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Discovery of Imidazole Glycerol Phosphate Dehydratase Inhibitors through 3-D Database Searching

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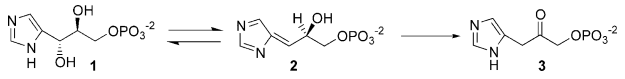
Abstract—Imidazole glycerol phosphate dehydratase (IGPD) has become an attractive target for herbicide discovery since it is present in plants and not in mammals. Currently no knowledge is available on the 3-D structure of the IGPD active site. Therefore, we used a pharmacophore model based on known inhibitors and 3-D database searches to identify new active compounds. In vitro testing of compounds from the database searches led to the identification of a class of pyrrole aldehydes as novel inhibitors of IGPD.

Discovery of new herbicidal targets continues to be important as the need for chemicals with lower use rate, toxicity, and environmental impact grows. The histidine biosynthetic pathway is an attractive target for herbicide discovery since it is absent in mammals, yet is critical for plant growth. The histidine pathway is not only crucial for histidine production, but has also been linked to purine biosynthesis, thus emphasizing its importance.[1] The histidine pathway enzyme recently identified as an herbicide target is imidazole glycerol phosphate dehydratase (IGPD).[2–4] Although the reaction mechanism for this interesting dehydration reaction has not been clearly delineated, it is proposed to go through a diazafulvene intermediate **2**.[5,6]



Several laboratories have discovered potent inhibitors of IGPD through biorational design.[2–4,7] Most inhibitors mimic the proposed diazafulvene reaction intermediate, **2**, and as such typically contain three proposed binding groups: (1) a nitrogen containing heterocyclic ring, (2) a hydrogen bond donor/acceptor, and (3) a phosphate or phosphonate.[6,8]

We investigated two approaches to potential herbicide discovery. One involved biorational design and synthesis based on known inhibitors.[9] The second relied on computational chemistry and database searching to identify candidates that may be structurally distinct from known inhibitors. This report will focus on the latter approach.

Pharmacophore generation in combination with 3-D database searching has become a powerful tool in the discovery of novel potent enzyme inhibitors. Several examples of using 3-D searching to identify novel potent enzyme inhibitors are reported in the literature.[10–12] One advantage that 3-D database searching has over de novo design is the ability to identify compounds that can be easily acquired or for which a synthesis route is known.

With no crystal structure of the IGPD protein available, we relied on published biological data to develop a pharmacophore model. This model was then used to search available 3-D databases. A pharmacophore model typically consists of a set of functional groups deemed essential for activity in a specific spatial orientation. These functional groups often contain sites for hydrogen bonding and hydrophobic interactions.

We began with a conformational analysis of a subset of potent inhibitors from Zeneca, Ciba-Geigy, and AgrEvo patents[13–15] and the natural substrates (Fig. 1). The patented inhibitors typically contain a triazole ring in place of the imidazole ring of the substrate, variations on the length or substitution pattern of the alkyl chain,

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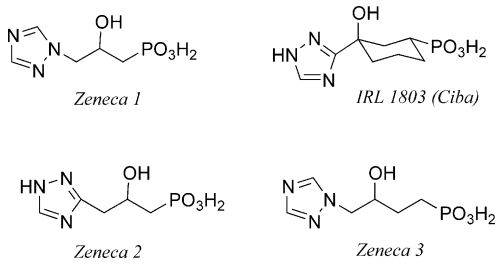


Figure 1. Structures of representative triazole inhibitors.

and a phosphonic acid, a more stable replacement for the phosphate group. Results from conformational analyses are consistent with literature data, in which the triazole and phosphonic acid are held in extended conformation relative to each other.[16] It is assumed that these fully optimized conformations of the molecules represent the biologically active forms.

We measured the distances between key atoms of the most active triazole inhibitors, Zeneca 1 ($K_i = 0.6$ nM) and IRL 1803 ($K_i = 40$ nM). The location of each functional group was determined for structures that were energy minimized using GALAXY.[17] The interatomic distances for each inhibitor are shown in Table 1. We chose distances from Zeneca 1 to build our query for the 3-D database search since it was the most active and flexible molecule.

The query shown in Figure 2 was constructed somewhat broadly to increase the chances of finding compounds that matched the constraints of the query. Since some of the proposed mechanisms need one proton donor within the ring,[6,7] the heterocyclic portion of the query consisted of a five-membered ring where four positions were either a carbon or nitrogen and the fifth position was fixed as a nitrogen. Also, the bonds within the ring were set to be either single or double bonds. The hydrogen bond donor/acceptor function contained a hydroxyl, thiol, or amine group. Finally the ionic group was constructed to contain either a phosphorus, sulfur, or carbon with one doubly-bonded oxygen and one singly-bonded oxygen. The distances between the groups were fixed to be ± 0.5 Å of the measured values.

Using the model described above and MDL's ISIS software[18] we searched two commercial 3-D databases,

Table 1. Interatomic distances in angstroms

Compd	N1–O3	N2–O3	N3–O3	N1–O4	N2–O4
Zeneca 1	4.7	3.1	—	5.5	6.1
IRL 1803	2.8	4.0	3.7	—	—
	O3–P	O3–O4	N1–P	N2–P	N3–P
Zeneca 1	4.0	4.6	—	—	—
IRL 1803	4.6	5.7	6.0	7.0	6.0

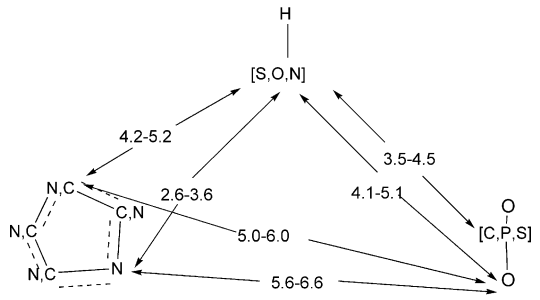


Figure 2. 3-D query with interatomic distances in angstroms.

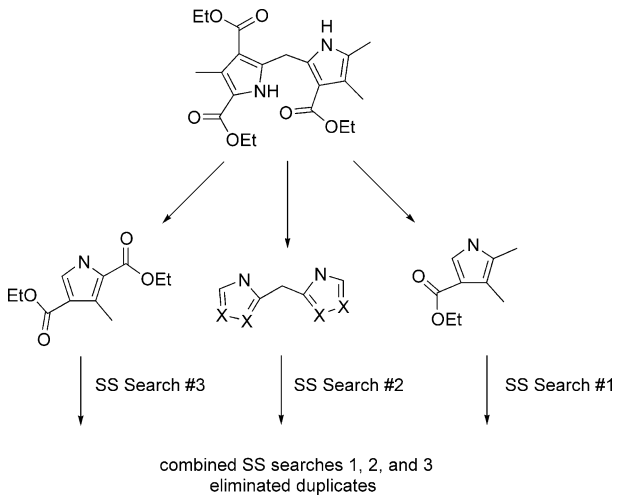


Figure 3. Substructure searching using bispyrrole cores.

the Available Chemical Directory (ACD3D) and the National Cancer Institute (NCI3D) databases, a total of ~370,000 compounds. The Conformationally Flexible Search (CFS) module in ISIS Base was used. This module allows for a fitting of each compound in the database to the query by rotation around single bonds.

The initial searching of both databases resulted in about 1200 hits. This hit list was refined by eliminating compounds with a molecular weight of >500 and compounds that had a cost of $>\$50$ per sample. A subset of the remaining compounds was acquired based on availability and tested for biological activity.

A total of 140 compounds were assayed in the imidazole glycerol phosphate dehydratase endpoint assay. IGPD activity was determined by monitoring production of imidazole acetol phosphate by its UV absorbance at 290 nm in the presence of a base.[19] Some common structural themes emerged from the actives including di- and triphosphate nucleotides, pyrroles, amino acids, and amino acid analogues.

Several compounds from the CFS search were interesting from an activity standpoint. However, one class of compounds was interesting from a chemistry perspective as well. These were the bispyrroles. The majority of the inhibitors we found were amino acids, nucleotides and nucleosides, often containing multiple chiral sites. The bispyrroles offered a somewhat simpler structure that was synthetically approachable.

Using various cores of the active bispyrroles, we performed 2-D substructure searches with the ISIS Base Substructure Searching (SS) module. Representative queries are shown in Figure 3.

We searched both commercial (ACD and NCI) and proprietary databases (~600,000 compounds). Hundreds of compounds matched the queries, however constraints in time and the nature of the screen permitted us to acquire and test only a limited number of compounds. Hence, a total of 94 pyrroles were tested for biological activity.

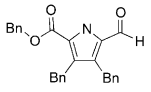
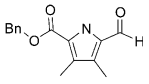
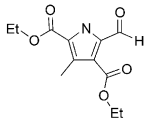
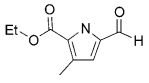
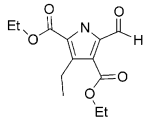
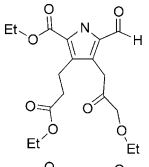
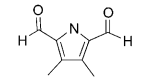
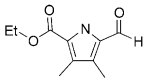
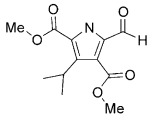
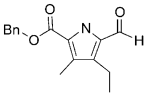
As a result of substructure searching, we found an active class of monopyrrole aldehydes. These structures represent a new class of IGPD inhibitors with activity in the low micromolar range. IC_{50} data were collected for the most active compounds and are shown in Table 2.

It is interesting to note that the monopyrrole aldehydes do not completely satisfy the original 3-D pharmacophore query. Therefore, these compounds may adopt a unique binding mode within the enzyme.

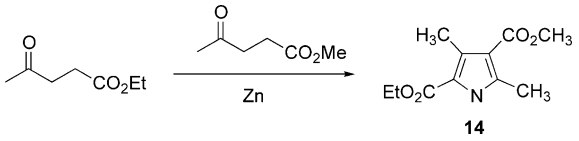
Patents and publications do exist for a class of pyrrole herbicides, but no mode of action was reported.[21,22] To verify that we had discovered a class of pyrroles distinct from the known herbicides, we synthesized and tested one of the reported compounds, **14**. This compound showed no activity in the IGPD enzyme assay.

We have shown that pharmacophore development in combination with 3-D database searching is an effective tool in discovery of novel lead chemistry. The monopyrrole aldehyde inhibitors identified through 3-D database and substructure searching are unique and could provide a starting point for future structure–activity studies and design of additional inhibitors.

Table 2. IGPD inhibition by pyrrole aldehydes[a]

Compd	Structure	IC_{50} (μM)	Compd	Structure	IC_{50} (μM)
4		4.0	**9**		8.9
5[b]		5.1	**10**		9.2
6		5.6	**11**		12.6
7		7.1	**12**		17.6
8		8.3	**13**		24.0

[a]Structure and purity confirmed by LC–MS and LC–ELSD (evaporative light scattering detection).
[b]See ref 20.



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