PHOTOPHYSICAL AND BIOLOGICAL STUDIES ON NEWLY SYNTHESIZED NOVEL ARYL-SUBSTITUTED PYRAZOLONE ANALOGUE

Abstract

In the present work, a new pyrazoles derivative namely ethyl 2-(2-(ethoxycarbonyl)hept-1-en-3-yl)6,7-dihydro-3-hydroxy-2H-pyrazolo[4,3c]Pyridine5(4H)carboxylate (EPP) was synthesized and studied their biological and photophysical properties. The EEP structure was confirmed by ^{1}H NMR, FT-IR and LCMS analyses. EPP show good inhibitor properties for ***S. aureus*** and ***C. albicans.*** The photophysical properties are estimated using density functional theory in gaussian-9w software. The ground state dipole moment, HOMO-LUMO and molecular electrostatic potential map are estimated using basis set B3LYP-311G. The GCRD parameters suggest that, molecule is highly chemical reactive. The results suggest that synthesized compound shows antioxidant, antibacterial, antifungal and chemosensor application.

Key words: Pyrazolones, In vitro antifungal activity, antibacterial activity, antioxidant study

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I. INTRODUCTION

Organic compounds (Heterocycles) are used in wide range of physical, chemical and biological applications. The heterocyclic organic molecules have plays dynamic role in human metabolism because their structural subunits exist in many natural products, like antibiotics, alkaloids hormones, pharmaceuticals and many others. In addition, it is also having physiological and pharmacological properties [1]. Heterocyclic compounds have scaffolds site on which pharmacophores can easily arrange and generate selective drugs and also enhanced solubility and salt-formation properties that enable their oral absorption and bioavailability [2]. There re several different kind of heterocyclic compounds re used in biological application in that nitrogen substituted heterocycles re widely used in numerous applications in chemistry, biology and other sciences [3]. Nitrogen substituted heterocycles re the building blocks of life due to their wide occurrence in nature and central roles in the chemical reactions that occur in all organisms [4].

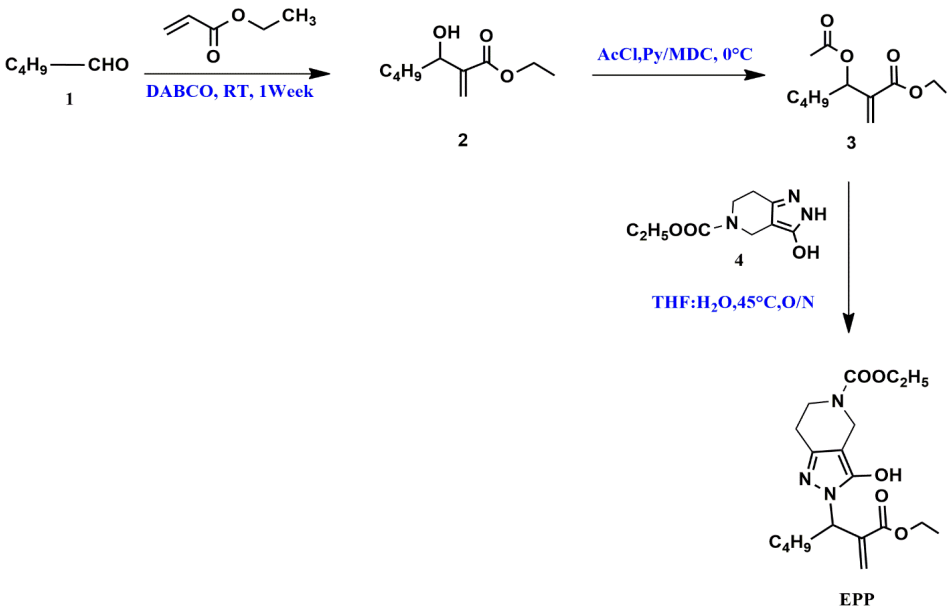
Among the various nitrogen substituted heterocyclic compounds, pyrazole re one of the most widely used organic compound in biological optoelectronic applications. The pyrazole re five membered ring structure composed of two nitrogen and three carbon atoms in adjacent position. This gave great impetus to the search for potential pharmacologically active drugs carrying pyrazole scaffold and also Herpes simplex virus inhibitor activity [5-6], MAP kinase inhibitory, analgesic, anti-HIV, apoptosis-inducing activity, anti-arrhythmic, anticancer, anti-proliferative, antimalarial, anti-inflammatory anti-leishmania, anticonvulsant and antimicrobial activities [7-21].

In view of the above we have synthesized pyrazole derivative using Baylis-Hillman acetate and studied their photophysical and biological activities like antifungal, antimicrobial and antioxidant.

II. EXPERIMENTAL

1. **Material and Methods:** The high purity chemical regent was commercial received samples from Sigma ldrich Pvt Ltd. lumina silica gel 60F254 (Fischer) plate was used to measured thin layer chromatography of organic molecule. The Buchi pparatus was used to measured melting point. The ^{1}H NMR, ^{13}C NMR nd IR spectra were recoded using Bruker C-400F nd Nicolet-Impact-410 FT-IR spectrometer respectively. LCMS nalysis were performed on by using gilent1200 series instrument.

2. **Synthesis of N/O-substituted pyrazolone derivatives:** The aryl substituted pyrazolone derivatives was synthesized s shown in the **scheme 1**. highly functionalized adduct (2) was obtained by mixing aldehyde and activated alkene in the presence of tertiary base. This adducts was converted to acetates (3) in the presence of catalytic mount of pyridine and dichloromethane s the solvent. Further, acetate (3) was treated with pyrazolones (4) to produce the desired target molecules (EPP). The ^{1}H NMR, LC-MS, and IR spectroscopy were used to confirm the synthesized compounds.



Scheme 1: synthesis of the EEP

Ethyl 2-(2-(ethoxycarbonyl)hept-1-en-3-yl)6,7-dihydro-3-hydroxy-2H-pyrazolo[4,3-c]Pyridine-5(4H)-carboxylate (**EPP**)

Yellow liquid, % of yield: 95; ^{1}H NMR (400MHz, d_6-DMSO): δ= 9.78 (s, 1H, OH), 6.21(s, 1H, cis CH_2), 5.73 (s, 1H, CH), 4.82 (q, 1H, trans CH_2), 4.14-4.03 (m, 6H, $3CH_2$), 3.63-3.56 (m, 2H, CH_2), 2.58 (t, 2H, CH_2), 2.05 (m, 1H, CH), 1.85 (m, 1H, CH), 1.25 (m, 9H, $3CH_3$), 1.17 (m, 1H, CH), 0.82 (t, J = 8Hz, 3H, CH_3); MS cald. for $C_{19}H_{29}N_3O_5$: 379.21, Found: 380.1; IR (ν cm^{-1}): 2955(C-H), 1693(C=O); Elem. anal. cald (found): C: 60.14(60.12), H: 7.70(7.69), N: 11.07(11.05).

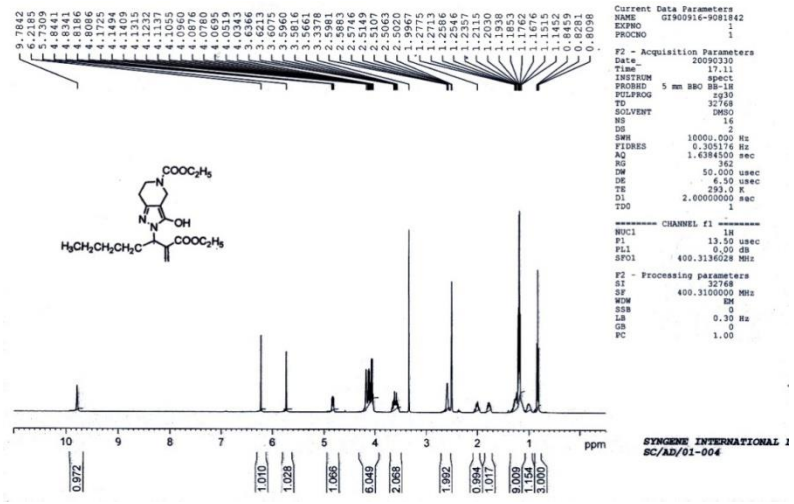


Figure 1: H NMR spectrum of EPP

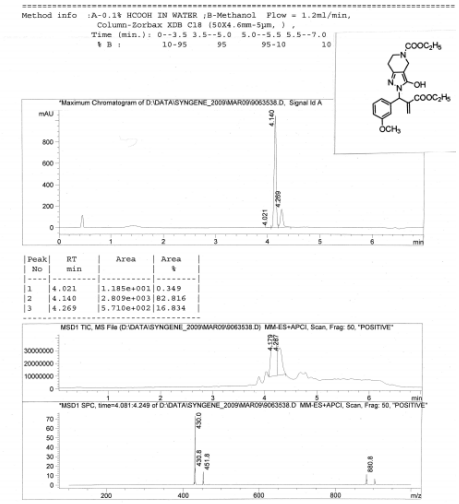


Figure 2: LCMS spectrum of EPP

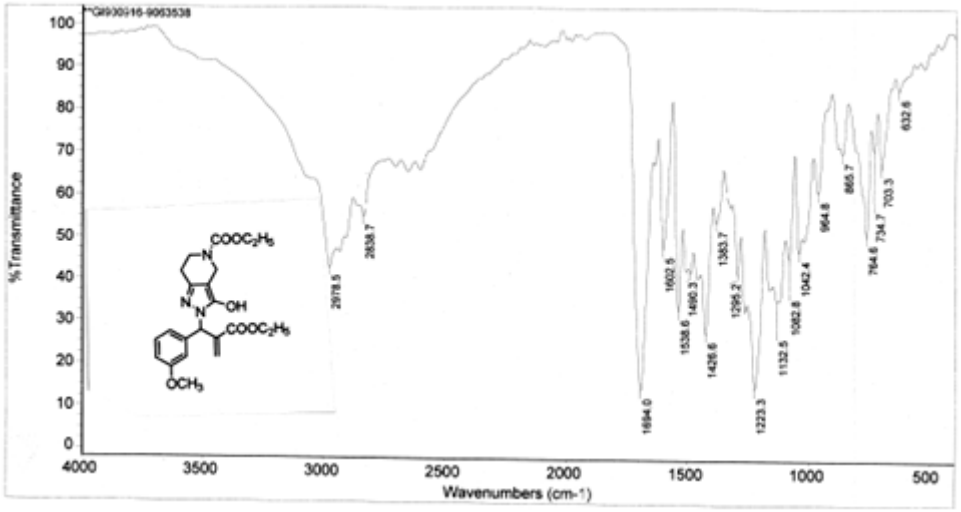


Figure 3: IR spectrum of EPP

- **In vitro antifungal studies:** The antifungal ctivity of newly synthesised of pyrazolones were screened using *niger* and *C. lbicans* in DMSO (serial plate dilution method). Initially, using peptone (1g), D-glucose (4g) nd gar (2g) dissolved in 100 mL distilled water and prepared Sabourands agar media (approximate pH 5.7). The suspension of spores fungal strain for lawning was prepared by dissolved fungal strain in 3 mL of normal saline. The freshly prepared gar media (20ml) was poured into petri dish and dried using incubator t 37 °C for 1h. finally, punch wells were made on seeded gar plates, and 10-50 µg/mL of EPP was added into each well labelled. control was also prepared for plates in the same way using solvent DMSO. Reference and sample petri dishes were prepared in triplicate and maintained t 37 °C for 3-4 days. antifungal activity was determined by measuring the inhibition zone and results were compared with the standard Flucanazole [22].

- **In vitantibacterial studies:** The antibacterial activity of the newly synthesized pyrazolones was screened using *S ureus, B subtilis, E coli a*nd *S typhi bacterial* strains by disc diffusion method. The Whatman no.1 filter paper were punched in circular shape (6.25 mm in diameter) and placed in the standard petri discs. Batches of 100 discs were dispensed to each screw-capped bottle nd sterilized by dry heat t 140 °C for n hour. The different concentration of EPP were prepared in DMSO and added each discs. Finally, these disc re triplicate and placed in nutrient gar medium separately seeded with fresh bacteria and entire systems re incubated t 37 °C for 24 h. Solvent and growth controls were kept and the zones of inhibition and minimum inhibitory concentrations were noted. The results were compared with the standard Ciprofloxacin [23]

III. RESULTS AND DISCUSSION

The ^{1}H NMR spectrum of EPP were recorded using Bruker C-400F, 400MHz, spectrometer in DMSO-d_6 and $CDCl_3$ using TMS s n internal standard with resonance frequency of 400 MHz and 100 MHz and shown in fig.1. The ^{1}H NMR of EPP spectra showed broad signal t 9.78 δ_{ppm} is due to -OH proton of pyrazole ring, The signal t δ 6.21 (s, 1H) and 5.73 (s, 1H) account for the pyrazole ring proton and methine proton of the molecule. The protons of cyclohexane ring resonated from 2.58 (t, 2H, CH_2), 2.05 (m, 1H, CH), 1.85 (m, 1H, CH), account for the 11 protons present in the molecule. The signals t 1.17(q, 2H) and 1.25(t, 3H) accounts for the ethyl protons of the molecule. The LCMS spectrograph exhibited peak t 433.3 which matched with the calculated mass of the molecule 379.21 with molecular formula $C_{19}H_{29}N_3O_5$.

Computational photophysical properties: The ground state optimization of the EPP in gas phase was carried out using density functional theory (DFT) with basis sets B3LYP/311G. Further, ground state optimization of EPP in solvent phase was carried using combined with integral equation formalism variant-Polarizable Continuum Model (IEFPCM) solvation model. The optimized molecular geometry of the EPP in the gas phase is shown in Fig.4.

The ground-state dipole moment of the EPP in the gas nd solvent phase were calculated nd re result re presented in Table 1 and it is observed that ground state dipole moment in gas phase is lower than solvent phase. The higher value of dipole moment is due to the consideration of solvent environmental effects on solute

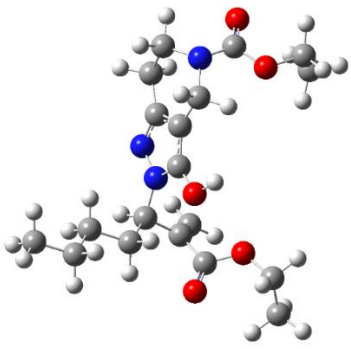


Figure 4: Ground-state optimized molecular geometries of EEP

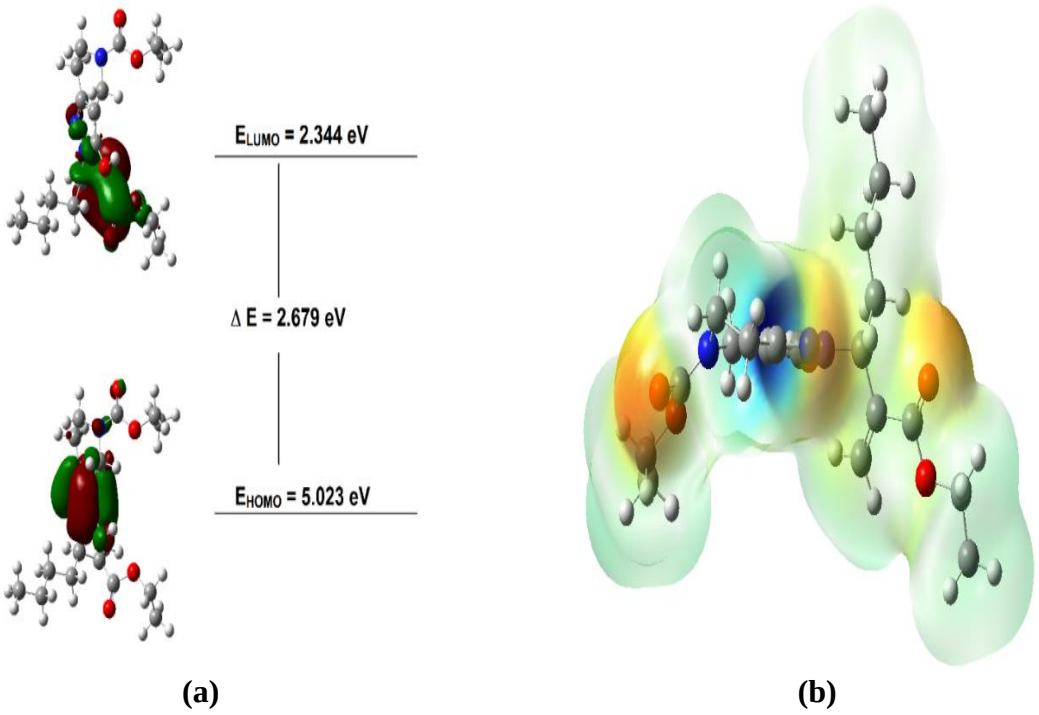


Figure 5: a) 3D plots of HOMO-LUMO with energy levels and b) Molecular electrostatic potential plots of EPP

Table 1 Theoretical ground state dipole moment (μ_g) in different solvents

Solvent	μ_g (D)	HOMO eV	LUMO eV	ΔE eV
Benzene	5.442	-5.122	-2.088	3.034
Toluene	5.531	-5.123	-2.101	3.022
DEE	5.664	-5.121	-2.159	2.962
Ethyl acetate	5.745	-5.118	-2.206	2.912
Methyl chloride	5.843	-5.126	-2.245	2.881
Isoproponol	5.912	-5.114	-2.235	2.879
Ethanol	5.993	-5.131	-2.245	2.886
Methanol	6.052	-5.999	-2.965	3.034
DMF	6.112	-5.983	-3.117	2.866
Acetonitrile	6.213	-5.981	-3.225	2.756
DMSO	6.304	-5.977	-3.566	2.411

The HOMO-LUMO energy levels of EPP with energy band gap re shown in the Fig.5a. The HOMO electron cloud re strongly spread over pyrozoles in molecule which suggest that, EPP has stabilized molecular and also the better electron-donor to the one with the better electron-acceptor substituent. LUMO electron cloud mainly spread our

pyrazole and aliphatic side chain these results suggests that, molecules has more number delocalized electron. The energy band gap between HOMO and LUMO is found to be 2.411-3.034 eV. Using theoretical estimated HOMO-LUMO value global chemical reactivity descriptors (GCRD) are calculated. The GCRD parameters gives important knowledge about structure stability, chemical hardness, softness, nucleophilic, electrophilic and chemical reaction, and stability of the molecule with the help of molecular orbitals. The GCRD parameter such s chemical hardness ($\eta = (IP - EA)/2$), electronegativity ($\chi = (IP + EA)/2$), chemical potential ($\mu = -\chi$), chemical softness ($S = 1/2\eta$) nd electrophilicity index ($\omega = \mu^2/2\eta$) were calculated from E_{HOMO} and E_{LUMO} values, where, ionization potential, $IP = -E_{HOMO}$ and electron affinity, $EA = -E_{LUMO}$. [24] and values are given in Table 2. From the Table 2, it is found that EPP molecule chemically soft in nature and very high electronegativity, excellent chemical strength and stability.

Table 2: The GCRD parameter of the EPP

Molecule	E_{HOMO} (eV)	E_{LUMO} (eV)	ΔE (eV)	η	μ	S	ω
EPP	5.023	2.344	2.679	1.339	3.683	0.373	5.064

Molecular electrostatic potential (MEP) map of EPP was estimated using DFT with basis sets B3LYP/311G and re shown Fig.5b. The MEP plot give the knowledge about molecular shape and negative and positive charge distribution on molecules in terms of colour grading. In addition, MEP give the information bout internal charge transfer and favorable molecular geometries for chemical reaction. From Fig. 5b it is observed that nucleophilic site (blue colour) re mainly located in pyrazolone molecules and negative phase (red colour) mainly located on oxygen molecules. The neutral green colour spread over the molecule which indicate that molecule re chemical stable long with molecules also have donor acceptor groups. Further, variance in the MEP surfaces shows n increase in the positive potential due to the proton environment in the molecule.

- **In vitro antimicrobial activity:** The newly synthesized EPP were subjected to antimicrobial studies. Interestingly the compounds exhibited very good antibacterial activity against *S.aureus, B.subtillis, S.typhi* nd *E.coli.* The better activity of the products could be due to the presence of biologically active pyrazole and propyl acetate group which resembles hydrogen with respect to steric requirements t enzyme receptor sites. The presence of propyl acetate enhances the rate of absorption by its increased lipid solubility. The high aliphophilic character of propyl acetate group is significant in improving pharmacological activity. The EPP antimicrobial data is given in Table 2.

- **In vitro antifungal activity:** The compound exhibited very good antifungal activity against *niger* nd *C. lbicans.* The reason for the excellent activity could be same s that of antibacterial activity. The EPP exhibited moderate activity towards fungal

organism. The value of the antifungal data is given in Table 2. and minimum inhibitory concentration (MIC) is given in the parenthesis.

- **Antioxidant activity:** The newly synthesized EPP showed moderate to excellent radical scavenging capacity t the tested concentration of 15 µg/mL in comparison with the standard edaravone (73.16) it shows 56.72. The variation exhibited in DPPH scavenging capacity could be attributed to the effect of substitutions in pyrazolo moiety. The antioxidant activity results re in the range s observed in the previous results [23].

Table 2: Antimicrobial and antifungal activity of the newly synthesized pyrazolones

Compound	***S.aureus***	***B. subtilis***	***S.typhi***	***E.coli***	***A.niger***	***C.albicans***
EPP	24(40)	21(40)	22(40)	23(40)	22(30)	25(40)
Standard[a]	24	23	23	25	25	24
Control[c] DMSO	0	0	0	0	0	0

Note

- Standard drug used: Bacteria (Ciprofloxacin), Fungal (Flucanazole) were in 40 µg in 100µL and R: Resistance
- Compounds used: (40 µg in 100µL) and zone of inhibition in mm, MIC in µg/mL given in parenthesis.
- Control: DMSO (Dimethyl sulphoxide)

IV. CONCLUSION

A highly functionalized novel pyrazolone derivatives (EPP) were synthesized by the reaction of Baylis-Hillman acetate with newly synthesized pyrazolones and studied their photophysical and biological activities. The molecular structural was confirmed using ^{1}H NMR, FT-IR and LCMS spectrometer. The photophysical parameters like dipole moment, HOMO-LUMO and MESP re estimated using DFT in gaussian-9w software and results re suggest the molecule has good optoelectronic properties. Further, newly prepared compound was screened for their *in-vitro* biological activities and results exhibited that, EPP has good antifungal, antibacterial and antioxidant applications.

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