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Synthesis, characterization and molecular docking studies of N-(4-Bromo-2-fluorophenyl) malonamide

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ABSTRACT

The novel N-(4-bromo-2-fluorophenyl) malonamide has been efficiently synthesized from diethyl malonate (DEM) and NH_3. Computational studies were undertaken to test the inhibitory effect of the synthesized molecule on protein kinase PKnB from Mycobacterium tuberculosis. Based on the virtual screening and molecular docking we found that N-(4-bromo-2-fluoro phenyl)malonamide showed good binding affinities with the active site pocket (comprising of VAL-95) of the PknB.

Keywords: tuberculosis, synthesis, molecular docking, malonamide.

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INTRODUCTION

The problem of tuberculosis (TB) drug resistance and the continuing rise in the disease incidence has prompted the research on new drug development as well as on increasing the understanding of the mechanisms of drug resistance. Molecular docking was performed to study the binding activity of synthesized malonamide onto the active site of *Mycobacterium tuberculosis* protein kinase B (PKnB) in an effort to increase the understanding of the action and resistance of synthesized malonamide in this bacterium. Protein kinases B (PKnB) plays an important role in mammalian cellular signaling. Mycobacterium tuberculosis PknB is an essential receptor-like protein kinase involved in cell growth control. M. tuberculosis PKnB is a trans-membrane Ser/Thr protein kinase (STPK) highly conserved in Gram-positive bacteria and apparently essential for mycobacterial viability. We have attempted with the help of a docking approach to elucidate the extent of specificity of protein kinase B towards synthesized compound, as anti-tubercular agent. Malonamide compounds possess anti-tubercular,[1-3] anti-convulsant,[4] and fungicidal activity.[5] They also act as antagonists,[6] HIV-1 integrase inhibitor,[7] and modulator of chemokine activity.[8] Therefore, the present work is limited to the synthesis, characterization, virtual screening and molecular docking of N-(4-bromo-2-fluorophenyl)malonamide.

MATERIALS AND METHODS

All chemicals were of analytical grade. Melting Points was determined in an open glass capillary on Electro-thermal apparatus and is uncorrected. The purity of the compounds is monitored by TLC technique using silica-gel-coated Al plates (Merck). The structure of the compound is confirmed on the basis of Infra red spectra (IR) using KBr discs, on a Perkin Elmer Spectrum RX1 infra red spectrophotometer. ^{1}H NMR spectra was recorded in DMSO on Bruker DRX-300 (300 MHz); chemical shift (δ) are reported in ppm using TMS as an internal reference. Elemental analysis was performed on Elementor Vario EL III. It gave satisfactory microanalysis. N-(4-bromo-2-fluorophenyl) malonamide is docked into the nucleotide-binding pocket of the M. tuberculosis PKnB structure (PDB ID 2FUM) [9] using the program AutoDock4. [10] The grid spacing was set to 0.375A in each spacing & each grid map consisted of a 60x60x60 grid point. During docking experiment 10 runs were carried out & the rest of the parameters were set as the default value.

Experimental

A mixture of 4-Bromo-2-fluoroaniline (9.50 gm, 0.05mol.) and freshly distilled diethyl malonate (DEM) (16.0 ml, 0.1mol) was gently refluxed in a round bottom flask using an upright air condenser for 1 hour and 45 min. After cooling it was filtered. The filtrate was taken in a round bottom flask containing 10.0 ml ethanol and liquor NH_3 (d-0.88) (17.0 ml, 1.0 mol) was added to it. The flask was tightly corked and vigorously shaken for 30 min, later then the flask (tightly corked) was left over night. The white crystalline solid obtained was then filtered and

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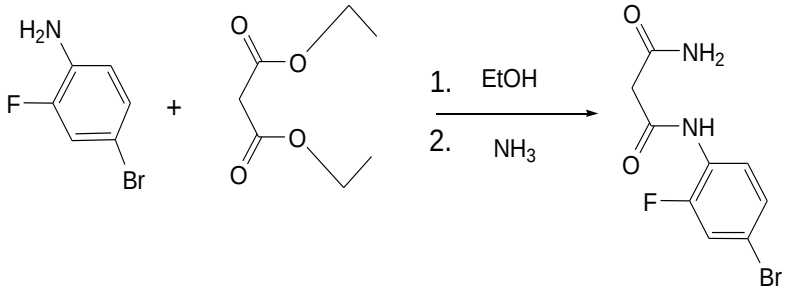
purified by recrystallization from abs. ethanol. On analysis, it was found to be N-(4-bromo-2-fluorophenyl) malonamide, white solid (Yield-85.33%).

Scheme 1



(A) (B) (C)

M.p: 191-192^{o}C

IR (KBr) v: 3256-3109, 3023, 2925, 1666, 1524, 1352, 1188, 1066 cm^{-1}.

^{1}H-NMR (300 MHz, DMSO-D_6) δ ppm: 2.50 (DMSO), 3.34 (DMSO water), 3.68 (s, 2H, -CH_2), 6.23 (s, 2H, -NH_2), 7.38-8.00 (m, 3H, Ar-H), 10.12 (s, 1H, -CONH).

Mass spectrum: Base peak m/z 273.

Anal. calc. For ($C_9H_8BrFN_2O_2$) C-39.27%, H-2.90%, N-10.18%,

found:C-39.31%,H-2.89%,N-10.20%.

RESULT AND DISCUSSION

In order to expose the specificity of the Protein Kinase PKnB from Mycobacterium Tuberculosis towards the compound synthesized, a docking approach was carried out. Docking was used to predict the binding orientation of drugs to their protein target in order to in-turn predict the affinity and activity of a drug, which includes docking of ligand to a set of grids describing the target protein. The synthesized malonamide was docked into the nucleotide-binding pocket of the *M. tuberculosis* PKnB structure using the program AutoDock4. The active site VAL 95 in the protein interacts with ligand of the substrate and gives rise to the catalytic activity to test ligand that helps in determining the binding pattern of the ligand to the active site of PKnB.

Based on the virtual screening and molecular docking we found that N-(4-bromo-2-fluoro phenyl)malonamide showed better binding affinities (min. binding energy is -5.86 kcal/mol, max. binding energy is -5.64 kcal/mol, Log P value is 1) with the active site pocket (comprising of VAL-95) of the PknB which also act as the substrate binding site.

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Fig. 1 and Fig. 2

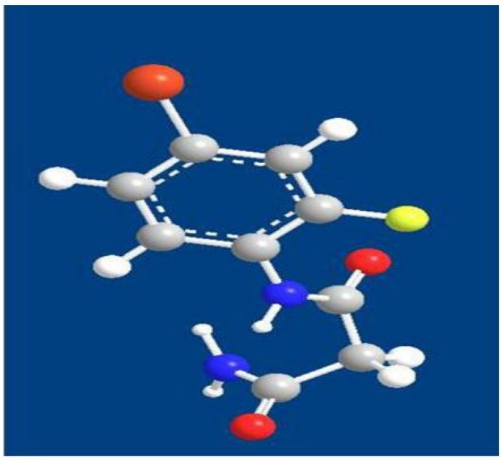

Fig1: 3D Structure of N-(4-bromo-2-fluoro phenyl) malonamide

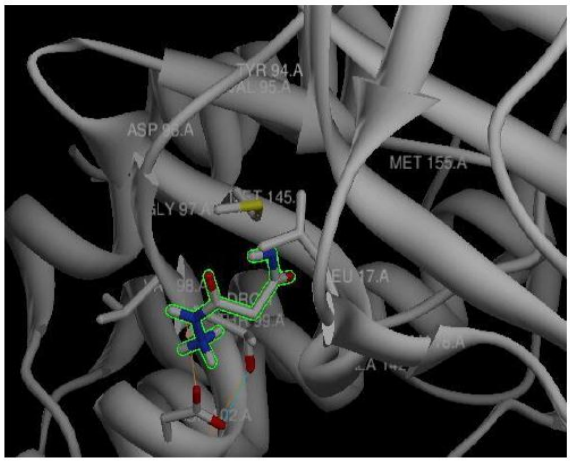

Fig2: 3D model of PKnB , malonamide form hydrophobic bond with VAL 95

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