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Media effects in modulating the conformational equilibrium of a model compound for tumor necrosis factor converting enzyme inhibition

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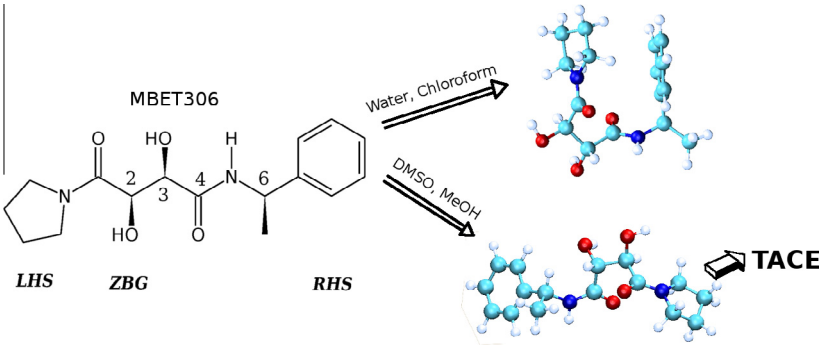
HIGHLIGHTS

- We studied the media effects on MBET306, a synthetic precursor for TACE inhibition.
- We sampled the conformations of MBET306 in various solvent using REM simulations.
- TD-DFT simulated spectra were obtained from conformational sampling.
- Experimental excitation/emission spectra were interpreted using TD-DFT results.
- Extended (TACE binding) structures of MBET306 are favored by amphiphatic solvents.

GRAPHICAL ABSTRACT



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ABSTRACT

Small-molecule inhibitors of Tumor Necrosis Factor α Converting Enzyme (TACE) are a promising therapeutic tool for Rheumatoid Arthritis, Multiple Sclerosis and other autoimmune diseases. Here we report on an extensive chemical–physical analysis of the media effects in modulating the conformational landscape of MBET306, the common scaffold and a synthetic precursor of a family of recently discovered tartrate-based TACE inhibitors. The structural features of this molecule with potential pharmaceutical applications have been disclosed by interpreting extensive photophysical measurements in various solvents with the aid of enhanced sampling molecular dynamics simulations and time dependent density functional calculations. Using a combination of experimental and computational techniques, the paper provides a general protocol for studying the structure in solution of molecular systems characterized by the existence of conformational metastable states.

Introduction

Autoimmune diseases comprise more than 50 syndromes including Rheumatoid Arthritis (RA), Multiple Sclerosis (MS), Crohn's disease and Lupus Erythematosus [1]. Despite the wide array of affected organs and clinical manifestations, research has highlighted a key and common role played in most of these diseases by the pro-inflammatory cytokine Tumor Necrosis Factor alpha (TNFα) [2]. In this regard, expensive "biologic" TNFα inhibitors, such as Xpro1595, directly targeting the soluble TNFα trimer have already been proved [3] to be therapeutic in animal models

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of Multiple Sclerosis promoting axon preservation and remyelination. Etanercept and Lenercept, two other bio-pharmaceutical interfering with TNFα, have been approved in 1999 for the treatment of RA in humans [4]. An alternative route for indirect TNFα blocking is that of inhibiting with small drugs the Zinc(II) matrix metalloproteinase Tumor Necrosis Factor α converting enzyme (TACE), i.e. the sheddase that cleaves the homotrimeric membrane precursor TNFα releasing the soluble form (sTNFα) and eliciting, in autoimmune diseases, the auto-immune reaction. Due the multifunctional nature of the TACE proteinase, implicated in fighting infection, eradicating tumors, as well as in the inflammatory response, and because of the prohibitive cost of manufacturing and administering anti-inflammatory biologics like Lenercept [5] or Xpro1595 [3], inhibition of TACE using small inexpensive molecules has recently raised immense interest in the academic community as well as in the pharmaceutical industry [6–13]. In this respect, a novel promising family of TACE inhibitors is represented by bis-amides of L-tartaric acid first developed by Rosner et al. [14,15] and later on by Dai and co-workers [16,13]. These compounds feature a tartrate core linking a left hand side (LHS) and right hand side (RHS) hydrophobic substituent through amide bonds. The central tartrate moiety binds the catalytic Zinc(II) on the groove of the TACE catalytic domain, while fitting the RHS in the deep primed TACE hydrophobic pocket [17].

Recent experimental and theoretical evidence [18] showed that in aqueous solution a synthetic precursor of the Dai compounds, MBET306, binds the free Zinc divalent cation very likely in a bidentate arrangement, favored by a compact conformation of the drug exposing the hydroxyl groups of the tartrate scaffold [19]. This results is at variance with the fact that in the experimentally analyzed TACE co-crystals [16,15,14], the Dai inhibitors bind the catalytic Zinc(II) in a unique tridentate manner, with the drug assuming an extended conformation. The extended structure of TACE-bound tartrate inhibitors could be hence favored by Zinc(II) binding and/or by the reduced local dielectric constant experienced by the ligand in the binding hydrophobic pocket [19].

In this study we examine the media effects on the average molecular structure of MBET306 in solution. To this end we have characterized the structural features of the molecule in solvents with disparate dielectric constants using a combination of experimental photophysical techniques and advanced computational tools such as enhanced sampling molecular dynamics simulations and *ab initio* calculations of excited states. More in detail, we use multicanonical molecular dynamics simulation by enforcing Hamiltonian Replica Exchange with torsional scaling [19] to sample efficiently the conformational landscape of MBET306. The methodology rapidly leads to the identification of the most populated conformational metastable states in each solvent. The manifold of conformational states is then used for the calculation of the absorption spectra in solution using Time Dependent Density Functional calculations with the Polarizable Continuum Model (PCM), providing a powerful interpretative clue of the experimental excitation and emission spectra of MBET306 in DMSO, methanol and chloroform and, at the same time, a convincing validation of conformational landscape revealed by the molecular dynamics simulations. Results showed that, while stable compact structures are important in water and chloroform (i.e. the two solvent at the extremes of the polarity scale) extended conformational states, as those exhibited by MBET306 synthetic sequels in the TACE co-crystals, are favored in the amphiphilic solvents DMSO and methanol, hence suggesting that binding on the TACE protein is likely to occur in a mixed hydrophobic–hydrophilic environment.

The paper is organized as follows. In the Section "Materials and methods" we provide details on the principal computational tools and experimental techniques used in our contribution. In the section "Results and discussion", we characterized the conformational landscape of MBET306 in various solvents as obtained from Replica Exchange simulations. Based on this analysis, we then proceed in a systematic comparison of theoretical and experimental absorption/excitation spectra. In the Section "Conclusions" we finally draw some conclusive remarks of our work.

Materials and methods

Experimental part

MBET306 (Fig. 1) is a synthetic precursor of the powerful TACE inhibitor drug-38 (PDB ID: 3O64) designed by Dai et al. [16] MBET306 was synthesized as previously reported [18]. All the starting materials for the ligand synthesis were obtained commercially and used as received. DMSO, methanol and chloroform for spectroscopic measurements on ligand solutions were purchased from Sigma Aldrich, Italy. UV–vis absorption spectra were recorded on a Lambda900 spectrophotometer (Perkin Elmer, Italy). Fluorescence spectra were recorded on a Perkin Elmer LS 50B luminescence spectrometer, with excitation and emission slits set to 10 nm. Emission measurements were performed using different excitation wavelengths in the range of 230–350 nm, excitation spectra were run using different emission wavelengths in the range of 350–450 nm. All spectroscopic measurements were performed at 298 K. Data points for UV–vis absorption and fluorescence are the average of at least two separate experiments. Emission spectra of the corresponding ligand-free samples, i.e. of the pure solvent, were subtracted from each recorded fluorescence spectra. A 35 mM stock solution of MBET306 was stored in DMSO solvent. The desired ligand concentration in the samples for spectroscopic experiments was obtained by appropriate dilution of the ligand stock solution in the desired solvent, with a final DMSO concentration in the sample lower than 1%. We used [MBET306] = 1 μM in all experiments unless otherwise stated.

Computational part

Molecular dynamics simulations: For the DMSO molecule, the force field was taken from Ref. [20] while for methanol and chloroform, the force field was assigned according to General Amber Force Field (GAFF) using the Antechamber program [21]. The force field for the MBET306 molecule is also based on the GAFF-AMBER parameterization [21] and is taken from Ref. [18]. The atomic charges of MBET306 used in the polar methanol ($\epsilon \simeq 33$) and DMSO ($\epsilon \simeq 47$) solvents are identical to those used in Ref. [18] for the simulation of MBET306 in water environment and were standardly computed, according to the prescription given in Ref. [22], from *ab initio* calculations *in vacuo* with the 6-31G basis set and the electrostatic potential fit with restraints (RESP). Due to reduced polarity of $CHCl_3$ ($\epsilon \simeq 5$), the atomic charges used in the simulations in chloroform were re-computed on the optimized

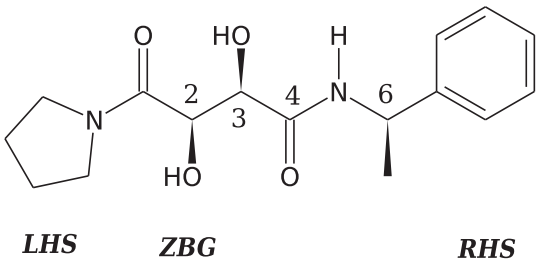

Fig. 1. Chemical structure of MBET306.