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Design, Synthesis, Characterization, Docking studies and Antibacterial evaluation of Novel Bipyrazole derivatives

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ABSTRACT

Bis-heterocycles especially those containing pyrazole moiety display much better antibacterial activity than monoheterocycles. So, a new series of Bi pyrazole derivatives were synthesized by using 4-substituted acetophenone and phenyl hydrazine. The newly synthesized compounds were characterized by IR, ^{1}H NMR, and ^{13}C NMR analytical data. And evaluated for Anti-bacterial activity, based on the *in-silico* evaluation of docking study on Fabh using 4VB as a control inhibitor, among all the four compounds the 7b has shown more significance in the activity than the others. That have been confirmed by Anti-bacterial *in-vitro* study.

Keywords: Bipyrazoles, Fabh-*in silico,* Anti-bacterial activity

INTRODUCTION

Heterocyclic Chemistry

In organic chemistry, heterocyclic compounds comprise major part and given important attention to chemists because of two reasons. Firstly, in nature at least one or more of heterocyclic rings present in most of the organic compounds and display useful biological properties. Secondly, the metabolically important molecules are made of heterocyclic rings and hence development and delivery of drugs using the understanding and imitating their chemistry.

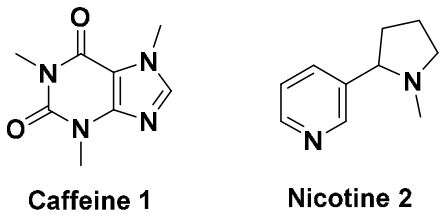

Figure 1

History of 1, 3-dipolar cycloaddition

The addition of a 1, 3-dipole to an alkene for the synthesis of five-membered rings is a classical reaction in organic chemistry. The 1, 3-dipolar cycloaddition (1, 3-DC) reactions are used for the preparation of molecules of fundamental importance for both academia and industry.

Basic aspects

A 1,3-dipole is defined as an "**a-b-c**" structure that undergoes 1,3-DC reactions and is portrayed by a dipolar structure as outlined in **Figure 2.**[1,2] Basically, 1,3-dipoles can be divided into two different types: the allyl anion type and the propargyl/allenyl anion type. The allyl anion type is characterized by four electrons in three parallel p_z orbital's perpendicular to the plane of the dipole and that the 1, 3-dipole is bent. Two resonance structures in which the three centers have an electron octet, and two structures in which "**a**" or "**c**" has an electron sextet, can be drawn. The central atom "**b**" can be nitrogen, oxygen, or sulfur

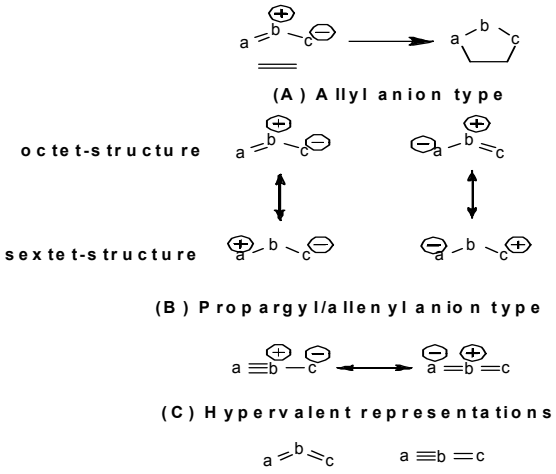

Figure 2. The basic resonance structure of 1, 3-dipoles

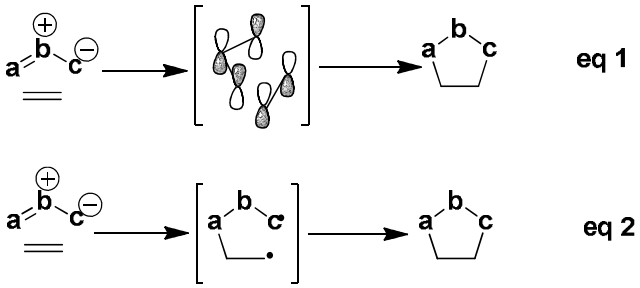

Figure 3

DOCKING STUDY

Major steps involved in molecular docking

Step 1-Building the receptor

In this step the 3D structure of the receptor should be downloaded from PDB and modified. This should include removal of water molecules from the cavity, stabilizing charges, filling in the missing residue, generation of side chain etc., according to the parameters available. After modification the receptor should be biologically active and stable.

Step 2-Identification of active site

After the receptor is build, the active site within the receptor should be identified. The receptor may have many active sites but the one of the interests should be selected.

Step 3-Ligand preparation

Ligand can be obtained from various databases like ZINC, PubChem or can be sketched using tools like chemsketch. While selecting the ligand, the Lipinski rule of 5 should be applied. The rule is very important for drug development where the pharmacologically active lead structure is optimized stepwise for increased activity and selectivity as well as drug like properties.

Step 4-Docking

This is a last step, where the ligand is docked into receptor and the interaction are checked. The scoring function generates depending on which ligand with best fit was selected.

Molecular docking study

In order to gain more insight on the binding mode of the compounds with Transferase docking studies using Auto Dock 4.0.1 were carried out. Top scoring molecules from the largest cluster were considered for interaction studies.

SCHEME OF WORK

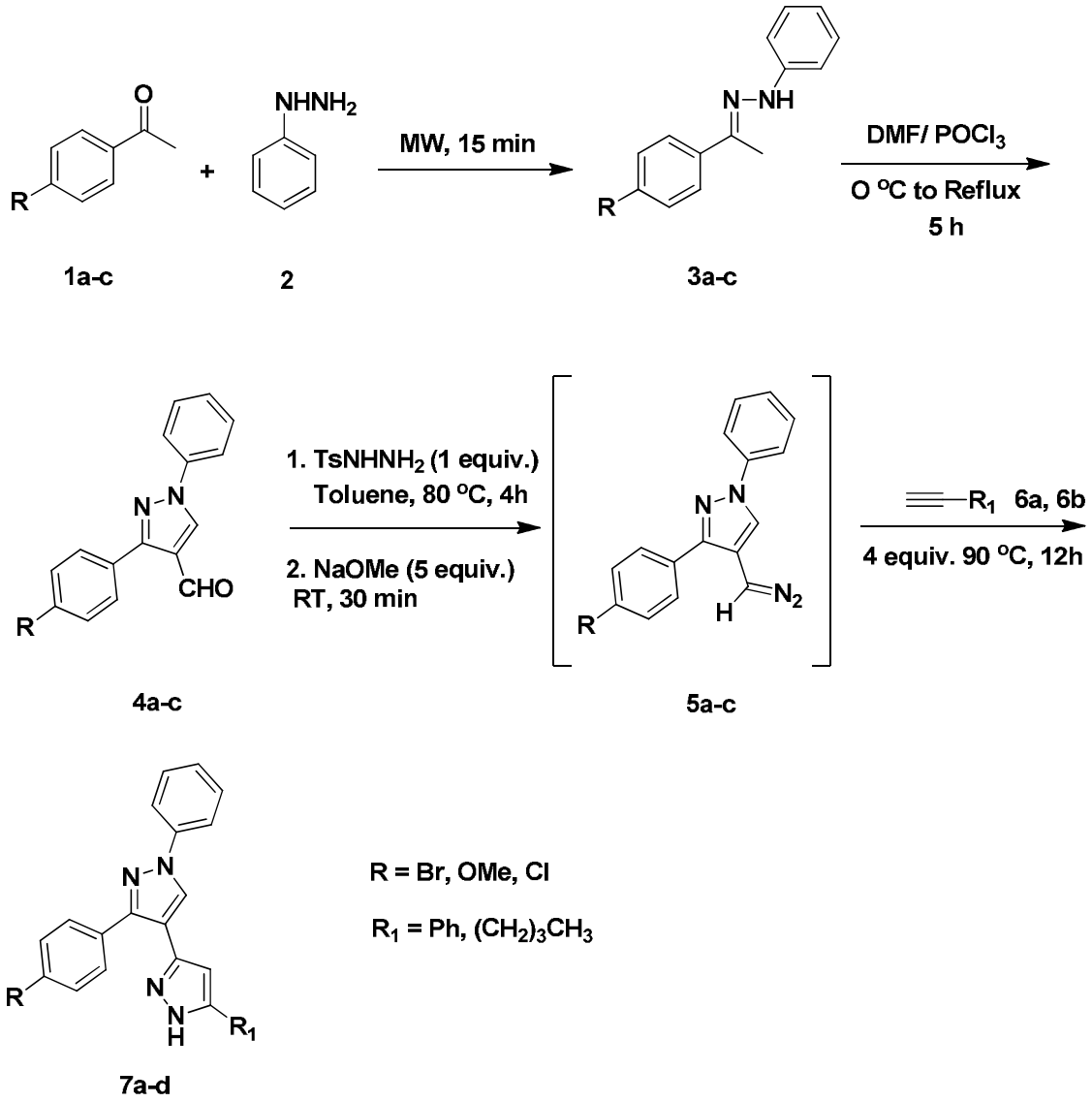

Figure 4

EXPERIMENTAL SECTION

General methods and materials

All chemicals and solvents were purchased from Sigma-Aldrich Pvt. Ltd. and Avra Synthesis Pvt. Ltd. Infrared (IR) spectra for all compounds were recorded as neat by Attenuated Total Reflectance (ATR) mode on a JASCO 6300 FTIR spectrometer. Column chromatography was performed using thick-walled glass columns along with a mixture of petroleum ether and ethyl acetate on silica gel (100-200 mesh, SRL, India). The relative proportions of solvents in chromatography solvent mixtures refer to the volume-to-volume ratio. Analytical TLC was performed on percolated plastic sheets of silica gel G/UV-254 of 0.2 mm thickness (Macherey-Nagel, Germany) using analytical grade solvents and visualized with iodine spray (10% w/w I_2 in silica gel), UV light (λ = 254 and 365 nm) and alkaline $KMnO_4$ solution. All melting points were measured on open capillaries and are uncorrected. ^1H and ^{13}C NMR spectra were recorded in deuterated chloroform

($CDCl_3$) using Bruker spectrometer (^{1}H NMR: 500 MHz; ^{13}C NMR: 125 MHz). Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as an internal standard and are expressed in parts per million (ppm). The number of protons (n) for a given resonance is indicated as NH. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiple). Coupling constants (*J*) are given in hertz (Hz).

Prediction of ADMET

The designed compounds in the drug library were subjected to preADMET software for the prediction of ADMET and pharmacokinetic properties. It is an important CADD tool by which the adsorption, distribution, metabolism, excretion, and toxicity of the designed molecules were analyzed. Hereby, determining this factor helpful for the prediction of the designed molecule were orally active or not [25-27]. Along with bidirectional approach on CaCo-2 (human colon carcinoma cell line and MDCK (Mardin-Darby-canine kidney) for the prediction of cellular permeability (endothelial and epithelial cells) lead to intestinal absorption and efficient for active transport were investigated [28-29]. On the other hand, toxicity prediction by drug conception on carcinogenicity and mutagenicity were also determined [30].

CHARACTERIZATION OF COMPOUNDS

Spectral data for synthesized compounds

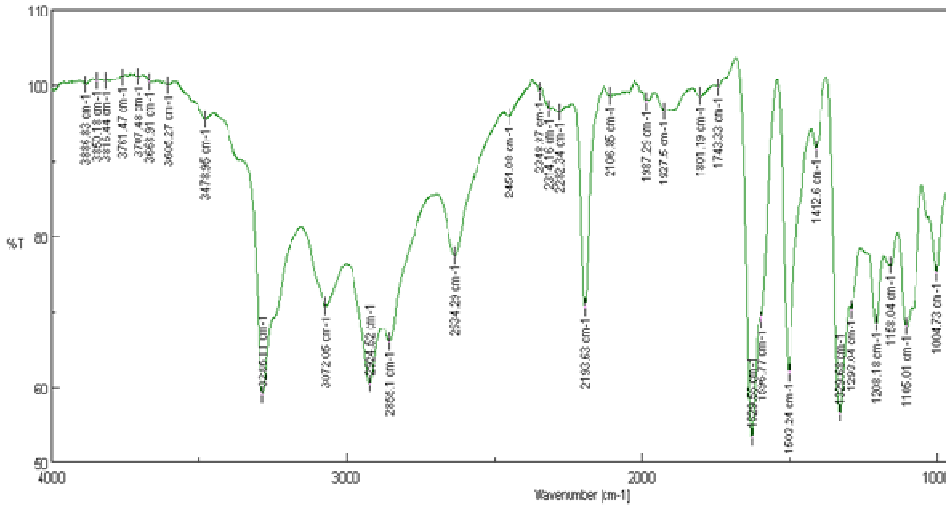

Figure 5IR spectrum of 3'-(4-bromophenyl)-1', 5-diphenyl-1H, 1'H-3, 4'-bipyrazole (**7a**)

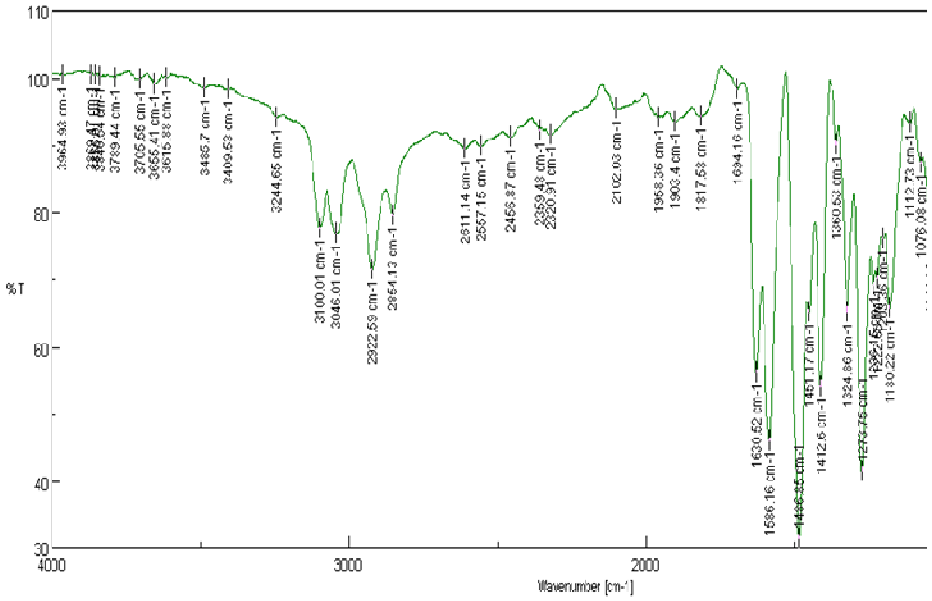

Figure 6 IR spectrum of 3'-(4-methoxyphenyl)-1', 5-diphenyl-1H, 1'H-3, 4'-bipyrazole (7b)

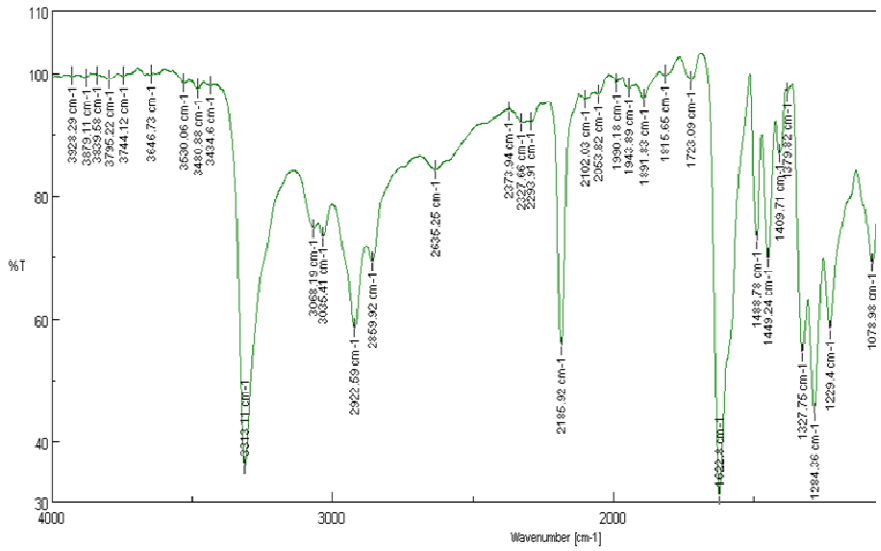

Figure 7 IR spectrum of 3'-(4-chlorophenyl)-1', 5-diphenyl-1H, 1'H-3, 4'-bipyrazole**(7c)**

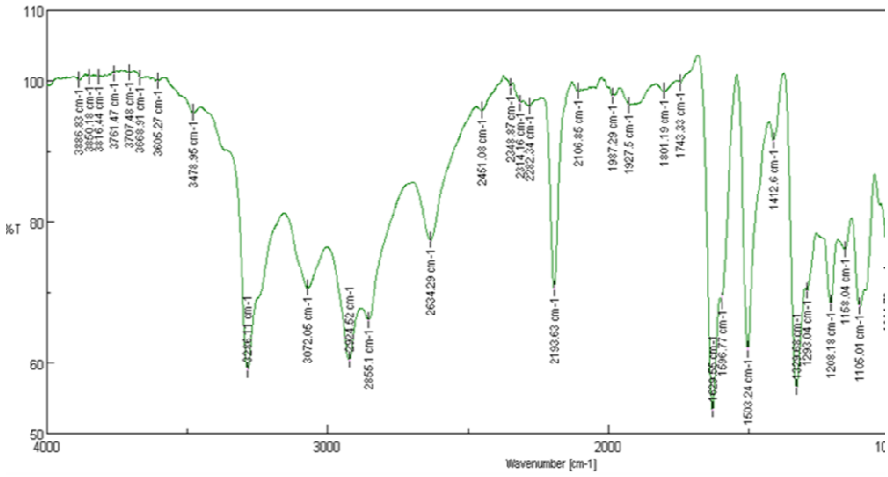

Figure 8 IR spectrum of 5-butyl-3'-(4-chlorophenyl)-1'-phenyl-1H, 1'H-3, 4'-bipyrazole (**7d**)

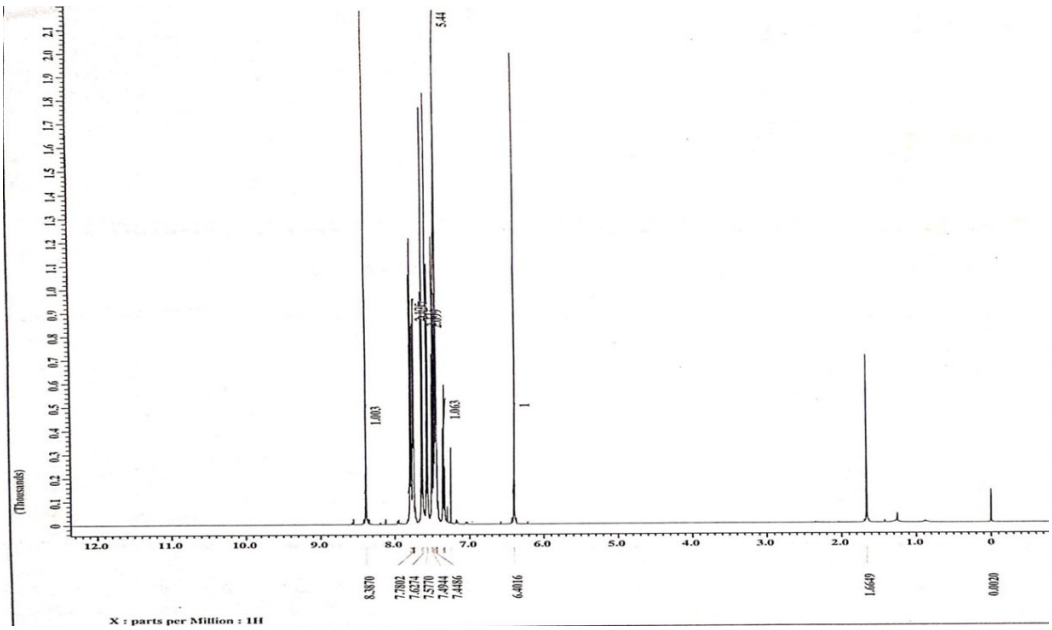

Figure 9 ^{1}H NMR spectrum of 3'-(4-bromophenyl)-1', 5-diphenyl-1H, 1'H-3, 4'-bipyrazole (**7a**)

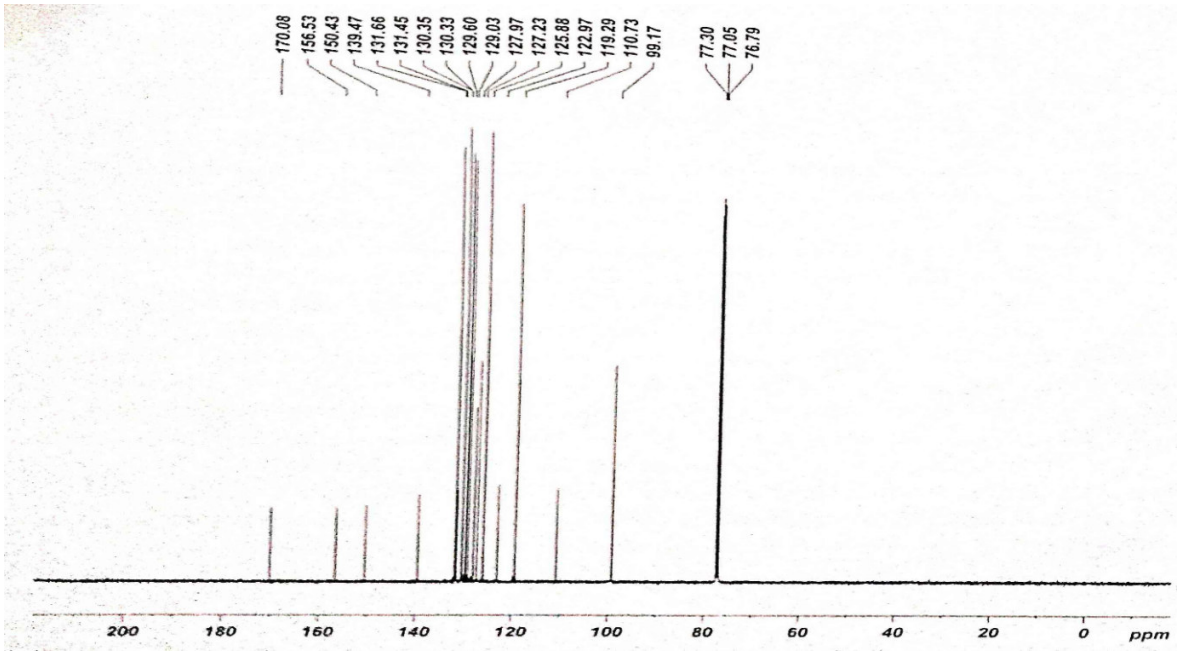

Figure 10 ^{13}C NMR spectrum of 3'-(4-bromophenyl)-1',5-diphenyl-1H,1'H-3,4'-bipyrazole (**7a**)

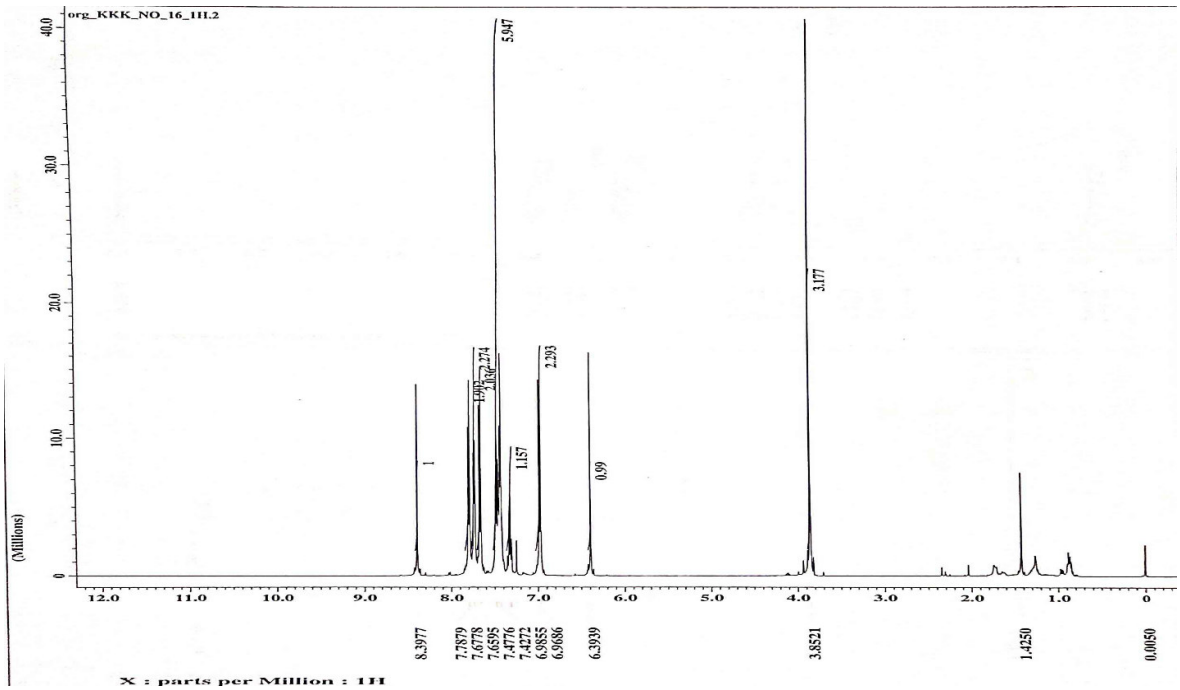

Figure 11 ^{1}H NMR spectrum of 3'-(4-methoxyphenyl)-1',5-diphenyl-1H,1'H-3,4'-bipyrazole (**7b**)

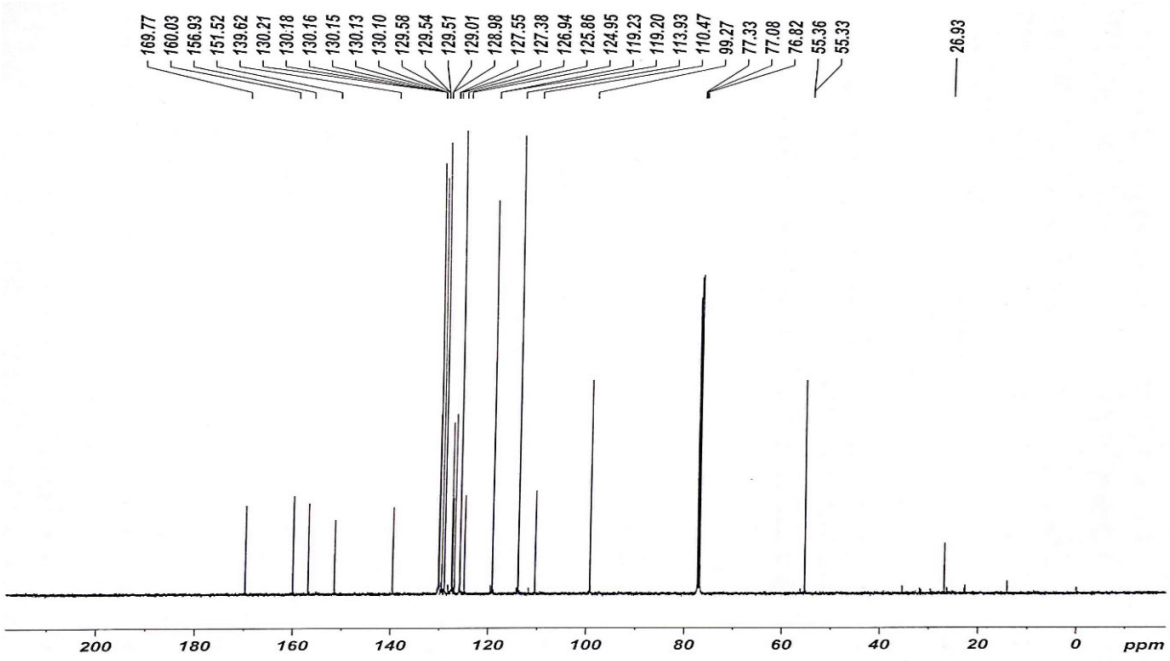

Figure 12[13]C NMR spectrum of 3'-(4-methoxyphenyl)-1',5-diphenyl-1H,1'H-3,4'-bipyrazole (**7b**)

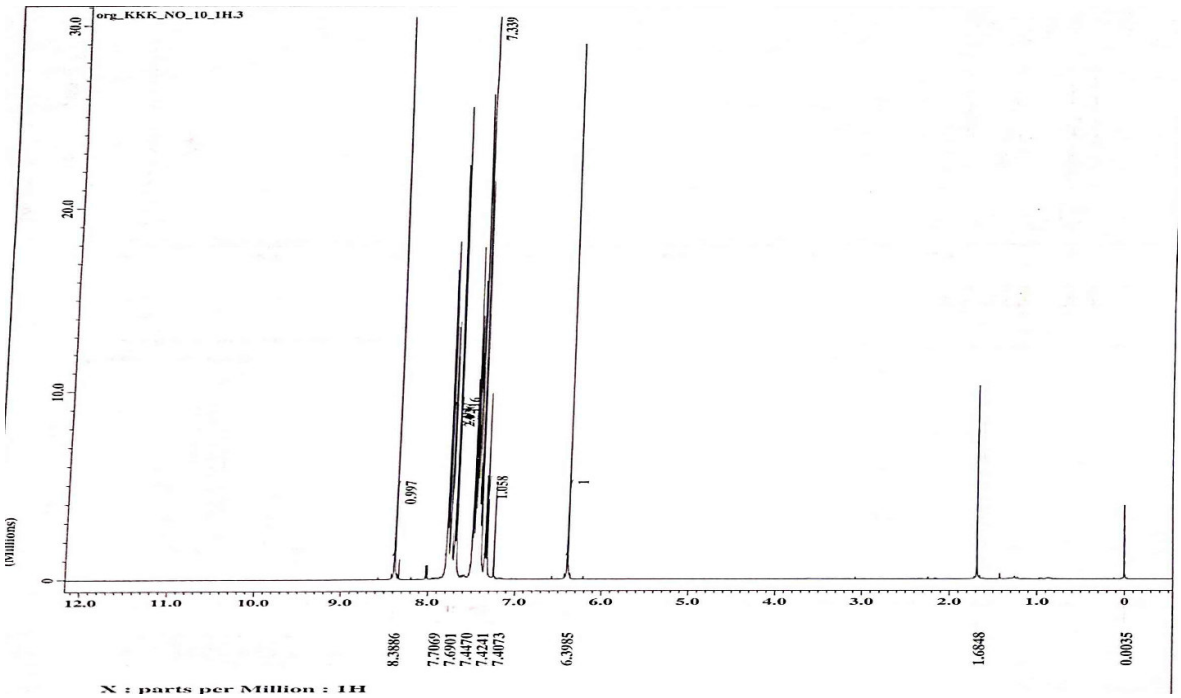

Figure 13[1]H NMR spectrum of 3'-(4-chlorophenyl)-1',5-diphenyl-1H,1'H-3,4'-bipyrazole (**7c**)

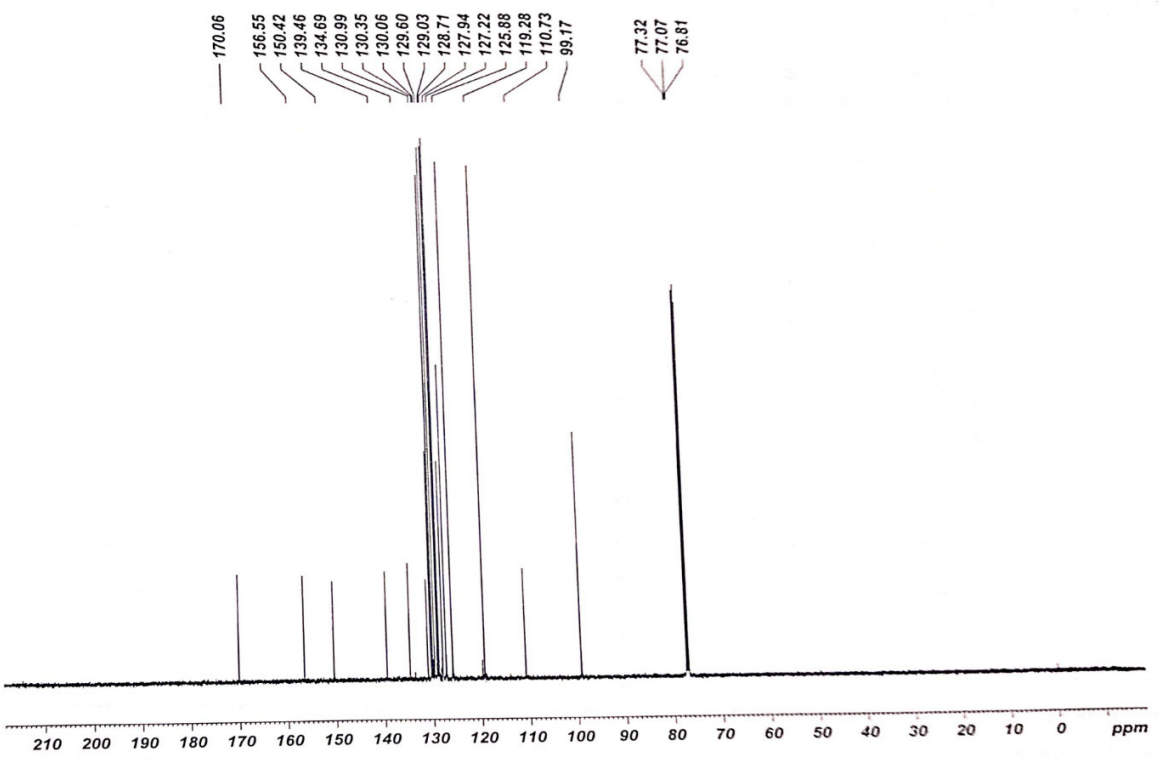

Figure 14 ^{13}C NMR spectrum of 3'-(4-chlorophenyl)-1',5-diphenyl-1H,1'H-3,4'-bipyrazole **(7c)**

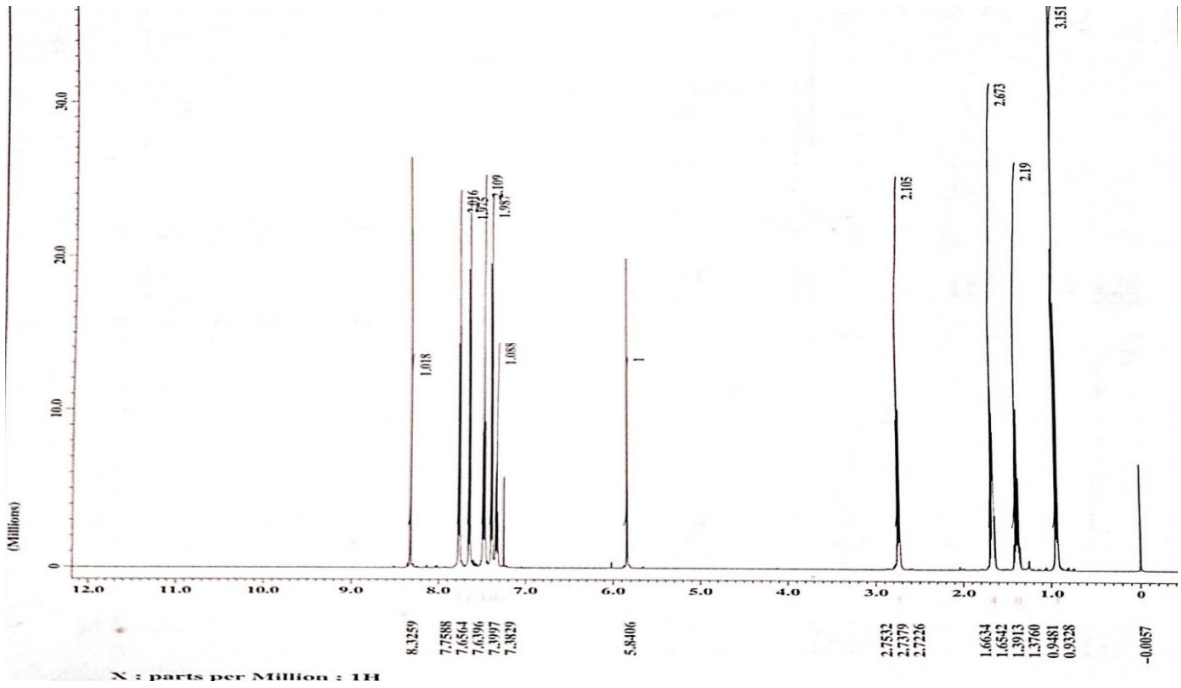

Figure 15^{1}H NMR spectrum of 5-butyl-3'-(4-chlorophenyl)-1'-phenyl-1H,1'H-3,4'-bipyrazole (**7d**)

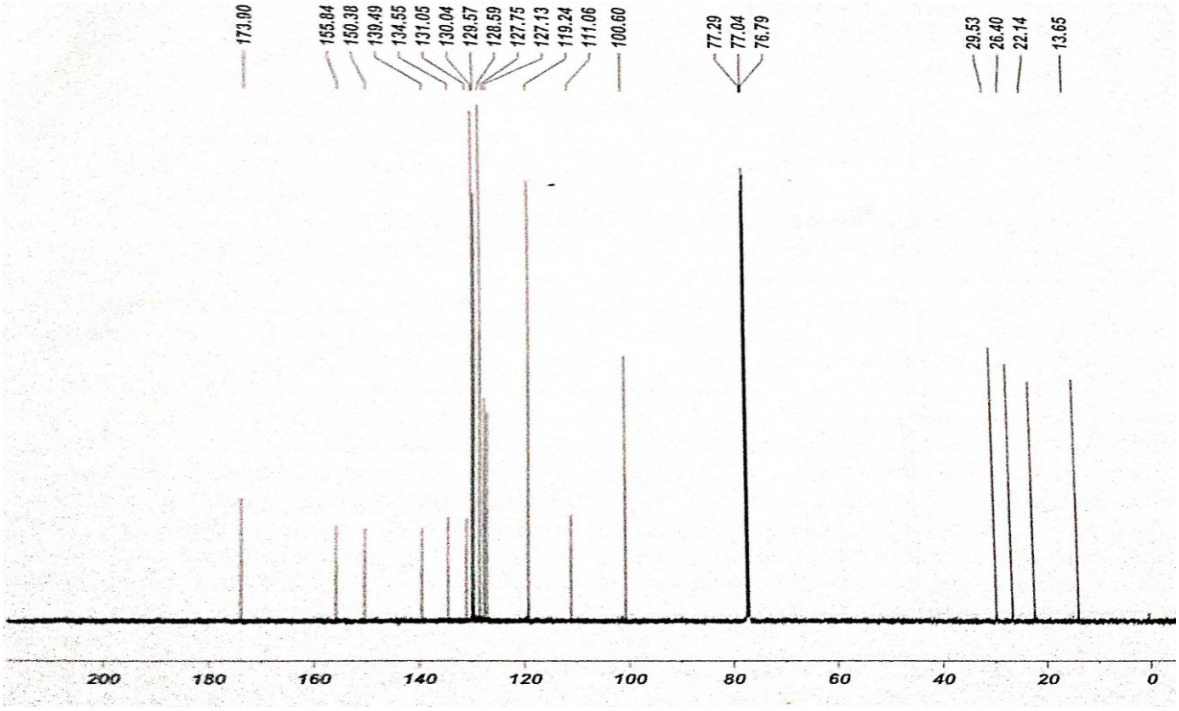

Figure 16 ^{13}C NMR spectrum of 5-butyl-3'-(4-chlorophenyl)-1'-phenyl-1H,1'H-3,4'-bipyrazole (**7d**)

CHARACTERIZATION

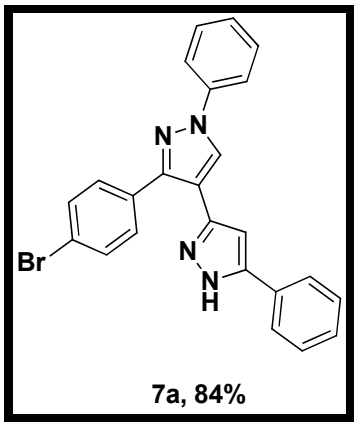

Figure 17: 3'-(4-bromophenyl)-1',5-diphenyl-1*H*,1'*H*-3,4'-bipyrazole (7a)

Colourless solid; mp 148-150 ºC; IR (cm^{-1}): 3055,3072,2924,2855,1630; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.38 (1H, s), 7.79 (2H, d, *J* = 7.7 Hz), 7.75 (2H, dd, *J* = 7.6, 1.5 Hz), 7.64 (2H, d, *J* = 8.4 Hz), 7.57 (2H, d, *J* = 8.4 Hz), 7.43-7.51 (5H, m), 7.34 (1H, t, *J* = 6.9 Hz), 6.40 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1, 156.5, 150.4, 139.5, 131.7, 131.5, 130.4, 130.3, 129.6, 129.0, 127.9, 127.3, 125.9, 122.9, 119.3, 110.7, 99.2.

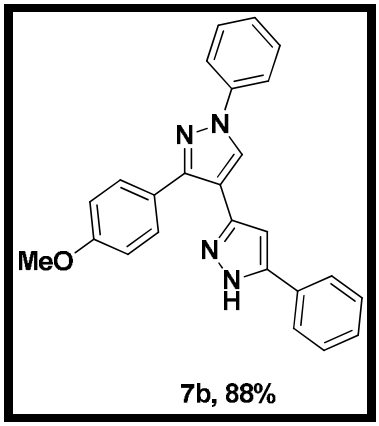

Figure 18: 3'-(4-methoxyphenyl)-1',5-diphenyl-1*H*,1'*H*-3,4'-bipyrazole (7b)

Colourless solid; mp 132-134 ºC; IR (cm^{-1}): 3100,3046,2922,2864,1673,; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.39 (1H, s), 7.79 (2H, d, *J* = 8.4 Hz), 7.74 (2H, d, *J* = 7.7 Hz), 7.67 (2H, d, *J* = 9.2 Hz), 7.42 – 7.49 (5H, m), 7.32 (1H, t, *J* = 6.9 Hz), 6.98 (2H, d, *J* = 8.5 Hz), 6.39 (1H, s), 3.85 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.8, 160.0, 156.9, 151.5, 139.6, 130.2, 130.1, 129.5, 129.0, 127.6, 127.4, 126.9, 125.9, 124.9, 119.2, 113.9, 110.5, 99.3, 55.4.

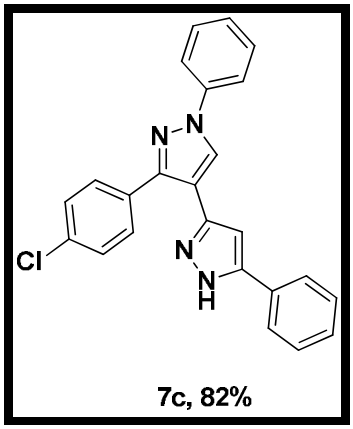

Figure 19: 3'-(4-chlorophenyl)-1',5-diphenyl-1*H*,1'*H*-3,4'-bipyrazole (7c)

Colourless solid; mp 110-112 ºC; IR (cm^{-1}): 3313,3068,3036,2856,1625; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.39 (1H, s), 7.79 (2H, d, *J* = 7.7 Hz), 7.74 – 7.76 (2H, m), 7.70 (2H, d, *J* = 8.4 Hz), 7.40-7.51 (7H, m), 7.35 (1H, t, *J* = 7.7 Hz), 6.39 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1, 156.6, 150.4, 139.5, 134.7, 130.9, 130.4, 130.1, 129.6, 129.0, 128.7, 127.9, 127.2, 125.9, 119.3, 110.7, 99.2.

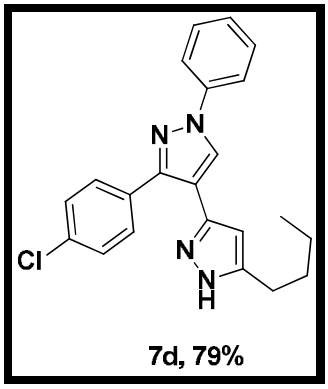

Figure 20: 5-butyl-3'-(4-chlorophenyl)-1'-phenyl-1*H*,1'*H*-3,4'-bipyrazole (7d)

Colourless solid; mp 94-96 ºC; IR (cm^{-1}): 3276,3072,2924,2856,1626; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.32 (1H, s), 7.76 (2H, d, *J* = 7.7 Hz), 7.65 (2H, d, *J* = 8.4 Hz), 7.48 (2H, t, *J* = 7.7 Hz), 7.39 (2H, d, *J* = 8.5 Hz), 7.33 (1H, t, *J* = 7.6 Hz), 5.84 (1H, s), 2.73 (2H, t, *J* = 7.7 Hz), 1.64-1.68 (2H, m), 1.38 (2H, sextet, *J* = 6.9 Hz), 0.93 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.9, 155.8, 150.4, 139.5, 134.6, 131.1, 130.0, 129.6, 128.6, 127.8, 127.1, 119.2, 111.1, 100.6, 29.5, 26.4, 22.1, 13.7.

DOCKING STUDIES

Figures and tables (significant compounds)

Docked pose of Compound 7b and 5BNS binding site

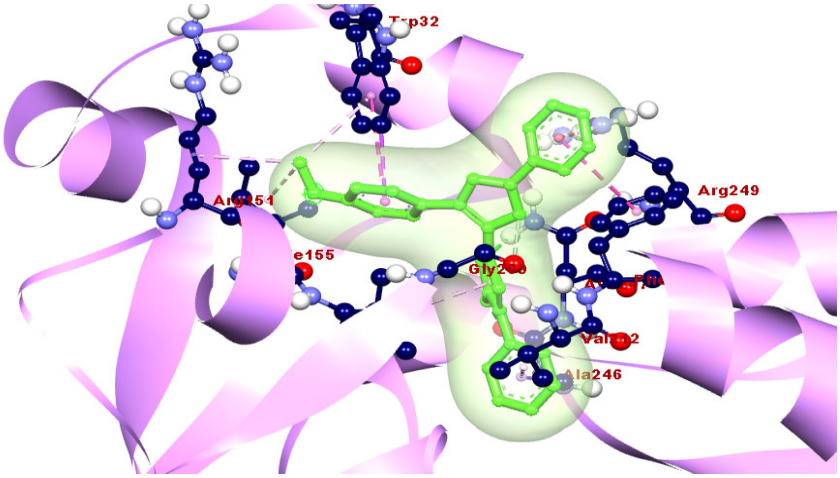

Figure 21: Hydrogen bond interactions on Compound 7b and 5BNS (green dashed lines) and Hydrophobic bonds (Pink dashed lines) and other amino acid residues

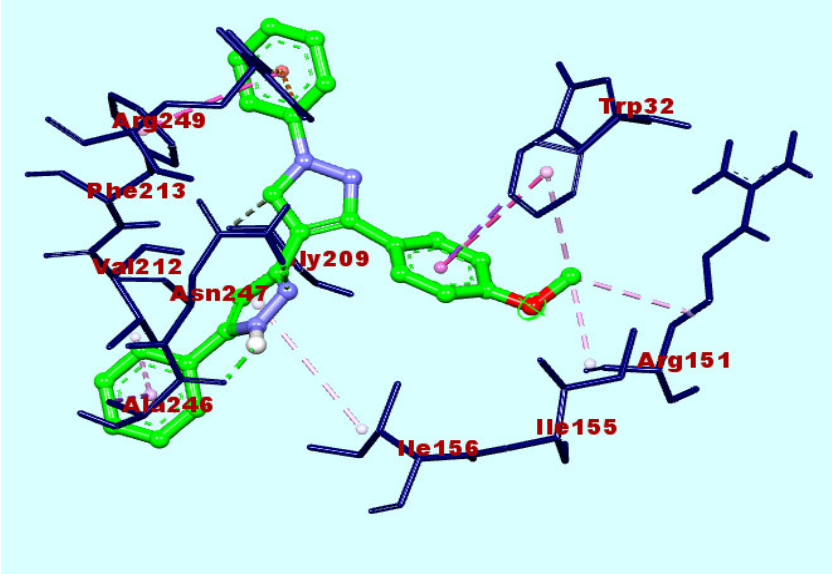

Figure 22

Table 1. Docking score of synthesized compounds (7a-7d) on 5NBS

Ligands	Docking score (kcal/mole) (5BNS)
7a	-8.66
7b	**-8.88**
7c	-8.52
7d	-8.29
Control Inhibitor – 4VM	-9.56

4VM = 1-{5-[2-fluoro-5-(hydroxymethyl) phenyl] pyridin-2-yl}-N-(quinolin-6-ylmethyl) piperidine-4-carboxamide)

Table.2 ADME (Absorption, Distribution, Metabolism, Excretion) properties and drug-likeness scores of the synthesized compounds

Compound code	BBB[a]	PPB [b]	HIA [c]	CaCo-2 [d]	MDCK [e]
7a	12.96	100.00	94.78	49.93	0.17
7b	9.09	100.00	94.55	34.32	0.97
7c	12.79	100.00	94.63	50.35	3.22
7d	13.10	100.00	94.28	50.92	1.46

[a] Blood-Brain Barrier penetration, [b] Plasma Protein Binding, [c] Human Intestinal Absorption, [d] Caco-2 cell permeability, [e] MDCK cell permeability

Table.3. Toxicity prediction of synthesized compounds

Compound code	Ames test mutagenicity	Mouse carcinogenicity	Rat carcinogenicity
7a	Mutagen	Positive	Positive
7b	Non-mutagen	Negative	Negative
7c	Mutagen	Positive	Negative

7d	mutagen	Positive	Negative

IN VITRO ANTIBACTERIAL STUDY

Methods for antimicrobial stability test

The standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial activity. *Escherichia coli*, aurous was inoculated in nutrient broth and incubated at overnight. After overnight incubation, the bacterial culture was swapped into solidified Muller Hinton agar plate and made wells of 6mm diameter were punched in the plate. Add different concentration of compounds, was dispensed in separate wells. The plates were incubated at 37° c for 24 hour. After incubation, the diameter of zone of inhibition around the wells were measured and recorded.

Standard drug used: Ciprofloxacin
Solvent used: DMSO

Table4: Antibacterial study of Bipyrazole compound

Micro organisms	Compounds	250µg/ml	500µg/ml	1000µg/ml	Ciprofloxacin (1 mg/ml)
	7(a)	15mm	16mm	18mm	25
	7(b)	14mm	18mm	20mm	24
	7(c)	14mm	16mm	17mm	24
	7(d)	15mm	16mm	18mm	25
Escherichiae Coli					

Antibacterial activity of 7(a-d) -in 250µg, 500µg, 1000µg, standard drug 1mg

RESULTS AND DISCUSSION

Synthetic methodology

A series of novel class of Synthesis of (3'-(4-substitutedphenyl)-1'-phenyl-5-substituted-1*H*,1'*H*-3,4'-bipyrazoles were synthesized from the mixtures of 4-substituted acetophenone, phenyl hydrazine, by microwave irradiation method followed by conventional method with good yield (between 80-90%)

Characterization of synthesized compounds

- The purity of synthesized compounds was confirmed by melting point and TLCUsing Cyclohexane and Ethyl acetate (80:20)as mobile phase.
- The structures of synthesized compounds were confirmed by FT-IR, ^{1}H-NMR,^{13}C-NMR spectral analysis results were correlated with the expected structure.

Docking study

The newly designed molecules are energy minimized and the resulting molecules are considered for docking analysis for action on Transferase enzyme using auto dock 4.0.1. Auto dock is employed to study the docking molecules within active site region of E.coliFabh with small molecules inhibitor -2 (PDB ID: 5BNS) interaction. Docking score and ADMET properties are represented in table 1and 2 among the studied compounds, compound 7b have highest binding score when compared with standard drugs ciprofloxacin.

AND found that the synthesized compounds have shown the significant score, among them 7b have shown more significance.

The designed compounds in the drug library were subjected to preADMET software for the prediction of ADMET and pharmacokinetic properties, determining this factor helpful for the prediction of the designed molecule were orally active or not for the prediction of cellular permeability (endothelial and epithelial cells) lead to intestinal absorption and efficient for active transport were investigated. By which the synthesized compounds were posses minimum permeability in the range of 0.1 to 3.22 were clearly depicted that those compounds were orally active drugs and enhances the bioavailability.

On the other hand, toxicity prediction by drug conception on carcinogenicity and mutagenicity were also determined. These titled compounds were possess middle absorption range as 9.0 to

13.10 values the toxicity prediction of synthesized compounds was mentioned, in which Ames test was incorporated to assessing the mutagenic property of the drug molecules. This test was generally used for the determination of bacteria in which the chemical molecule caused DNA mutations or not. By which the mutagenic property of synthesized molecules was predicted, in which 7b were nonmutagenic and existing are mutagenic to the Ames test were observed. Along with carcinogenicity of mouse and rat were predicted in which positive result was noted that those were no evidence of carcinogenicity *i.e.,* the compounds did not exhibit carcinogenic by nature.

In vitro antibacterial study

The antibacterial properties of the Bipyrazole compound are identified by disk diffusion method by using ciprofloxacin as standard drug. The results indicate that, the title compound showed inhibition of growth against tested microorganisms significantly, among all the compound 7(b) shows more significance.

CONCLUSION

The present study deals with designing of 3'-(4-substituted)-1'-phenyl-5-substituted- 1*H*,1'*H*-3,4'-bipyrazolesDerivatives on the basic reaction between 4 – substituted acetophenone (1a-c, 1.0m mol), phenyl hydrazine (2,1.0mmol). The synthesized compound were characterized by IR, ^{1}H NMR, ^{13}C NMR. All the compounds obey the Lipinski rule of 5. Solubility characters of synthesized compounds were carried out by using various solvents. The tested compounds are freely soluble in DMSO, soluble in Chloroform and Methanol, slightly soluble in Acetone, Ethyl Acetate and Ethanol and Insoluble in Water. The Structures of the synthesized compounds were studied using Auto dock software against target enzymes. The docking results showed that Compounds 7a, 7b, 7c, 7d were found to have significant binding score against target enzyme E.coliFabh compared to standard control Inhibitor 4VM.

REFERENCE

[1]. Padwa, A. In Comprehensive Organic Synthesis; Trost, B. M., Flemming, I., Eds.; Pergamon Press: Oxford, 4, 1991, 1069.
[2]. Wade, P. A. In Comprehensive Organic Synthesis; Trost, B. M., Flemming, I., Eds.; Pergamon Press: Oxford, 4, 1991, 1111.
[3]. Worrell B. T., Malik J. A and Fokin V. V., Direct Evidence of a Dinuclear Copper Intermediate in Cu(I)-Catalyzed Azide-Alkyne Cycloadditions. Science, 340, 2013, 457-460.
[4]. Horne W.S., Stout C. S., Ghadiri M. R. A Heterocyclic Peptide Nanotube. *J Am ChemSoc* 125, 2003, 9372-9376.
[5]. EisensteinM. Drug development: holding out for reinforcements.*Nature*. 484(7395), 2012, S16-8.
[6]. ZhuS, WangJ, ChandrasekharG, SmithE, LiuX, ZhangY. Synthesis and evaluation of 4-quinazolinone compounds as potential antimalarial agents. *EurJMedChem*. 45(9), 2010, 3864-9.
[7]. ZhuS, ChandrasekharG, MengL, RobinsonK, ChatterjiD. Febrifugine analogue compounds: synthesis and antimalarial evaluation.*BioorgMedChem.* 20(2), 2012, 927-32.
[8]. SchuckDC, JordãoAK, NakabashiM, CunhaAC, FerreiraVF, GarciaCRS. Synthetic indole and melatonin derivatives exhibit antimalarial activity on the cell cycle of the human malaria parasite Plasmodium falciparum.*EurJMedChem.* 78, 2014, 375-82.
[9]. MishraM, MishraVK, SengerP, PathakAK, KashawSK. Exploring QSAR studies on 4-substituted quinazoline derivatives as antimalarial compounds for the development of predictive models. *MedChemRes*. 23(3), 2014, 1397-405.
[10]. JoshiA, Viswanathan.Anti-infect. Agents.*J MedChem.* 5, 2006, 105.
[11]. DahlgrenMK, GarciaAB, HareAA, Tirado-RivesJ, LengL, BucalaR, JorgensenWL. Virtual screening and optimizationyieldlow-nanomolarinhibitors of the tautomeraseactivity of Plasmodium falciparummacrophage migration inhibitory factor. *JMedChem.* 55(22), 2012, 10148-59.

[12]. KaurK, JainM, KhanSI, JacobMR, TekwaniBL, SinghS, SinghPP, JainR. Extended side chain analogues of 8-aminoquinolines: synthesis and evaluation of antiprotozoal, antimicrobial. β-hematin inhibition and cytotoxic activities. *J MedChemComm*. 2, 2011, 300-7.
[13]. CasagrandeM, BasilicoN, RusconiC, TaramelliD, SparatoreA. Synthesis, antimalarial activity, and cellular toxicity of new arylpyrrolylaminoquinolines.*BioorgMedChem*. 18(18), 2010, 6625-33.
[14]. CurtiusT. Ueber dies Einwirkung von salpetrigerSäure auf salzsaurenGlycocolläther. BerDtschChemGes. 16(2), 1883, 2230-1.
[15]. BuchnerE. Einwirkung von Diazoessigäther auf die AetherungesättigterSäuren.*BerDtschChemGes*. 21, 1988, 2637.
[16]. BuchnerE, FritschM, PapendieckA, WitterH. Synthese der pyrazoline-3, 5-dicarbonsäure. LiebigsAnnChem. 273, 1993, 214.
[17]. BeckmannE, von PyrrolderivatenNB. BerDtschChemGes. 23, 1990, 3331.
[18]. HoukKN, Gonzalez J, LiY. Pericyclicreactiontransitionstates: passions and punctilios, 1935-1995. AccChemRes. 28(2), 1995, 81-90.
[19]. DielsO, AlderK.LiebigsAnnChemB; 460:98.
[20]. HuisgenR. In: PadwaA, editor. 1,3-Dipolar cycloadditionchemistry. Vol. 1.New York: Wiley; 1984, 1.
[21]. RidealEK. Ozone.London: Constable and Co. LTD;1920.
[22]. WolfmanDS, LinstrumelleG, CooperCF. Thechemistry of diazonium and diazogroups.New York: John Wiley & Sons;1978.
[23]. HuisgenR. 1.3-Dipolare Cycloaddition.AngewChem. 75(13), 1963, 604-37.
[24]. WoodwardRB, HoffmannR. The conservation of orbitalsymmetry.Weinheim: VerlagChemie;1970.
[25]. Ma, X.; Chen, C.; Yang, J. Predictive model of blood-brain barrier penetration of organic compounds. *Acta Pharm. Sin*., *26*, 2005, 500–512.
[26]. Mannhold, R. Molecular Drug Properties: Measurement and Prediction; Wiley-VHC Verlag GmbH & Co. KGaA: Weinheim, Germany, 2008.
[27]. Zhao, Y.H.; Le, J.; Abraham, M.H.; Hersey, A.; Eddershaw, P.J.; Luscombe, C.N.; Boutina, D.; Beck, G.; Sherborne, B.; Cooper, I.; et al. Evaluation of human intestinal absorption data and subsequent derivation of a Quantitative Structure-Activity Relationship (QSAR) with the abraham descriptors. *J of Pharma Sci*., *90*(6), 2001, 749–784.
[28]. Hou, T.J.; Zhang,W.; Xia, K.; Qiao, X.B.; Xu, X.J. ADME Evaluation in drug discovery. 5. correlation of caco-2 permeation with simple molecular properties. *Jl of ChemlInfor and Comp Sci*., *44*(5), 2004, 1585–1600.
[29]. Irvine, J.D.; Takahashi, L.; Lockhart, K.; Cheong, J.; Tolan, J.W.; Selick, H.E.; Grove, R. MDCK (Madin-Darby Canine Kidney) cells: A tool for membrane permeability screening. *J of Pharma Sci*., *88*(1), 1999, 28–33.
[30]. Ames, B.N.; Gurney E.G.; Miller, J.A.; Bartsch, H. Carcinogens as frameshift mutagens: metabolites and derivatives of 2-acetylaminofluorene and other aromatic amine carcinogens. Proceedings of the National Academy of Sciences. *69*(11), 1972, 3128-32.
[31]. Parepally, J.M.; Mandula, H.; Smith, Q.R. Brain uptake of nonsteroidal anti-inflammatory drugs: ibuprofen, flurbiprofen, and indomethacin. *PharmaResea*, *23*(5), 2006, 873-81.