CHAPTER 2

EXPERIMENTAL

2.1 Synthetic Procedure for 2,4-Diphenyl-5a,9a,10,10 a-tetrahydrobenzo[4,5]imidazo[1,2-A]pyrimidine Derivatives (1a-1g)

A mixture of 2-aminobenzimidazole (1 mmol) and substituted aromatic aldehyde (1 mmol) was dissolved in toluene (10 ml) in a 50 ml round bottomed flask and 10 mol% PTSA was added into the solution. The mixture was then heated to ambient temperature for about 30 min. Then phenylacetylene (1.3 mmol) and (20 mol %) Cu catalyst were added and heated to 90 °C for about 6-8 h. The course of reaction was monitored by TLC. When the reaction was completed, the reaction mixture was cooled and concentrated by rotary evaporation under reduced pressure. The crude compound was purified by column chromatography using ethyl acetate-hexane mixture.

2.2 Synthetic Procedure for Bispyrazoline Derivatives

2.2.1 4-[[5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl] (phenyl)methyl]-5-methyl-2-phenyl-1H-pyrazol-3(2H)-ones (2a-2j)

To a mixture of phenylhydrazine (1 mmol), methyl acetoacetate (1.2 mmol) and 20 mol % Ce-MCM-48 were added to water (3 ml) and stirred at 60-80 °C for 5 min, then aromatic aldehyde (0.5 mmol) was added and stirring was continued at the same temperature for about 15 min. After completion of the reaction (monitored by TLC), the reaction mixture was filtered, then the precipitate was dissolved in ethanol and the catalyst was removed by filtration. The crude product was obtained by evaporation of the solvent followed by recrystallization in ethanol afforded the bispyrazolines in pure form.
2.2.2 4-(5-hydroxy-1,3-diphenyl-1H-pyrazol-4-yl) (phenyl) methyl-2,5-diphenyl-1H-pyrazol-3(2H)-ones (2k-2n)

To a mixture of phenylhydrazine (2 mmol), ethyl benzoylacetate (2 mmol) and 20 mol % Ce-MCM-48 were added to water (3 ml) and stirred at 60-80 °C for 5 min, then aromatic aldehyde (1 mmol) was added and stirring was continued at same the temperature for about 15 min. After completion of the reaction (monitored by TLC), the reaction mixture was filtered, then the precipitate was dissolved in ethanol and the catalyst was removed by filtration. The crude product was obtained by evaporation of the solvent followed by recrystallization in ethanol afforded the bispyrazolines in pure form.

2.3 Synthetic Procedure for (E)-1-(benzo[d][1,3]dioxol-5-yl)-3-phenylprop-2-en-1-one derivatives (3a-3h)

Phenylpropenone derivatives were synthesized by mixing stochiometric amounts of 3,4-methylenedioxy acetophenone (1 mmol) and substituted aromatic aldehyde (1 mmol) in the molar ratio of 1:1 (Scheme 23). The reactants were dissolved in ethanol, thoroughly mixed using a magnetic stirrer for 10 min, and 10% NaOH solution was added dropwise at 30 °C. After stirring for 2 h, the contents of the flask were poured into ice-cold water. The solid precipitate formed was collected by filtration, dried and purified by recrystallization process using ethanol as a solvent (yield: 90 %). The completeness of the reaction was monitored by thin layer chromatography.

2.4 Materials and methods

2.4.1 Procedure for recording FT–IR, $^1$H, $^{13}$C NMR and 2D–NMR spectra

All the solvents used for recrystallization and thin layer chromatography were of analytical grade and used without further purification. All reactions were monitored by thin layer chromatography on silica gel precoated aluminum sheets (Type 60 GF254, Merck). The melting points were recorded in open capillaries and are uncorrected. FT–IR spectra were recorded on an AVATAR–330
FT-IR spectrometer (Thermo Nicolet) using KBr (pellet form). Mass spectra were recorded on a Varian Saturn 2000 GC–MS/MS spectrometer using electron impact technique. Samples were prepared by dissolving about 1 mg of compound in 5 mL of spectral grade methanol/acetone. $^1$H and $^{13}$C NMR spectra for all the compounds were recorded at 400 / 500 MHz and 100 / 125 MHz, in a JEOL-JNM ECP–400 MHz / Bruker instrument, using deuterated chloroform/deuterated DMSO as the solvent by taking about 10 mg and 50 mg of compound respectively for recording $^1$H NMR and $^{13}$C NMR spectra. Tetramethylsilane (TMS) was used as an internal reference for all NMR spectra, with chemical shifts reported in $\delta$ units (parts per million) relative to the standard. $^1$H NMR splitting patterns are designated as singlet (s), broad singlet (bs), doublet (d), doublet of doublet (dd), triplet (t) and multiplet (m). Coupling constants are expressed in Hertz (Hz).

2.4.2 Procedure for single crystal X-ray diffraction analysis

Single crystal X-ray diffraction analysis was carried out at 298 K using a three circle Bruker SMART–APEX CCD area detector system under Mo Kα (\(\lambda = 0.71073 \text{ Å}\)) graphite monochromated X-ray beam with a crystal to detector distance of 60 mm and a collimator of 0.5 mm. The total number of reflections was equal to 5582. The structural refinement was done using SHELXL 97 by full–matrix least–squares method with anisotropic temperature parameter for all non hydrogen atoms.

2.4.3 Procedure for theoretical calculations

Theoretical calculations were done by considering the crystal structure of compound (2a) as the initial structure and for compounds 4a-4f & 4h using DFT method and 6–31G (d,p) as the basis set in Gaussian 09 package to optimize the structure. The lowest unoccupied molecular orbital (LUMO) and highest occupied molecular orbital (HOMO) energy differences for the molecule were calculated.
with this method. The other properties like Mulliken charge distribution pattern and NBO analysis are also calculated using the same package. Gauss View program is used to visualize the results obtained from calculations, which were made by Gaussian 09 program.

2.4.4 Procedure for Molecular Docking and Prediction of Pharmacokinetic Properties

All the newly synthesized were subjected to docking studies using MAESTRO v 9.3.5\textsuperscript{132} implemented in the Schrödinger software.

2.4.4.1 Ligand preparation

The 2D structures of compounds (.mol files) were imported to the project table of Maestro. The chemical structures were minimized using OPLS-2005 (Optimized Potential for Liquid Simulations) force field\textsuperscript{133}. The possible stereoisomers of all ligands were generated and geometrically refined using LIGPREP module.\textsuperscript{134} The number of stereoisomers generated was limited to 32 per ligand. The EPIC\textsuperscript{135} program was used to neutralize the charge groups and for ionization and tautomeric states of the compounds.

2.4.4.2 Protein preparation

The 3D co-ordinates of crystallographic structure of all proteins were downloaded from Brookheaven Protein Data Bank (www.rscb.com). The protein complex was pre-processed and prepared by PROTEIN PREPARATION WIZARD\textsuperscript{136} in Maestro of Schrödinger software. The unwanted protein chains, water molecules and heterocyclic groups were deleted in Review and Modify panel of the preparation wizard. The minimization of the complex was continued using OPLS-2005 force field until the root mean square deviation (RMSD) reached the value of 0.3 Å. The protein grid was generated using Receptor grid generation panel which was implemented in the GLIDE\textsuperscript{137} application.
2.4.4.3 Molecular docking

The computationally prepared ligands and the proteins were used for the molecular docking using Glide program. Out of three docking procedures (i) HTVS – High Throughput Virtual Screening, (ii) SP – Standard Precision and (iii) XP - Extra precision, XP docking was used to identify the interactions between the proteins and the novel compounds. Molecular Dynamics stimulations studies were carried out to clarify the docking interactions by DESMOND \(^{138}\) module.

2.2.4.4 Insilico determination of ADME properties

A preliminary test of the drug-likeness of the compounds was calculated in accordance with Lipinski’s rule of five. \(^{139-140}\) All the newly synthesized compounds were subjected to a computational program using QIKPROP \(^{141}\) module of Schrödinger software for the insilico determination of pharmacokinetic properties such as absorption, distribution, metabolism and excretion (ADME).

The Lipinski’s rule of five values of compounds indicates that the compounds are endowed with drug like properties. To obey the Lipinski’s rule of five, the compounds requires molecular weight (mol_MW) of less than 500 amu, not more than 5 and 10 hydrogen bond donors (Donor HB) and hydrogen bond acceptors (Accpt HB) respectively, and the partition coefficient between octanol and water (QPlogPo/w) be less than 5. The compounds which have more than one violation of these rules are not considered as orally active molecule.