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Synthesis and Biochemical Testing of 3-(Carboxyphenylethyl)-imidazo[2,1-*f*][1,2,4]triazines as Inhibitors of AMP Deaminase

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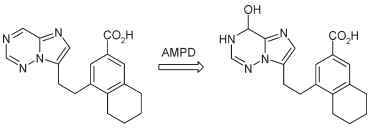
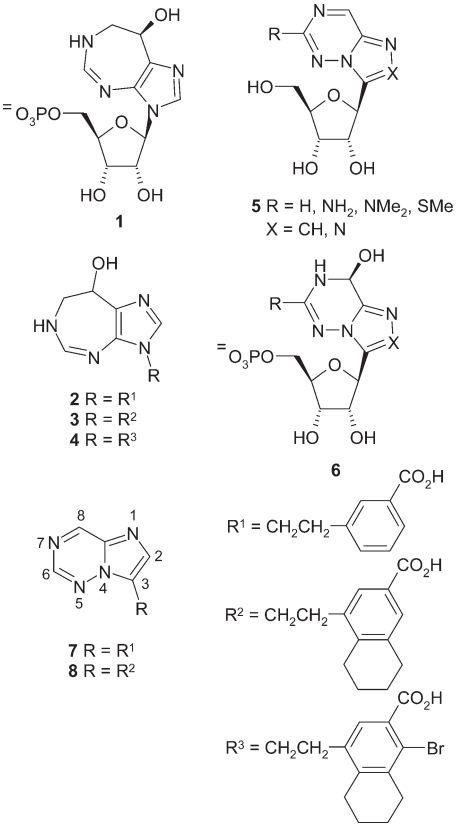
ABSTRACT *C*-Ribosyl imidazo[2,1-*f*][1,2,4]triazines and 3-[2-(3-carboxyphenyl)-ethyl]-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ols represent two classes of known AMP deaminase inhibitors. A combination of the aglycone from the former class with the ribose phosphate mimic from the latter led to the 3-[2-(3-carboxyphenyl)ethyl]imidazo[2,1-*f*][1,2,4]triazines, which represent a new class of AMP deaminase inhibitors. The best compound, 3-[2-(3-carboxy-5,6,7,8-tetrahydronaphthyl)-ethyl]imidazo[2,1-*f*][1,2,4]triazine (**8**), was a good inhibitor of all three human AMPD recombinant isozymes (AMPD1, AMPD2, and AMPD3; IC_{50} = 0.9−5.7 μM) but a poor inhibitor of the plant recombinant enzyme (*Arabidopsis FAC1*; IC_{50} = 200 μM).

KEYWORDS AMP-deaminase, adenylate-deaminase, imidazotriazine, AMPD inhibitor, AMPD inhibition



The synthesis and biological testing of inhibitors of the enzyme adenosine 5′-monophosphate deaminase (AMPD, EC 3.5.4.6) is of interest within both the agrochemical and the pharmaceutical industries. Inhibition of AMPD in plants causes an increase in intracellular adenylate nucleotide concentrations, setting off a chain of physiological events that lead to a strong herbicidal effect.[1,2] In mammals, AMPD inhibition under anoxic conditions leads to an increased intracellular concentration of adenosine, which it has been proposed could be beneficial to ischemic tissue.[3,4] Coformycin 5′-phosphate (**1**) is an extremely potent AMPD inhibitor (K_i = 5.5 pM[5]); however, the physicochemical properties of such polar-charged molecules are not conducive to efficient cellular uptake.[3] In an effort to improve bioavailability, Kasibhatla and co-workers succeeded in replacing the polar ribose phosphate moiety of compound **1** with a much less polar 3-carboxyphenylethyl group, as exemplified by inhibitors **2** {K_i [porcine heart enzyme (AMPD3)] = 510 nM[6]}, **3** [K_i (human AMPD3 recombinant enzyme) = 15 nM[7]], and **4** {K_i (human AMPD3 recombinant enzyme) = 2 nM;[7] IC_{50} [human skeletal muscle enzyme (AMPD1)] = 500 nM[4]}. In our own work, we have found that certain modified *C*-nucleosides containing pyrazolopyrimidine,[8] imidazotriazine,[9] and triazolotriazine[10,11] aglycones possess interesting herbicidal activity. It is believed that this activity is due to inhibition of AMPD, following 5′-phosphorylation and covalent hydration of the aglycone ring, which converts nucleosides of type **5** into the nucleotide inhibitors **6**.[12] In this paper, we report the synthesis of the novel AMPD inhibitors **7** and **8**, which result from combining the ribose phosphate mimics from inhibitors **2** and **3** with the aglycone from our own imidazotriazine inhibitors **5** (R = H; X = CH).



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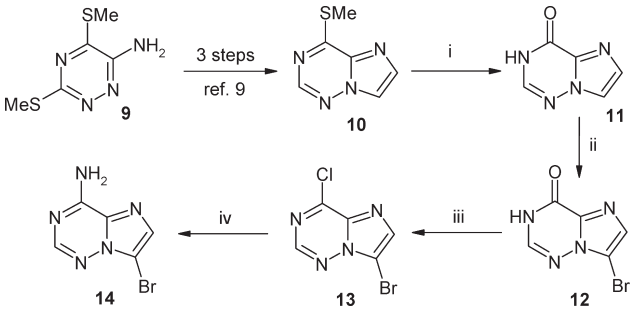
Scheme 1[a]



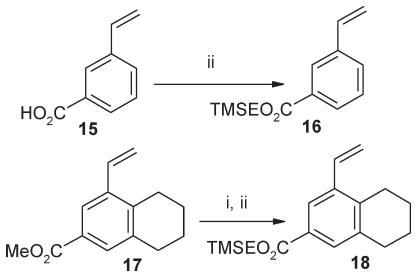
[a] Reagents, conditions, and yields: (i) 30% H_2O_2/H_2O, AcOH, 70°C, 5 h, 40%. (ii) NBS (2 equiv), DMF, room temperature, 4 h (85%). (iii) 0.15 M solution in $POCl_3$, *N*,*N*-dimethylaniline (3.5 equiv), reflux, 4 h. (iv) 2 M NH_3/*i*-PrOH (15 equiv), *i*-PrOH, 0 °C to room temperature, 16 h (76% over two steps).

Our previous attempts to synthesize inhibitors related to compounds **7** and **8** had been hampered by the presence of 6- and 8-methylsulfanyl groups in the imidazotriazine ring system, which interfered with an attempted transition metal-catalyzed hydrogenation reaction.[13] In addition, the carboxyl group of the ribose mimic had been protected as a methyl ester, which we were unfortunately unable to hydrolyze at the end of the synthesis without concomitant degradation of the imidazotriazine aglycone. Consequently, in the current work, we determined to remove the methylsulfanyl groups early on in the synthesis and to protect the acid as a more labile trimethylsilylethyl ester. We envisaged accessing the target molecules **7** and **8** via a convergent route involving a Heck coupling reaction between a 3-vinylphenycarboxy ester and a 3-bromoimidazotriazine.

The imidazotriazinone **11** was selected as a promising sulfur-free starting point for further aglycone elaboration. This key intermediate can be obtained from the 8-methylsulfanyl imidazotriazine **10**, itself accessible in three steps from the previously described aminotriazine **9**,[9,14] by treatment with 30% hydrogen peroxide in acetic acid (Scheme 1).[15] Alternatively, compound **11** can be synthesized in three steps from imidazole 2-carboxylic acid using the recently published procedure of Babu et al.[16] Bromination of the imidazotriazinone **11** with *N*-bromosuccinamide in DMF proceeded in good yield to give the bromide **12**. Chlorination of compound **12** with phosphorus trichloride oxide and *N*,*N*-dimethylaniline yielded the chloride **13**, which was treated with alcoholic ammonia to give the bromo amine **14** in 76% yield over two steps.

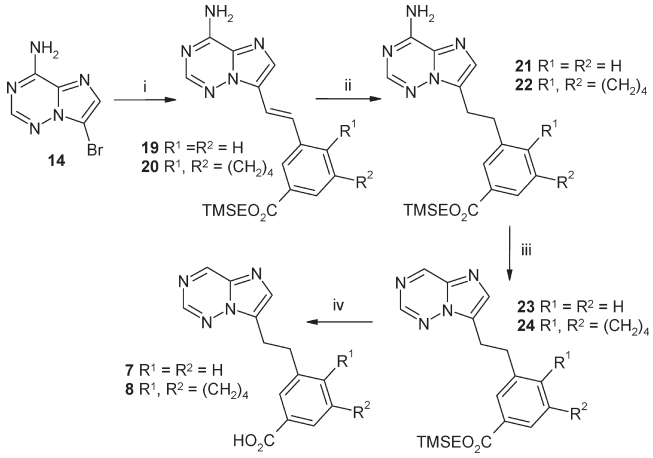
The Heck coupling partners, styrenes **16** and **18**, were simply prepared from the commercially available acid **15** and the previously described methyl ester **17**[13] as detailed in Scheme 2. Several different conditions were tried to achieve the Heck coupling between the styrene **16** and the bromide **14**.[17,18] Thus, the effect of varying the palladium source [$Pd(OAc)_2$ and $Pd(dba)_2$], ligand [none, PPh_3, and P(*o*-Tol)$_3$], base (Et_3N, $NaHCO_3$, and K_2CO_3), and additive (*n*-Bu_4NBr and *n*-Bu_4NCl) was investigated. The best conditions used palladium acetate, tri*o*-tolylphosphine, triethylamine, and tetra-*n*-butylammonium chloride to give the desired coupling

Scheme 2[a]



[a] Reagents, conditions, and yields: (i) 1 M NaOH, MeOH, room temperature, 16 h. (ii) $Me_3SiCH_2CH_2OH$ (1.5 equiv), BOP [benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexa-fluorophosphate] (1.2 equiv), Et_3N (3.4 equiv), CH_2Cl_2, room temperature, 16 h (69% for **16**; 59% over two steps for **18**). TMSE = trimethylsilylethyl.

Scheme 3[a]



[a] Reagents, conditions, and yields: (i) **16** or **18** (3 equiv), $Pd(OAc)_2$ (0.1 equiv), (*o*-Tol)$_3$P (0.2 equiv), Et_3N (3 equiv), *n*-Bu_4NCl (1 equiv), Me_2N-CO·Me, 100 °C, 16 h (39% for **19**; 41% for **20**). (ii) H_2, 10% Pd/C, MeOH/DMF 1:1, room temperature, 16 h (66% for **21**; 24% for **22**). (iii) *n*-BuONO (3 equiv), 1,4-dioxane, 80 °C, 5 h (49% for **23**); *n*-BuONO (10 equiv), 1,4-dioxane, 100 °C, 2 h (38% for **24**). (iv) (a) CsF (1.1 equiv), DMF, 60 °C, 4 h; (b) 4 M HCl/1,4-dioxane (1.1 equiv), 1,4-dioxane (99% for **7** and **8**). TMSE = trimethylsilylethyl.

product **19** in 39% yield (Scheme 3). Repeating the reaction but with the tetrahydronaphthyl styrene **18** yielded the coupling product **20** in 41% yield. Subsequent hydrogenation over palladium on charcoal gave the amines **21** and **22**, which were reductively deaminated using *n*-butylnitrite in dioxane[19] to give the esters **23** and **24**. Finally, the trimethylsilylethyl esters were cleaved using cesium fluoride followed by acidification to give the target acids **7** and **8** in quantitative yield. Compounds **7** and **8** are actually pro-inhibitors of AMPD, which need to undergo covalent hydration to give the proposed active inhibitors **7a** and **8a**. Enthalpy of hydration calculations indicated that the imidazotriazine ring system should readily undergo covalent addition of water,[11] and NMR experiments conducted on the previously reported[9] parent heterocycle **25** provided experimental support for the required hydration. The proton NMR spectrum of the

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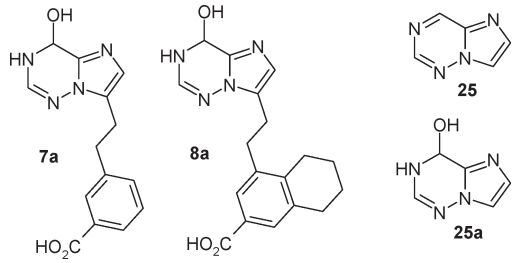
Table 1. Inhibition of Different AMPD Isozymes by Compounds **7** and **8**

	IC_{50} (μM)			
inhibitor	human AMPD1 (ΔM54)[20]	human AMPD2 (1A/2)[21]	human AMPD3 (1b)[22]	plant AMPD (ΔV211M)
7	310	100	370	1400
8	5.7	2.3	0.9	200

heterocycle **25** in $CDCl_3$ showed that it existed entirely in the 10π aromatic form **25**, whereas in D_2O it existed as a 95:5 mixture of **25** and the covalent hydrate **25a**. The addition of 1 equiv of DCl to the D_2O solution shifted the equilibrium entirely over in favor of the hydrate **25a**.



Biochemical testing of compounds **7** and **8** for inhibition of plant *Arabidopsis* recombinant AMPD and the human AMPD1, AMPD2, and AMPD3 recombinant isozymes showed interesting differences in inhibition levels (Table 1). In particular, both compounds were found to be significantly better inhibitors of all three human isozymes than of plant AMPD. This selectivity seems reasonable, bearing in mind that the ribose mimics in inhibitors **7** and **8** come from a research program directed toward discovering inhibitors of human AMPD.[3,6,7] As expected, by analogy with the literature inhibitors **2**, **3**, and **4**,[7] compound **8** was a more powerful inhibitor than compound **7**. The inhibition levels shown by compound **8** against our human AMPD1 (IC_{50} = 5.7 μM) and AMPD3 (IC_{50} = 900 nM) recombinant isozymes are around an order of magnitude higher than the values reported for the related inhibitor **4** against the human skeletal muscle enzyme (AMPD1; IC_{50} = 500 nM)[4] or inhibitor **3** against the human AMPD3 recombinant enzyme (K_i = 15 nM).[7] This suggests that the hydrated imidazotriazine ring system in compound **8a** binds less strongly to AMPD than the diazepinol ring system in compounds **3** and **4**. Nonetheless, the levels of inhibition achieved in Table 1, taken together with the improved hydrolytic stability of the imidazotriazine ring system (as compared to the diazepinol in compounds **1**−**4**),[23] mean that inhibitor **8** is an interesting lead structure worthy of further investigation.

In summary, the first examples of AMPD inhibitors combining an imidazotriazine ring with a carboxyphenylethyl ribose mimic, compounds **7** and **8**, have been successfully synthesized in a convergent manner that would be suitable for analogue synthesis. Biochemical studies indicate that compound **8** is a good inhibitor of human AMPD3 (IC_{50} = 900 nM) but only a very modest inhibitor of the plant enzyme (IC_{50} = 200 μM).

SUPPORTING INFORMATION AVAILABLE

Experimental details for the synthesis and characterization of compounds **7**, **8**, **11**−**14**, **18**, **20**, **22**, and **24**; protocol describing the preparation of the *Arabidopsis* ΔV211M recombinant enzyme; and procedure used for the AMP deaminase assay and IC_{50} value determinations. This material is available free of charge via the Internet at http://pubs.acs.org.

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