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Antiviral Research 71 (2006) 149–153
Antiviral Research
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Mini-review

Aryl furano pyrimidines: The most potent and selective anti-VZV agents reported to date

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Received 25 January 2006; accepted 3 April 2006

Dedicated to Prof. Erik De Clercq on the occasion of reaching the status of Emeritus-Professor at the Katholieke Universiteit Leuven in September 2006.

Abstract

Bicyclic aryl furano pyrimidines represent the most potent anti-VZV agents reported to date. Lead compounds have EC_{50} values in vitro as low as 0.1 nM and selectivity index values exceeding one million. They have an absolute requirement for VZV thymidine kinase (TK) and most likely act as their phosphate forms. Some structural modification, such as aryl substitution, is tolerated, while little sugar modification is acceptable. We herein summarise their biological profiles and structure activity relationships as discovered to date.

Keywords: Varicella zoster virus; Bicyclic nucleoside analogue; Shingles

Contents

1. Introduction

Bicyclic nucleoside analogues (BCNAs) with a furanopyrimidine structure (**1**) have been known for over 20 years as unwanted by-products from the Pd-catalysed coupling of terminal alkynes with 5-iodo nucleosides, primarily deoxyuridine related (Robins and Barr, 1981, 1983; Crisp and Flynn, 1993). This by-product could be reduced by the use of dimethylformamide (DMF) as alternate reaction solvent (Robins et al., 1990), or could be enhanced by treatment of the (intended) 5-alkynyl nucleoside with copper(I) and triethylamine (Robins and Barr, 1983). Until our recent work, biological evaluation of the bicyclic by-products (**1**) (Fig. 1) had been limited to the parent compound (**1**, R = H) which was noted to be inactive as an antiviral against herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), cytomegalovirus (HCMV) and varicella zoster virus (VZV) (Kumar et al., 1991, 1996). Thus, it was surprising to us to note in 1999 the highly potent and specific anti-VZV activity of longer chain homologues of **1** (McGuigan et al., 1999). Activity peaked at R = C8(*n*-octyl), with a potency of 0.008–0.024 μM against VZV OKA and YS in vitro (Table 1). This compared to 1.9–2.1 μM for acyclovir in the same assay

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0166-3542/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2006.04.001

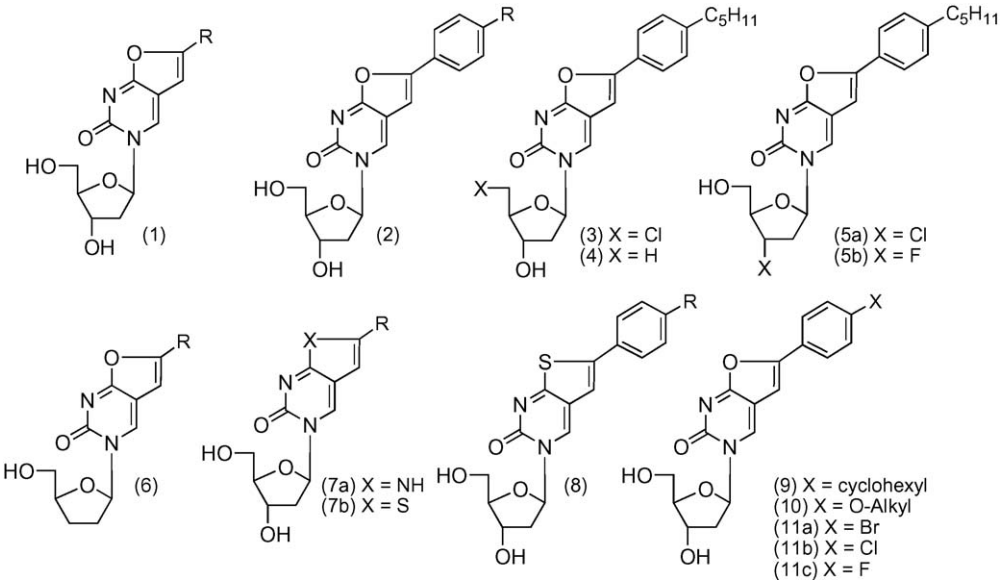

Fig. 1. Structures of BCNA nucleoside analogues.

(McGuigan et al., 1999). Thus, the lead octyl compound is ca. 300-times more potent than acyclovir vs. VZV. The whole family of agents of type **1** were all highly specific to VZV with no other detectable antiviral action, including the closely related herpes simplexvirus types 1 and 2 and Simian varicella virus (Sienaert et al., 2004). They were all rather non-cytotoxic (CC_{50} values >50 μM).

Subsequently, with a view to restricting the conformational freedom of the alkyl chain, we reported the preparation and evaluation of the corresponding phenyl systems (**2**) with a range of *p*-alkyl substituents (R = H–*n*-octyl) (McGuigan et al., 2000). These were noted to be highly potent against VZV, with the optimal compounds (R = pentyl–hexyl) displaying a ca. 50-fold boost over the optimal leads of type **1**. Thus, the *n*-pentyl compound in series (**2**), R = C_5H_{11}, shows an EC_{50} in vitro of ca. 0.1–0.5 nM versus VZV and is non-toxic at 200 μM. In our view this represents the most potent agent reported to date against VZV and amongst the most potent of antivirals in any area. Thus, **2**, R = nPnt is ca. 10,000 times more potent than acyclovir versus VZV in vitro and ca. 10–50 times more potent than BVDU (E-5-(2-Bromovinyl)-2′-deoxyuridine) (McGuigan et al., 2000).

In 2001, we reviewed the data to date on analogues of **1** and **2** and the structure activity relationships (SARs) and mechanism of action as we then knew it (McGuigan et al., 2001). We now update this review with a particular emphasis on the aryl family (**2**).

Table 1
Anti-VZV activity and cytotoxicity of representative BCNA compounds

	EC_{50} (μM)		MCC (μM)	CC_{50} (μM)
	OKA	YS		
1, R = C_8H_{17}	0.008	0.010	>50	>50
2, R = C_5H_{11}	0.0003	0.0001	>200	>200
3	>5	–	20	>50
4	>5	>5	20	95
5a	>20	–	≥20	>200
5b	>2	>2	5	5.2
6, R = C_8H_{17}	>5	>5	20	112
7a, R = C_8H_{17}	0.15	0.38	≥20	>50
7b, R = C_8H_{17}	0.003	0.005	20	53
8, R = C_5H_{11}	0.014	0.025	50	>200
9	1.4	3.0	≥20	>200
10, R = C_5H_{11}	0.001	0.001	40	>200
11a	0.29	0.2	≥5	96
11b	0.09	0.08	35	>200
11c	>50	>50	≥200	171

2. Sugar modifications

In the parent alkyl family (**1**) we have previously noted little tolerance for structural modification in the deoxyribose sugar (McGuigan et al., 2000a). Thus, for a range of alkyl compounds we found that conversion to the ribo- and arabino-analogues lead to significant (ca. 150–3000-fold) loss of antiviral activity. The importance of the deoxy ribose structure has more recently been further probed by the replacement of the 3′- and 5′-hydroxyl groups in the aryl family (**2**) by other functionalities. Thus, the 5′-deoxy-5′-chloro compound (**3**) was prepared via the usual alkyne coupling and cyclisation on 2′,5′-dideoxy-5′-chloro-5-iodo-2′-deoxyuridine (Luoni et al., 2003). The product was found to be very poorly active against VZV in vitro ($EC_{50} \geq 5$ μM), with a more than 10,000-fold reduction in potency versus **2**, R = pentyl. This supports the notion of VZV TK-mediated phosphorylation as an essential step in activation of these agents (Sienaert et al., 2002; Balzarini and McGuigan, 2002). This conclusion was further supported by the preparation and evaluation of the 5′-deoxy parent compound (**4**) (Luoni et al., 2004) which was again poorly active/inactive. On the basis of the essential requirement for VZV TK, the requirement for a free 5′-hydroxyl group is to be anticipated. However, the 3′-hydroxyl group is less obviously essential. Thus, it was of interest to test the corresponding 3′-chloro (**5a**) species. Again this was poorly active or inactive (Luoni et al., 2004). Similarly, we found that the 3′-fluoro

analogue (**5b**) was also inactive (McGuigan et al., 2004). In the alkyl family (**1**) we have noted that 3′-deoxygenation to the 2′,3′-dideoxy compounds (**6**) leads to a complete loss of anti-VZV activity but, interestingly, the introduction of anti-HCMV activity (McGuigan et al., 2004a). Remarkably, full deoxygenation of **6** to the 2′,3′,5′-trideoxy species gives full retention of the anti-HCMV activity (Bidet et al., 2004) clearly showing that this anti-HCMV family functions by a new, 'non-nucleoside'—mode of action (MoA) (McGuigan et al., 2004a). Thus, the data to date indicate that an intact deoxyribose sugar or very close analogue is essential for the potent anti-VZV activity of these compounds. Both the 5′ and 3′ hydroxyl groups appear essential for activity.

3. Base modifications

In the alkyl family (**1**), we have previously noted that pyrrolo analogues (**7a**) lose almost all of the anti-VZV activity of furano parents (**1**) (McGuigan et al., 2000b), while the thieno analogues (**7b**) retained full activity (Brancale et al., 2001). More recently, we have noted that the thieno analogues in the aryl family (**8**) suffer a significant reduction in potency versus their furano parents, being ca. 100-fold for the *n*-pentyl leads (Angell et al., 2004). Indeed, contrary to the usual pattern, in the thieno series, the alkyl family exceeds the aryl family (slightly) in potency. The reason that the furano to thieno substitution is tolerated in one family (**1**) and not the other (**2**) is currently unclear, but may correspond to differential efficiencies of phosphorylation, or in recognition at the polymerase level.

4. Aryl side chain modification

We have previously noted (McGuigan et al., 2000) the clear variation in anti-VZV potency with *p*-alkyl side chain length; activity rises from H (EC_{50} 0.1–0.3 μM) to *n*-pentyl/hexyl (0.1–0.5 nM) and then declines to *n*-octyl (EC_{50} ca. 30 nM). This correlates to an optimal lipophilicity, based on calculated log *P* values (*C* log *P*) of ca. 3.0–3.5. To some extent such a lipophilicity has been predictive of a potent anti-VZV effect in both series (**1**) and (**2**) (McGuigan et al., 2000a). Whether this apparent optimal lipophilicity corresponded to optimal membrane transport or to the 'ideal' side chain geometry that corresponded with molecules of such a log *P* was unclear. Thus, it was of interest to modify the *p*-alkyl side chain, retaining *C* log *P* values, but modifying the alkyl geometry. We did this by branching of the alkyl side chain. A series of compounds were reported by us, the most notable being the cyclohexyl analogue (**9**). This has an identical *C* log *P* value to the lead (**2**, R = *n*-pentyl) (3.0) and yet is ca. 3000-fold less active (Luoni et al., 2005). This implies that the linear, or in any case more flexible, *n*-pentyl side chain is important for extreme anti-VZV potency. However, the precise pattern of activity versus branch point in branched open chain alkyl systems emerged as rather complex; activity increases over the family isopropyl, isobutyl, isohexyl, but then decreases dramatically for the isoheptyl compound (Luoni et al., 2005). Although the isohexyl compound is active at 10–17 nM, there is no case where a branched system is more active than a linear analogue. This implies that the increased steric bulk along the side chain may be disadvantageous, or that the resulting log *P* to achieve a pentyl-like length on the longest chain, may be too high. To further probe the tolerance to alkyl side chain modification and in fact primarily to seek to boost the rather low water solubility of the aryl family (**2**) we have reported the corresponding phenolic ethers (**10**). We have reported that, in general, these compounds retain high antiviral potency, comparable with, if a little less than that of the alkyl parents. Thus, the pentyloxy system (**10**, R = *n*-pentyl) is active at 1 nM and thus 3–10-fold less active than the lead (**2**, R = *n*-pentyl). Similarly, the optimal chain length in the phenolic alkyloxy series (**10**) was noted to be pentyl–hexyl, just as in the alkyl family (McGuigan et al., 2002). This is in contrast to earlier work in the alkyl series (**1**) where introduction of an ether oxygen along the chain lead to a significant (ca. 100-fold) reduction in potency (Brancale et al., 2000). This may correspond to different tolerances in the two families or to a specific property of the phenolic ethers as opposed to alkyl ethers. Structural isomers of **10** currently in preparation may help address this.

5. Aryl substitution

We have previously noted that *p*-bromo or *p*-chloro substitution (**11**) in the aryl family (**2**) leads to a retention in potency of the tolyl parent compound (**2**, R = Me) (McGuigan et al., 2001). Lacking the pentyl chain as noted above these compounds are not exquisitely potent, sub-nanomolar compounds but are active at 80–300 nM. They are thus still 10–50 times more potent than acyclovir versus VZV. It is interesting to note that the *C* log *P* values for **11a**,**b** of ca. 1.6–1.8 are just slightly higher than the tolyl system (**2**, R = Me) and that these compounds share rather similar potencies. Interestingly, however, the *p*-fluoro analogue (**11c**) is *inactive* as an antiviral at the highest concentration tested and thus at least two to three orders of magnitude less active than **11a**,**b** (McGuigan et al., 2003). Although the *p*-fluoro compound is calculated (and expected) to be less lipophilic than **11a**,**b**, with a *C* log *P* of 1.0, it is as lipophilic as the parent phenyl compound (**2**, R = H) and yet is >100-fold less active. Surprisingly, this does not correspond to rather poor phosphorylation of the *p*-fluoro analogue by VZV TK, since the *p*-fluoro derivative is an excellent substrate for VZV TK (Balzarini and McGuigan, 2002). By contrast, fluorine is tolerated at the *ortho* and *meta* positions to preserve antiviral activity; indeed the *ortho*-monofluoro analogue is active at 40 nM and thus more active than the phenyl parent and >1000-fold more active than **11c**. The ortho chloro- and bromo-compounds are also potent, with the meta analogues less so, and in these cases, less so than the *para* compounds (**11a**,**b**) (McGuigan et al., 2003).

6. Pharmacology of BCNAs

The prototype BCNA derivatives have been shown to consistently inhibit a variety of clinical VZV isolates with exquisite potency (Andrei et al., 2005). The observations that the BCNAs, including the prototype series of compounds **1** and **2** completely lose their anti-VZV efficacy against both laboratory and clin-

ical virus isolates that have a deficiency in TK points to the VZV-encoded TK as the activating enzyme for these compounds (Sienaert et al., 2002). Indeed, a pronounced affinity of the compounds was found for recombinant VZV TK, and this susceptibility for the enzyme highly depends on the nature of the substituents present on the BCNAs (Sienaert et al., 2002). It was striking to notice that there was no close correlation between affinity for VZV TK and antiviral activity, pointing to a different structure–activity relationship for the eventual antiviral target of the BCNAs. In this respect, it should be mentioned that the closely related SVV TK also recognizes the BCNAs as a substrate, whereas SVV is insensitive to the inhibitory activity of the BCNAs (Sienaert et al., 2004). Also the dTMP kinase activity associated with VZV TK recognizes the BCNA-5′-monophosphates as a substrate. Interestingly, both human and bacterial (*E. coli*) thymidine phosphorylase (TPase) do not recognize BCNAs and they are thus not subject to cleavage to their free (inactive) base (Balzarini et al., 2002). Whether VZV DNA polymerase is the eventual target for the anti-VZV activity of the BCNAs needs still to be demonstrated.

7. Conclusions

This brief SAR survey has highlighted the exquisite potency and selectivity of the lead aryl BCNA family, particularly for the *p*-pentyl phenyl compound. Little structural modification in the sugar is tolerated without loss to activity, while some base and aryl side chain modification is acceptable. However, in no case to date is the potency of the parent system enhanced. This lead pentyl system shows highly promising properties as an anti-VZV agent and is under pre-clinical development by FermaVir Pharmaceuticals of New York.

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