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Mechanism and structure based design of inhibitors of AMP and adenosine deaminase

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A B S T R A C T

Inhibitors of the enzyme adenosine monophosphate deaminase (AMPD) show interesting levels of herbicidal activity. An enzyme mechanism-based approach has been used to design new inhibitors of AMPD starting from nebularine (**6**) and resulting in the synthesis of 2-deoxy isonebularine (**16**). This compound is a potent inhibitor of the related enzyme adenosine deaminase (ADA; IC_{50} 16 nM), binding over 5000 times more strongly than nebularine. It is proposed that the herbicidal activity of compound **16** is due to 5́-phosphorylation *in planta* to give an inhibitor of AMPD. Subsequently, an enzyme structure-based approach was used to design new non-ribosyl AMPD inhibitors. The initial lead structure was discovered by *in silico* screening of a virtual library against plant AMPD. In a second step, binding to AMPD was further optimised *via* more detailed molecular modeling leading to 2-(benzyloxy)-5-(imidazo[2,1-f][1,2,4]triazin-7-yl)benzoic acid (**36**) (IC_{50} 300 nM). This compound does not inhibit ADA and shows excellent selectivity for plant over human AMPD.

1. Introduction

The herbicidally active natural product carbocyclic coformycin (**1**)[1] has been shown to undergo phosphorylation *in planta* to give the phosphate **2**, which is a powerful inhibitor of adenosine monophosphate deaminase (AMPD; *FAC1*; EC 3.5.4.6) (IC_{50} 20 nM).[2] The enzyme AMPD converts adenosine monophosphate (AMP) into inosine monophosphate (IMP) *via* the tetrahedral high energy intermediate **5** and plays an important role in maintaining the adenylate energy charge.[3] Herbicidal activity has also been reported for the closely related natural product coformycin (**3**)[4] and the phosphate **4** of this compound is an extremely potent inhibitor of AMPD (K_i 55 pM).[5] The carbocyclic structures **1** and **2** lack the labile glycosyl bond present in **3** and **4**, meaning they are not subject to degradation by nucleosidases and phosphorylases. Consequently, even though the phosphate **2** is a less potent inhibitor of AMPD than compound **4**, its improved metabolic stability means that the herbicidal effect of carbocyclic coformycin (**1**) is far superior to that of coformycin (**3**).[6]

The interesting herbicidal activity of carbocyclic coformycin (**1**) led to the initiation of a synthesis program in which we sought to identify simpler herbicidally active analogues. However, the chemistry proved to be very challenging due to the lability of the diazepine ring system and even very close analogues were much less active as inhibitors and herbicides.[7] Consequently, following a search for more synthetically accessible AMP deaminase inhibitors, we chose nebularine phosphate (**7**) (K_i 6.5 μM; rabbit muscle AMPD)[5] as a promising lead structure. The corresponding nucleoside, nebularine (**6**), is not herbicidally active, again probably due to rapid metabolism of the glycosyl bond. The phosphate **7** is a substrate analogue which undergoes covalent hydration within the AMPD active site to give the covalent hydrate **9**.[8] Inhibitors **2**, **4** and **9** can all be considered as stable mimics of the high energy AMPD reaction intermediate **5**, or of the transition states leading to or from it.

The equilibrium constant for the hydration of nebularine (**6**) to hydrate **8** has been estimated to be 1.1×10^{-7} meaning that the enzyme has to work hard to stabilise the hydrate **9** in the AMPD active site.[9] It therefore follows that aglycones capable of forming more stable covalent hydrates will require less stabilisation and should give more potent AMPD inhibitors. In the current report we describe the application of this enzyme mechanism-based design approach leading to the synthesis of 2-deoxy isonebularine (**16**). Based upon the very promising biochemical and biological results obtained for compound **16**, we subsequently followed an enzyme structure-based design approach which led to the synthesis of the new plant specific non-ribosyl AMPD inhibitor lead structures **36** and **39**. Enzyme structure-based herbicide design has considerable potential for improving herbicide discovery and is a field of growing interest within the agrisciences.[10] The worldwide spread of weeds exhibiting resistance to established herbicide modes of action poses an increasingly serious threat to efficient food production.[11]

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Consequently, the discovery and development of herbicides with novel modes of action, such as AMPD, is of great importance in order to ensure the future sustainability of modern agriculture.

2. Enzyme mechanism-based inhibitor design

A synthesis program looking at carbocyclic and *C*-nucleoside based analogues of nebularine (**6**) was undertaken which resulted in the discovery of the experimental herbicide deaminoformycin (**10**).[12] This compound showed interesting lead levels of herbicidal activity which were shown to be attributable to AMPD inhibition and the corresponding phosphate **11** was a good inhibitor of AMPD (K_i 600 nM; *Arabidopsis* enzyme).[13] In support of our design hypothesis, enthalpy of hydration calculations indicated that **11** and especially its N(2)H tautomer **11a**, should be more readily hydrated than nebularine phosphate (**7**) (Table 1).[12] Co-crystallization of AMPD with phosphate **11** followed by X-ray crystallographic analysis confirmed the presence of the covalent hydrate **13** in the AMPD active site (Figures 1 and 2). These results encouraged us to search for aglycones which could build even more stable covalent hydrates and resulted in the synthesis of nucleoside **14**. Compound **14** was predicted to react exothermically with water (Table 1) and in agreement with these calculations existed to the extent of 90% as the covalent hydrate **15** in aqueous solution.[14] However, the aglycone ring of **14** was too reactive and unstable to allow synthesis of the corresponding 5́-phosphate derivative for AMPD testing and the compound was herbicidally less active than deaminoformycin (**10**).

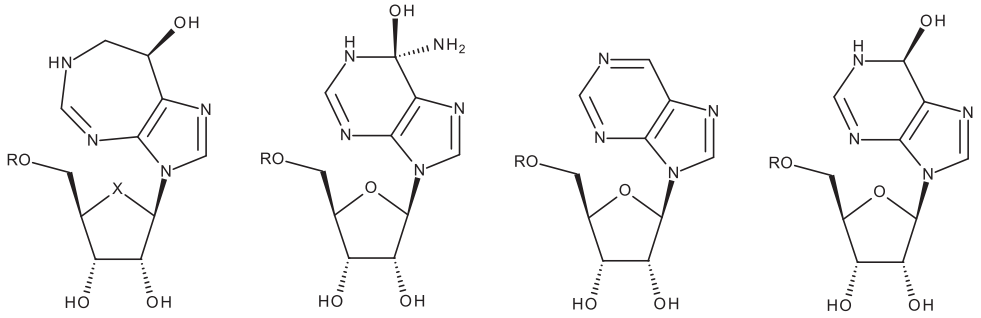
Enthalpy of hydration calculations indicated that an imidazotriazine aglycone, as present in the proposed new target **16**, should be able to form a covalent hydrate **18** of greater stability than seen for the pyrazolopyrimidine in **10** but should be less electrophilic and hopefully, therefore, more stable than the triazolotriazine in **14** (Table 1).[14] Nonetheless, the C(4) = N double bond in **16** retains a high level of reactivity towards nucleophilic and basic reagents[15,16,17] which made the synthesis very challenging. Many strategies were tried over a period of several years before the successful synthesis shown in Scheme 2 was achieved. The synthesis of the imidazotriazine aglycone (Scheme 1) is a modification of that published by Babu and co-workers.[18] We found that the use of the dimethoxybenzyl protecting group facilitated isolation and purification of the early intermediates, especially on larger scales. The key step in the proposed synthesis of **16** was to be a palladium catalyzed coupling of an imidazotriazine with the thymidine derived furanoid glycal **25**[19] which we hoped to achieve by adapting methodology published by Daves[20] and Raboisson.[21] Ideally, we would have used the iodide **24** but the second step in the proposed synthesis involved a borohydride reduction under acidic conditions[22] which would have also reduced the unprotected C(4) = N double bond.[15] Consequently, we coupled glycal **25** with the amino iodide **23** to give ketone **26** which was then stereospecifically reduced to give the alcohol **27** (Scheme 2). Compounds **26** and **27** are both 92:8 mixtures of the β-(shown) and α-*C*-nucleosides. The concluding four steps of the synthesis involved acylation, *N*-deacylation, radical deamination[23] and deprotection to give 2-deoxy isonebularine (**16**) as the pure β-*C*-nucleoside following HPLC purification. The ^{1}H NMR spectrum in D_2O indicated that **16** existed entirely in the 10π aromatic form. However, addition of one equivalent of DCl resulted in complete conversion to the covalent hydrate, as evidenced by the upfield shift of the *C*4-H signal in the proton NMR from δ9.23 in **16** to δ6.43 in **18**. Under similar acidic conditions the purine aglycone of **6** showed no detectable hydrate formation. Although the imidazotriazine ring system in **16** proved to be substantially more stable than the triazolotriazine in **14**, it was still too reactive to allow synthesis of the 5́-phosphate derivative **17** using similar methodology to that successfully used to synthesise **2**[2] and **11**.[12] The C(4) = N double bond of **16** rapidly reacts with nucleophiles, even under very mildly acidic or basic conditions, leading to ring opening and decomposition.

Table 1
Enthalpy of hydration calculations indicating the ease with which different aglycones are expected to be able to form covalent hydrates.

Hydration reaction	Calculated ΔH(hydration)[a] Kcal mol^{-1}
6 to **8**	7.1
10 to **12**	5.5
10a to **12a**	1.7
14 to **15**	−2.1
16 to **18**	−1.6

[a] Calculations were performed as described in reference 14 without taking entropic or explicit H-bonding factors into account. The ΔH values are consequently only approximate but are adequate for comparison of similar structures.

As expected, the nucleoside **16** was not an inhibitor of AMPD and since we were unable to synthesise the phosphate **17** we could not directly assess the inhibitory potential of our isonebularine analogue against AMPD. Instead we chose to look at inhibition of the mechanistically closely related enzyme adenosine deaminase (ADA; EC 3.5.4.4) which converts adenosine to inosine. The aglycone binding pockets of ADA and AMPD have been shown to be highly conserved[8] and as might be expected, nucleoside-based ADA inhibitors give AMPD inhibitors following 5́-monophosphorylation to the nucleotides.[5] Thus, compounds **1**, **3**, **6** and **10** are ADA inhibitors, while the corresponding phosphates **2**, **4**, **7** and **11** are AMPD inhibitors. Upon testing **16** proved to be a potent ADA inhibitor with an IC_{50} value of 16 nM (calf intestinal mucosa enzyme), meaning that it binds more strongly than nebularine (**6**) and carbocyclic coformycin (**1**) which have IC_{50} values of 90 μM[14]



1 X = CH_2, R = H
2 X = CH_2, R = PO_3^{2-}

3 X = O, R = H
4 X = O, R = PO_3^{2-}

5 R = PO_3^{2-}

6 R = H
7 R = PO_3^{2-}

8 R = H
9 R = PO_3^{2-}

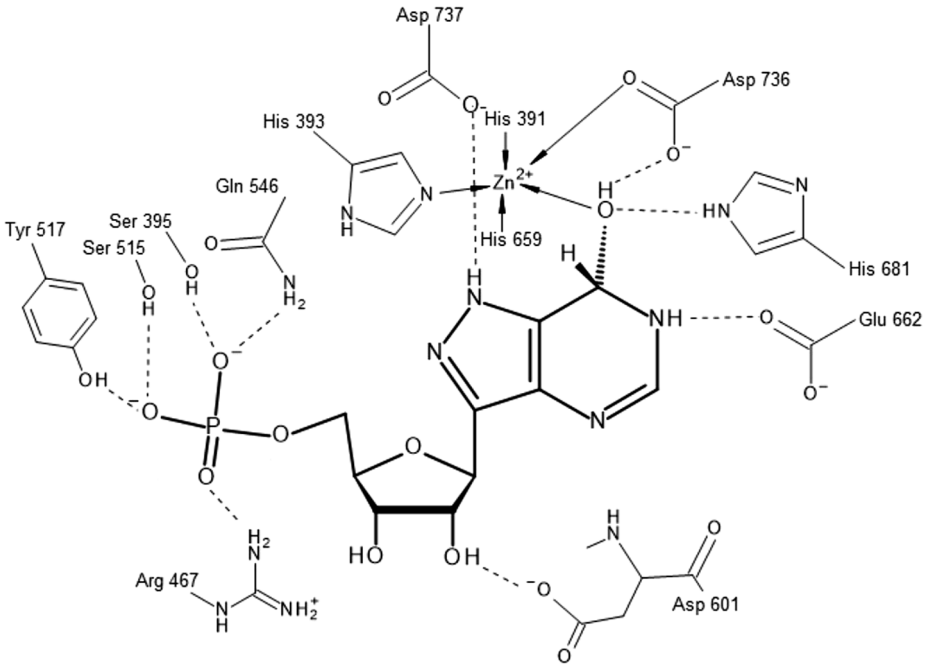


Figure 1. Schematic representation of the observed binding mode of the covalent hydrate **13** in the active site of *Arabidopsis* AMPD. H-bonds (maximum donor–acceptor distance = 3.2 Angstroms) are shown as dashed lines. Arrows indicate coordination bonds to the zinc ion. The amino acids are numbered according to the UniProt entry O80452.

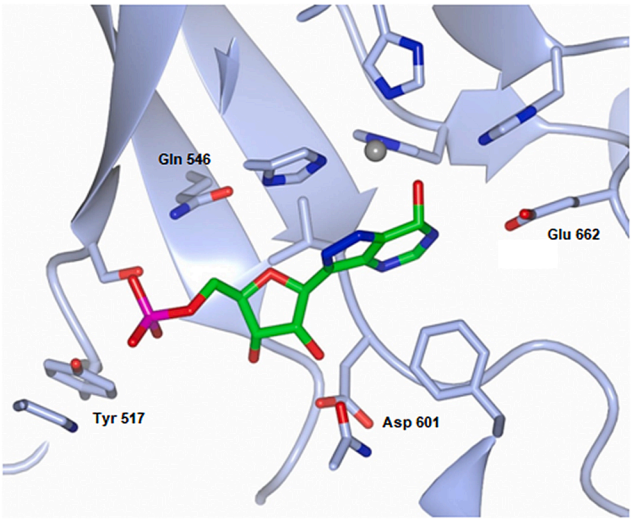


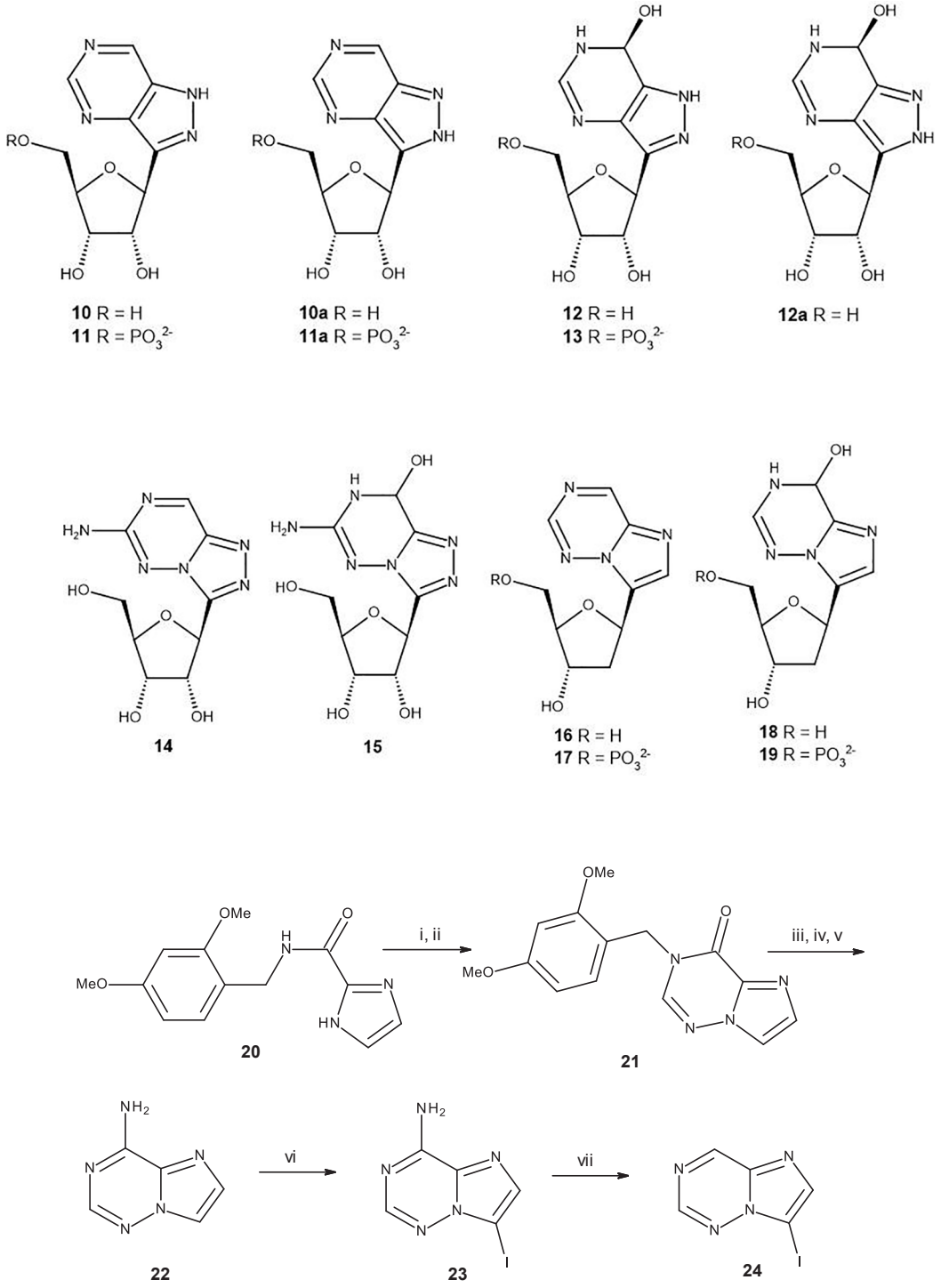
Figure 2. 3D-Representation showing the observed binding mode of the covalent hydrate **13** in the *Arabidopsis* AMPD active site. The zinc atom is shown as a grey ball.

and 25 nM,[2] respectively. Even more encouraging were the results from our herbicidal screens which showed that **16** possessed post emergence herbicidal activity at a level which was much superior to that shown by deaminoformycin (**10**) and was on a par with carbocyclic coformycin (**1**). The best results were shown by the diacetyl derivative **29**, which exhibited post-emergence herbicidal activity at rates of 20–80 g ha^{-1} (depending on the species), probably due to the improved uptake of this less polar compound. Both **1** and **10** have been shown to exert their biological activity *via in planta* phosphorylation to give the AMPD inhibitors **2**[2] and **11**[12,13] and we propose that a similar mechanism is responsible for the herbicidal activity of **16**. Inhibition of ADA does not play a role in the herbicidal activity seen for **16** because this enzyme is not present in plants.[2,13]

3. Enzyme structure-based inhibitor design

Although 2-deoxy isonebularine (**16**) is a good herbicide lead structure, its structural complexity and lack of specificity mean that it would be advantageous to discover simpler inhibitors which bind selectively to plant AMPD and not to ADA. Inspired by the results of Kasibhatla *et al*[24] we replaced the ribosyl moiety of our inhibitors with an aromatic carboxylic acid, as exemplified by structure **30**. Although **30** was a good inhibitor of human AMPD3 (IC_{50} 900 nM) it was a rather poor inhibitor of the plant enzyme (IC_{50} 200 μM).[17] The ribose phosphate mimic present in structure **30** arose from a research program directed towards discovering inhibitors of human AMPD[24] which might explain the poor affinity for plant AMPD. The availability of Bayer in-house plant AMPD structures containing **4** and **13** bound at the active site encouraged us to try and use a protein structure-based approach to design new improved plant specific AMPD inhibitors.

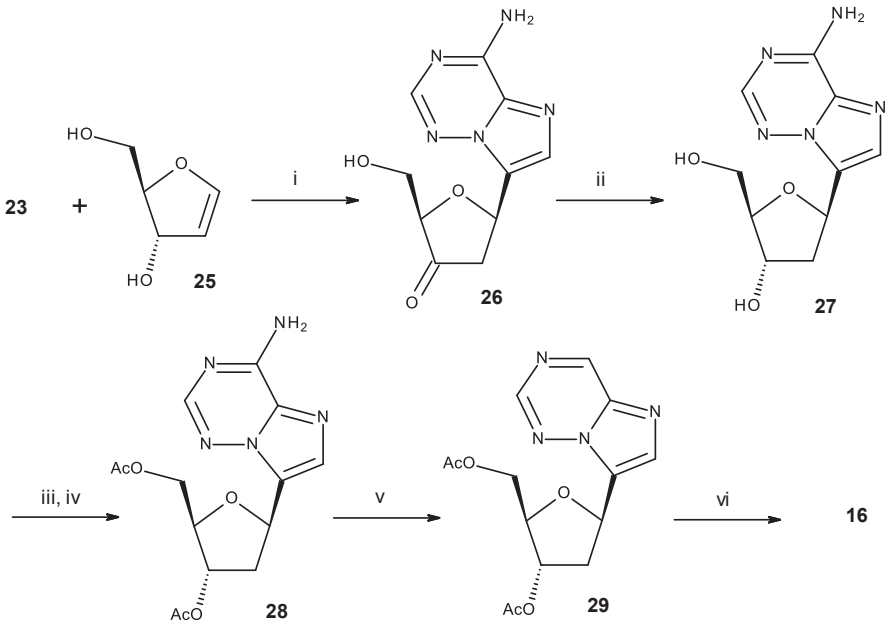
A virtual library of 91 compounds based upon the general structure **31** was docked into the AMPD binding site and potential binding affinities were assessed using force field calculations. Solvation effects were not taken into account for this library. The resulting binding scores are only a very approximate measure of a ligands free energy of binding. Nonetheless, the ranking between any two ligands shows which should bind more strongly. About 45% of the structures were predicted to bind to AMPD as well or better than the covalent hydrate of compound **30**. Several of the best ranked compounds were quite closely related to **30** but it was decided not to follow these up due to the poor biological performance observed for previously synthesised compounds of this type.[16,17] Compounds with a benzoic acid moiety directly attached to the imidazotriazinyl aglycone also had a good ranking and were considered more interesting due to their structural novelty and expected ease of synthesis *via* Suzuki coupling chemistry. Seven compounds (Table 2) from this structural class were subsequently prepared and tested for AMPD inhibition and herbicidal activity. None of the compounds were herbicidally active, however, four were better inhibitors of plant AMPD that **30**. In particular, the simple benzoic acid derivative **32** bound to plant AMPD nearly 7 times more strongly than **30** and was considered an interesting new lead structure. Although a comparison of



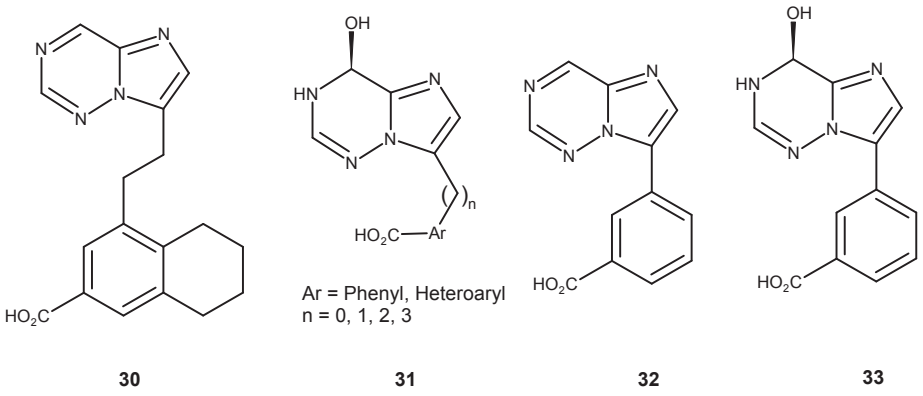
Scheme 1. Synthesis of the imidazotriazine aglycones **23** and **24**. Reagents, conditions and yields: (i) $PhP(O)ONH_2$ (1.3 equiv), NaH (1.2 equiv), DMF, rt, 4 h. (ii) $HC(OET)_3$, HCO_2H, reflux, 10 h (86% over 2 steps). (iii) CF_3CO_2H, 50 °C, 5 h (94%). (iv) $POCl_3$, $PhNMe_2$, 160 °C, 4 h. (v) NH_3, *sec*-butanol/*i*-propanol, 0 °C to rt, 16 h (84% over 2 steps). (vi) *N*-iodosuccinimide (3 equiv), DMF, rt, 4 days (76%). (vii) *n*-BuONO (10 equiv), dioxane, 100 °C, 2 h (38%).

the calculated binding energy and observed AMPD inhibition was only possible for three compounds, we were encouraged to see that the ranking had been correctly predicted (Table 2).

Subsequently, the binding of the putative covalent hydrate **33**, derived from **32**, was investigated in more detail with a view to further improving potency. The x-ray crystal structures of **4** and **13** bound to plant AMPD were used as templates for optimising the binding pose of the hydrate **33**. The coordination bond between the hydrate hydroxyl group and the zinc atom, together with the H-boding interactions between the aglycone heterocyle and Glu 662, His 681, Asp 736 and Asp 737 were maintained in all structures. In addition to force field energies, the HYDE score[25] was determined and used to calculate an improved binding score. HYDE takes solvation and entropic effects implicitly into consideration which results in a more accurate estimation of potential ligand binding energy. Two promising areas for improving the binding of **33** were identified:



Scheme 2. Synthesis of the 2-deoxy isonebularine (**16**). Reagents, conditions and yields: (i) **23** (1 equiv), **25** (2 equiv), $Pd(dba)_2$ (0.1 equiv), Ph_3As (0.2 equiv), DMA/Et_3N, 80 °C, 16 h (51%). (ii) $NaBH(OAc)_3$ (1.2 equiv), MeCN/AcOH, −24 °C, 30 min (87%). (iii) Ac_2O (12 equiv), pyridine, rt, 4 h (iv) EtOH, 70 °C, 21 h (61% over 2 steps). (v) *n*-BuONO (25 equiv), dioxane, 100 °C (27%). (vi) MeOH/Et_3N, 64 °C, 2 h (38%).



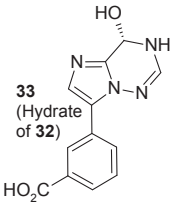
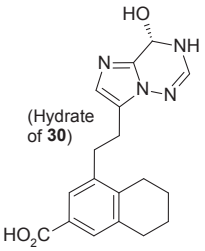
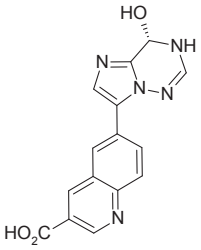
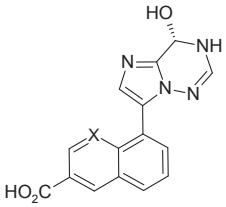
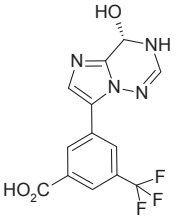
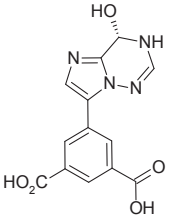
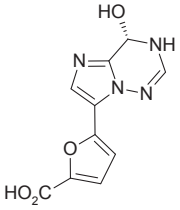
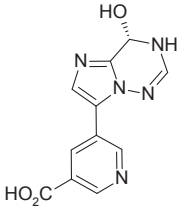
i) The unsubstituted side of the phenyl ring in **33** appears to be oriented towards the entrance to the AMPD active site which is lined with mostly lipophilic residues. This suggested that a lipophilic substituent positioned *ortho* or *meta* to the carboxylic acid might improve binding as shown in structures **34**.

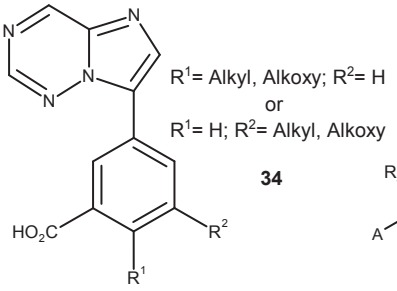
ii) The carboxylic acid moiety of **33** does not appear to be able to make any good H-bonding interactions within the phosphate binding pocket of AMPD. Our modelling results suggested that a two-atom spacer inserted between the phenyl ring and the carboxylic acid should allow better access to this binding region as depicted in structures **35**.

Around 25 analogues based upon structures **34** were synthesised and tested for biochemical and herbicidal activity. The best compound was the benzyl ether **36** (Table 3), which was a good inhibitor of plant AMPD (IC_{50} 300 nM), binding about 100 times more strongly than the unsubstituted compound **32** (Table 3). The analogue **37** containing an *ortho*-hexyl ether was a less potent inhibitor (IC_{50} 10 μM) as was the analogue **38** in which the benzyloxy group was *meta* to the carboxylic acid (IC_{50} 25 μM). The methyl ester derivative of **36** did not inhibit AMPD indicating that the free acid was important for AMPD binding. Compounds **36** and **37** showed herbicidal activity at 1 kg/ha, whereas compounds **38** was inactive at this dosage. The proposed binding mode of compound **36** to plant AMPD is shown in Figure 3a. Even though the carboxylic acid of **36** appears to be essential for binding it does not appear to have any direct H-bonding interactions with the AMPD protein. However, indirect H-bonding interactions mediated through a water molecule may well be present. The extra binding potency compared to **32** is proposed to come predominantly from lipophilic interactions between the benzyl group and the lipophilic channel leading to the active site.

In the second series based upon structures **35**, around ten analogues were synthesised. The best compound was the phenoxy acetic acid derivative **39** (IC_{50} 1.3 μM) which binds about 23 times more strongly to AMPD than compound **32** (Table 2). It is interesting to note that the phosphonate analogue **40** was a weaker inhibitor of AMPD (IC_{50} = 40 μM) than the **39**, even though a phosphonate might be expected to be a better phosphate mimic than a carboxylic acid. Neither **39** nor **40** were herbicidally active but activity at 1 kg/ha was shown by the methyl ester derivative of **39**. The proposed binding mode of compound **39** is shown in Figure 3b. In contrast to **32** and **36**, the acid in compound **39** is

Table 2
Results from *in silico* screening of a 91 member virtual library against plant AMPD. *Top row*: Three arylimidazotriazines, including compound **32**, identified for synthesis, plus the reference compound **30**. *Bottom row*: An additional four analogues, not included in the virtual library, were synthesised in order to better explore the SAR. All compounds are shown as the covalent hydrates.

Structure	 **33** (Hydrate of **32**)	 (Hydrate of **30**)		
Binding Score (kcal mol^{-1})	−68	−62	−56	−46 (for X = CH)
IC_{50} (AMPD) (μM)	30	200	>500	250 (for X = N)
Structure				
Binding Score (kcal mol^{-1})	n.d.	n.d.	n.d.	n.d.
IC_{50} (AMPD) (μM)	100	120	190	>500



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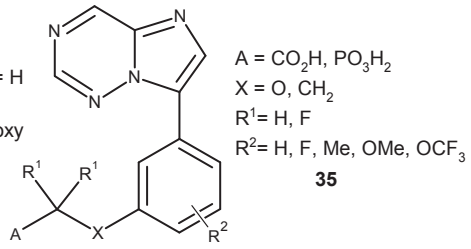

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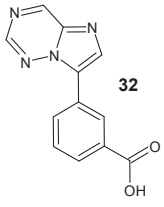
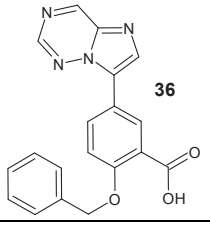
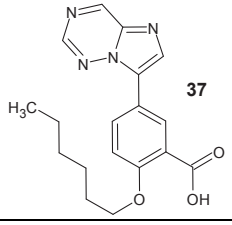
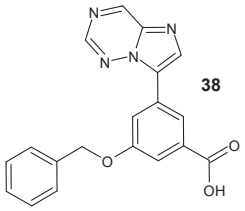
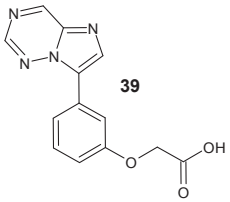
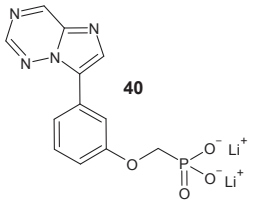
Table 3
Results from the structure-based optimisation of compound **32** showing calculated binding score to plant AMPD and measured inhibition levels against the plant and human AMPD isozymes.

Structure	 **32**	 **36**	 **37**
Binding Score (kcal mol^{-1})	−66	−86	−88
Plant AMPD (ΔV211M)[17] IC_{50} (μM)	30	0.3	10
Human AMPD3 (1b)[26] IC_{50} (μM)	n.d.	400	400
Structure	 **38**	 **39**	 **40**
Binding Score (kcal mol^{-1})	−62	−87	−86
Plant AMPD (ΔV211M)[17] IC_{50} (μM)	25	1.3	40
Human AMPD3 (1b)[26] IC_{50} (μM)	200	650	200

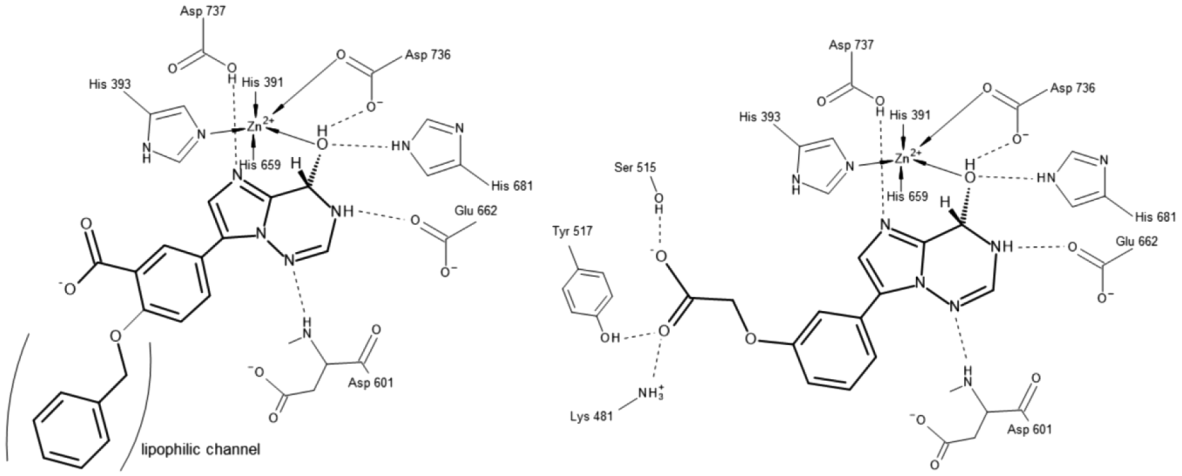


Figure 3. (a) (left). Schematic representation of the proposed binding mode of the covalent hydrate derived from compound **36** to the active site of *Arabidopsis* AMPD. **(b) (right).** Schematic representation of the proposed binding mode of the covalent hydrate derived from compound **39** to the active site of *Arabidopsis* AMPD. In both cases, H-bonds are shown as dashed lines and arrows indicate coordination bonds to the zinc ion. The amino acids are numbered according to the UniProt entry O80452.

predicted to be able to make good H-bonding interactions in the AMPD phosphate binding pocket with Lys 481, Ser 515 and Tyr 517.

The synthesis of inhibitors **32**, **36** and **39** was achieved in four steps as shown in Scheme 3, the key step being a Suzuki coupling reaction between a pinocolboronicacid ester and the imidazotriazine **24**. All six compounds[27] shown in Table 3 are believed to bind to AMPD as the corresponding covalent hydrates (as has been shown for **11**/**13**) but unfortunately, we were unable to obtain protein–ligand co-crystal structures to confirm this. The high selectivity shown by **36** and **39** for plant AMPD over human AMPD3 is remarkable in comparison to the results previously obtained for compound **30**.[17] None of the compounds shown in Table 3 inhibited the related enzyme ADA.

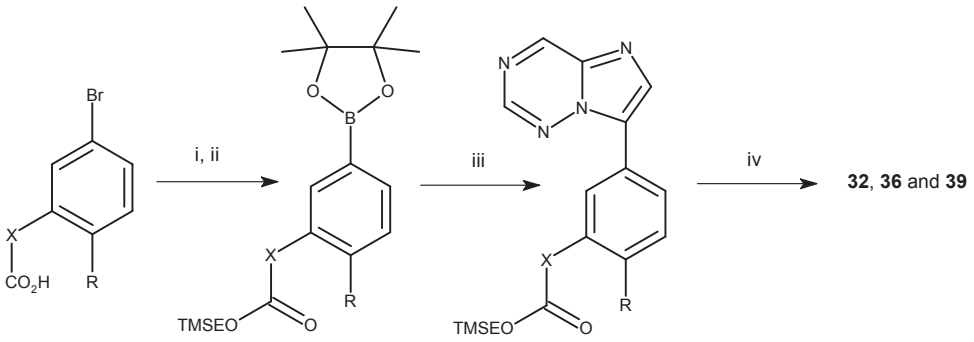
4. Summary

The herbicidally active natural product carbocyclic coformycin (**1**) is an inhibitor of ADA which undergoes 5́-phosporylation *in planta* to give a potent inhibitor **2** of AMPD.[2] An enzyme mechanism-based approach has been used to design new inhibitors starting from the known inhibitor nebularine (**6**). The movement of a single aglycone ring nitrogen by one position in **6** to give 2-deoxy isonebularine (**16**) has a remarkable effect upon the ability of the aglycone ring system to form a stable covalent hydrate (Table 1), so that **16** binds over 5000 times more strongly than **6** to the closely related enzyme ADA. Since ADA is not present in plants,[2,13] ADA inhibition cannot play a role in the herbicidal activity of **16** and it is proposed that **16** undergoes 5́-phosporylation *in planta* to give the phosphate **17**, which is expected to be an strong inhibitor of AMPD. The herbicidal nucleosides carbocyclic coformycin (**1**) and deaminoformycin (**10**) have been shown to exert their biological activity *via* this mechanism.[2,13] Unfortunately, the comparative instability of **16** has prevented an experimental confirmation of this hypothesis in the current case.

Starting from **16** and the X-ray crystal structure of the covalent hydrate **13** bound to AMPD, an enzyme structure-based approach has been used to design new non-ribosyl AMPD inhibitors. This approach began by *in silico* screening of a virtual library against plant AMPD which led to the synthesis of a small number of compounds including the new lead inhibitor **32**. In a second step, more detailed structure-based molecular design starting from **32** led to the synthesis of stronger inhibitors including the benzoic acid derivative **36** and the phenoxyacetic acid **39**. Overall a good correlation between calculated AMPD binding score and the measured IC_{50} value was observed. The best compounds exhibited herbicidal activity, albeit at much lower levels than observed for compounds **1** and **16**. In future research it would be interesting to investigate whether hybrid molecules, containing the lipophilic side chain from **36** and the extended acid side chain from **39**, possess improved biological activity.

5. Experimental

Commercially available reagents were used without further purification and solvents were dried using standard procedures as required.



X = bond, R = H for **32**; X = bond, R = OBn for **36;** X = OCH_2, R = H for **39**

Scheme 3. Synthesis of AMPD inhibitors **32**, **36** and **39**: Reagents, conditions and yields: (i) $TMSCH_2CH_2OH$ (1.5 equiv), BOP (1.2 equiv). *i*-Pr_2EtN (3 equiv), CH_2Cl_2, rt, 16 h (73–88%). (ii) Bispinacolborane (1 equiv), $(dppf)PdCl_2$ (0.1 equiv), KOAc (3 equiv), DMSO, 80 °C, 4 h (33–62%). (iii) **24** (1 equiv), $PdOAc_2$ (0.1 equiv), o-Tol_3P (0.2 equiv), Cs_2CO_3 (3 equiv), dioxane/H_2O, 80 °C, 3.5 h (57–70%). (iv) CsF (1.2 equiv), DMF, 60 °C, 12 h then HCl (1.2 equiv), rt (46–50%). TMSE = trimethylsilylethyl.

Analytical thin layer chromatography was carried out using glass-backed plates coated with silica gel 60 containing a fluorescent indicator and plates were visualised under UV light (254, 366 nm). Preparative chromatography was carried out on silica gel 60H columns using a Biotage Isolera One apparatus. Preperative HPLC was performed using a Gilson system equipped with a Merck Luna C18 column (10 μm particle size, 100 Å pore size). ^{1}H NMR spectra were recorded at 400 or 600 MHz and ^{13}C NMR spectra at 100 or 150 MHz on a Bruker DRX 400 or Bruker Avance III 600 spectrometer at 25 °C. Accurate mass measurements were determined under electrospray ionization conditions on a Waters QTOF Premier spectrometer.

The biochemical assay conditions used for determining enzyme inhibition against AMPD[13] and ADA[2] were as previously described. The biological assay conditions used for determining herbicidal activity and experimental details describing the synthesis of compounds **10**, **11** and **12** have also been previously reported.[28]

5.1. N-(2,4-dimethoxybenzyl)-1H-imidazole-2-carboxamide 20

A mixture of ethyl imidazole-2-carboxylate[29] (20.0 g, 138 mmol) and 2,4-dimethoxybenzylamine (22.3 mL, 145 mmol) was heated using an oil bath at 200 °C for 20 h. The crude product was crystallised from EtOH/MeOH, filtered and washed with hexane. A second crop was obtained by crystallisation of the mother liquors. The two batches were combined to give the title amide **20** as a colourless solid (31.8 g, 122 mmol, 88%); (Found: MH^+, 262.1192. $C_{13}H_{16}N_3O_3$ requires 262.1192); δ_H (400 MHz; $CDCl_3$) 3.79 (3H, s), 3.83 (3H, s), 4.55 (2H, d, J = 7.5), 6.42 (1H, dd, J = 1.0, 9.5), 6.48 (1H, d, J = 1.0), 7.12 (2H, d, J = 11.0), 7.24 (1H, d, J = 9.5), 7.67 (1H, bs), 11.33 (1H, bs). δ_C (150 MHz; $CDCl_3$) 38.68 (CH_2), 55.42 (2 × CH_3), 98.58 (CH), 103.90 (CH), 118.27 (C), 119.02 (CH), 129.63 (CH), 130.44 (CH), 141.25 (C), 158.51 (C), 158.67 (C), 160.68 (C).

5.2. 3-(2,4-Dimethoxybenzyl)imidazo[2,1-f][1,2,4]triazin-4(3H)-one 21

Sodium hydride (60% oil dispersion, 1.84 g, 45.9 mmol) was added to a solution of the amide 20 (10.0 g, 38,3 mmol) in dry dimethylformamide (DMF, 300 mL) at rt under an inert nitrogen atmosphere. The suspension was stirred at rt for 1 h, *O*-(diphenylphosphinoyl)hydroxylamine (11.6 g, 49.8 mmol) was added and the mixture was stirred at rt for a further 4 h. The mixture was filtered and the solid washed twice with DMF. The combined filtrate was evaporated under reduced pressure to yield 1-amino-N-(2,4-dimethoxybenzyl)-1H-imidazole-2-carboxamide. Triethylorthoformate (133 mL) followed by formic acid (0.58 mL) were added and the mixture was heated with stirring in an oil bath at 160 °C for 10 h. The mixture cooled to rt, diluted with EtOAc (180 mL) and stored at 5 °C for 2 days. The precipitated solid was filtered off, washed thrice with EtOAc and dried to yield the title compound **21** (9.45 g, 33 mmol, 86%); (Found: MH^+, 287.1151. $C_{14}H_{15}N_4O_3$ requires 287.1144); δ_H (400 MHz; $CDCl_3$) 3.80 (3H, s), 3.85 (3H, s), 5.07 (2H, s), 6.47 (2H, m), 7.45 (1H, d, J = 9.5), 7.52 (2H, d, J = 11.0), 7.92 (1H, s). δ_C (150 MHz; $CDCl_3$) 44.54 (CH_2), 55.49 (CH_3), 55.61 (CH_3), 98.70 (CH), 104.56 (CH), 115.09 (C), 118.71 (CH), 132.12 (CH), 133.09 (CH), 133.46 (C), 141.95 (CH), 151.92 (C), 158.59 (C), 161.62 (C).

5.3. Imidazo[2,1-f][1,2,4]triazin-4(3H)-one

The triazinone **21** (5.0 g, 17.5 mmol) was dissolved in trifluoroacetic acid (120 mL) and heated at 50 °C for 6 h. The reaction mixture was cooled to rt and diluted with diethyl ether (250 mL) with stirring. The precipitated solid was filtered off, resuspended in diethyl ether (200 mL) with stirring and filtered off again. The solid was washed thrice with diethyl ether and dried to yield the title compound (2.24 g, 16.5 mmol, 94%). ^{1}H and ^{13}C NMR identical to those previously obtained.[17]

5.4. Imidazo[2,1-f][1,2,4]triazin-4-amine 22

Phosphoryl trichloride (90 mL) and *N,N*-dimethylaniline (6.0 mL) were added to imidazo[2,1-f][1,2,4]triazin-4(3H)-one (1.82 g, 13.4 mmol) and the mixture was heated at reflux (160 °C) for 4 h under an inert nitrogen atmosphere. The excess $POCl_3$ was evaporated under reduced pressure to give 4-chloroimidazo[2,1-f][1,2,4]triazine. Dry butan-2-ol (45 mL) was added to the chloroimidazotriazine and the solution was immediately cooled to 0 °C under an inert nitrogen atmosphere. A 2 M solution of ammonia in propan-2-ol (100 mL) was added dropwise over 10 min and the mixture was allowed to warm to rt. After 16 h the solvent was removed under reduced pressure and the residue was dissolved in EtOAc (50 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (150 mL). The aqueous phase was extracted five times with EtOAc (100 mL). The combined organic extracts were dried (MgSO4) and evaporated under reduced pressure. The resulting solid was suspended in *t*-butyl methyl ether (100 mL) and stored at 5 °C for 1 day. The solid product was filtered off, washed twice with *t*-butyl methyl ether and dried to yield the title compound **22** (1.52 g, 11.2 mmol, 84%); (Found: MH^+, 136.0623. $C_5H_6N_5$ requires 136.0623); δ_H (600 MHz; DMSO-d_6) 7.58 (1H, s), 8.03 (2H, s), 8.57 (2H, bd, $J = 42$ Hz); δ_C (150 MHz; DMSO-d_6) 117.69 (CH), 128.39 (C), 130.92 (CH), 149.10 (CH), 153.86 (C).

5.5. 7-Iodoimidazo[2,1-f][1,2,4]triazin-4-amine 23

N-iodosuccinimide (7.94 g, 35 mmol) was added to a solution of the amine **22** (1.59 g, 11.8 mmol) in dry DMF (65 mL) under an inert nitrogen atmosphere. After 4 days at rt, the solvent was removed under reduced pressure, CH_2Cl_2 (65 mL) was added and the mixture was heated with stirring at 40 °C for 30 min. The solid was filtered off, washed thrice with CH_2Cl_2 (65 mL) and dried to yield the title compound **23** (2.35 g, 9.00 mmol, 76%); (Found: MH^+, 261.9591. $C_5H_5N_5I$ requires 261.9590); δ_H (600 MHz; DMSO-d_6) 7.70 (1H, s), 8.14 (1H, s), 8.25 (2H, bd, $J = 24$ Hz); δ_C (150 MHz; DMSO-d_6) 72.91 (C), 130.79 (C), 136.76 (CH), 149.67 (CH), 153.60 (C).

5.6. 7-iodoimidazo[2,1-f][1,2,4]triazine 24

n-Butylnitrite (2.15 mL, 18.4 mmol) was added to a solution of the iodide **23** (480 mg, 1.84 mmol) in dry 1,4-dioxane (23 mL) at rt under an inert nitrogen atmosphere. The reaction mixture was transferred to a pre-heated oil bath and heated at 100 °C for 2 h. The mixture was cooled to rt, silica gel was added (ca. 1 g) and the volatiles were removed under reduced pressure. The resulting solid was added to the top of a silica gel chromatography column and eluted with EtOAc (0–100%) in heptanes. Fractions containing product were combined and evaporated under reduced pressure to yield the title compound **24** (173 mg, 0.70 mmol, 38%); (Found: MH^+, 246.9478. $C_5H_4N_4I$ requires 246.9481); δ_H (600 MHz; DMSO-d_6) 8.20 (1H, s), 9.07 (1H, s), 9.34 (1H, s); δ_C (150 MHz; DMSO-d_6) 75.16 (C), 138.21 (C), 141.92 (CH), 149.27 (CH), 150.18 (CH).

5.7. (2R,5R)-5-(4-Aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-2-(hydroxymethyl)dihydrofuran-3(2H)-one 26

Triphenylarsine (89 mg, 0.28 mmol) was added to a solution of *bis* (dibenzylideneacetone)palladium(0) (82 mg, 0.14 mmol) in dry *N,N*-dimethylacetamide (8 mL) at rt under an inert nitrogen atmosphere. This mixture was stirred at rt for 20 min and then added to a suspension of the aminoiodide **23** (370 mg, 1.42 mmol), 1,4-anhydro-2-deoxy-D-*erythro*-pent-1-enitol **25**[16] (329 mg, 2.84 mmol) and triethylamine (0.40 mL, 2.84 mmol) in dry N,*N*-dimethylacetamide (8 mL) at rt under an inert nitrogen atmosphere. The mixture was stirred at 80 °C for 16 h, evaporated to dryness under vacuum and purified by column chromatography (8–25% MeOH/CH_2Cl_2) to yield the title compound **26** as a

92:8 mixture of the β- and α-*C*-nucleosides (179 mg, 0.72 mmol, 51%); (Found: MH^+, 250.0937. $C_{10}H_{12}N_5O_3$ requires 250.0940); δ_H (400 MHz; DMSO-d_6) 2.91 (2H, m), 3.60 (2H, m), 4.03 (1H, t, 2.7 Hz), 4.89 (1H, t, J = 6.5 Hz, OH), 5.68 (1H, dd, J = 6.5, 10.5 Hz), 7.80 (1H, s), 8.09 (1H, s), 8.24 (2H, bd, J = 27 Hz, NH_2). The ketone **26** was rather unstable in our hands and was not stored but immediately reduced to the diol **27**.

5.8. (1R)-1-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-1,4-anhydro-2-deoxy-D-erythro-pentitol 27

Sodium cyanoborohydride (468 mg, 2.21 mmol) was added to a cooled solution of ketone **26** (458 mg, 1.84 mmol) in MeCN (18 mL) and glacial AcOH (18 mL) at −24 °C. The mixture was stirred at −24 °C for 30 min and at −18 °C for 15 min, then ethanol (30 mL) was added. The mixture was allowed to warm to rt, evaporated to dryness under vacuum and purified by column chromatography (15–25% MeOH/CH_2Cl_2) to yield the title compound **27** as a 92:8 mixture of the β- and α-*C*-nucleosides (402 mg, 1.60 mmol, 87%); (Found: MH^+, 252.1096. $C_{10}H_{14}N_5O_3$ requires 252.1097); δ_H (400 MHz; D_2O) 2.29 (1H, m), 2.53 (1H, m), 3.60 (2H, m), 4.01 (1H, bs), 4.42 (1H, bd, J = 5.5 Hz), 5.52 (1H, dd, J = 6.5, 10.5 Hz), 7.60 (1H, s), 8.03 (1H, s); δ_C (150 MHz; DMSO-d_6) (CH_2 at δ 39–40 hidden by DMSO-d_6), 62.15, 69.52, 71.98, 87.57, 128.75, 129.08, 129.60, 148.90, 153.89.

5.9. (1R)-3,5-di-O-acetyl-1-(4-aminoimidazo[2,1-f][1,2,4]triazin-7-yl)-1,4-anhydro-2-deoxy-D-erythro-pentitol 28

Acetic anhydride (0.51 mL, 5.4 mmol) was added to a stirred solution of the diol **27** (113 mg, 0.45 mmol) in dry pyridine (4 mL) at rt. After 4 h, ethanol (5 mL) was added and the mixture was stirred for a further 1 h. The solvent was evaporated under vacuum and the mixture of acetylated products was dissolved in ethanol (30 mL) and heated at 70 °C for 21 h. The solvent was evaporated *in vacuo* and the crude product was purified by column chromatography (5–20% MeOH/CH_2Cl_2) to yield the title compound **28** as a 94:6 mixture of the β- and α-*C*-nucleosides (93 mg, 0.28 mmol, 61%); (Found: MH^+, 336.1314. $C_{14}H_{18}N_5O_5$ requires 336.1308);); λ_m (H_2O) 232, 270 nm; δ_H (400 MHz; $CDCl_3$) 2.09 (3H, s), 2.13 (3H, s), 2.47 (1H, m), 2.58 (1H, m), 4.22 (1H, dd, J = 3, 10.5 Hz), 4.31 (1H, m), 4.35 (1H, dd, J = 3, 10.5 Hz), 5.33 (1H, d, J = 7.5 Hz), 5.60 (1H, dd, J = 6.5, 10.5 Hz), 5.90–6.80 (2H, NH_2), 7.57 (1H, s), 8.15 (1H, s); δ_C (150 MHz; $CDCl_3$) 21.07 (CH_3), 21.15 (CH_3), 64.10 (CH_2), 71.18 (CH), 76.12 (CH), 82.67 (CH), 128.92 (C), 129.25 (C), 129.94 (CH), 148.94 (CH), 153.62 (C), 170.54 (C), 170.64 (C).

5.10. (1R)-3,5-di-O-acetyl-1,4-anhydro-2-deoxy-1-imidazo[2,1-f][1,2,4]triazin-7-yl-D-erythro-pentitol 29

A solution of the amine **28** (65 mg, 0.19 mmol) in dry 1,4-dioxane (2.0 mL) under an inert argon atmosphere was heated in an oil bath to 100 °C. Neat *n*-butylnitrite (0.60 mL, 4.85 mmol) was added in one go, causing the reaction mixture to noticeably foam. After 40 min a further quantity of *n*-butylnitrite (0.1 mL, 0.81 mmol) was added. The mixture was heated at 100 °C for a further 15 min, cooled to rt and purified by column chromatography (60–100% EtOAc/heptane) to yield the title compound **29** as a 94:6 mixture of the β- and α-*C*-nucleosides (17 mg, 0.053 mmol, 27%); (Found: MH^+, 321.1191. $C_{14}H_{17}N_4O_5$ requires 321.1199); λ_m (H_2O) 228, 328 nm; δ_H (400 MHz; $CDCl_3$) 2.09 (3H, s), 2.15 (3H, s) 2.57 (2H, m), 4.25 (1H, dd, J = 3, 10.5 Hz), 4.35 (2H, m), 5.35 (1H, d, J = 7.5 Hz), 5.60 (1H, dd, J = 7.5, 10.5 Hz), 7.97 (1H, s), 8.82 (1H, s), 9.26 (1H, s). δ_C (150 MHz; $CDCl_3$) 20.84 (CH_3), 21.06 (CH_3), 63.91 (CH_2), 71.06 (CH), 76.01 (CH), 82.80 (CH), 128.71 (C), 134.55 (CH), 136.68 (C), 148.38 (CH), 150.77 (CH), 170.50 (C), 170.59 (C).

5.11. (1R)-1,4-anhydro-2-deoxy-1-imidazo[2,1-f][1,2,4]triazin-7-yl-D-erythro-pentitol 16

A solution of the diacetate **29** (12 mg, 0.038 mmol) in methanol (8 mL) and triethylamine (2 mL) was heated at 64 °C for 2 h. The mixture was cooled to rt and evaporated under vacuum. Dry toluene 10 mL) was added and the mixture was evaporated *in vacuo* to give the title compound **16** (8 mg, 0.034 mmol, 91%). Purification by reversed phase HPLC under neutral conditions (MeCN/water gradient) gave the pure β-*C*-nucleoside **16** as a colourless solid (4 mg, 0.017 mmol, 38%; 98.9% purity as assessed by analytical HPLC in 2% MeCN/98% water); (Found: MH^+, 237.0983. $C_{10}H_{13}N_4O_3$ requires 237.0988); $[\alpha]_D^{20}$ = –22° (c = 0.22 g/100 mL, H_2O); λ_m (H_2O) 226, 329 nm; δ_H (400 MHz; D_2O) 2.38 (1H, m), 2.57 (1H, m), 3.64 (2H, m), 4.05 (1H, m), 4.48 (1H, m), 5.71 (1H, dd, J = 6.5, 10.5 Hz), 8.02 (1H, s), 8.82 (1H, s), 9.23 (1H, s); δ_C (150 MHz; D_2O) 37.67 (CH_2), 62.19 (CH_2), 69.88 (CH), 72.55 (CH), 87.22 (CH), 129.23 (C), 134.33 (CH), 136.49 (C), 147.92 (CH), 150.35 (CH).

5.12. 3-(imidazo[2,1-f][1,2,4]triazin-7-yl)benzoic acid 32

The title compound **32** was prepared from 3-bromobenzoic acid and the iodide **24** in an analogous manner to that described below for the synthesis of compound **36**. Analytical data for the caesium salt derivative of **32**; (Found: $[M-H]^-$, 239.0579. $C_{12}H_7N_4O_2$ requires 239.0569); δ_H (600 MHz; D_2O) 7.54 (1H, t, J = 8 Hz), 7.87 (1H, d, J = 8 Hz), 8.08 (1H, d, J = 8 Hz), 8.25 (1H, s), 8.41 (1H, s), 8.79 (1H, s), 9.20 (1H, s); δ_C (150 MHz; D_2O) 126.32 (C), 127.66 (CH), 128.90 (CH), 129.39 (C), 129.60 (CH), 129.79 (CH), 135.17 (CH), 136.30 (C), 136.80 (C), 148.01 (CH), 150.04 (CH), 174.59 (C).

5.13. 2-(benzyloxy)-5-(imidazo[2,1-f][1,2,4]triazin-7-yl)benzoic acid 36

5.13.1. 2-(trimethylsilyl)ethyl 2-(benzyloxy)-5-bromobenzoate

(Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (865 mg, 1.96 mmol), 2-(Trimethylsilyl)-ethanol (0.35 mL, 2.44 mmol) and *N*-Ethyl-N-(propan-2-yl)propan-2-amine (0.85 mL, 4.89 mmol) were added sequentially to a solution of 2-(benzyloxy)-5-bromobenzoic acid[30] (500 mg, 1.63 mmol) in dry CH_2Cl_2 (25 mL) at rt. The mixture was left at rt for 16 h and then evaporated to dryness under vacuum. Aqueous 1% hydrochloric acid (10 mL) was added and the mixture was extracted three times with ethyl acetate (20 mL). The combined organic extracts were washed with saturated aqueous NaHCO3 solution (10 mL), dried over MgSO4 and evaporated to dryness *in vacuo*. The crude product was purified by column chromatography (0–40% EtOAc/heptane) to yield the title compound as a colourless oil (484 mg, 1.19 mmol, 73%); (Found: MNa^+, 429.0515. C19H23O3Br-SiNa requires 429.0498); δH (600 MHz; CDCl3) 0.07 (9H, s), 1.08 (2H, m), 4.38 (2H, m), 5.16 (2H, s), 6.88 (1H, d, J = 8.8 Hz), 7.32 (1H, t, J = 7.5 Hz), 7.38 (2H, t, J = 7.5 Hz), 7.47 (2H, d, J = 7.5 Hz), 7.50 (1H, dd, J = 2.5, 8.8 Hz), 7.91 (1H, d, J = 2.5 Hz); δC (150 MHz; CDCl3) −1.53, 17.39, 63.60, 70.77, 112.60, 115.51, 122.97, 126.95, 127.95, 128.54, 134.16, 135.68, 136.21, 157.02, 165.19.

5.13.2. 2-(trimethylsilyl)ethyl 2-(benzyloxy)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate

Potassium acetate (180 mg, 1.83 mmol) was added to a solution of 2-(trimethylsilyl)ethyl 2-(benzyloxy)-5-bromobenzoate (249 mg, 0.61 mmol) in dry DMSO (2 mL) at rt under an inert nitrogen atmosphere. The mixture was stirred at rt for 20 min and then bis-(pinakolato)-diborane (155 mg, 0.61 mmol) and [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II).CH2Cl2 (50 mg, 0.061 mmol) were added, taking care to keep everything under nitrogen. The mixture was heated with shaking at 80 °C for 4 h and then allowed to cool to rt. Water (10 mL) was added and the mixture was extracted three times with EtOAc

(15 mL). The combined organic extracts were washed with saturated brine solution (10 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography (0–30% EtOAc/heptane) to yield the title compound (172 mg, 0.38 mmol, 62%); (Found: MNa^+, 477.2256. $C_{25}H_{35}BO_5SiNa$ requires 477.2245); δ_H (600 MHz; $CDCl_3$) 0.07 (9H, s), 1.10 (2H, m), 1.34 (12H, s), 4.38 (2H, m), 5.20 (2H, s), 6.99 (1H, d, $J = 8.8$ Hz), 7.30 (1H, t, $J = 7.5$ Hz), 7.38 (2H, t, $J = 7.5$ Hz), 7.49 (2H, d, $J = 7.5$ Hz), 7.85 (1H, d, $J = 8.8$ Hz), 8.22 (1H, s); δ_C (150 MHz; $CDCl_3$) −1.48 (CH_3), 17.45 (CH_2), 24.84 (CH_3), 63.12 (CH_2), 70.19 (CH_2), 83.83 (C), 112.63 (CH), 120.92 (C), 126.92 (CH), 127.79 (CH), 128.49 (CH), 136.54 (C), 138.26 (CH), 139.75 (CH), 160.15 (C), 166.62 (C); the ^{13}C-signal for the boron-bonded C5 atom was not observed due to ^{13}C-^{11}B spin–spin coupling.

5.13.3. 2-(trimethylsilyl)ethyl 2-(benzyloxy)-5-(imidazo[2,1-f][1,2,4] triazin-7-yl)benzoate

A solution of caesium carbonate (346 mg, 1.06 mmol) in water (0.5 mL) was added to a solution of 2-(trimethylsilyl)ethyl 2-(benzyloxy)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (220 mg, 0.46 mmol) and the iodide **24** (87 mg, 0.35 mmol) in 1,4-dioxane (3.5 mL) at rt under an inert nitrogen atmosphere. The mixture was shaken for 25 min and then palladium (II) acetate (8.0 mg, 0.036 mmol) and *Tris*(*o*-tolyl)phosphine (22 mg, 0.072 mmol) were added and the mixture was heated with shaking at 80 °C for 3.0 h. The mixture was added to ice-cooled water (10 mL) and the mixture was extracted thrice with EtOAc (15 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography (0–40% EtOAc/heptane) to yield the title compound (90 mg, 0.20 mmol, 58%); (Found: MH^+, 447.1852. $C_{24}H_{27}N_4O_3Si$ requires 447.1852); δ_H (600 MHz; $CDCl_3$) 0.09 (9H, s), 1.11 (2H, m), 4.45 (2H, m), 5.25 (2H, s), 7.18 (1H, d, $J = 8.8$ Hz), 7.34 (1H, t, $J = 7.5$ Hz), 7.40 (2H, t, $J = 7.5$ Hz), 7.52 (2H, d, $J = 7.5$ Hz), 8.22 (1H, s), 8.25 (1H, dd, $J = 2.0$, 8.8 Hz), 8.52 (1H, d, $J = 2.0$ Hz), 8.87 (1H, s), 9.27 (1H, s); δ_C (150 MHz; $CDCl_3$) −1.47 (CH_3), 17.41 (CH_2), 63.63 (CH_2), 70.63 (CH_2), 113.98 (CH), 119.51 (C), 121.89 (C), 126.98 (CH), 127.99 (CH), 128.45 (C), 128.58 (CH), 130.56 (CH), 131.66 (CH), 134.92 (CH), 136.24 (C), 136.55 (C), 148.52 (CH), 150.55 (CH), 158.26 (C), 166.12 (C).

5.13.4. 2-(benzyloxy)-5-(imidazo[2,1-f][1,2,4]triazin-7-yl)benzoic acid 36

Caesium fluoride (37 mg, 0.24 mmol) was added to a solution of 2-(trimethylsilyl)ethyl 2-(benzyloxy)-5-(imidazo[2,1-f][1,2,4]triazin-7-yl)benzoate (90 mg, 0.20 mmol) in dry N,*N*-dimethylformamide (2 mL) at rt under an inert nitrogen atmosphere. The mixture was shaken at 60 °C for 8 h and then evaporated under vacuum to yield the caesium salt of the title compound. Hydrogen chloride in 1,4-dioxane (0.6 M, 2.8 mL, 1.68 mmol) was added dropwise to the crude caesium salt in 1,4-dioxane (2.5 mL), followed by water (0.6 mL) to give a clear solution. The mixture was evaporated under vacuum and the solid was washed twice with water (10 mL) to give the title compound as a colourless solid (35 mg, 0.10 mmol, 51%); (Found: $[M-H]^-$, 345.0985. $C_{19}H_{13}N_4O_3$ requires 345.0988); δ_H (400 MHz; DMSO-d_6) 5.30 (2H, s), 7.33 (1H, m), 7.40 (3H, m), 7.52 (2H, d, $J = 7.5$ Hz), 8.25 (1H, dd, $J = 2.0$, 8.8 Hz), 8.53 (1H, d, $J = 2.0$ Hz), 8.54 (1H, s), 9.06 (1H, s), 9.47 (1H, s), 12.95 (1H, bs, OH); δ_C (150 MHz; DMSO-d_6 + CD_3OD 1:1) 70.66 (CH_2), 114.84 (CH), 120.22 (C), 122.19 (C), 127.87 (CH), 128.42 (CH), 128.62 (C), 129.04 (CH), 129.77 (CH), 131.74 (CH), 135.41 (CH), 137.26 (C), 137.58 (C), 149.37 CH), 151.26 (CH), 157.96 (C), 168.51 (C; v. broad).

5.14. [3-(imidazo[2,1-f][1,2,4]triazin-7-yl)phenoxy]acetic acid 39

5.14.1. 2-(trimethylsilyl)ethyl (3-bromophenoxy)acetate

(Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (7.72 g, 17.45 mmol), 2-(Trimethylsilyl)-ethanol (3.13 mL, 21.81 mmol) and *N*-Ethyl-*N*-(propan-2-yl)propan-2-amine (7.60 mL, 43.63 mmol) were added sequentially to a solution of 2-(benzyloxy)-5-bromobenzoic acid[31] (3.60 g, 14.54 mmol) in dry CH_2Cl_2 (135 mL) at rt. The mixture was left at rt for 16 h and then evaporated to dryness under vacuum. Aqueous 1% hydrochloric acid (100 mL) was added and the mixture was extracted three times with methyl-*t*-butyl ether (100 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ solution (100 mL), dried over $MgSO_4$ and evaporated to dryness *in vacuo*. The crude product was purified by column chromatography (0–20% EtOAc/heptane) to yield the title compound as a colourless oil (4.26 g, 12.86 mmol, 88%); (Found: $[M-H]^-$, 329.0206. $C_{13}H_{18}O_3BrSi$ requires 329.0209); δ_H (600 MHz; $CDCl_3$) 0.05 (9H, s), 1.03 (2H, m), 4.31 (2H, m), 4.58 (2H, s), 6.84 (1H, m), 7.07 (1H, m), 7.14 (2H, m); δ_C (150 MHz; $CDCl_3$) −1.63 (CH_3), 17.26 (CH_2), 63.86 (CH_2), 65.47 (CH_2), 113.48 (CH), 118.05 (CH), 122.74 (C), 124.77 (CH), 130.54 (CH), 158.47 (C), 168.45 (C).

5.14.2. 2-(trimethylsilyl)ethyl [3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy]acetate

Potassium acetate (889 mg, 9.06 mmol) was added to a solution of 2-(trimethylsilyl)ethyl (3-bromophenoxy)acetate (1.00 g, 3.02 mmol) in dry DMSO (9 mL) at rt under an inert nitrogen atmosphere. The mixture was stirred at rt for 20 min and then *bis*-(pinakolato)-diborane (766 mg, 3.02 mmol) and [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II).CH_2Cl_2 (20 mg, 0.025 mmol) were added, taking care to keep everything under nitrogen. The mixture was heated with shaking at 80 °C for 4 h and then allowed to cool to rt. Water (10 mL) was added and the mixture was extracted three times with EtOAc (15 mL). The combined organic extracts were washed with saturated brine solution (10 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography (0–20% EtOAc/heptane) to yield the title compound (385 mg, 1.02 mmol, 34%); (Found: MNH_4^+, 396.2358. $C_{19}H_{35}BNO_5Si$ requires 396.2378); δ_H (600 MHz; $CDCl_3$) 0.05 (9H, s), 1. 30 (2H, m), 1.34 (12H, s), 4.30 (2H, m), 4.63 (2H, s), 7.05 (1H, m), 7.30 (2H, m), 7.44 (1H, d, $J = 7.5$ Hz); δ_C (150 MHz; $CDCl_3$) −1.62 (CH_3), 17.24 (CH_2), 24.76 (CH_3), 63.57 (CH_2), 65.45 (CH_2), 83.77 (C), 118.53 (CH), 119.46 (CH), 128.08 (CH), 128.97 (CH), 157.24 (C), 169.02 (C); the ^{13}C-signal for the boron-bonded C3 atom was not observed due to ^{13}C-^{11}B spin–spin coupling.

5.14.3. 2-(trimethylsilyl)ethyl [3-(imidazo[2,1-f][1,2,4]triazin-7-yl) phenoxy]acetate

A solution of caesium carbonate (397 mg, 1.22 mmol) in water (2 mL) was added to a solution of 2-(trimethylsilyl)ethyl [3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy]acetate (154 mg, 0.41 mmol) and the iodide **24** (100 mg, 0.41 mmol) in 1,4-dioxane (18 mL) at rt under an inert nitrogen atmosphere. The mixture was shaken for 25 min and then palladium (II) acetate (9.1 mg, 0.041 mmol) and *Tris*(*o*-tolyl)phosphine (25 mg, 0.082 mmol) were added and the mixture was heated with shaking at 80 °C for 3.5 h. After cooling to rt, water (10 mL) was added and the mixture was extracted twice with EtOAc (15 mL). The combined organic extracts were washed with saturated brine solution (10 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography (0–20% EtOAc/heptane) to yield the title compound (87 mg, 0.23 mmol, 57%); (Found: MH^+, 371.1534. $C_{18}H_{22}N_4O_3Si$ requires 371.1539); δ_H (400 MHz; $CDCl_3$) 0.05 (9H, s), 1.05 (2H, m), 4.33 (2H, m), 4.70 (2H, s), 7.00 (1H, dd, $J = 8.5$, 2.5 Hz), 7.46 (1H, t, $J = 8.5$ Hz), 7.72 (1H, d, $J = 8.5$ Hz), 7.79 (2H, d, $J = 7.5$ Hz), 8.25 (1H, s), 8.86 (1H, s), 9.28 (1H, s); δ_C (150 MHz; $CDCl_3$)) −1.51 (CH_3), 17.41 (CH_2), 63.96 (CH_2), 65.64 (CH_2), 113.78 (CH), 115.08 (CH), 120.51 (CH), 128.41 (C), 129.03 (C), 130.13 (CH), 135.59 (CH), 136.88 (C), 148.51 (CH), 150.71 (CH), 158.19 (C), 168.82 (C).

5.14.4. [3-(imidazo[2,1-f][1,2,4]triazin-7-yl)phenoxy]acetic acid 39

Caesium fluoride (40 mg, 0.26 mmol) was added to a solution of 2-(trimethylsilyl)ethyl [3-(imidazo[2,1-f][1,2,4]triazin-7-yl)phenoxy]

acetate (81 mg, 0.22 mmol) in dry N,*N*-dimethylformamide (2 mL) at rt under an inert nitrogen atmosphere. The mixture was shaken at 60 °C for 12 h and then evaporated under vacuum to yield the caesium salt of the title compound (100 mg). This material was dissolved by sequential addition of 1,4-dioxane (4.5 mL), hydrogen chloride in 1,4-dioxane (4 M, 0.43 mL, 1.72 mmol) and water (0.6 mL). The mixture was evaporated under vacuum and the solid was washed twice with water (10 mL) to give the title compound as a colourless solid (30 mg, 0.11 mmol, 51%); (Found: $[M-H]^-$, 269.0679. $C_{13}H_9N_4O_3$ requires 269.0675); δ_H (400 MHz; DMSO-d_6) 4.79 (2H, s), 7.02 (1H, dd, $J = 8.5$, 2.5 Hz), 7.50 (1H, t, $J = 8.5$ Hz), 7.85 (2H, m), 8.61 (1H, s), 9.07 (1H, s), 9.50 (1H, s), 13.05 (1H, s, OH); δ_C (150 MHz; DMSO-d_6) 65.08 (CH_2), 113.71 (CH), 114.90 (CH), 119.95 (CH), 128.25 (C), 128.74 (C), 130.47 (CH), 136.20 (CH), 137.19 (C), 149.20 (CH), 151.50 (CH), 158.44 (C), 170.53 (C).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data (Copies of the LC/MS, ^1H and ^{13}C NMR spectra for compounds **16**, **32**, **36** and **39**. Details relating to the X-ray crystallography and molecular modelling experiments, including the structures and binding scores of the 91 member virtual library used for the virtual screening against AMPD and binding energies for the structure-based optimisation work.) to this article can be found online at https://doi.org/10.1016/j.bmc.2021.116272.

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27 Compounds **37** and **38** were synthesised in an analogous manner to compounds **32** and **36**. Compound **40** was prepared by reacting 3-bromophenol with diethyl phosphonomethyltriflate (Phillion, D. P.; Andrew, S. S. Tetrahedron Letters, 1986, 27, 1477) under basic conditions (5 equiv. K_2CO_3, MeCN, rt). The resulting bromide was converted to the corresponding pinocolboronicacid ester which was then coupled with **24** as described for **32** and **36**. The final step involved deprotection of the diethylphosphonate using TMSBr (McKenna, C. E.; Schmidhauser, J. J. Chem. Soc. Chem. Comm., 1979, 739) to give **40**, which was isolated as the dilithium salt.
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