NOVEL FIXED-RING ANALOGUES OF TAMOXIFEN: SYNTHESIS AND BIOLOGICAL EVALUATION

Maria A. Chiacchio,a Laura Legnani,a Agata Campisi,*,a Bottino Paola,a Lanza Giuseppe,a Daniela Iannazzo,b Lucia Veltri,c Salvatore Giofrèd and Roberto Romoed

a Dipartimento di Scienze del Farmaco, Università di Catania, Viale A. Doria 6, 95125 Catania, Italy.
b Dipartimento di Ingegneria, Università di Messina, Contrada Di Dio, 98166 Messina, Italy.
c Dipartimento di Chimica e tecnologie chimiche, Università della Calabria, Via P. Bucci 12/C, 87036 Arcavacata di Rende, Italy.
d Dipartimento di Scienze chimiche, biologiche, farmaceutiche ed ambientali, Università di Messina, Via S.S. Annunziata, 98168 Messina, Italy.

Table of Contents

Molecular Docking Studies
'H and 'C Attached-Proton-Test (APT) NMR of compounds 14a-k
M06/cc-pVTZ Energies and Cartesian Coordinates
**Molecular Docking Studies**

**Receptor and Ligands Preparation**

The X-ray crystal structure of ERα in complex with 4-OH-TAM (PDB code: 3ERT) was retrieved from the protein data bank database (http://www.rcsb.org/pdb). The AutoDock Tools software has been used to remove ligands and water molecules, adding all the hydrogen atoms, and to calculate Gaisteiger charges for each atom of the macromolecules. The chemical structures of compounds were generated using ChemOffice v12.0 Ultra software package and optimized with ADT 4.2 for docking studies. The determination of ligand interactions for each pose within the binding pocket of receptor were analysed by BIOVIA Discovery Studio 2017.

**Docking Protocol**

Molecular Docking was carried out using AutoDock 4.2. Grid Maps were centered on the ERα active site with 44 points per dimension and a step size of 0.375. The Lamarckian genetic algorithm implemented in AutoDock has been employed to dock compounds 14a-k. We have carried out comparative docking experiments of compounds 14a-k. Each docking experiment consisted of 100 docking runs with 150 individuals and 2.5 x 106 energy evaluations. Other parameters were left to their default values. 100 highest-scoring docking poses were saved and binding affinity of the best mode was selected. The cluster with the lowest free energy of binding was visually analyzed using Python Molecular Viewer 1.4.3 (PMV). The Autodock docking parameters were validated to ensure that the ligand orientation and the position obtained from the docking studies represent valid reasonable binding modes of inhibitors. The ligand linezolid was extracted and docked back into the corresponding binding pocket. The results of docking simulation predicted the binding conformation of 4-OH-TAM with a root mean square deviation (RMSD) of 1.9 Å, compared with conformation of co-crystallized structure, thus indicating a valid docking protocol.
$^1$H and $^{13}$C Attached-Proton-Test (APT) NMR of compound 14a
$^1$H and $^{13}$C Attached-Proton-Test (APT) NMR of compound 14b
$^1$H and $^{13}$C Attached-Proton-Test (APT) NMR of compound 14c
$^1$H and $^{13}$C Attached-Proton-Test (APT) NMR of compound 14d
$^1$H and $^{13}$C Attached-Proton-Test (APT) NMR of compound 14e
$^1$H and $^{13}$C Attached-Proton-Test (APT) NMR of compound 14f
$^1$H and $^{13}$C Attached-Proton-Test (APT) NMR of compound $^{14}$g
$^{1}H$ and $^{13}C$ Attached-Proton-Test (APT) NMR of compound 14h
$^{1}$H and $^{13}$C Attached-Proton-Test (APT) NMR of compound 14i
$^1$H and $^{13}$C Attached-Proton-Test (APT) NMR of compound 14j

[Diagram of NMR spectra showing H-Ph, H3, H14-H16, C-Ph, C14-C16, C3, C9, C12, C18]
$^1$H and $^{13}$C Attached-Proton-Test (APT) NMR of compound 14k
M062X/cc-pVTZ Energies and Cartesian Coordinates

12b
E = -631.850927
G= -631.680305
Imaginary Frequencies: 0

Imaginary Frequencies: -263.03

13a
E = -604.210549
G= -604.139547
Imaginary Frequencies: 0

TS3
E = -1236.08668
G= -1235.819529
Imaginary Frequencies: -263.03
TS4
E = -1236.038093
G= -1235.772465
Imaginary Frequencies: -256.50

E = -1236.022598
G= -1235.756068
Imaginary Frequencies: -318.54

TS5
E = -1236.07495
G= -1235.810121
Imaginary Frequencies: -377.59