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Design and synthesis of fluorescent and biotin tagged probes for the study of molecular actions of FAF1 inhibitor

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ABSTRACT

To study the molecular action of ischemic Fas-mediated cell death inhibitor, we prepared fluorescent-tagged and biotin-tagged probes of the potent inhibitor, KR-33494, of ischemic cell death. We used the molecular modeling technique to find the proper position for attaching those probes with minimum interference in the binding process of probes with Fas-mediated cell death target, FAF1.

Ischemia is caused by contraction or occlusion of the blood vessels.[1] Although the mechanisms of cell death after ischemic attack are not fully understood yet, Fas and FasL are known to be important factors in the pathology of ischemia together with **FAF1** protein.[2–4] Through the extensive designing and derivatizing process we have identified **KR-33493** (Fig. 1) as a potent inhibitor for Fas-mediated cell death, **FAF1**.[5,6]

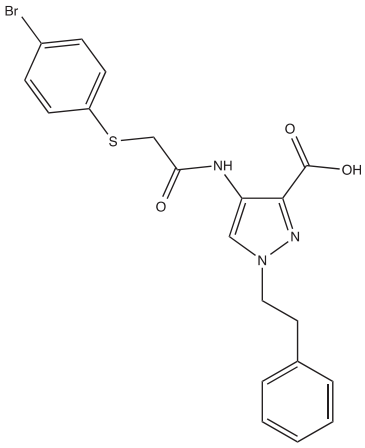

Figure 1. The structure of KR-33493.

In order to study the molecular action of **FAF1** inhibitor, KR-33493, in the ischemic condition, we need to prepare fluorescent-tagged and biotin-tagged probes. First, we needed to find

Figure 2. Molecular model for the complex of FAF1 (green) and KR-33493 compound (orange), which is obtained through the hierarchical protein structure modeling approach, based on secondary-structure enhanced Profile–Profile threading Alignment (PPA) and the iterative implementation of the Threading ASSEmbly Refinement (TASSER) program. The Phenyl rings of Tyr^{225} and Trp^{240} play an important role in interaction with CIF forming π–π stacking interaction.

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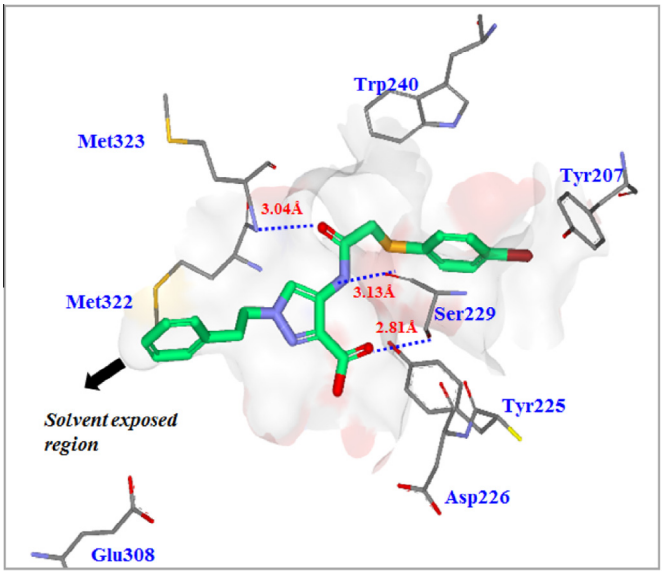
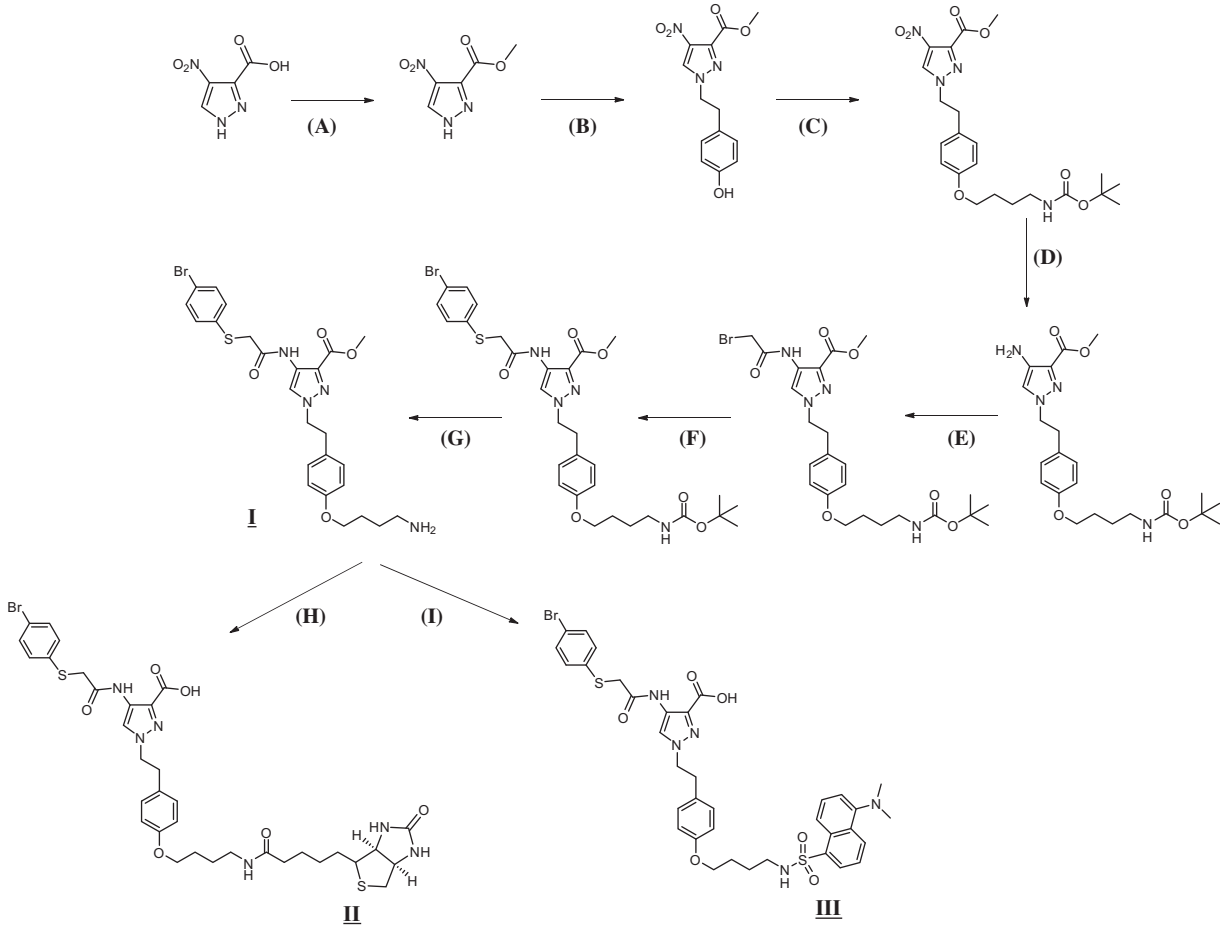


Figure 3. One of possible models for binding interaction of **KR-33493** with **FAF1**.

the position for the attachment of fluorescent and biotin components. The most preferred position to attach these components would be the one that has a least interference in the binding interaction of **KR-33493** with **FAF1** protein. To find the binding mode of **KR-33493** with FAF1, we initially constructed the 3D structure of FAF1 and then carried out the docking experiments as shown in Figures 2 and 3. As the sequence similarity of FAF1 with other proteins is less than 20%, we used hierarchical protein structure modeling approach based on the secondary-structure enhanced Profile–Profile threading Alignment (PPA) and the iterative implementation of the Threading ASSEmbly Refinement (TASSER) program.[7,8] Through this program, we obtained five candidate models for 3D structure of FAF1 and thus carried out molecular dynamics simulation using CHARMM force field (version 27.0) and default parameters interfaced with Accelrys Discovery Studio3.5. To decide binding sites, we used the binding site defining protocol in Accelrys Discovery Studio3.5 and defined the binding site from receptor cavity. Then, we docked our compounds including KR-33493 and an inactive compounds from our previous study,[9] and thus examined whether or not match SAR study for the compounds, and finally selected one binding model for FAF1 and an active compound as shown in Figure 1. In this binding mode the carbonyl group of Ser229 acts as a hydrogen bonding acceptor to form a hydrogen-bonding with the amide group of **KR-33493** and the hydroxyl group on the side chain of Ser229 forms another hydrogen bonding with the carboxyl group of **KR-33493**. The amine group of Met323 acts as a hydrogen-bonding donor to form hydrogen bonding with the amide carbonyl group of **KR-33493**. Especially, Met323 together with Met322 seems to provide a rather narrow hydrophobic environment.



Scheme 1. Reagents and condition: (A) concd sulfuric acid, MeOH, rt, 92%; (B) 4-(2-bromo-ethyl)-phenol, Cs_2CO_3, DMF, rt, 56%; (C) t-boc aminobutyl bromide, Cs_2CO_3, DMF, rt, 97%; (D) 10% Pd/C, H_2, MeOH, rt, 97%; (E), (F) bromoacetyl bromide, *p*-bromobenzenethiol, NEt_3, THF, rt, 76%; (G) trifluoroacetic acid, CH_2Cl_2, 0 °C, 89%; (H) (i) D-biotin, N-succinimide, EDC·HCl, DMAP, NEt_3, DMF, rt (ii) 6 N NaOH, MeOH, rt, 73%; (I) (i) dansyl chloride, NEt_3, CH_2Cl_2, 0 °C, (ii) 6 N NaOH, MeOH, rt, 84%.