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Probing gorge dimensions of cholinesterases by freeze-frame click chemistry

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ABSTRACT

Freeze-frame click chemistry is a proven approach for design *in situ* of high affinity ligands from bioorthogonal, reactive building blocks and macromolecular template targets. We recently described *in situ* design of femtomolar reversible inhibitors of fish and mammalian acetylcholinesterases (EC 3.1.1.7; AChEs) using several different libraries of acetylene and azide building blocks. Active center gorge geometries of those AChEs are rather similar and identical triazole inhibitors were detected *in situ* when incubating the same building block libraries in different AChEs. *Drosophila melanogaster* AChE crystal structure and other insect AChE homology models differ more in their overall 3D structure than other members of the cholinesterase family. The portion of the gorge proximal to the catalytic triad and choline binding site has a ~50% reduction in volume, and the gorge entrance at the peripheral anionic site (PAS) is more constricted than in the fish and mammalian AChEs. In this communication we describe rationale for using purified recombinant *Drosophila* AChE as a template for *in situ* reaction of tacrine and propidium based libraries of acetylene and azide building blocks. The structures of resulting triazole inhibitors synthesized *in situ* are expected to differ appreciably from the fish and mammalian AChEs. While the latter AChEs exclusively promote synthesis of *syn*-substituted triazoles, the best *Drosophila* AChE triazole inhibitors were always *anti*-substituted. The *anti*-regioisomer triazoles were by about one order of magnitude better inhibitors of *Drosophila* than mammalian and fish AChEs. Moreover, the preferred site of acetylene + azide reaction in insect AChE and the resulting triazole ring formation shifts from near the base of the gorge to closer to its rim due to substantial differences of the gorge geometry in *Drosophila* AChE. Thus, in addition to synthesizing high affinity, lead inhibitors *in situ*, freeze-frame, click chemistry has capacity to generate species-specific AChE ligands that conform to the determinants in the gorge.

1. Introduction

Click chemistry has recently being defined as a "chemical philosophy introduced by K. Barry Sharpless in 2001 and describes chemistry tailored to generate substances quickly and reliably by joining small units together. This is inspired by the fact that nature also generates substances by joining small modular units" [1]. In recent years freeze-frame click chemistry has been tested on number of macromolecular biological templates evolving into an established approach for design *in situ* of high affinity ligands from bioorthogonal, reactive building blocks. Representative examples of successful applications of click chemistry include *in situ* design of a 200 pM carbonic

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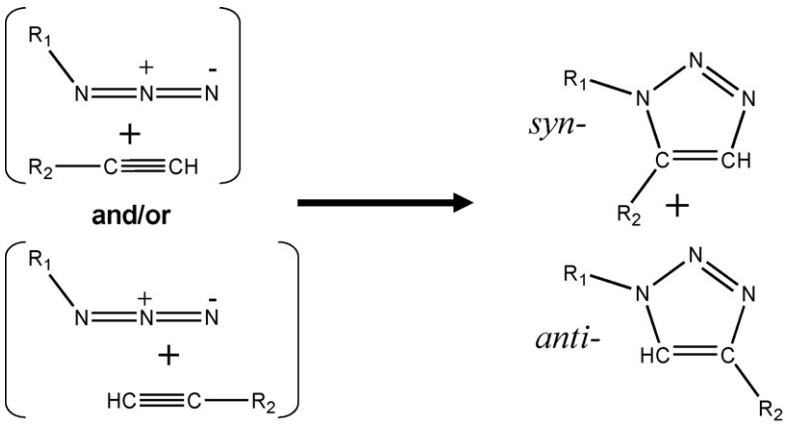

Scheme 1.

anhydrase (EC 4.2.1.1) inhibitor [2], 1.7 nM HIV-1 protease (EC 3.4.23.16) inhibitor [3], 62 nM α-1,3-fucosyltransferase (EC 2.4.1.214) inhibitor [4], and as a chronologically first and most noteworthy design of femtomolar acetylcholinesterase (AChE; EC 3.1.1.7) tight binding reversible inhibitors [5–7].

Joining acetylene and azide derivative building blocks in the course of a cycloaddition "click" reaction can inherently produce two different isomeric substitutions of a resulting 1,2,3-triazole heterocyclic ring, *syn*- and *anti*- (Scheme 1).

In the absence of a macromolecular template or a small molecular metal catalyst both substitutions appear as equally likely reaction product. The influence of the binding site geometry of a macromolecular template or interaction with metal catalyst [8] results in preferential formation of one of the products.

In this communication we analyze products of cycloaddition "click" reaction formed in AChEs and their potency for inhibition of AChE from different species, with aim of designing species-specific AChE inhibitors.

2. Results and discussion

Comparison of active center gorge geometries of various AChEs reveals high level of similarity between mammalian (mouse) and fish (*Torpedo californica*) AChEs, while larger differences in both shape and volume (Fig. 1) are observed for insect (*Drosophila melanogaster*) AChE.

It is therefore not surprising that *in situ* "click" chemistry screening using the same libraries of acetylene and azide building blocks yields identical products when mammalian and fish AChEs are used as reaction templates [5–7]. All "*in situ*" generated triazole inhibitors of mammalian or fish AChE were "*syn*-substituted" reflecting the curvature of their long and narrow active center gorges. In contrast "*anti*-substituted" triazoles, inherently more elongated in shape, appeared as two to three orders of magnitude weaker inhibitors (Table 1). This very large difference in inhibitory potency was entirely due to a markedly compromised fit of compounds within the active center gorge shape resulting in reduction of their mean residence times in the gorge and acceleration of their dissociation rates from the enzyme (Table 1).

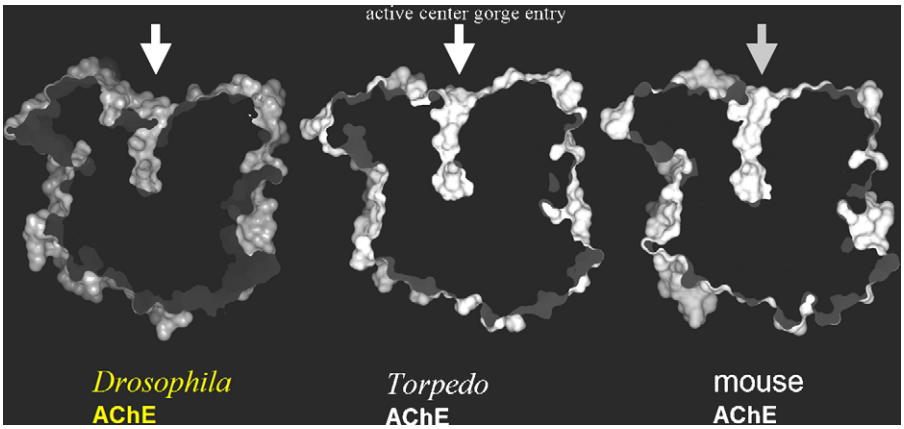

Fig. 1. Solvent accessible Connolly surfaces generated (using 1.4 Å probe radius) for *Drosophila melanogaster*, *Torpedo californica* and mouse AChEs. Cross-section through the center of each molecule reveals different geometries of the centrally located active center gorge.