Bioorganic & Medicinal Chemistry 17 (2009) 3025–3027

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Alkenyl substituted bicyclic nucleoside analogues retain nanomolar potency against Varicella Zoster Virus

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ARTICLE INFO

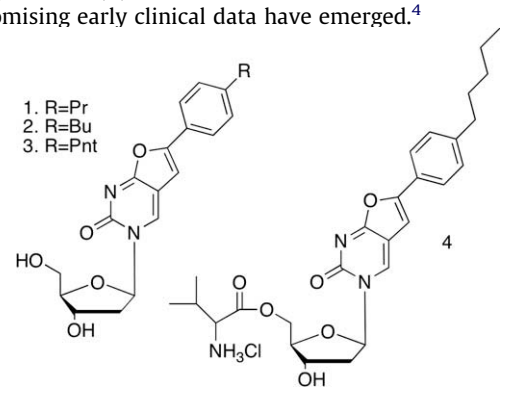
Article history:
Received 24 November 2008
Revised 9 March 2009
Accepted 10 March 2009
Available online 14 March 2009

Keywords:
BCNAs
VZV
Antiviral

ABSTRACT

Novel alkenyl substituted aryl bicyclic furano pyrimidines have been prepared and evaluated in vitro against Varicella Zoster Virus (VZV). The *para*-substituted analogues retain the nanomolar potency we have reported for *p*-alkyl analogues, while the *ortho*- and *meta*- alkenyl systems lose 3–4 orders of potency.

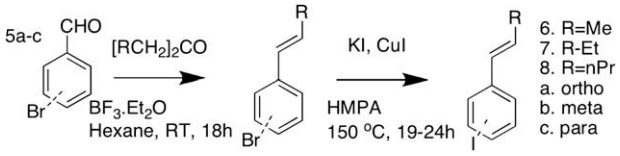
We have previously reported the discovery in our laboratories of the bicyclic furano pyrimidine family of antivirals (the BCNAs), which display unique potency and selectivity versus Varicella Zoster Virus (VZV).[1] We had originally reported the alkyl family but subsequently noted substantial increases in potency for the phenyl analogues, particularly with *p*-alkyl substitution (**1**–**3**).[2] We believe the *p*-pentyl analogue to be the most potent inhibitor of VZV reported to date, with in vitro efficacy at ca. 0.5 nM, and little or no detectable toxicity.[3] Indeed, as its orally bioavailable 5′-valyl prodrug, FV100, (**4**) has entered clinical trials for VZV shingles, and promising early clinical data have emerged.[4]



We have described some of the structure–activity relationships surrounding lead compound (**3**).[5] In particular, activity increases ca. 1000-fold on *para*-*n*-pentyl substitution of the phenyl ring.[6] There are few reports of alternative substitution patterns on the phenyl ring, although we have noted that *ortho* halogens are tolerated while *meta* are not.[7] In this paper we report the effect of unsaturation in the *p*-alkyl side chain at the position adjacent to the phenyl ring, and also the effect of moving the alkenyl chain to the *ortho* and *meta* positions of the phenyl.

The synthetic route to the desired target compounds was based around the need for a regio- and stereo- selective access. We chose to follow the one-pot method of Kabalka[8] where *E*-1-arylalkenes are prepared in high selectivity from ketones and aromatic aldehydes. Kabalka suggests a tandem Aldol-Grob sequence mechanism for this reaction.[9] Thus, the *ortho*- (**5a**) *meta*- (**5b**) and *para*- (**5c**) bromo benzaldehydes were reacted with symmetrical ketones pentan-3-one, heptan-4-one and nonan-5-one in the presence of boran-trifluoride to generate the corresponding *E*-1-alkenylaryl bromides (Scheme 1). Yields ranged from 12% to 81% and were higher for longer chain homologues. Pure *E* stereochemistry was confirmed by a ca. 15 Hz olefinic coupling constant.

Following poorly satisfactory attempts to convert these bromophenyl systems to their trimethysilylacetylenes for coupling to 5-iodo nucleoside, we decided instead to prepare the



Scheme 1.

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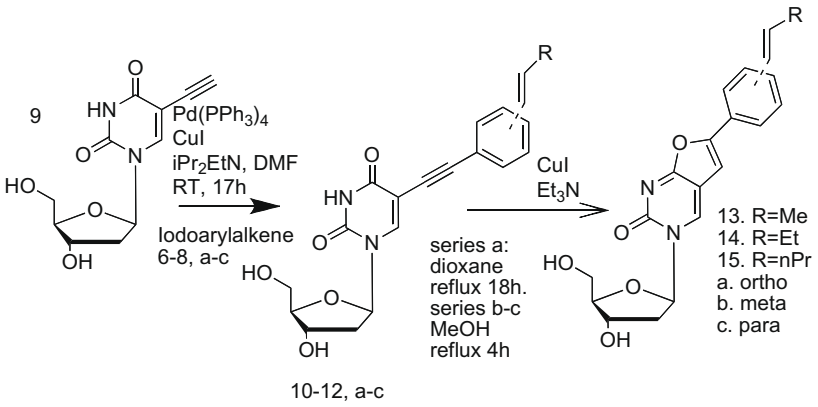
doi:10.1016/j.bmc.2009.03.022



Scheme 2.

Table 1
Anti-VZV activity of compounds **13–15a–c**

	C3 EC_{50} C3 (μM)					C4 EC_{50} (μM)					C5 EC_{50} (μM)				
	Compd	OKA	YS	MCC (μM)	CC_{50} (μM)	Compd	OKA	YS	MCC (μM)	CC_{50} (μM)	Compd	OKA	YS	MCC (μM)	CC_{50} (μM)
o	**13a**	12	—	400	103	**14a**	33	—	400	102	**15a**	8.4	—	80	41
m	**13b**	13	58	>200	>200	**14b**	1.9	2.3	⩾20	68	**15b**	1.1	1.3	⩾200	>200
p	**13c**	0.004	0.004	⩾50	>200	**14c**	0.0006	0.0007	⩾5	>200	**15c**	0.0003	0.0008	>5	>200
p-Alkyl	**1**	0.011	0.003	⩾50	188	**2**	0.0016	0.0005	⩾50	>200	**3**	0.0006	0.0005	⩾50	>200

5-ethynyl nucleoside and couple to the iodo phenyl system. Thus, we carried out halogen (iodine for bromine) exchange on the aryl bromides using copper iodide in hexamethyl phosphoric acid and elevated temperature.[10] Thus, products **6–8a–c** were isolated in good yield (67–93%) after flash column chromatography. The substitution of bromine by iodine was most evident in the ^{13}C NMR Spectra (δc Ar-I 95 ppm, Ar-Br 120 ppm), and by changes in the phenyl hydrogen pattern in the ^{1}H NMR.

The necessary nucleoside synthon 5-ethynyl-2′-deoxyuridine (**9**) was prepared from the 5-iodo nucleoside via Sonogoshira coupling to trimethylsilyl acetylene,[11] and this was coupled to the iodophenyl species **6–8a–c** under Pd(0) and Cu(I) catalysis in DMF to yield the intermediate 5-alkynyl species **10–12a–c**, Scheme 2. These were then cyclised to give the desired bicyclic systems **13–15a–c**. The products were characterised by ^{1}H and ^{13}C NMR, mass spectrometry and microanalysis, all data confirming the structure and purity of the products.[12]

The target compounds were assayed for their ability to inhibit two strains of VZV in vitro, OKA and YS, with data being shown in Table 1.[13] Data on the comparative alkyl analogues (*para* only) **1–3** are included for comparison.

For the *para* series **13–15c** a very potent anti-VZV effect is noted ranging from ca. 4 nM (C3) to ca. 0.3–0.8 nM (C4, C5). Data on this family mirrors closely that of the parent alkyl series **1–3**.

In all cases, compounds lost completely activity versus thymidine kinase-deficient virus mutants (data not shown) confirming their complete dependence on VZV TK for activation. By comparison to the *para* family the *ortho*- and *meta* series were noted to be poorly active, being ca. 3–4 orders of magnitude less potent than the *para* series. For the C3–C4 homologues the *meta* compounds were slightly more active than the *ortho* and indeed were equiactive with acyclovir in these assays but still ca. 2000-fold less potent than their *para* isomers.

Lastly, slight cytotoxicity was seen for some of the *ortho* and *meta* compounds but not the *para* regioisomers.

Flexible alignment simulation[14] (Fig. 1) of **3** (red chain) and **15c** (green chain) showed a good alignment for these two compounds,

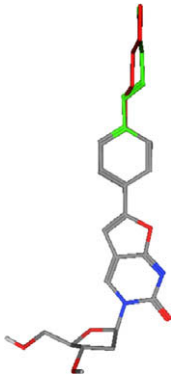


Figure 1. Flexible alignment simulation of compounds **3** and **14c**.

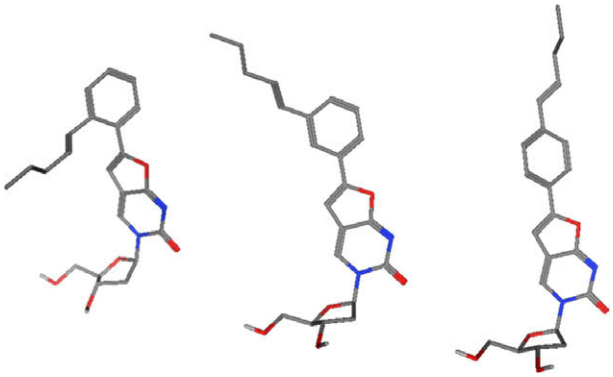


Figure 2. Energy minimized structures of compounds **14a–c**.

supporting their similarly high potencies, while the *ortho* and the *meta* derivatives showed completely different structures (Fig. 2), supporting the poor activity for these latter compounds.

Table 2
Inhibition of VZV TK-catalysed dThd phosphorylation by test compounds

Compound	IC_{50}[a] (μM)
13a	0.31 ± 0.04
13b	2.6 ± 1.0
13c	1.9 ± 1.5
14a	3.8 ± 0.4
14b	23 ± 7
14c	2.4 ± 0.2
15a	119 ± 73
15b	397 ± 34
15c	2.3 ± 0.4
1	25 ± 3
2	4.7 ± 0.2
3	4.1 ± 0.5

[a] 50% Inhibitory concentration required to inhibit VZV TK-catalysed dThd (1 μM) phosphorylation by 50%.

The compounds have also been investigated for their affinity against recombinant VZV thymidine kinase (TK) (Table 2). The assays were performed by measuring the inhibition of VZV TK-catalysed [^{3}H]thymidine phosphorylation by the test compounds. The 50% inhibitory concentrations ranked between 0.33 and 416 μM and did not correlate within the C3 (**13**), C4 (**14**), C5 (**15**) series nor with their antiviral activity.

For example, compound **13a** (ortho analogue with C3 side chain) was more inhibitory (IC_{50}: 0.33 μM) than its *meta* and *para* counterparts (IC_{50}: 2.7–3.2 μM), whereas the *para* derivative in the C5-series was more inhibitory (IC_{50}: 2.6 μM) than the ortho and *meta* derivatives (IC_{50}: 160–416 μM). Lack of correlation between the antiviral activity of the BCNAs and their affinity for VZV TK has been noted earlier,[15] and indicates a different structure–activity relationship of the compounds for VZV TK and the eventual antiviral target.

While the precise mode of action of the BCNAs versus VZV remains unclear, the data in this manuscript support our earlier conclusions[3] that phosphorylation of the compounds by the VZV-encoded thymidine kinase is a pre-requisite for their antiviral action. Interestingly, we have found[16] that although the TK from Simian Varicella Virus (SVV) can phosphorylate the BCNAs, the agents are not inhibitory to SVV in vitro. This implies that the eventual target of the BCNAs may be specific to VZV. We are now further pursuing such a target by utilising a tritium labelled (**3**) prepared by the catalytic addition of tritium to compound (**15c**) described above. The results of these studies will be reported in due course.

In conclusion, *p*-1-alkenyl phenyl BCNAs are noted to be highly potent anti-VZV agents, being roughly equipotent with their *p*-alkyl parents. By contrast their *ortho* and *meta* 1-akenyl analogues are rather poorly active. There was, however, no correlation between affinity of the compounds for VZV TK and the eventual anti-VZV activity.

Acknowledgements

The authors would like to thank Helen Murphy for excellent secretarial assistance. Mrs. Lizette van Berckelaer, Mrs. Lies Van den Heurck, Mrs. Anita Camps and Mr. Steven Carmans for excellent technical assistance. Financial support was provided by the K.U.Leuven (GOA 05/19).

References and notes

1. McGuigan, C.; Yarnold, C. J.; Jones, G.; Velazques, S.; Barucki, H.; Brancale, A.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **1999**, *42*, 4479.
2. McGuigan, C.; Barucki, H.; Blewett, S.; Carangio, A.; Erichsen, J. T.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **2000**, *43*, 4993.
3. Sienaert, R.; Naesens, L.; Brancale, A.; De Clercq, E.; McGuigan, C.; Balzarini, J. *Mol. Pharmacol.* **2002**, *61*, 249.
4. Canas, S. M.; Wasgin, B.; Boehlecke, B.; Henson, G.; Patti, J. M.; Morris, A. M. 21st ICAR, Montreal, April 13–17, 2008, p 40.
5. McGuigan, C.; Pathirana, R.; Migliore, M.; Adak, R.; Angell, A.; Henson, G.; Snoeck, R.; Andrei, G.; De Clercq, E.; Balzarini, J. *Antiviral Res.* **2006**, *70*, A31.
6. McGuigan, C.; Pathirana, R. N.; Migliore, M.; Adak, R.; Luoni, G.; Jones, A. T.; Diez-Torrubia, A.; Camarasa, M.-J.; Velazquez, S.; Henson, G.; Verbeken, E.; Sienaert, R.; Naesens, L.; Snoeck, R.; Andrei, G.; Balzarini, J. *J. Antimicrob. Chemother.* **2007**, *60*, 1316.
7. McGuigan, C.; Jukes, A.; Blewett, S.; Barucki, H.; Erichsen, J. T.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J. *Antiviral Chem. Chemother.* **2003**, *14*, 165.
8. Kabalka, G. W.; Tejedor, D.; Li, N. S.; Mallah, R. R.; Trotman, S. *J. Org. Chem.* **1998**, *63*, 6438.
9. Kabalka, G. W.; Li, N. S.; Tejedor, D.; Malladi, R. R.; Trotman, S. *J. Org. Chem.* **1999**, *64*, 3157.
10. Suzuki, H.; Kondo, A.; Ogawa, T. *Chem. Lett.* **1985**, *4*, 11.
11. (a) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, *50*, 4467; (b) Robins, M. J.; Barr, P. J. *J. Org. Chem.* **1983**, *48*, 1854.
12. *Typical procedure and data for* (**13b**): To a stirred solution of 5-ethynyl-2′-deoxyuridine (500 mg, 1.98 mmol) in dry dimethylformamide (8 mL), at room temperature under a nitrogen atmosphere, were added dry diisopropylethylamine (2.64 mmol, 0.46 mL), 1-*E*-(1-propenyl)-3-iodobenzene (322 mg, 1.32 mmol), tetrakis (triphenylphosphine) palladium(0) (153 mg, 0.132 mmol) and copper(I) iodide (50 mg, 0.264 mmol). The reaction mixture was stirred at room temperature, for 18 h. Copper(I) iodide (50 mg, 0.264 mmol), triethylamine (10 mL) were then added to the mixture, which was heated at 80 °C, for 6 h. The reaction mixture was concentrated in vacuo, and the resulting residue was dissolved in methanol and dichloromethane (1:1) (50 mL) whereupon an excess of Amberlite IRA-400 (hydrogen carbonate form) was added and stirred for 1 h at room temperature. The resin was filtered, washed with methanol, and the combined filtrate was evaporated to dryness to obtain a brown residue. The crude product was purified by flash column chromatography on silica gel to offer a yellow solid. The product was recrystallised in hot methanol to give a white solid (205 mg, 28%).^{1}H NMR (DMSO-d_6; 300 MHz) δ 8.89 (1H, s, H-4), 7.82 (1H, m Ar-H), 7.65 (1H, m, Ar-H), 7.44 (2H, m, Ar-H), 7.33 (1H, s, H-5), 6.48 (2H, m, H-c1 and H-c2), 6.19 (1H, app t, J = 6.0 Hz, H-1′), 5.32 (1H, d, J = 4.3 Hz, OH-3′), 5.21 (1H, t, J = 5.1 Hz, OH-5′), 4.26 (1H, m, H-3′), 3.94 (1H, m, H-4′), 3.68 (2H, m, H-5′), 2.41 (1H, m, H-2′), 2.15 (1H, m, H-2′), 1.88 (3H, d, J = 6.9 Hz, H-c3); ^{13}C NMR (DMSO-d_6; 75 MHz) δ 171.4 (C-7a), 154.1, 153.9 (C-2, C-6), 138.6 (C-4), 138.5 (C-c), 130.5, 129.7, 127.3, 126.9, 123.3, 122.0 (6 × C: C-b, C-d, C-e, C-f, C-c1, C-c2), 129.0 (C-a), 107.1 (C-4a), 100.1 (C-5), 88.5 (C-4′), 88.0 (C-1′), 69.8 (C-3′), 60.9 (C-5′), 41.6 (C-2′), 18.7 (C-c3); MS (ES+) m/e 391 (MNa^+, 100%); Accurate mass: $C_{20}H_{20}N_2O_5Na$ requires 391.1270; found 391.1274; Anal. Calcd for $C_{20}H_{20}N_2O_5 \cdot H_2O$: C, 65.21; H, 5.47; N, 7.60. Found: C, 64.99; H, 5.04; N, 7.21.
13. Confluent HEL cells grown in 96-well microtiter plates were inoculated with VZV at an input of 20 PFU (plaque-forming units/well). After a 1–2 h incubation period, residual virus was removed and the infected cells were further incubated with MEM (supplemented with 2% inactivated FCS, 1% L-glutamine and 0.3% sodium bicarbonate) containing varying concentrations of the compounds. Antiviral activity was expressed as EC_{50} (50% effective concentration), or compound concentration required to reduce viral plaque formation after 5 days by 50% compared to the untreated control.
14. Minimisation and flexible alignment simulation were performed using MOE 2007.09 (molecular operating environment) (MOE 2007.09. Chemical Computing Group, Inc., Montreal, Que., Canada. http://www.chemcomp.com).
15. Balzarini, J.; McGuigan, C. *Biochim. Biophys. Acta* **2002**, *1587*, 287.
16. Sienaert, R.; Andrei, G.; Snoeck, R.; De Clercq, E.; Luoni, G.; McGuigan, C.; Balzarini, J. *Antiviral Res.* **2004**, *62*. 17th ICAR, Tucson AZ, May 2–6, abstract 94.