Synthesis, Characterization, Molecular Docking, and Biological Evaluation of Novel Schiff Base of Pyrazoline Derivatives as Potential Anti-Tubercular Agents

[1]Debaprasad Ghosh [2]Dr Kalpana Devi [3]Rakesh Panda

[1] Student Department of Pharmaceutical Chemistry, Al Ameen College of Pharmacy, Lalbagh Main Gate, Bangalore -27
[2] Professor Department of Pharmaceutical Chemistry, Al Ameen College Of Pharmacy, Lalbagh Main Gate, Bangalore- 27
[3] Associate Professor Department of Pharmaceutical Chemistry, Al Ameen College of Pharmacy, Lalbagh Main Gate, Bangalore- 27

Email: [1]bubaipharma1@gmail.com, [2]Kalpanadevi6@gmail.com, [3]rakeshpanda555@gmail.com

Corresponding Address
Debaprasad Ghosh
Al-Ameen College of Pharmacy
Housur Road (Near lal bagh Main Gate) Bangalore-560027
E-Mail: bubaipharma1@gmail.com

Abstract

In this present experiment, two new series of pyrazoline containing Schiff base were synthesized by reaction of p-amino acetophenone with iso nicotinyl chloride (1DKA1 to 1DKA3) and reaction between substituted benzaldehyde and para-amino benzaldehyde (2DKA1 to 2DKA8). The Claisen-Schmidt condensation reaction involving substituted aldehydes results in the formation of various substituted chalcones. These newly formed chalcones were cyclized with 99% pure hydrazine hydrate with the help of formic acid to form substituted pyrazoline containing carbaldehyde, which further reacted with primary amines to form the corresponding Schiff's bases. All the newly formed compounds were characterized by Infrared and Proton NMR, Carbon NMR, and MS spectra data, then further study shows the biological in-vitro studies, which are concerned with anti-tubercular MABA (Multiplate Alamar Blue assay) to detect activities of those novel Schiff base pyrazoline derivatives. The compound from the 2nd scheme showed a significant anti-tubercular activity(2DKA1).

Keywords: Pyrazoline, Schiff's base *in- silico* study. Anti-tubercular activity, MABA (Multiplate Alamar Blue Assay).

1. Introduction

Schiff bases derived from pyrazolines are significant pharmacologically active compounds and represent an important class of heterocyclic compounds due to their wide range of biological properties. These properties include antimicrobial, antitubercular, antithrombotic, antitumor, anti-inflammatory, antioxidant, and antidiabetic activities.[1]

In the running clinical scenario, the universal armamentarium of antitubercular drugs remains inadequate to address the rising populations of patients with multidrug-resistant and extensively drug-resistant Mycobacterium tuberculosis. As per the World Health Organization, tuberculosis (TB) is the second leading cause of mortality from an infectious disease. [2]

The limitations of the present anti-TB drugs are numerous, along with their drug resistance, the serious adverse effects, lack of drug efficacy, and the treatment of resistant strains that requires continuation treatments that employ more toxic drugs, increasing the economic load, which makes TB a vicious cycle. These factors necessitate the hunt for new antimycobacterial agents that are easy to synthesize and have low adverse effects. Therefore, it is unavoidable to discover new drug entities for the treatment of TB, which has spread globally. However, powerful new anti-TB drugs with a new mechanism of action have not been developed in the last 40 years. Despite severe toxicity on repeated dosing of isoniazid (INH), it is still considered to be a firstline drug for chemotherapy of tuberculosis.[3]

Schiff bases are prepared through the reaction between a carbonyl compound and a primary amine with glacial acetic acid (refer to samples 1DKA1 to 1DKA3 and 2DKA1 to 2DKA8). The Schiff bases of pyrazoline derivatives have been reported to exhibit various activities, including anti-tubercular, antioxidant, antimicrobial, anti-inflammatory, and antidiabetic effects. Pyrazoline derivatives are versatile compounds essential for synthesizing new organic intermediates in medicinal, pesticide, and coordination chemistry.[4]

Chalcones are precursor compounds of flavonoid biosynthesis in plants, and they can also be synthesized in the laboratory. Chalcones have a broad spectrum of biological activities, including antioxidative, antibacterial, anthelmintic, amoebicidal, antiulcer, antiviral, insecticidal, antiprotozoal, anticancer, cytotoxic, and immunosuppressive. Chalcone is a class of compounds that has many applications in different fields. Chalcone α, β-unsaturated carbonyls are used as key intermediates for the preparation of several heterocyclic compounds such as thiazine, oxazines, isoxazoles, pyrazoles, and pyrazoline. [5]

The term Schiff's base derives from the name of the German chemist Hugo Schiff, who, in 1864, was the first to describe the products resulting from the reaction of primary amines with carbonyl compounds. [6]

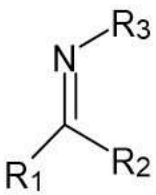


Figure 1. The Schiff bases, where R1, R2, and R3 are alkyl or aryl groups. R1 or /R2 can also be hydrogen.

Schiff bases are compounds characterized by a double bond between carbon and nitrogen[7]. Schiff bases can form a very good complex structure with the metal ions[8]. They act as L-type ligands, providing two-electron donors without electron changes in their valence shells[9]. The Schiff bases can be found in nature as well as can be synthesized in the laboratory. Schiff bases are known for their pharmacological activity and are used in food manufacturing, dye production, and analytical chemistry. They exhibit various biological activities, including antituberculosis, antifungal, antimalarial, antibacterial, and anti-inflammatory properties[10]. Schiff base synthesis is frequently performed by refluxing a mixture containing equimolar amounts of primary amines and aldehyde within non-aqueous organic solvents for 8-12 hours, which is catalysed by acids like glacial acetic acid.[11]

2. Result and Discussion

1. Chemistry

The introduction chapter of this article shows the information on the synthesis, activities, chalcone, and Schiff base of pyrazoline derivatives. As part of this work is to create new therapeutically active Schiff bases of pyrazoline, we have synthesized various substituted Schiff bases of pyrazoline derivatives with the help of two different types of schemes.

Scheme 1

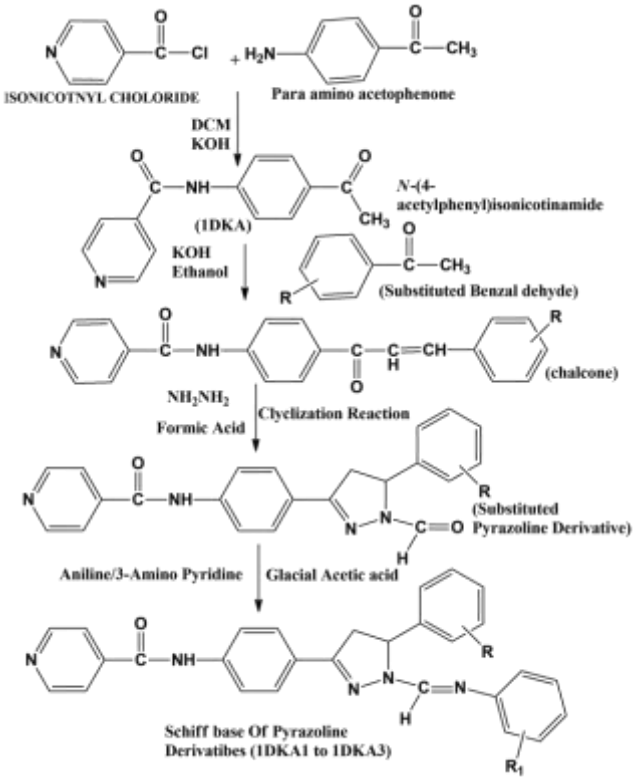
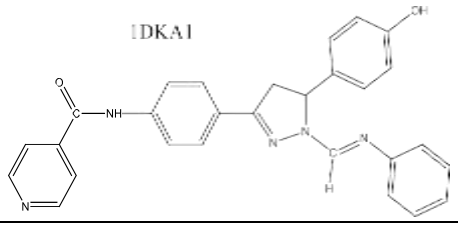
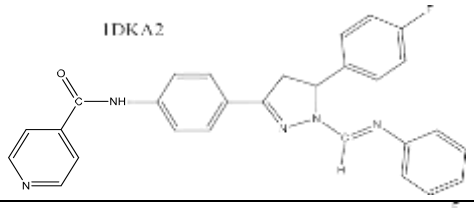
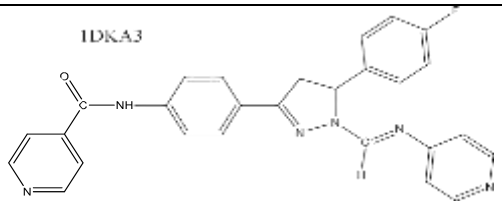


Table 1: Structure of synthesized Schiff Base of The Pyrazoline Derivatives

Compound code	Structure
1DKA1	
1DKA2	
1DKA3	

General method of preparation of the (1DKA) N-(4-acetyl-phenyl)-isonicotinamide (DKA) (Scheme 1) (Amide Intermediate)

Iso nicotinyl chloride was dissolved in dichloromethane (DCM) and stirred in a round-bottom flask placed in an ice bath. A solution of p-aminoacetophenone in DCM was subsequently added, followed by potassium hydroxide (KOH). The mixture was stirred for one hour. After stirring, it was cooled, and the product was filtered and recrystallized using isopropyl alcohol.

General Method of the synthesis of Synthesis of N- {4- [3-(substituted phenyl)-acryloyl]phenyl}-isonicotinamide (OH and F Substituted)

Equimolar quantity of and substituted aromatic benzaldehyde were dissolved in absolute alcohol and 40% KOH solution was added slowly with stirring. After complete addition, stirring was continued for 9hrs and kept overnight. The reaction mixture was poured in ice water and acidified with 10% HCL to obtain product. Crude product was crystallized from ethanol. Reaction was monitored by TLC using different ration of n-hexane and ethyl acetate.

Synthesis of N-(4-(5-(Substituted phenyl)-1-formyl-4,5-dihydro-1H-pyrazol-3-yl) phenyl) isonicotinamide

A solution of chalcones (0.008M), hydrazine hydrate (0.016M) in 4ml of formic acid was heated under reflux for 4-6 hrs, then poured into crushed ice. The solid was separated by

filtration, washed with water, and recrystallized from ethanol to obtain the respective pyrazoline derivatives.

Synthesis of Schiff base of the Pyrazoline containing Schiff base derivatives

The equivalent quantity of the Pyrazoline and primary amine (Aniline and 3-aminopyridine) react with each other in the presence of a few drops of Glacial Acetic acid under reflux conditions, the temperature should be maintained from 80 to 100 degrees centigrade for 6 to 8 hours.

N-(4-acetyl-phenyl)-isonicotinamide (DKA) (Scheme 1) (Amide Intermediate)

Physical Properties: Mol formula & weight: $C_{14}H_{12}N_2O_2$, 240 gm/mole, colour: Off white, Melting point 106 -108 c, Rf value 0.79,solubility-Methanol, DMSO, Percentage of yield 95%
IR (KBr)cm^{-1} : 3338 (NH str), 2765 (Ar -CH str), 1725 (C=O str), 1586 (C=N str), 1408(C=C Ar str).

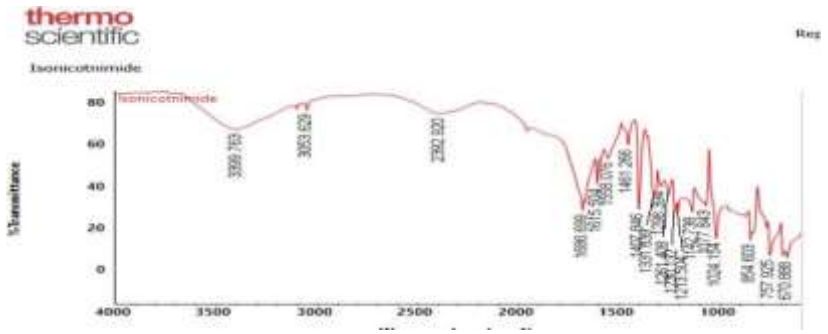


Fig 2: IR of N-(4-acetyl-phenyl)-iso nicotinamide (DKA) (Scheme 1) (Amide Intermediate)

Physical properties and Spectral Characterization of 1DKA1((E)-N-(4-(5-(4hydroxyphenyl)-1-((phenylimino)methyl)-4,5-dihydro-1H-pyrazol-3-yl) phenyl) isonicotinamide)
Physical Properties: Mol formula & weight: $C_{28}H_{22}N_5O_2$, 4643 gm/mole, colour: off white, melting point 196 -201 c, Rf value 0.74, solubility-Methanol, ethanol, DMSO, Percentage of yield 72%
IR(KBr)cm^{-1n}:3441.5cm^{-1}(OH-Str),3219.6cm^{-1}(N-HStr),2930.8,2833.9cm^{-1}(=CH=),1628.7cm^{-1}(C=N,867.4cm^{-1} (Aromatic C-H),**HNMR** (H-$_{23}$,5.1)(H-$_{21}$,10.45),(Pyrazole H-1,2 6.604)(H_{14}-$H1_{8-7}$.4.52),**CNMR** C-$_{17}$ 162.15, C-8 58.40.C-9 40.40, C-1to C-6 (120 to 150), **MS:** (m/z):363 [M$^+$]

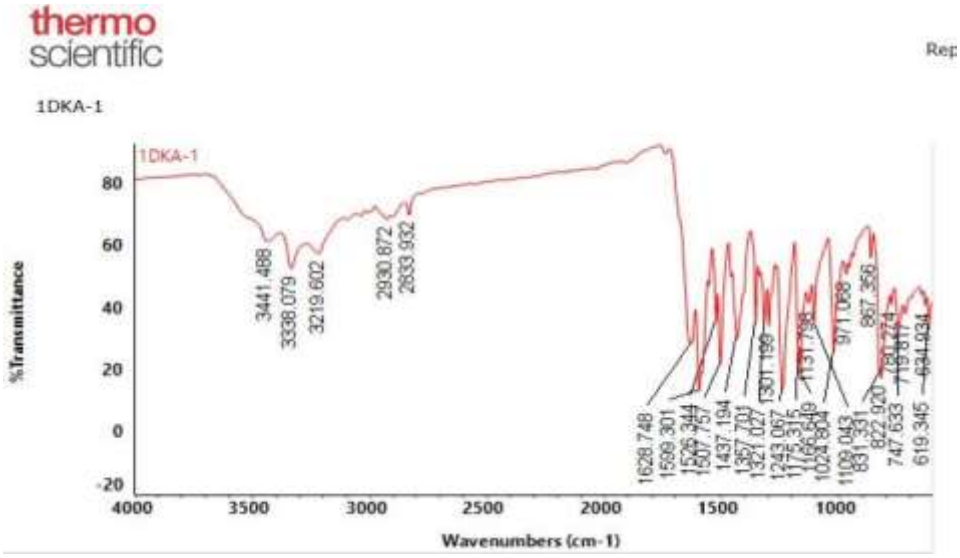


Fig:3 IR of (E)-N-(4-(5-(4-hydroxyphenyl)-1-((phenylimino)methyl)-4,5-dihydro-1H-pyrazol-3-yl) phenyl) isonicotinamide (1DKA2)

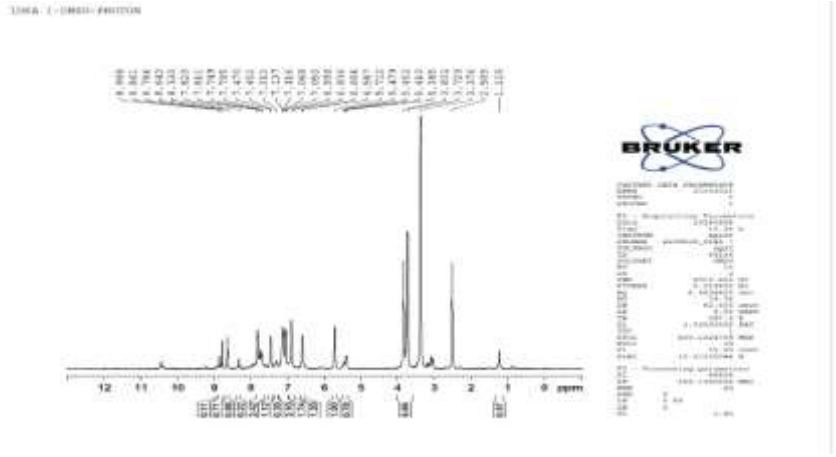


Fig: 4 HNMR of (E)-N-(4-(5-(4-hydroxyphenyl)-1-((phenylimino)methyl)-4,5-dihydro-1Hpyrazol-3-yl) phenyl) iso nicotinamide (1DKA1)

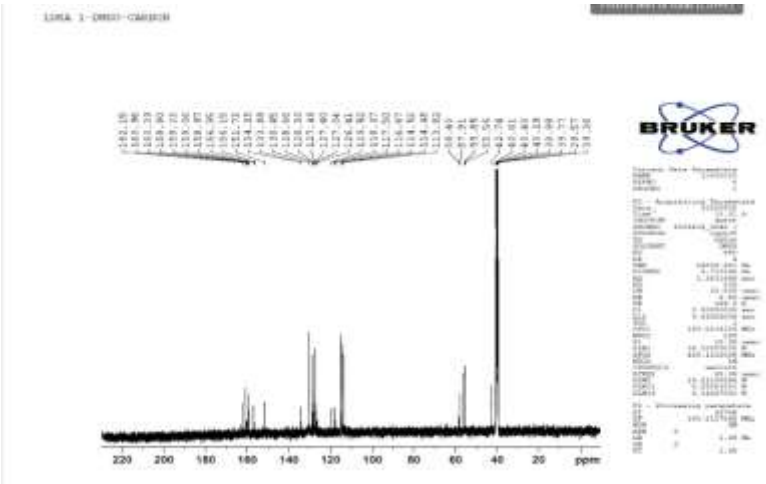


Fig: 5 CNMR of (E)-N-(4-(5-(4-hydroxyphenyl)-1-((phenylimino)methyl)-4,5-dihydro-1Hpyrazole-3-yl) iso nicotinamide(1DKA1)

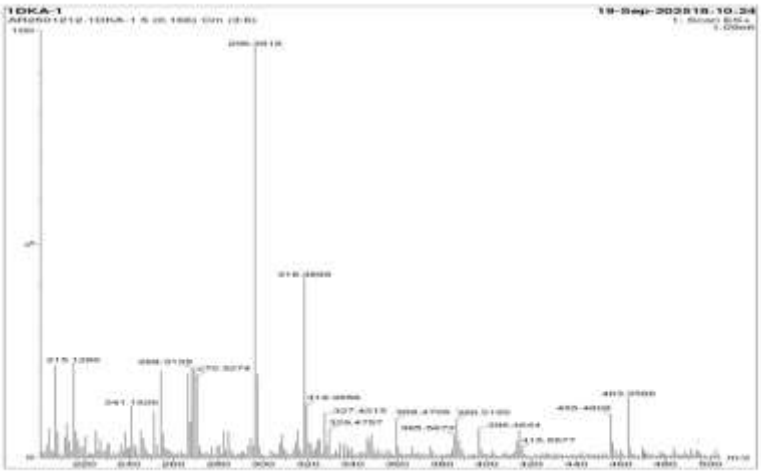


Fig:6 MASS spectra of (E)-N-(4-(5-(4-hydroxyphenyl)-1-((phenylimino)methyl)-4,5-dihydro-1H-pyrazol-3-yl) phenyl) iso nicotinamide(1DKA1)

Physical properties and Spectral Characterization of (1DKA2) ((E)-N-(4-(5-(4hydroxyphenyl)-1-((phenylimino)methyl)-4,5-dihydro-1H-pyrazol-3yl) phenyl) is nicotinamide)

Physical Properties: Mol formula & weight: $C_{28}H_{22}N_5O_2$,464 gm/mole, Colour: off white, Melting point 196 -205 c, Rf value 0.75, solubility-Ehanol, DMSO, percentage of yield 55%, **IR** 3340 and 3234 cm^{-1}(N- H),2921,2852cm^{-1}(C=H, C-H),1743cm^{-1} (N-H), 1691 cm^{-1} (N=H)1319-1064 cm^{-1} (Fluroride Ring)

Physical properties and Spectral Characterization of 1DKA3 ((E)-N-(4-(5-(4fluorophenyl)-1-((phenylimino)methyl)-4,5-dihydro-1H-pyrazol-3yl)phenyl)isonicotinamide)

Physical Properties: Mol formula & weight $C_{28}H_{22}FN_2O$,463 gm/mole, Colour: off white, Melting point 201 -211 c, Rf value 0.75, Solubility-DMSO, percentage of yield 55%, **IR:**3445cm^{-1} (N=H),3340cm^{-1}2928cm^{-1} (Ar-C-H,Ar-C=H),1691cm^{-1}(NH),1357cm^{-1}(Fluorine ring)

Scheme 2

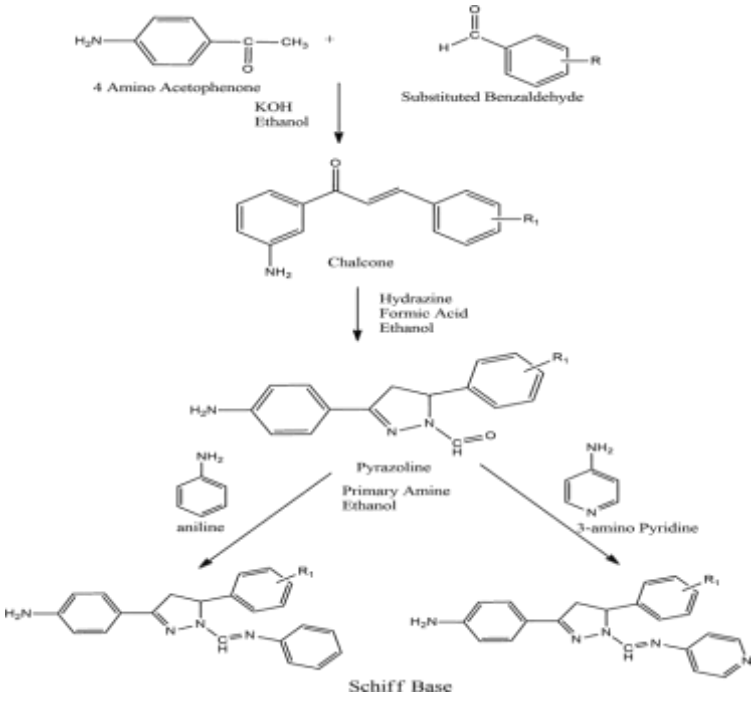
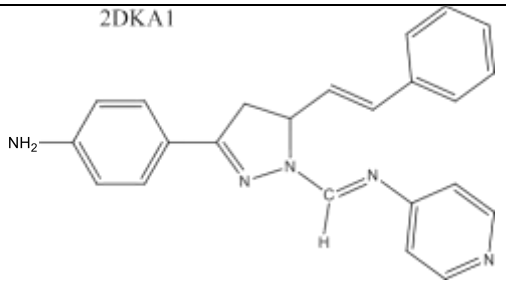
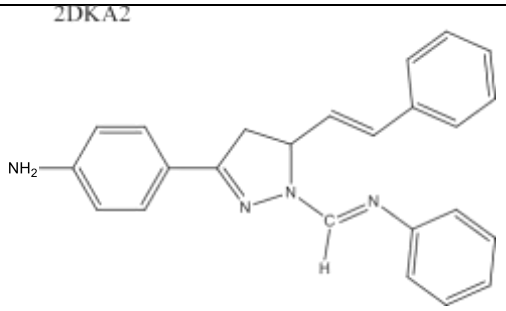
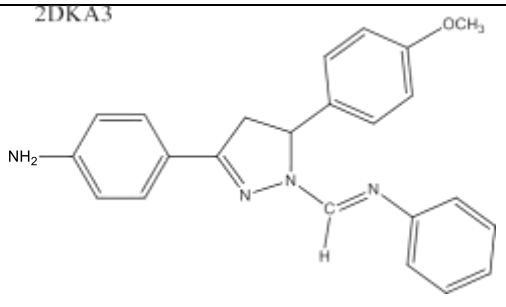
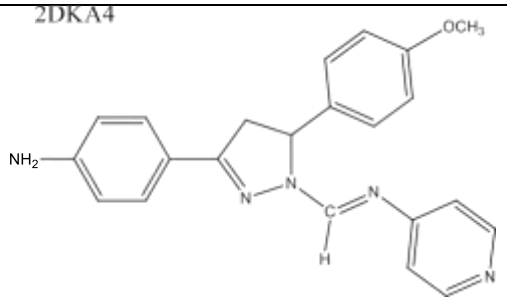
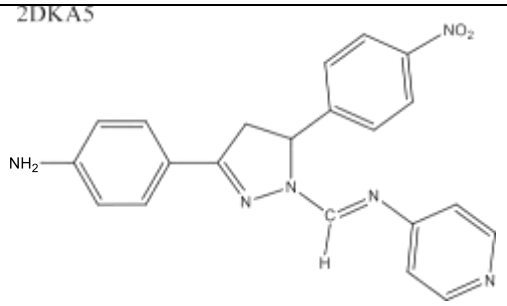
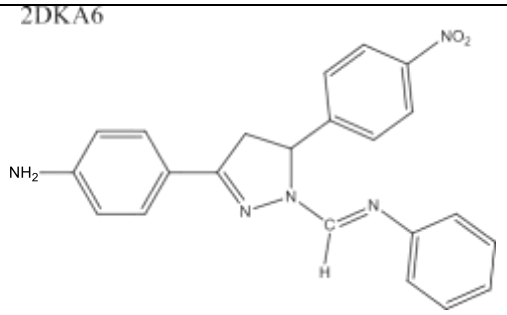
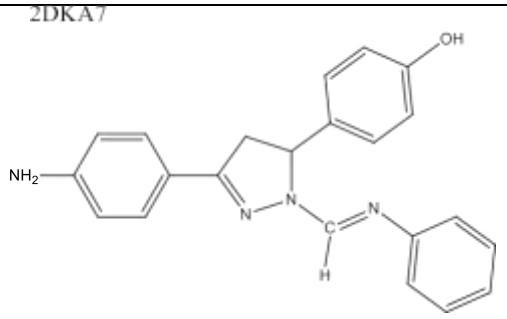
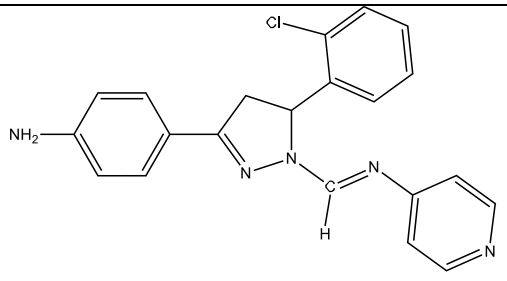


Table 2: List of Novel Schiff bases of pyrazoline derivatives

Compounds	Structure
2DKA1	
2DKA2	
2DKA3	

2DKA4	
2DKA5	
2DKA6	
2DKA7	
2DKA8	

Step 1: General method of the Synthesis of substituted chalcone

An equimolar quantity of Para amino acetophenone and substituted aromatic benzaldehyde was dissolved in absolute alcohol, and a 40% KOH solution was added slowly with stirring. After complete addition, stirring was continued for 9 hours and kept overnight. The reaction mixture was poured into ice water and acidified with 10% HCL to obtain the product. The crude product

was crystallized from ethanol. Reaction was monitored by TLC using different ratios of n-hexane and ethyl acetate.

Step 2: Preparation of the pyrazoline Schiff base derivatives (2DKA1 to 2DKA8)
The equivalent quantity of the pyrazoline derivatives and Primary amine reacts with each other in the presence of a few drops of glacial acetic acid under reflux conditions, the temperature should be maintained from 80 to 100 °C for 6 to 8 hrs.

Physical properties and Spectral Characterization of 2DKA1 ((E)-N-((3-(4-aminophenyl)-5-((E)-styryl)-4,5-dihydro-1H-pyrazol-1-yl)methylene)pyridin-4-amine)

Physical Properties: Molecular weight 367 gm/mol
IR:3348-3049cm-1(NH2),2941,2870 cm-1 (CH) 1689 cm-1(Schiff Base)HNMR: H_{-15} 8.367, H_{-1},H_{-2} 5.501, 3.534,H_{-4} to H_{-7} H_{-16} 8.313,7.791,H_{-3} 6.167 CNMR ; C_{-15} 8.367.C_{-2} 5.501,3.524,C_{-4}.C_{-6} 8.313-7.791,H_{-3} 6.167
MASS : (m/z):368 [M^+]

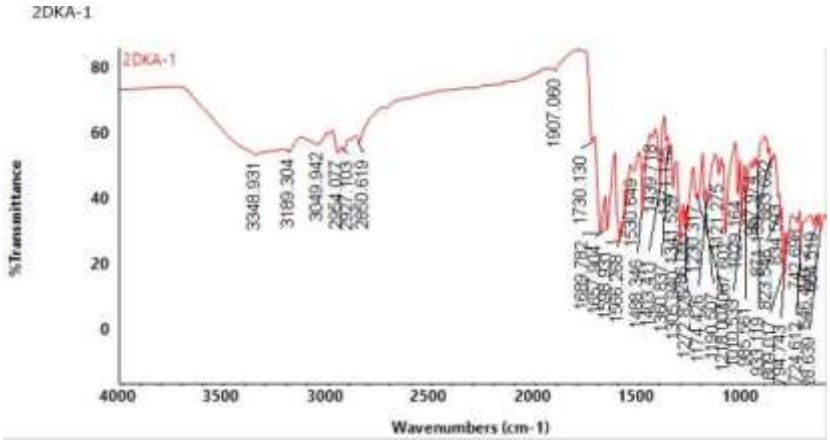


Fig 7: IR spectrum of compound (2DKA1) (E)-N-((3-(4-aminophenyl)-5-((E)-styryl)-4,5-dihydro1H-pyrazol-1-yl) methylene)pyridin-4-amine

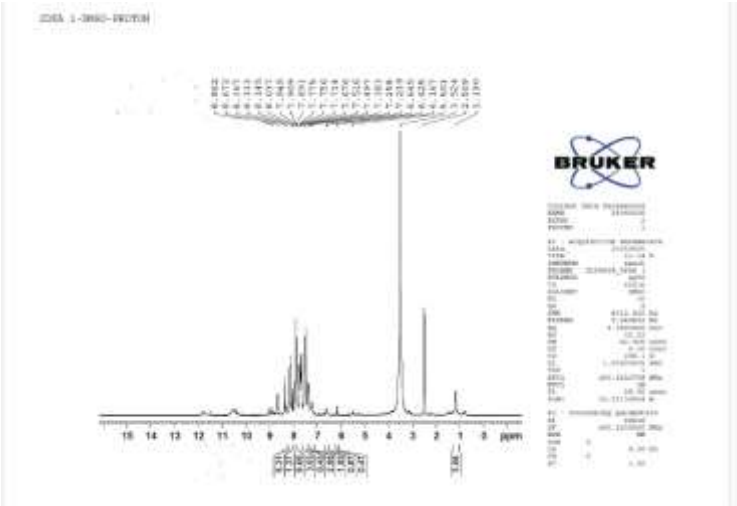


Fig 8: ^{1}H NMR spectrum of compound 2DKA1- (E)-N-((3-(4-aminophenyl)-5-((E)-styryl)-4,5-dihydro-1H-pyrazol-1-yl) methylene) pyridin-4-amine

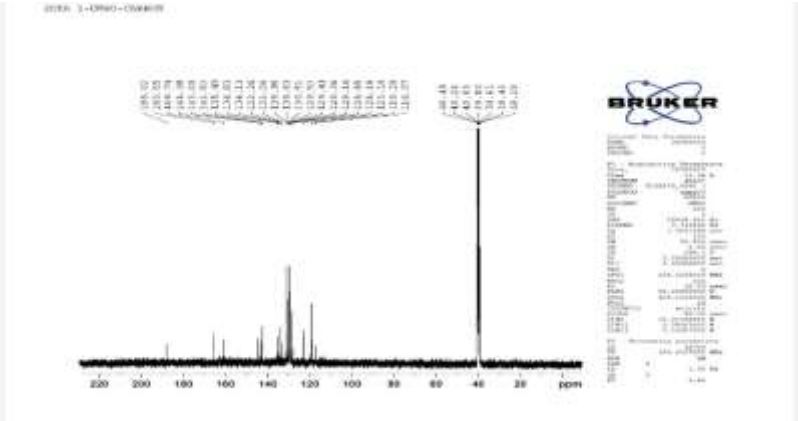


Fig 9: ^{13}C NMR spectrum of compound 2DKA1- (E)-N-((3-(4-aminophenyl)-5-((E)-styryl)-4,5dihydro-1H-pyrazol-1-yl) methylene) pyridin-4-amine

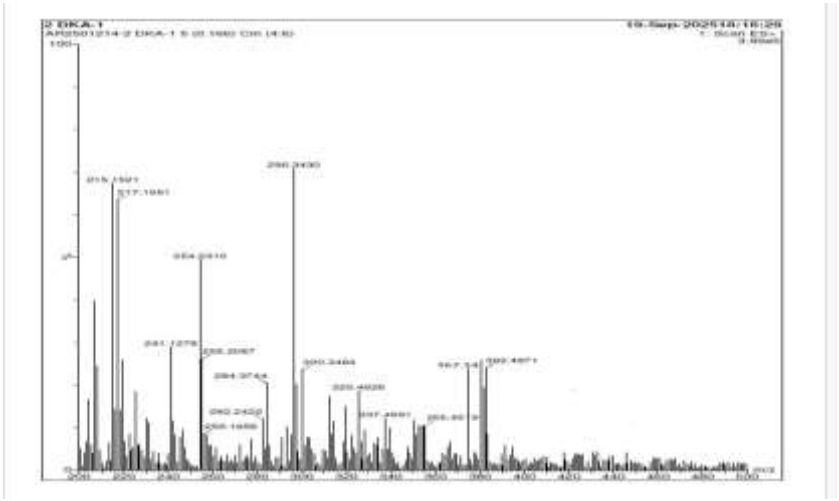


Fig:10 Mass of (E)-N-(4-(5-(4-hydroxyphenyl)-1-((phenylimino)methyl)-4,5-dihydro-1Hpyrazol-3-yl) phenyl) iso nicotinamide

Physical properties and Spectral Characterization of 2DKA2((E)-N-((3-(4-aminophenyl)-5-((E)-styryl)-4,5-dihydro-1H-pyrazol-1-yl)methylene)aniline)

Physical Properties: Molecular Formula $C_{24}H_{22}N_4$, Molecular Weight 366 gm/mole, Colour Grey, Melting Range 205-209, Rf Value 0.76, solubility Ethanol, DMSO,% of yield 73%, **IR KBr (cm^{-1})** 3339, 3219cm^{-1}(NH2),2925,2921 cm^{-1}(CH),1649(C=O).**Physical properties and Spectral Characterization of 2DKA3((E)-N-((3-(4-aminophenyl)-5-(4-methoxyphenyl)4,5-dihydro-1H-pyrazol-1-yl) methylene) aniline)**

Physical Properties: Molecular Formula and molecular weight $C_{24}H_{22}N_4$, 370 gm/mole, Colour- White, Melting Range 203-205 c, Rf-0.73, Solubility Ethyl acetate, DMSO, % of yield 62%, IR 3345 cm^{-1}(NH),2925,2852 cm^{-1}(C-H, C=H),1628 cm^{-1}(N=H),1384cm^{-1}(-OCH_3)

Physical properties and Spectral Characterization of 2DKA4((E)-N-((3-(4-aminophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)methylene) pyridin-4-amine)

Physical Properties: Molecular Formula and weight $C_{22}H_{21}N_5O$,373gm/mole, Colour yellow, Melting Range 207-209, Rf value 0.761, Solubility DMSO, Ethyl Acetate, % Of yield 58%, **IR**

3108(=CH- str),2940(-N-CH_3str),1627 (-N= str), 1595(-C=C- str), 807(-C-Br str),732(-C-I str)

Physical properties and Spectral Characterization of 2DKA5((E)-N-((3-(4-aminophenyl)-5-(4nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)methylene)pyridin-4-amine)

Physical Properties: Molecular Formula and mol weight $C_{21}H_{18}N_6O_2$,386, Colour Brown, Melting range 208-210, Solubility Ethyl Acetate DMSO, % of yield 62%IR 3076(NH str), 1615(-C=N str), 1470(-C-H str), 1489(-C=C str), 1399(-C-H str) 1064(-C-F str).

Physical properties and Spectral Characterization of 2DKA6((E)-N-((3-(4-aminophenyl)-5-(4nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl) methylene) aniline)

Physical Properties: Molecular formula and weight $C_{22}H_{16}N_5O_2$, 385g/mole , Colour White, Melting Range 208-211, Rf value 0.65, Solubility DMSO,% of yield 52%, 3336(NH str)IR 2939 (CH_3), 2838(-O-CH_3), 1615(-C=C- str), 1258(-C-O str), 743(-C-I str).

Physical properties and Spectral Characterization of 2DKA7 ((E)-4-(3-(4-aminophenyl)-1-
((phenylimino)methyl)-4,5-dihydro-1H-pyrazol-5-yl)) Phenol

Physical Properties: Molecular Formula and Molecular Weight $C_{22}H_{20}N_4O$ and 356,Colour White,
Melting Range 208-211, Rf Value 0.79, Solubility DMSO ,% of yield 52%,IR 3229cm^{-1}(Ar-OH),3045 cm^{-1}(N=H),2921,2853 cm^{-1}(C-H,C=H str),1674 cm^{-1}(N=H)

Physical properties and Spectral Characterization of 2DKA8 ((E)-N-((3-(4-aminophenyl)-5-
(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)methylene)pyridin-4-amine)

Physical Properties: Molecular Formula and Molecular Weight $C_{21}H_{18}ClN_5$ 375 gm/mole, Colour Light Yellow, Melting Range 200-201 Rf Value-0.74.Solubility: Ethanol, Methanol, DMSO, % of yield 72%.IR 3243 cm^{-1}(NH-str),3090,2986,2922 cm^{-1}(-C-H-,C=H)1698 cm^{1}(N=H),1315 cm^{-1}(Ar-OH Ring),HNMR H-$_{6,7}$ 3.503,2.509, H-$_5$ 7.891, H-$_{20}$ 6.25, H-$_{18,19}$ 8.367, H-
$5_{\text{ to }19}$ 7.891-6.25H-$_{15}$ 3.524,CNMR C-$_{17}$ 162.15, C-$_8$ 58.40.C-$_9$ 40.40, C-$_{1to6}$ (120 to 150), **MS** (m/z):376 [M^+]

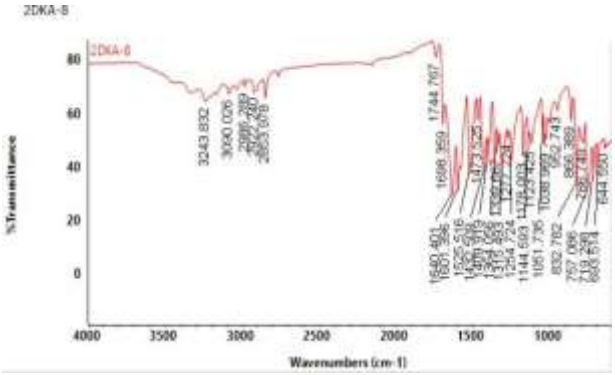


Fig: 11 IR of the 2DKA8 (E)-N-((3-(4-aminophenyl)-5-(2-chlorophenyl)-4,5-dihydro-1Hpyrazol-1-yl) methylene)pyridin-4-amine

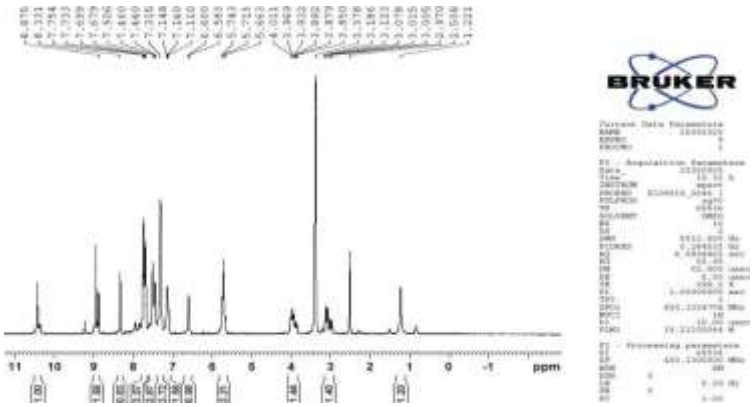


Fig 12 HNMR of the 2DKA8 (E)-N-((3-(4--aminophenyl)-5-(2-chlorophenyl)-4,5dihydro-1H-pyrazol-1-yl) methylene)pyridin-4-amine

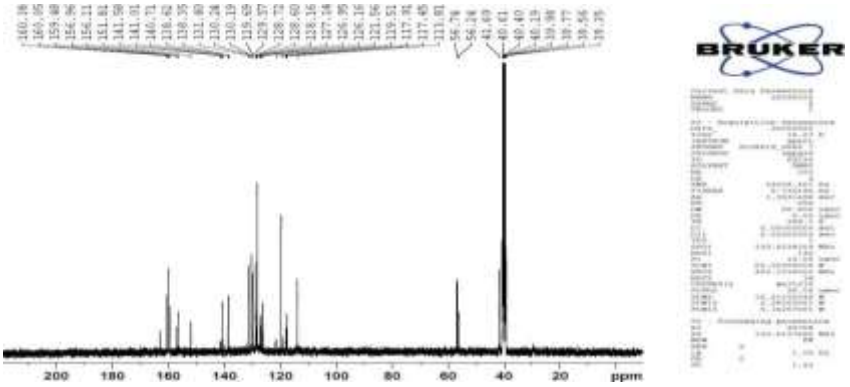


Fig 13 CNMR of the 2DKA8 (E)-N-((3-(4--aminophenyl)-5-(2-chlorophenyl)-4,5-dihydro1H-pyrazol-1-yl) methylene)pyridin-4-amine

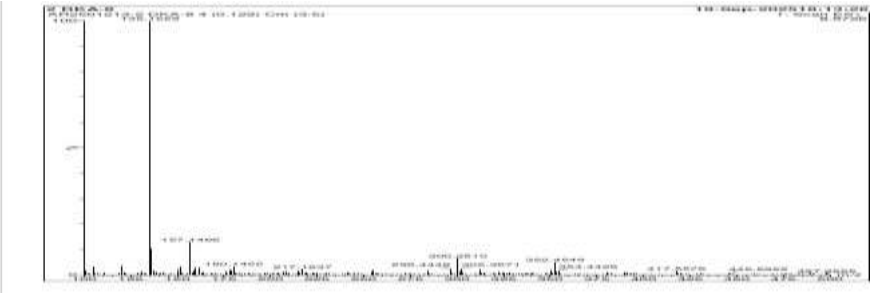


Fig 14 Mass of the 2DKA8 (E)-N-((3-(4--aminophenyl)-5-(2-chlorophenyl)-4,5-dihydro1H-pyrazol-1-yl) methylene) pyridin-4-amine

1. In-silico Physiochemical study of the Novel drugs

A. In-silico ADME STUDY Swiss- ADME and Molilslinsperision

In-silico methods of determination of the physicochemical descriptors and properties is a key role in drug development and target identification. The web tools, such as SwissADME, give the different properties of a drug based on pharmacokinetic properties, druglike nature, and medicinal chemistry friendliness of one or multiple small molecules.

Table 3: *In-silico* ADME properties of the compounds (1DKA1to 1DAK3 and 2DKA1 to 2DKA8) obtained from Swiss-ADME

Compound code	1DK A1	1DK A2	2DK A1	2DK A2	2DK A3	2DK A4	2DK A5	2DK A6	2DK A7	2DKA 8
Num. heavy atoms	35	35	28	28	25	24	28	26	28	26
Num. Arom. Heavy atoms	24	24	18	18	18	18	16	18	16	18
Num. Rotatable bonds	7	07	5	5	5	5	5	5	5	5
Num.H-bond acceptors	5	6	3	2	2	3	2	3	2	3
Num. of H-bond donors	2	1	1	1	1	1	1	1	1	1
Molar refractivity	144.7 9	0.5 2	2.8 9	5.0 9	5.0 9	126.1	5.0 9	5.5 2	125.8 9	126.6
Total Polar Surface Area(Å)	90.18	82.84	66.87	53.98	52	54.84	55.92	56.98	58.86	57.32
Log Po/w (ilogp)	2.78	3.02	3.06	3.65	3.36	3.39	3.36	3.56	3.25	3.02
Water solubility	Poorly soluble	Moderatel y soluble	soluble	Soluable	Mode rately lubl e	Mode rately lubl e	Mode rately uble e	Mode rately lubl e	Mode rately lubl e e	Moder ately soluble
GI absorption	High	High	high	High	High	High	High	High	High	High
BBB permeant	No	no	yes	yes	yes	yes	yes	yes	yes	yes
Drug likeness (violation)	Yes; (0)	Yes; (0)	Yes; (0)	Yes: (1)	Yes; (0)	Yes; (0)	Yes; (0)	Yes; (0)	Yes; (0)	Yes;(0)
Lead likeness (violation)	No;	No; (1),	No; (1),	No; (1),	No; (1),	No; (1),	No; (1),	No; (1),	No; (1),	No; (1),

Molinspiration is also a web tool that provides the calculation of important molecular properties (logP, polar surface area, number of hydrogen bond donors and acceptors and others), as well as prediction of bioactivity score for the most important drug targets.

Table 4: *In-silico* Physicochemical Studies of compounds obtained from Molinspiration

Compound Code	*miLogP*	*TPSA*	*natoms*	*MW (g/mol)*	*nON*	*nOHNH*	*n violation*	*n rotb*	*Volume*
1DAK1	*5.62*	*73.11*	*36*	*473.58*	*6*	*2*	*1*	*7*	*440.92*
1DKA2	*3.59*	*82.59*	*35*	*464.50*	*7*	*1*	*0*	*6*	*408.37*
2DKA1	*3.65*	*66.88*	*28*	*367.88*	*5*	*2*	*0*	*5*	*343.50*
2DKA2	*4.95*	*53.99*	*28*	*366.47*	*4*	*2*	*0*	*5*	*347.66*
2DKA3	*4.64*	*53.99*	*27*	*355.46*	*4*	*2*	*0*	*4*	*336.46*
2DKA4	*3.71*	*74.22*	*27*	*356.43*	*5*	*3*	*0*	*4*	*328.26*
2DKA5	*3.75*	*99.81*	*30*	*399.45*	*7*	*2*	*0*	*4*	*360.38*
2DKA6	*3.37*	*99.82*	*31*	*399.70*	*7*	*2*	*0*	*4*	*361.38*
2DKA7	*3.71*	*74.22*	*27*	*356.43*	*5*	*3*	*0*	*4*	*328.26*
2DKA8	*3.53*	*66.88*	*27*	*375.86*	*5*	*2*	*0*	*4*	*329.62*

B. *In-silico* toxicity studied (Protox)

The ProTOX-II web server is a prediction scheme that is classified into different levels of toxicity, such as oral toxicity, organ toxicity (hepatotoxicity), toxicological endpoints (such as

mutagenicity, carcinogenicity, cytotoxicity, and immunotoxicity), toxicological pathways (AOPs), and toxicity targets, thereby providing insights into the possible molecular mechanism behind such toxic responses.

Procedure:

The structures of synthesized compounds were drawn using Chemsketch and the smiles notation was generated.The generated smiles were pasted in the respective Protox-II web-tools and were runner. The results of toxicity over different organs were tabulated.

All the synthesized compounds exhibited level 4 toxicity. All compounds are exhibited level 4 toxicity, with the highest predicted LD_{50} of 1000mg/kg

Table 5: *In-silico* Toxicity studies of the compounds obtained from Protox ii

Compound code	***Predicted LD50 (mg/kg)***	***PredictedToxicity Class***	***epatotoxicity***	***Carcinoge-nicity***	***Immunoto-xicity***	***Mutageni-city***	***Cytotoxicity***
1DAK1	1000	4	+	+	-	-	-
1DAK2	1000	4	+	+	-	-	-
2DAK1	1000	4	+	+	-	+	-
2DAK2	1000	4	+	+	-	+	-
2DAK3	1000	4	+	+	-	+	-
2DKA4	1000	4	+	+	-	+	-
2DKA5	1000	4	+	+	-	+	-
2DKA6	1000	4	+	+	-	+	-
2DKA7	1000	4	+	+	-	+	-
2DKA8	1000	4	+	+	-	+	-

“+” =Active, “-” = Inactive

c. **Online ADR Study Pass Online softwear**

PASS (Pharmacovigilance Adverse Drug Reaction Surveillance System) is an online software designed to collect, monitor, and analyze adverse drug reaction (ADR) data efficiently. It enables healthcare professionals and researchers to report and evaluate drug safety information in real time, supporting effective pharmacovigilance practices.

Tabel 6: Online ADR

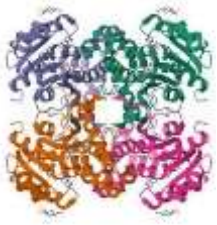
1DKA1	Acneiform eruption Ulcer, aphthous Nail discoloration Palpitation Pure red cell aplasia Optic neuritis
1DKA2	Nail discoloration Acneiform eruption Pseudoporphyria
1DKA3	Nail discoloration Acneiform eruption Pseudoporphyria Pure red cell aplasia
2DKA1	Nail discoloration Allergic dermatitis Ototoxicity Fatty liver

2DKA2	Nail discoloration Allergic dermatitis Ototoxicity Fatty liver
2DKA3	Pseudoporphyria Nail discoloration Fatty liver Weight loss
2DKA4	Nail discoloration Pure red cell aplasia Fatty liver Ulcer, peptic
2DKA5	Pure red cell aplasia Allergic dermatitis Nail discoloration Weight loss
2DKA6	Pure red cell aplasia Allergic dermatitis Carcinogenic, group 3 Dyspnea
2DKA7	Nail discoloration Allergic dermatitis Pure red cell aplasia Fatty liver Ulcer, aphthous
2DKA8	Pure red cell aplasia Nail discoloration Visual acuity impairment Toxic, respiration

C. ***Molecular Docking***

Molecular docking is a bioinformatic modelling method that studies the interactions between two or more molecules to form stable adducts. It predicts the 3D structure of complexes based on ligand and target binding interactions. Molecular Docking generates various possible adduct structures, which are ranked using a scoring function. Docking simulations aim to identify the optimized docked conformer based on the total system energy. However, challenges such as ligand chemistry, receptor flexibility, and effective scoring functions persist.

In-silico docking of Synthesized for their anti-Tubecular activity: Table 7: Protein Taken

PDB ID : 6SQB(Anti- tubular) Crystal structure of M. tuberculosis InhA in complex with NAD+ and 3-(3-chlorophenyl) propanoic acid	

Procedure:

The protein was downloaded from the RCSB website in PDB format and pre-processed using Discovery Studio. Next, water molecules and unwanted entities were removed, missing polar hydrogen atoms were added, and energy minimization was performed to optimize the structure. The 2D structures of the ligand were created using Chem Draw and saved as mdl.mol files. Next, Chem

3D's energy minimization technique was applied to optimize the structures for the lowest possible energy

Prepared proteins and ligands were imported into PyRx, assigned as macromolecules and ligands, and saved in. pdbqt format. The docking was conducted for each ligand, recording

binding affinity and RMSD values. The complex was saved in. pdbqt to analyse interactions. The 2D interactions of the ligands with the amino acids of target proteins were visualized using Bio-via Discovery Studio. **The *in-silico* screening was conducted to evaluate the potential anti-tubercular activity of the synthesized compounds. These compounds were evaluated for their interactions with the (PDB ID 6SQB) receptor, which revealed promising interactions**.

Table 8: Binding energy of the compounds with target protein PDB ID: 6SQB

Compound code	Binding Affinity(kcal/mol) (6SQB)
1DKA1	-11
1DKA2	-9.3
1DKA3	-9.9
2DKA1	--9.8
2DKA2	-10
2DKA3	-9.6
2DKA4	-9.2
2DKA5	-9.2
2DKA6	-9.7
2DKA7	-9.7
2DKA8	-9.6
NAD+Crystallize	-7.3
Isoniazide	-5.3

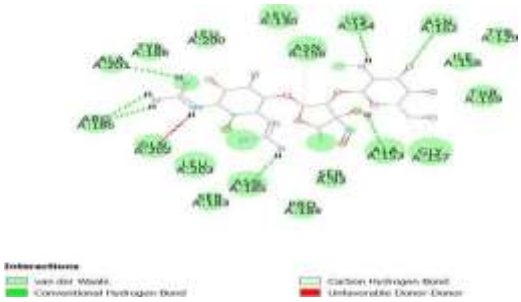


Fig15: 2D interaction of Isoniazid having binding affinity of -5.3 kcal/mol, respectively with 6SQB receptor

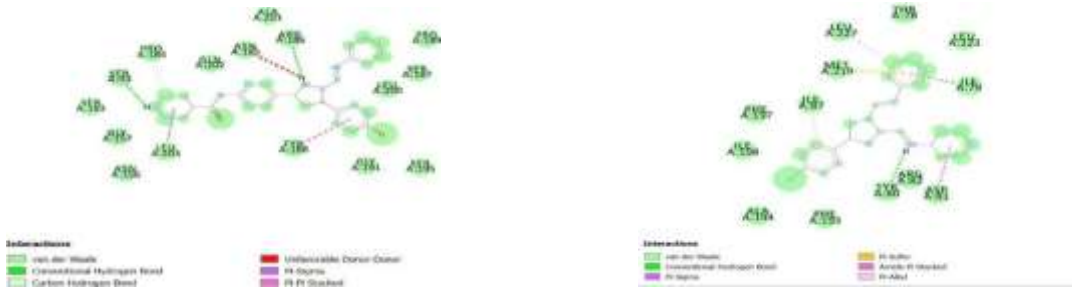


Fig16: 2d model of the binding affinity of the 1DKA1 and 2DKA2 with 6sqb Protein

Compounds 1DKA1 and 2DKA2 ashowed binding affinity of -11, -10, and -9.7 kcal/mol, respectively. 1DKA1 showed the conventional hydrogen bond with SER A:93 and ARG A:186. The compound 2DKA2 established the conventional hydrogen bonding with ILE A:79 and ARG A:82, Whereas the standard drug Isoniazid shows the conventional hydrogen bond with ASN A:185 and ALA A:153.

D. <u>In-vitro Anti-tubercular activity:</u>

Test and Method Used: Anti-TB test by MABA Assay

Standard Strain used: ***Mycobacteria tuberculosis*** (Vaccine strain, H37 RV strain): ATCC No 27294

Procedure:

The anti-Mycobacterial activity of compounds was performed against *M. tuberculosis* using the microplate Alamar Blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent, and shows good correlation with the proportional and BACTEC radiometric method. Briefly, 200µl of sterile deionized water was added to all outer perimeter wells of the sterile 96-well plate to minimize evaporation of medium in the test wells during incubation. The 96-well plate received 100 µl of the Middlebrook 7H9 broth containing *Mycobacterium tuberculosis*. Serial dilutions of compounds were made directly on the plate. The final drug concentrations tested were 100 to 0.2 µg/Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% Tween 80 was added to the plate and incubated for 24 hrs. A blue colour in the well was interpreted as no bacterial growth, and a pink colour was scored as growth. The MIC was defined as the lowest drug concentration that prevented the colour change from blue to pink.

Standard values for the Anti-Tb test, which was performed.

Isoniazid – 1.6µg/ml. Ethambutol – 1.6µg/ml, Pyrazinamide- 3.125µg/ml, Rifampicin – 0.8µg/ml, Streptomycin- 0.8µg/ml

Table 9: Results

SL/No	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	1DKA1	S	S	S	S	R	R	R	R
2	2DKA2	S	S	S	S	S	S	R	R

3	**2DKA6**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**

Note:
S- Sensitive R- Resistant Table

10: MIC Table

Sl. No	Sample	Mic Value
1	**1DKA1**	**12.5 µg/ml**
2	**2DKA1**	**3.12 µg/ml**
3	**2DKA6**	**25 µg/ml**

Picture:

3. Conclusion

The research paper aims to synthesize some novel pyrazoline Schiff bases. This project was carried out by using two different types of schematic methodology.
In first methodology, derivatives are prepared by using iso nicotinyl chloride and paraamino benzophenone in the presence of KOH and form N-(4-acetyl-phenyl)- iso nicotinamide as starting materials to yield as the first intermediate-(4-acetyl-phenyl)isonicotinamide reacts with the substituted benzaldehyde in the presence of KOH and Ethanol to form the N- {4- [3-(substituted phenyl)-acryloyl]-phenyl}-iso nicotinamide derivatives. This the N- {4- [3-(substituted phenyl)- acryloyl]-phenyl}-iso nicotinamide reacts with the 90% hydrazine hydride and formic acid undergoes cyclization reaction to form substituted pyrazoline derivatives of N-(4-(5-(Substituted phenyl)-1-formyl- 4,5dihydro-1H-pyrazol-3-yl) phenyl) iso nicotinamide, Then of N-(4-(5-(Substituted phenyl)1-formyl-4,5- dihydro-1H-pyrazol-3-yl) phenyl) iso nicotinamide reacts with the primary amines like (Aniline and 3 amino pyridine) in presence of some amounts of glacial acetic acid to form corresponding Schiff bases of pyrazoline (1DKA1 to 1DKA3).
In 2nd methodology derivatives are prepared by using substituted benzaldehyde and Para amino acetophenone in presence of the KOH and form substitutes chalcone This substituted chalcone reacts with the 90% hydrazine hydrate and formic acid undergoes cyclization reaction to form

substituted pyrazoline derivatives, then substituted pyrazoline reacts with the primary amines like (Aniline and 3 amino pyridine) to form corresponding Schiff bases of pyrazoline (2DKA1 to 2DKA3).

Lastly, for *in vitro* anti-tubercular activity (using the MABA Assay), compound 2DKA1 exhibited the highest minimum inhibitory concentration (MIC) when compared to the standard drugs Isoniazid, Ethambutol, Pyrazinamide, Rifampicin, and Streptomycin at 3.125µg/ml. The compound showed strong antitubercular activity because its pyridine and pyrazoline rings are similar to those in Pyrazinamide, allowing it to block mycolic acid synthesis in *Mycobacterium tuberculosis*. The styryl and amino groups improve enzyme binding and help the compound enter the bacterial cell easily

4. Reference

1. Özkınalı S, Gür M, Şener N, Alkın S, Çavuş MS. Synthesis of new azo Schiff bases of pyrazole derivatives and their spectroscopic and theoretical investigations. Journal of Molecular Structure. 2018 Dec 15; 1174:74-83.
2. K.D. Olczak, M. Daszkiewicz, K., Slawinska, D.B., Baginska, Gozdowski, P., Daszkiewicz, B. Fronc, K., Semczuk, Bacteria and Candida yeasts in inflammations of the oral mucosa in children with secondary immunodeficiency, J. Oral Pathol.

Med.41 (2012) 568–576.

3. Ahmad A, Husain A, Khan SA, Mujeeb M, Bhandari A. Synthesis, antimicrobial and antitubercular activities of some novel pyrazoline derivatives. Journal of Saudi Chemical Society. 2016 Sep 1;20(5):577-84.
4. X.Y. Yu, Q.Q. Zhao, J. Chen, W.J. Xiao, J.R. Chen, when light meets nitrogencentered radicals: from reagents to catalysts, Accounts Chem.Res 53(5) (2020)1066–

1083,

5. Rahman MA, Siddiqui AA. Pyrazoline derivatives: a worthy insight into the recent advances and potential pharmacological activities. *IJPSDR.* 2010;2(3):165– 175.
6. Schiff, H. Mittheilungen aus dem Universitäts-laboratorium in Pisa: 2. Eine neue Reihe organischer Basen [Communications from the university laboratory in Pisa: 2. A new series of organic bases]. Ann. Der Chem. Und Pharm. **1864**, 131, 118–119.
7. Pfeiffer, P.; Breith, E.; Llibbe, E.; Tsumaki, T. Tricyclische orthokondensierte Nebenvalenzringe. Justus Liebigs Ann. Chem. **1933**, 503, 84–130.
8. Lundgren, R.L.; Stradiotto, M. Ligand Design in Metal Chemistry: Reactivity and Catalysis; Key Concepts in Ligand Design: An Introduction; John Wiley & Sons, Ltd.: Chichester, UK; Hoboken, NJ, USA, 2016; pp. 1–13.
9. Samina KT, Abhijit AY and Ratnamala SB. Synthesis and characterization of a novel Schiff base of 1,2-diaminopraopane with substituted salicylaldehyde and its transition metal complexes. Journal of Molecular Structure, (2018) 1152: 223-231.
10. Thalla N, Devineni S R, Parimi B N, and Chamarthia N R. A Facile, Catalyst-free Green Synthesis for Schiff's Bases in Aqueous Medium under Ultrasonic Irradiation

Conditions and Their Antimicrobial Activity. Der Chemica Sinica, (2012), 3(4):808-

816

11. Bhusnure O G, Vibhute Y B, Giram P S, Vibhute A Y, Bhusnure O G. Innovative Green synthesis of Schiff bases and their Antimicrobial Activity. Journal of Pharmacy Research (2015),9(12),670-677
12. Hassan AS. Antimicrobial evaluation, in silico ADMET prediction, molecular docking, and molecular electrostatic potential of pyrazole-isatin and pyrazole-indole hybrid molecules. Journal of the Iranian Chemical Society. 2022 Aug;19(8):3577-89.
13. Ahamed A, Husain A, Khan SA, Mujeeb M, Bhandari A. Synthesis, antimicrobial and antitubercular activities of some novel pyrazoline derivatives. Journal of Saudi Chemical Society. 2016 Sep 1;20(5):577-84.
14. Upadhyay N, Tilekar K, Loiodice F, Anisimova NY et al., Pharmacophore hybridization approach to discover novel pyrazoline-based hydantoin analogs with anti-tumour efficacy. Bioorg Chem. 2020.