SPECIMEN FORMAT FOR THESES OF MONTH

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Department	:	Chemistry
Branch/ Area:	:	Organic Chemistry
Sub Subject Heading:	:	Synthesis of N-heterocyclic Compound
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Title of the thesis	:	Inverse Electron Demand Diels Alder Reaction of Pyrido[1,2-*a*]pyrimidine-2-ones - Experimental and Theoretical Investigation and Antibacterial Activity of Synthesized Adducts
(i) In Roman Script		INVERSE ELECTRON DEMAND DIELS ALDER REACTION OF PYRIDO[1,2-*A*]PYRIMIDINE-2-ONES - EXPERIMENTAL AND THEORETICAL INVESTIGATION AND ANTIBACTERIAL ACTIVITY OF SYNTHESIZED ADDUCTS
(ii) In roman Script		inverse electron demand diels alder reaction of pyrido[1,2-*a*]pyrimidine-2-ones - experimental and theoretical investigation and antibacterial activity of synthesized adducts
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Designation of Supervisor	:	Assistant Professor (SG)
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Abstract within 300 words: The IEDDA reaction of diene 2-oxo-2H-pyrido[1,2-*a*] pyrimidin-3 (4*H*)-ylidiene acetic acid with various electron rich dienophiles in DMF medium yielded moderate to good yields in the presence of Lewis acid catalyst indium (III) chloride. FT-IR, ^{1}H NMR, ^{13}C NMR, and Mass Spectroscopy investigations were used to characterize and confirm the structures of all the synthesized adducts. Except for 3-butoxy-9-methyl-2,3,4,5-tetrahydropyrano[2,3-*d*]pyrido[1,2-*a*]pyrimidine-4-carboxylic acid **(5)** and Methyl-2-Oxo-1-Phenyl-1,2,3,3*a*,4,5-hexa hydropyrazolo[1,5-*b*]Pyrido[1',2':1,2]Pyrimido[5,4-*e*][1,2]Oxazine-4-Carboxylic Acid **(7)** which showed 1:1 and 6:4 ratio and for other adducts the ratio was found to be 6:1, supporting the *endo* rule. The analysis of the LUMO and HOMO energies of diene and dienophiles helped to prove the IEDDA reaction pathway. NBO charges computed using DFT techniques gave justification for the regioselectivity provided by the reaction. The synchronous nature of the transition states and intrinsic reaction coordinates of the reaction of diene 2-oxo-2H-pyrido[1,2-*a*] pyrimidin-3(4H)-ylidiene acetic acid with dienophiles butyl vinyl ether and 1-methyl-1-cyclohexene by DFT method explained the observed diastereoisomeric ratio of 1:1 and 6:1 *endo* and *exo* ratio respectively. All synthesized compounds were tested for antibacterial activity, which showed moderate activity, however the compound 3*a*-methyl-2-oxo-1-phenyl-1,2,3,3*a*,4,5-hexahydropyrazolo[1,5 *b*]pyrido[1',2':1,2]pyrimido[5,4-*e*][1,2]oxazine-4-carboxylic acid was shown to be more powerful in both *in-silico* and *in-vitro* investigations.

Major objectives : To synthesis fused 2-oxo-2*H*-pyrido [1, 2-*a*] pyrimidin-3(4*H*)-ylidiene acetic acid by IEDDA reaction with different dienophiles such as butyl vinyl ether, 1-phenyl-3-methyl-pyrazolone,1-methyl-1-cyclohexene, 3, 4-Dihydro-2*H*-pyran, 1-morpholinocyclohexene using Lewis acid catalyst Indium (III) Chloride

✔ To characterize the compounds by FT-IR, ^{1}H NMR , ^{13}C NMR, ESI-MS

- ✔ To study the mechanistic details of the IEDDA reaction by computational method
- ✔ To study the antibacterial activity of the synthesized compound against the gram-positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli.*
- ✔ To carry out *in-silico* studies for the synthesized compounds with the target receptors *Staphylococcus aureus* (PDB-2W9S) and *Escherichia coli* (PDB-1MBT).
- ✔ To correlate the *in-vitro* studies with the *in-silico* studies

ii) Methodology :

Melting points were determined using Biochem melting point apparatus, and are uncorrected. Spectra were recorded on a Fourier transform infrared spectrometer using KBr pellet technique on an IR-AFFINITY-I. Absorption frequencies are quoted in reciprocal centimeter. Nuclear Magnetic Resonance (^{1}HNMR) spectra were determined by Bruker modern 600MHz and JEOL 600MHz NMR instrument in DMSO-d_6 and Trifluoroacetic acid-d_1 , with tetra methyl silane as the internal reference. The 600 MHz NMR machine was utilized to measure two dimensional spectra. Chemical shifts are reported in parts per million (ppm) relative to solvent peak. The mass spectrum was recorded using Jeol GC-mate–II spectrophotometer. All reagents and solvents were obtained from Sigma-Aldrich was used as received unless otherwise stated. Purification of the crude products was carried out using chromatographic columns packed with alumina.

Synthesis and characterization of pyrido [1,2-*a*] pyrimidin-2-ones derivatives General Procedure

Mixed 10 mmol of powdered $InCl_3$ with 5 ml of DMF and maintained at 50^0C. To this, added 1mmole of diene in 10 ml of DMF slowly with constant stirring. $InCl_3$ rapidly dissolved to form a slightly yellow clear solution. At this stage, 1mmole of dienophile was bubbled through the solution. The mixture was kept for refluxing in a water bath between 80^0C-90^OC. The resulting solution was cooled and treated with water, and the precipitate was filtered, collected and recrystallized using ethanol **.**

Computational studies

Gaussian 16W software package was used to perform DFT calculations. The frequency optimization was performed utilising density functional theory [DFT] with the B3LYP functional and a differentiated basis set based on atom type. Particularly, 6-311+G(d,p) was utilised for H, C, N, and

O, which has been proven to produce relatively accurate energetics for cycloadditions, while effective core potential basis set DEF2TZVP was used for indium chloride. Using the Integral equation formalism polarizable Continuum Model (IEFPCM) solvent model, the role of DMF solvent was included during optimizations

Antibacterial susceptibility testing

The *in-vitro* antibacterial activity screened for the synthesized compounds against *Staphylococcus aureus* and *Escherichia coli* bacterial strains by disc diffusion method against the standard drug **Kanamycin**.

Molecular docking

Docking calculations were performed using the software Autodock vina 1.5.6, and the binding energy of the protein—synthesized adducts was determined. Using the graphical interface programme 'MGL tools,' the ligand was then converted into PDBQT. The protein data bank coordinate file with the names 2W9S and 1MBT was used as an antibacterial receptor molecule. To run docking simulations, the grid box was modified with 'MGL tools.' The best docked conformation and binding affinity for drugs with proteins were determined using Autodock Vina

Lipinski rule of five (adme)

ADME parameters and molecular properties were computed for both the standard drug and test compounds an online portal called SwissADME.

ii. Findings:

The results pertaining to the present investigation on the IEDDA reaction of the diene 2-oxo-2H-pyrido [1, 2-*a*] pyrimidin-3(4*H*)-ylidiene acetic acid is summarized below

- Initially optimization of IEDDA reaction conditions such as catalyst, solvent, time and temperature were done using the diene 2-oxo-2H-pyrido[1,2-*a*] pyrimidin-3(4*H*)-ylidiene acetic acid and the dienophile butyl vinyl ether as model substrates. The results showed that, the reaction was unsuccessful in absence of catalyst and in the presence of catalyst aluminium chloride and zinc chloride.
- The IEDDAR reaction of diene 2-oxo-2H-pyrido[1,2-*a*] pyrimidin-3(4*H*)-ylidiene acetic acid with dienophiles butyl vinyl ether, 1-phenyl-3-methyl-pyrazolone, 1-methyl-1-cyclohexene, 3,4-Dihydro-2H-pyran, 1-morpholinocyclohexene in presence of Lewis acid catalyst indium(III)chloride in DMF medium provided the expected cyclo adducts with moderate to good yields.

- All the synthesized compounds were characterized by spectroscopic methods such as FT-IR, ^{1}H NMR, ^{13}C NMR, and Mass spectral studies.
- The reaction showed endo selectivity. The diastereoisomeric ratio of the adducts were found to be 6:1 for *endo* and *exo* isomers (**9**, **11** and **13**) except for 3-butoxy-9-methyl-2,3,4,5-tetrahydropyrano[2,3-*d*]pyrido[1,2-*a*]pyrimidine-4-carboxylic acid **(5)** and Methyl-2-Oxo-1-Phenyl-1,2,3,3*a*,4,5-hexa hydropyrazolo[1,5-*b*]Pyrido[1',2':1,2]Pyrimido[5,4-*e*][1,2]Oxazine-4-Carboxylic Acid **(7)** which showed 1:1 and 6:4 ratio respectively.
- The IEDDA reaction pathway was proved by the analysis of energies of LUMO and HOMO of diene and dienophiles. The reaction occurred by lowering of LUMO energy of diene by Lewis acid catalyst.
- The computed global reactivity indices, electronic chemical potential (μ), global electrophilicity (ω), and maximum charge transfer (ΔN max) also supported the IEDDA mechanism.
- The regioselectivity observed in the IEDDA reaction of diene 2-oxo-2H-pyrido[1,2-*a*] pyrimidin-3(4*H*)-ylidiene acetic acid with dienophiles was explained on the basis of NBO charges calculated by DFT methods.
- Analysis of the transition states and intrinsic reaction coordinates of the reaction of diene 2-oxo-2H-pyrido[1,2-*a*] pyrimidin-3(4*H*)-ylidiene acetic acid with dienophiles butyl vinyl ether and 1-methyl-1-cyclohexene by DFT method explained the observed diastereoisomeric ratio of 1:1 and 6:1 *endo* and *exo* ratio respectively. The synchronous nature of the transition states proved the proposed concerted mechanism of the IEDDA reaction.
- Synthesized compounds were tested for antibacterial activity and all the compounds exhibited moderate activity and the compound 3*a*-methyl-2-oxo-1-phenyl-1,2,3,3*a*,4,5-hexahydropyrazolo[1,5-*b*]pyrido[1',2':1,2]pyrimido[5,4-*e*][1,2]oxazine-4-carboxylic acid (**7a**) was found to be more potent in both *in-silico* and *in-vitro* studies.

To conclude we have demonstrated a successful IEDDA reaction for the synthesis of fused pyrido[1,2-*a*]pyrimidin-2-one by LUMO lowering strategy using Lewis acid catalyst indium chloride. This approach may be used to create highly substituted and functionalized pyrimidine fused heterocyclic scaffolds.

Examiners

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