46**. Treatment with $POCl_3$ resulted in formation of 7-chloro derivative **47**. Displacement of the chlorine with cyclopentylamine gave **48.** Removal of the thiomethyl group by treatment with Raney Nickel followed by iodination with NIS gave iodo derivative **49.** Stille coupling of **49** and tributylstannylpyrimidine **33** gave the C3-pyrimidine substituted derivative **50**. The 2-thiomethyl group was subsequently removed by an oxidation followed by cyclopentylamine displacement to give the desired pyrazolotriazine **51**.

Again in this case the intermediate **48** could be used for the synthesis of C3-pyridyl rather than C3-pyrimidinyl pyrazolotriazines via Suzuki coupling of **52** and **38** to give intermediate **53** as outlined in Scheme 5. The high temperature required for the cyclopen-



Scheme 4. Reagents and conditions: (a) Toluene, 0 °C to rt, 15 h (60%); (b) 2 N NaOH (aq), rt, 12 h (80%); (c) NaOH (aq), CH_3I, EtOH, rt, 3 h (81%); (d) $POCl_3$, diethylaniline, reflux, 3 h (86%); (e) cyclopentylamine (neat), 80 °C, 2 h (78%); (f) raney Ni, EtOH, reflux, 6 h (78%); (g) NIS, CH_2Cl_2, rt, 30 min (85%); (h) $PdCl_2(PPh_3)_2$, toluene, 100 °C, 24 h (i) *m*-CPBA, CH_2Cl_2, rt, 1 h; then cyclopentylamine (neat), 60 °C, 3 h (23% from **49**).



Scheme 5. Reagents and conditions: (a) NBS, CH_2Cl_2, rt, 30 min (82%); (b) **38**, $PdCl_2(PPh_3)_2$, Na_2CO_3, DMF, 100 °C, 12 h (33%); (c) cyclopentylamine (neat) in pressure vessel, 160 °C, 24 h (34%).

tylamine displacement of the fluorine in **53** also resulted in displacement of the thiomethyl group, thus giving the di-cyclopentylamino substituted derivative **54**.

Anti-HSV activity for pyrazolotriazines **51** and **54** is shown in Table 2. Pyrazolotriazine **51** is about 10-fold less potent than the corresponding pyrazolopyridine (**55**). However, the 5,7-di-cyclopentylamino substituted derivative **54** showed very good anti-HSV activity (similar activity to the analogous pyrazolopyridine **56**). Unfortunately, as compound **54** also has significantly increased molecular weight compared to alternative compounds with similar anti-HSV activity (such as **11**) we decided not to progress the pyrazolotriazines further.

Compound **11** was chosen for PK studies because of its potent anti-HSV activity. It was dosed in Sprague–Dawley rats at 1 mg/kg both iv and po. The concentration versus time curves are shown in Figure 2. A moderate clearance of 24.1 ml/min/kg was observed along with a 2.5 h half life and volume of distribution of 1.2 L/kg. The oral bioavailability was 24% with a C_{max} of 0.3 μM at 30 min post dosing. Further analogs have not been extensively examined in vivo but this initial data demonstrates reasonable evidence of oral bioavailability and potential for further development.

Previously, we had shown that the pyrazolo[1,5-*a*]pyridines in general showed better antiviral activity than the corresponding imidazo[1,2-*a*]pyridines.[12] Here, the pyrazolo[1,5-*c*]pyrimidine series turned out to be a viable alternative to the pyrazolo[1,5-*a*]pyridines, showing similar anti-HSV activity and cytotoxicity profile. Furthermore a number of the pyrazolo[1,5-*c*]pyrimidines showed similar or better anti-HSV activity than the current gold standard, acyclovir. The pyrazolo[1,5-*c*]pyrimidines are also slightly less lipophilic than the corresponding pyrazolo[1,5-*a*]pyridines. The pyrazolo[1,5-*a*]pyrimidines appear to have a diminished potency and smaller selectivity index (more cytotoxicity) than the pyrazolo[1,5-*c*]pyrimidines and pyrazolo[1,5-*a*]pyridines. Finally, the pyrazolo[1,5-*a*][1,3,5]triazines have demonstrated similar levels of antiviral activity to **1** however the large molecular weight and high lipophilicity of compound **54** makes it unattractive for further SAR studies. Finally, evidence of oral bioavailability for compound **11** suggests the pyrazolo[1,5-*c*]pyrimidine series has the potential for further optimization and development as an oral antiherpetic.

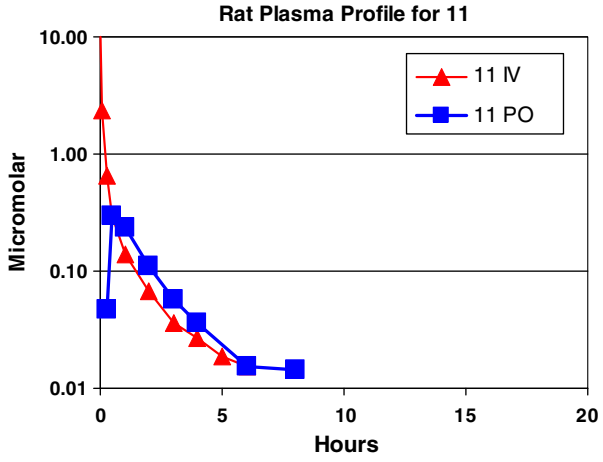


Figure 2. Rat pk profile for **11**.

Acknowledgements

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