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## ADENOSINE DEAMINASE INHIBITORS. SYNTHESIS AND BIOLOGICAL EVALUATION OF ARALKYLADENINES (ARADS)

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Abstract: Several 9-aralkyladenines have been prepared and their ADA inhibitory activity was determined. A minimum of two carbon atoms separating the aromatic ring from the adenine-bearing carbon (C3') was found essential for potent activity. © 1998 Elsevier Science Ltd. All rights reserved.

Adenosine deaminase (ADA, adenosine aminohydrolase, E.C. 3.5.4.4) is an enzyme in the purine catabolic pathway. It is responsible for the conversion of adenosine and 2'-deoxy adenosine to inosine and 2'-deoxyinosine, respectively.<sup>1</sup> ADA deficiency is associated with lymphotoxic elevations in the concentrations of 2'-deoxyadenosine and dATP, which result in a severe combined immunodeficiency syndrome (SCIDS).<sup>2</sup> This association of ADA deficiency with SCIDS has created an interest in the pharmacologic inhibition of ADA, as potential cytotoxic therapy of malignant lymphoproliferative diseases.<sup>3</sup> A benefit of ADA inhibition is to prevent deamination of important chemotheraputic agents that contain adenine bases such as arabinofuranosyladenine (ara-A), 8-azaadenosine, formycin A, and 2',3'-dideoxyadenosine.<sup>4</sup> Another potential use of ADA inhibition is protection of injured tissues in cerebral and myocardial ischemia that is the result of increased levels of adenosine, which appears to limit the extent of degeneration.<sup>5</sup> Two important ADA inhibitors are 2'-deoxycoformycin (pentostatin) and *erythro*-9-(2-hydroxy-3-nonyl)-adenine (EHNA).<sup>6</sup>



Preparation of analogs of EHNA and testing them for ADA inhibition is an ongoing program in our laboratory. We have reported the synthesis of C1' and nor-C1' derivatives of (+)-EHNA and 3-deaza-(+)-

EHNA.<sup>7</sup> We have also reported the synthesis and biological evaluation of chain hydroxylated analogs of (+)-EHNA, which were putative metabolites in (+)-EHNA metabolism.<sup>8</sup> The hydroxyl groups caused decreased inhibitory activity and confirmed the loss of hydrophobic interactions at the chain terminus with little interference with overall inhibitor binding.

Although ADA interaction with a tight binding transition state inhibitor has been obtained by X-ray analysis,<sup>9</sup> no comparable data for a semi-tight binding inhibitor such as EHNA are available. Neither the conformation of the chain nor its binding site have been elucidated. We have recently reported the effect of introducing unsaturation in the alkyl chain at the 5',6'-position.<sup>10</sup> Biological data showed that the *cis*-isomer had comparable activity to EHNA but was thirteen and fifty times more active than the corresponding *trans* and acetylene derivatives, respectively. Since *cis* olefins can be considered as a partial structure of an aromatic ring, it was decided to incorporate two or three atoms of the alkyl chain into a phenyl ring and determine the ADA inhibitory activity. In designing such compounds, the length and position of the alkyl substituent on the aromatic ring were chosen to maintain the nine-carbon chain length of the parent compound. Thus, a series of aralkyladenines was prepared following synthetic procedures outlined earlier for the preparation of unsaturated derivatives.<sup>10</sup>



As can be seen in the scheme, the first step involves the opening of epoxide 3 with the anion of a suitable aryl or aralkyl unit to give alcohol 4. This was accomplished by choosing either a Grignard or an organolithium reagent based on the convenience of its preparation. Incorporation of the purine ring at C3 made use of the Mitsunobu

reaction which took place with inversion of configuration at this center. The resultant 9-aralkyl-6-chloropurine derivatives could not be isolated in pure form due to their similar chromatographic behavior to dihydrodiisoproylazodicarboxylate (DIADH<sub>2</sub>), one of the by-products of the reaction. However, ammonolysis of the partially purified compounds allowed the isolation and purification of the corresponding 6-amino derivatives 5. The target compounds 6 were obtained by reductive debenzylation in good yield.<sup>11</sup>

The ADA inhibitory activity of the ARADS was determined following procedures outlined earlier.<sup>8</sup> The findings are listed in the table. It can be easily seen that the distance between C3' and the aromatic ring plays a significant role in ligand/enzyme binding. With only one carbon (**6a**, **6b**, **6c**) much reduced activity is observed. It is interesting to note that this finding corroborates similar results obtained by pulse ultrafiltration screens for receptor binding, a recently introduced technique to identify homologues with the highest affinity for a receptor.<sup>12</sup> Increasing the distance by one (**6d**), two (**6e**, **6f**), three (**6g**), and four (**6h**) carbon atoms imparted similar or more potent activity than (+)-EHNA.

The newly described ARADS provide an opportunity to probe further the binding requirements for adenosine deaminase. This is currently being pursued by a molecular modeling/synthesis approach. The findings will be communicated in due course.

| Compound     | n | R   | K <sub>i</sub> 10 <sup>.9</sup> M |  |
|--------------|---|---|-----------------------------------|--|
| ба           | 0 | 2-CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> | 302                               |  |
| 6 b          | 0 | 3-CH <sub>2</sub> CH <sub>3</sub>                 | 133                               |  |
| 6c           | 0 | 4-CH <sub>3</sub>                                 | 302                               |  |
| 6d           | 1 | 3-CH <sub>3</sub>                                 | 1.02                              |  |
| 6e           | 2 | Н   | 0.89                              |  |
| 6 f          | 2 | 2-CH <sub>3</sub>                                 | 0.51                              |  |
| 6 g          | 3 | Н   | 0.76                              |  |
| 6 h          | 4 | Н   | 0.95                              |  |
| (+)-EHNA (2) |   |   | 2.00                              |  |
|              |   |   |                                   |  |

Table 1: Inhibition of Adenosine Deaminase

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