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# Design, synthesis and inhibitory activity against human dihydroorotate dehydrogenase (*h*DHODH) of 1,3-benzoazole derivatives bearing amide units

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#### ABSTRACT

A series of 1,3-benzoazole derivatives possessing amide moieties were designed, synthesized and evaluated as inhibitors against human dihydroorotate dehydrogenase (*h*DHODH). Compounds **A11**, **A14** and **A26** exhibited good to excellent activities against *h*DHODH at the concentration of 10  $\mu$ M. In particular, compound **A14** displayed an IC<sub>50</sub> value of 0.178  $\mu$ M with 2-fold preference over **A771726**. The result implied that a proper degree of steric size and electron density of the C-6 amide moiety was necessary to retain the inhibitory activity of the synthesized compounds.

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Dihydroorotate dehydrogenase (DHODH) is an enzyme essential to the fourth and rate-limiting step in de novo pyrimidine biosynthesis<sup>1</sup> and it catalyzes the conversion of dihydroorotate (DHO) to orotate concurrent (ORO) with the reduction of ubiquinone.<sup>2,3</sup> The significance of pyrimidine bases for metabolism, cell and proliferation determines *h*DHODH as an attractive chemotherapeutic target for the development of new drug candidate in different biological and clinical applications for cancer, arthritis and malaria.<sup>4,5</sup>

Leflunomide (1) and brequinar (2) are the most successful examples of low-molecular weight inhibitors of *h*DHODH that have been in clinical development (Figure 1).<sup>6,7</sup> Leflunomide (1) is the first *h*DHODH inhibitor<sup>8</sup> that was approved for use in human medicine in the treatment of rheumatoid arthritis<sup>9-11</sup> and turns out to be a pro-drug to the active metabolite **A771726** (3),<sup>12-18</sup> while brequinar (2) is an antitumor and immunosuppressive agent which shows immunosuppressive activity.<sup>19-21</sup> Unfortunately, severe side effects like leukocytopenia, mucositis and abnormalities in liver enzymes have been observed during clinical use of brequinar and lefluonomide.<sup>22,23</sup> Consequently, more efficient *h*DHODH inhibitors are needed as potential prototypes for synthesis of new *h*DHODH inhibitors.

The application of heterocycles as amide bioisosteres is a significant utility in drug design as the surrogates may lead to compounds with enhanced pharmacokinetic and improved cell-based potency properties.<sup>24</sup> As an important biologically active nucleus, 1,3-benzoazole is documented to exhibit widespread potential pharmacological activities like anticancers, antiinflammatory agents, proton pump inhibitors, and *etc.*<sup>25-29</sup> Simultaneously, 1,3-benzoazole has evolved as an effective chemical isosteres of amide bonds and been widely used in modern molecular design.<sup>30</sup>

Moreover, it is well documented that pyridine pyrazole is a very important class of fused heterocycle due to its specific physiological activity and the structural similarity with indole and azaindole. *N*-aryl pyrazole analogs have shown potent DHODH inhibitory activity with IC<sub>50</sub> ranging from 13-100 nM.<sup>31-</sup>





Enlightened by all of the descriptions above, scaffold hopping based on bioisosteres were proposed to identify new possible chemotypes. The amide–aryl segment of the monocyclic series of leflunomide (1) was replaced by a bicyclic1,3-benzoazole scaffold and pyridylpyrazolyl was introduced into the benzimidazole core structure to investigate whether there would present some new beneficial pharmacokinetic properties (Figure 2). Herein, we described the molecular design, synthesis and initial findings on inhibitory activities against hDHODH.



Figure 2. Molecular design of title compound

As described in Scheme 1, under the action of 1-ethyl-3-(3dimethyllaminopropyl) carbodiimide hydrochloride and *N*hydroxybenzotriazole, the treatment of benzoic acids 4 with appropriately substituted amines afforded the benzamide derivatives 5 in 65 - 72% yields. The catalytic hydrogenation of intermediates 5 with 15% Pd/C in methanol afforded the key aniline derivatives 6 which were used for the further reaction without purification. The methyl of 3-methoxybenzamide 8 was removed in the presence of boron tribromide to yield the resulting compound 9. A sample of aldehyde 12 was generated by the reduction of pyrazole carboxylic acid 10 to 11 with lithium aluminum hydride in dry tetrahydrofuran and then oxidation of 11 to 12 with pyridinium chlorochromate. The intermediate carboxylic acid 10 was synthesized according to the methods reported in the literature. <sup>34, 35</sup>



Scheme 1. Synthesis of intermediates

The last synthetic route employed for the synthesis of the target compounds A (C) was outlined in Scheme 2. The mixture of aniline derivatives 6 and aldehyde 12 was heated at reflux overnight in 1, 2-dichloroethane to get the title compound A. The synthesis of compound B were similar to those of A3 in which 2-hydroxy-3-nitrobenzoic acid was used as the starting material. Surprisingly, the treatment of 9 and 12 generated the Schiff base 13 instead of the cyclization compound C under the same condition. And the target compound C was finally obtained by the oxidization of 13 with 3-dichloro-5,6-dicyano-1,4-benzoquinone in dichloromethane in good yield.



Scheme 2. The synthetic route for preparation of target compounds A, B and C.

In order to explore SAR preliminarily, the compounds with different groups at R position were synthesized and evaluated for enzyme inhibition assay. And A771726 (teriflunomide/aubagio) was used as a positive control. The inhibitory activities as well as the IC<sub>50</sub> of synthesized compounds were listed in Table 1. Firstly, compounds (A1 - C) derived from alkyl groups exhibited lower activity than the corresponding benzylated and phenylated counterparts (A4 - A26) in following rank order: alkyl< benzyl< phenyl. The results implied that the enzyme inhibition activity might be influenced by the size or electronic effect of the substituents. For comparison of benzoxazole and benzimidazole, compound B and C displayed no obvious improvement over analogue A3, suggesting that the skeleton had no apparent effect

on the activity. Further investigations were performed to study the effect of various substituted phenyls at R. Compounds A11 with o-methylphenyl and compound A14 with o-methoxy group dramatically showed higher enzyme inhibitory activities than that of the other mono-substituted counterparts, which implied that inhibitory activity might be influenced by the size of the orthosubstituent. Notably, the activity level of A14 rivaled that of A771726, making it to be the most potent compound. Given the potency observed with 2-methoxy analogs A14, additional compounds featuring this element were explored. Nevertheless, introducing electron-donating groups, electron-withdrawing groups, or halogen atoms at different positions all showed reduced potency compared to that A14. Among bis-substituted compounds, the activity data revealed a clear preference for the potency when substitution at C5-position was  $CF_3$  (A26) as compared to that of  $OCH_3$  (A24), which indicated that an electron-withdrawing group at the C5-position of 2methoxyphenyl contributed to the potency of synthesized compounds. And, replacement of phenyl group at R position with pyridine group to generate analogue A7 resulted in a sharp decline of potency. The pyridine unit probably was an ineffective group for activity. The biological activity data suggested that a proper degree of electron density and steric size at R position was necessary to retain the activity of the synthesized compounds, which paved the way for further optimizations.

Table 1. The inhibition activities against hDHODH of compounds A, B and C



Co	ompound	R	Inhibition (%) at 10 $\mu$ M	IC <sub>50</sub> (µM)
	A1	Ме	14.14	n.t.
	A2	Et.	24.85	n.t.
	A3	<i>n</i> -Pr	23.44	n.t.
	В		26.57	n.t.
	c	-	30.10	n.t.
	A4	Benzyl	34.94	n.t.
	A5	4-CF <sub>3</sub> -benzyl	32.04	n.t.
	A6	4-CH <sub>3</sub> -benzyl	22.74	n.t.
	A7	2-Picoline	27.58	n.t.
	A8	2,3,6-(F) <sub>3</sub> -benzyl	43.04	n.t.
	A9	4-Cl-benzyl	43.99	n.t.
	A10	4-OCH <sub>3</sub> -phenyl	36.06	n.t.
	A11	2-CH <sub>3</sub> -phenyl	73.88	$0.676 \pm 0.091$
	A12	Phenyl	49.44	n.t.
	A13	4-CF <sub>3</sub> -phenyl	36.29	n.t.

A14	2-OCH3-phenyl	60.51	$0.178 \pm 0.065$
A15	2-F-phenyl	38.83	n.t.
A16	2-Br-phenyl	27.63	n.t.
A17	2-I-phenyl	24.81	n.t.
A18	2,4-(F) <sub>2</sub> -phenyl	26.89	n.t.
A19	2,4-(OCH <sub>3</sub> ) <sub>2</sub> -phenyl	1.303	n.t.
A20	2,4-(CH <sub>3</sub> ) <sub>2</sub> -phenyl	48.76	n.t.
A21	2,6-(OCH <sub>3</sub> ) <sub>2</sub> -phenyl	0	n.t.
A22	3-CH <sub>3</sub> -phenyl	18.95	n.t.
A23	2,3,4-(F) <sub>3</sub> -phenyl	31.85	n.t.
A24	2,5-(OCH <sub>3</sub> ) <sub>2</sub> -phenyl	9.43	n.t.
A25	2-F-6-OCH <sub>3</sub> -phenyl	15.74	n.t.
A26	2-OCH <sub>3</sub> -5-CF <sub>3</sub> -phenyl	55.22	$4.850 \pm 0.230$
A771726		64.03	$0.356 \pm 0.075$
n.t.: not tested.			

In conclusion, a series of 1, 3-benzoazole derivatives possessing C-6 amide units were designed, synthesized and screened for their inhibitory activity against human dihydroorotate dehydrogenase (hDHODH). Compounds A11, A14 and A26 exhibited good to excellent activities against *h*DHODH at the concentration of 10  $\mu$ M. Even more remarkable, compound A14 displayed the highest inhibition activity for *h*DHODH with IC<sub>50</sub> value of 0.178  $\mu$ M, and was comparable to that of A771726. The results indicated that the differences in inhibitory activity might be due to variations in incorporation of steric size and electrical property of the C-6 amide substitute moieties. The relationships between structure and activity obtained in this study could be beneficial for discovering new hDHODH inhibitors and further chemo-biological optimization on benzoheterocycle derivatives is well ongoing in our laboratory.

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