RESEARCH PAPER

Synthesis, insilico studies and identification of Schiff base linked pyrazole scaffolds as potential anti-skin cancer and anti-melanogenic agents

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ABSTRACT
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INTRODUCTION

Cancer is a disease characterized by uncontrolled multiplication and spread of abnormal forms of the body's own cells. It is the second most common cause of death in the developed nations and one in three people will be diagnosed with cancer during their lifetime. Cancer is responsible for approximately one-quarter of all deaths in the UK, with lung and bowel cancer comprising the largest category, closely followed by breast and prostate cancer. There are three main approaches for treating established cancer—surgical excision, irradiation and drug therapy (often called chemotherapy)—and the relative value of each of these approaches depends on the type of tumor and the stage of its development. Chemotherapy may be used on its own or as an adjunct to other forms of therapy.
Skin cancers are the most common groups of cancers diagnosed worldwide, with more than 1.5 million new cases. In 2023, an estimated 330000 new cases of melanoma were diagnosed worldwide and almost 60000 people died from the disease. Melanoma of skin is the 13th most common in men and 15th in women.

Pyrazole plays an important role as antitumor agent because of its good inhibitory activity at various receptor sites such as, EGFR telomerase, ROS Receptor Tyrosine Kinase and Aurora- A Kinase. Several pyrazole derivatives have anticancer activity; other activities are also reported for pyrazole nucleus including antitubercular, anticonvulsant, anticancer, antimicrobial, anti-HIV, anti-hepatotoxic, anti-inflammatory, analgesic, and herbicidal. The substitution at pyrazole nitrogen decreases the basicity due to the steric hindrance to hydration. Because of the presence of diverse properties, easily accessible synthetic pathway and the wide range of biological activities, pyrazole is centre of attraction for organic chemists.

By recent research studies, it has been found that pyrazole is quite a promising scaffold for study in the area of anticancer drugs. This idea prompted us to design pyrazole-schiff bases as potent anti-melanogenic and anti-skin cancer agents.

Materials and methods

Experimental section includes four phases

- *In-silico* Studies
- Synthetic Studies
- Spectral analysis
- *In vitro* anti-tyrosinase and anti-skin cancer activity screening

IN-SILICO STUDIES

Softwares & Databases used

- RCSB Protein Data Bank (https://www.rcsb.org/)
- Online Smiles Translator (https://cactus.nci.nih.gov/translate/)
- Zinc Database (https://zinc.docking.org)
- ACD chem Sketch 2018 v2.5
- Schrodinger Maestro v12.6
- Glide v2.6
- QikProp

TARGET SELECTION

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The present study was focused on **anti-tyrosinase and anti-skin cancer activity**. Based upon various literature survey, binding site required for exhibiting anti-tyrosinase and anti-skin cancer activity was identified as **Tyrosinase** (**PDB ID: 6QXD**).The target was downloaded from RCSB Protein Data Bank.

LEAD IDENTIFICATION

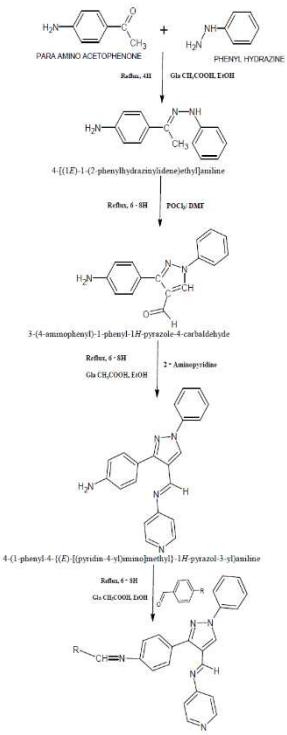
From various literatures reviewed, we understood that schiff bases of pyrazole heterocycles have been reported to exhibit cancer activity. Schiff bases linked with pyrazole moiety have been not so far reported for anti-tyrosinase and anti-skin cancer activity. Based on these observations, emphasis was given on schiff bases linked pyrazole derivatives which were designed and selected as lead molecules. Various schiff bases linked pyrazole analogs were designed and focus was given on its molecular modification by modifying the substituents and its position in the aromatic system.

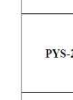
LEAD OPTIMISATION

Software used: **QikProp**

QikProp is a quick, accurate, easy-to-use absorption, distribution, metabolism, and excretion (ADME) prediction program designed by Professor William L. Jorgensen. QikProp predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules, either individually or in batches. In addition to predicting molecular properties, QikProp provides ranges for comparing a particular molecule's properties with those of 95% of known drugs.

SYNTHETIC STUDIES



Compound code	R
PYS-1	
PYS-2	
PYS-3	
PYS-4	
PYS-5	

Preparation of 3-[(1*E*)-1-(2-phenylhydrazinylidene)ethyl]aniline

A solution of *p*-amino acetophenone (0.01 mole) in ethanol (10 ml) was placed in a round bottomed flask. To the reaction mixture, phenyl hydrazine (0.01 mole) and 2-3 drops of glacial acetic acid were added slowly and refluxed for about 4 hours The mixture was allowed to cool to room temperature. The precipitate so obtained was recrystallized using ethanol and the purity of the compounds was determined by TLC.

Preparation of 5-(3-aminophenyl)-2-phenyl-2,3-dihydro-1H-pyrazole-4-carbaldehyde

To a cold solution of *N, N*-dimethyl formamide (0.02 mole), freshly distilled phosphorus oxychloride (0.01 mole) was added with stirring over a period of 30 minutes. After formylation, a solution of 3-[(1*E*)-1-(2-phenylhydrazinylidene)ethyl]aniline (0.01 mole) in N, N-dimethyl formamide (10 ml) was added drop-wise while maintaining the temperature at 0-50C. The resulting mixture was heated under reflux for 6-8 hours, cooled and poured with continuous stirring onto crushed ice. After 15 min, the formed precipitate was filtered and recrystallized from aqueous ethanol. The purity of the compounds was determined by TLC.

Preparation of 4-(3-phenyl-4-{(*E*)-[(pyridin-4-yl)imino]methyl}-2,5-dihydro-1*H*-pyrazol-1- yl)aniline

A solution of 5-(3-aminophenyl)-2-phenyl 2,3-dihydro-1*H*-pyrazole-4-carbaldehyde (0.01 mole) and 2-aminopyridine (0.01 mole) in ethanol (20 ml) and a catalytic amount of glacial acetic acid (2-3 drops) were refluxed for 6-8 hours. The reaction mixture was then cooled and poured with continuous stirring onto crushed ice. The solid product so obtained was filtered off and recrystallized from ethanol. The purity of the compounds was determined by TLC.

Preparation of Schiff bases:

A mixture of 4-(3-phenyl-4-{(*E*)-[(pyridin-4-yl)imino]methyl}-2,5-dihydro-1*H*-pyrazol-1- yl)aniline (0.01 mole) and 2-aminopyridine (0.01 mole) in ethanol (20 ml) and a catalytic amount of glacial acetic acid (2-3 drops) was refluxed for about 6-8 hours. The reaction mixture was then cooled and poured with continuous stirring onto crushed ice. The solid product so obtained was filtered off and recrystallized from ethanol. The purity of the compounds was determined by TLC.

Analytical studies

Melting Point was determined using MR-VIS visual melting point apparatus, LABINDIA and are uncorrected.

 Reactions were monitored by Thin Layer Chromatography (TLC) on a precoated silica gel G plate using chloroform: ethylacetoacetate as mobile phase and iodine vapours as visualizing agent.

 UV spectra were recorded on JASCO V-530 UV-Vis Spectrophotometer, Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore.

 IR spectra was recorded on SHIMADZU FT-IR Spectrophotometer, Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore.

 1H NMR&13C NMR spectroscopy was recorded on BRUKER, Centralized Sophisticated Instrument Facilities, Interdisciplinary Institute Of Indian System Of Medicine SRM Institute of Science and Technology, Chennai.

 MASS spectroscopy was recorded on SHIMADZU, Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore.

BIOLOGICAL SCREENING

Tyrosinase inhibition assay

The inhibition tyrosinase activity effect was determined by the dopachrome method according to the modified procedure. The reaction was conducted in a 96-well microplate. Kojic acid was used as a positive control. Firstly, the tyrosinase enzyme was pre-incubated with the varying concentrations of treatment samples. The 10.0 µg/ml of mushroom tyrosinase in 50 mM phosphate buffer pH 6.8 (100 µl) and 70 µl of deionized water were placed into wells of 96-well plate. Then, 10.0 µl of treatment samples were subjected and mixed with the enzyme. The plate was incubated at 37°C for 10 minutes. Next, 10.0 µl of 20.0 mM L-Dopa in 50 mM phosphate buffer pH 6.8 was added to the well and mixed gently. The plate was incubated at 37°C for 20 minutes. The absorbance value was measured at the wavelength of 495 nm of a microplate reader (Robonik, India). The percentage of tyrosinase inhibitory activity of test substances was calculated

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 105 cells/mL using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100 µL of the diluted cell suspension (1x 104 cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µL of different concentrations of test samples were added on to the partial monolayer in microtiter plates. The plate was then incubated at 37oC for 24 h in 5% CO2 atmosphere. After incubation the test solutions in the wells were discarded and 20 µL of MTT (2 mg/1 mL of MTT in PBS) was added to each well. The plate was incubated for 4 h at 37oC in 5% CO2 atmosphere. The supernatant was removed and 100 µL of DMSO was added and the plate was gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage of viability was calculated using the following formula, % viability = Sample abs/Control abs x 100

RESULTS AND DISCUSSION

Among several classes of anti-tyrosinase and anti-skin cancer drugs, the study was emphasized on schiff base linked pyrazole derivatives. Various schiff base-based drugs have been reported to exhibit anticancer activity. Pyrazole derivatives have also been reported to possess anticancer activity. Hence based on these observations, it was decided to link schiff base with pyrazoles to synergize the activity. Targeting, potent leads to possibility of increased biological activity. Therefore, it was decided to select schiff base linked pyrazole derivatives as lead molecules.

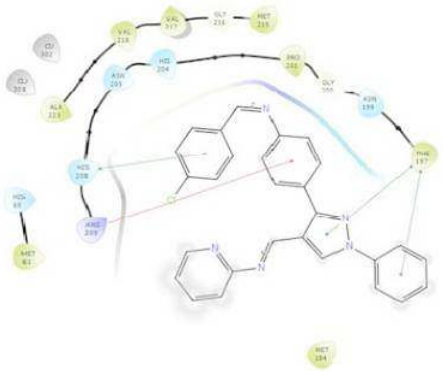
MOLECULAR DOCKING BINDING INTERACTIONS

The binding interactions of schiff base linked pyrazole derivatives at the target site, TYROSINASE (JKB inhibitor) **PDB ID: 6QXD**

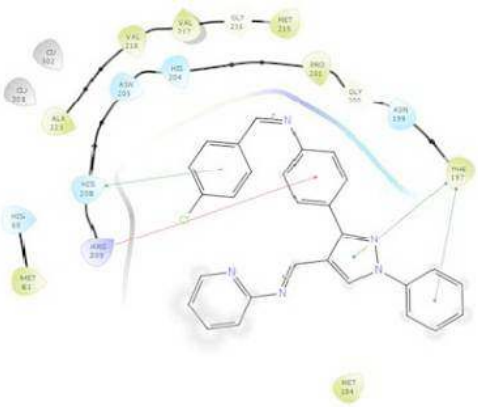
Binding Interactions of selected compounds

S.No	Compound code	Binding Interactions
1	PYS-1	ALA 221,ARG 209,HIS 208, MET 215, PHE 197, GLY 216
2	PYS-2	ALA 221,ARG 209, GLY 216, HIS 208, MET 215, PHE 197
3	PYS-3	ALA 221, ASN 205, HIS 208, MET 215, PHE 197, VAL 219
4	PYS-4	ARG 221, ASN 205, HIS 208, MET 215
5	PYS-5	ALA 221, GLY 216, HIS 60, MET 61, PHE 197, ASP 55, CU 301
6	Kojic acid	ALA 221, CU 301, GLY 216, HIS 208, MET 215

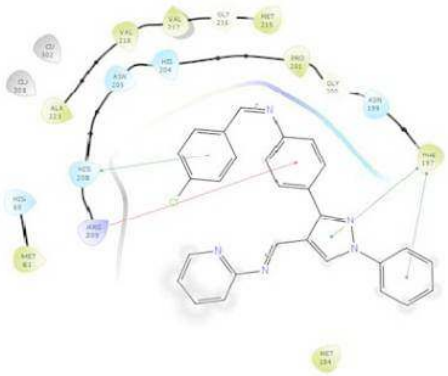
The molecular docking study was performed using Glide docking program of Maestro 13.1 (Schrodinger 2020-03). Among the docked compounds**, PYS-1, PYS-2, PYS-3, PYS-4, PYS-5** displayed good docking score compared to the standard Kojic acid on docking with the target **tyrosinase** (**PDBID:6QXD**) bounded with co-crystallised ligand JKB inhibitor. The standard revealed hydrogen bond interactions with amino acid residues **Ala 221, Met 215** in comparison with the compounds**.** The compound code (**PYS-1, PYS-2, PYS-3, and PYS-5**) shows similar hydrogen bonding interaction on amino acid residue, **His 208, Met 215** compared to the standard. Compound code **PYS-1, PYS-2, PYS-5** shows hydrogen binding interactions with amino acid residue **Gly216. PYS-5** shows hydrophobic bonding interaction with amino acid residue **Cu 301.** Compounds **PYS -1, PYS-2 and PYS-5** shows similar hydrophobic interactions in amino acid residues **Ala 221, Gly216**.



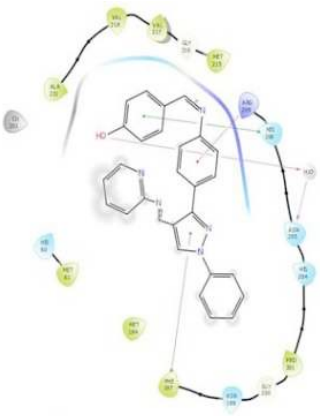
Binding interaction of docked compound PYS-1



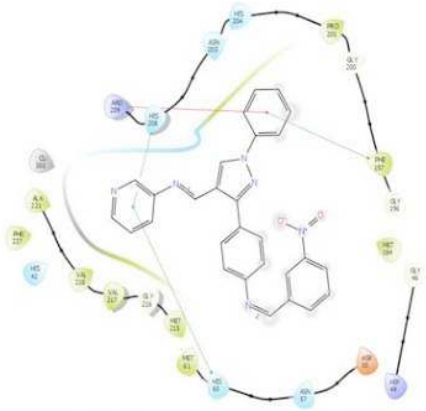
Binding interaction of docked compound PYS-2



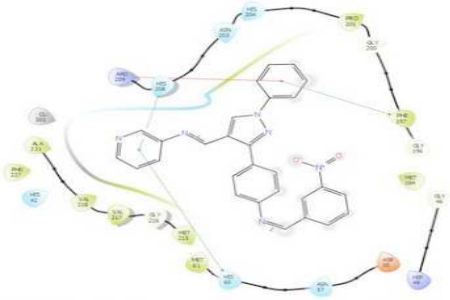
Binding interaction of docked compound PYS-3



Binding interaction of docked compound PYS-4



Binding interaction of docked compound PYS-5



Binding interaction of STANDARD KOJIC ACID

Physicochemical properties of the synthesized compounds

S.No	Compound code	Molecular formula	Molecular weight	Appearance	Color	% yield	Melting point range ^0C	Rf value
1.	**PYS-1**	$C_{28}H_{20}ClN_5$	461.953	Solid	Brown	54	130-136	0.86
2.	**PYS-2**	$C_{28}H_{21}N_5O_2$	459.509	Solid	Brown	55	168-172	0.84
3.	**PYS-3**	$C_{28}H_{20}FN_5$	445.501	Solid	Brown	56	135-137	0.91
4.	**PYS-4**	$C_{28}H_{21}N_5O$	443.510	Solid	Brown	58	146-148	0.72
5.	**PYS-5**	$C_{28}H_{20}N_6O_2$	472.508	Solid	Brown	53	180-182	0.88

UV SPECTROSCOPY

S.No	Compound code	λmax
1.	**PYS-1**	251
2.	**PYS-2**	237
3.	**PYS-3**	262
4.	**PYS-4**	237
5.	**PYS-5**	262

IR SPECTROSCOPY

PYS-1

S.No	Type of Vibration	Wavenumber(cm^{-1})
1.	(Aromatic) C-H Stretching	3326.61
2.	C=N Stretching	2360.44
3.	C=C Stretching	1681.62
4.	C-C Stretching	1599.66
5.	C-Cl Stretching	834.062

PYS-2

S.No	Type of Vibration	Wavenumber(cm^{-1})
1.	(Alcohol) OH Stretching	3325.64
2.	(Aromatic) C-H Stretching	2972.73
3.	C=N Stretching	1540.85
4.	C=C Stretching	1652.70
5.	C-C Stretching	1540.85

PYS-3

S.No	Type of Vibration	Wavenumber(cm^{-1})
1.	(Aromatic) C-H Stretching	3324.68
2.	C=N Stretching	2926.45
3.	C=C Stretching	1558.2
4.	C-C Stretching	1540.85
5.	C-F Stretching	879.381

PYS-4

S.No	Type of Vibration	Wavenumber(cm^{-1})
1.	(Alcohol) OH Stretching	3324.68
2.	(Aromatic) C-H Stretching	2972.73
3.	C=N Stretching	2926.45
4.	C=C Stretching	1588.2
5.	C-C Stretching	1540.85

PYS-5

S.No	Type of Vibration	Wavenumber(cm^{-1})
1.	(Aromatic) C-H Stretching	3325.64
2.	C=N Stretching	2972.73
3.	C=C Stretching	1588.2
4.	C-C Stretching	1540.85
5.	C-NO_2 Stretching	803.206

1H NMR SPECTROSCOPY

PYS-2

S.No	Type of Proton	δ ppm
1	Aromatic C-H	(m, 19H, 6.93 - 7.27)
2	-CH	(s, 1H, 2.51)
3	-CH	(s, 1H, 3.40)
4	- OH	(s, 1H, 9.70)
5	- OH	(s, 1H, 10.15)

PYS-4

S.No	Type of Proton	δ ppm
1	Aromatic C-H	(m, 20H, 6.57 - 7.96)
2	-CH	(s, 1H, 2.53)
3	-CH	(s, 1H, 3.40)
4	- OH	(s, 1H, 10.06)

13C NMR SPECTROSCOPY

PYS-2

S.No	Type of Proton	δ ppm
1	Aromatic C	112.34 -146.31
2	C=N	158.29
3	C=N	160.75

PYS-4

S.No	Type of Proton	δ ppm
1	Aromatic C	115.06 -129.50
2	C=N	146.51
3	C=N	152.76

MASS SPECTROSCOPY

PYS-1: m/z ratio: 461

PYS-4: m/z ratio: 445

The chemical structures of the synthesized Schiff base linked pyrazole derivatives (**PYS 1,2,3,4 and 5**) were established by FT-IR, MASS and 1H & 13CNMR spectral studies.

 The IR spectrum of all the compounds showed the characteristic band at range of 3324.68-3326.61 cm-1 which indicated the presence of **Aromatic** ring and C=N stretching of imines were observed in the range of 2972.27-2360.44cm-1. Characteristic bands at range of 1540.85-1618.62 cm-1 for the presence of C=C and C-C stretching absorption band were observed in the range of 1450.85-1599.66 cm-1.

 PYS-1 showed C-Cl stretching at 834.06 cm-1. PYS-2 confirms the presence of phenolic -OH stretching absorption band at 3325.64cm-1. PYS-3 confirms the presence of fluorine stretching absorption band at 879.381 cm-1. PYS-4 confirms the presence of phenolic -OH stretching absorption band at 3324.68 cm-1 . PYS-5 confirms the presence of nitro group stretching absorption band at 803.206 cm-1 .

 The 1HNMR spectrum of the compound, PYS-2 revealed the presence of aromatic protons as multiplet at the range of 6.93-7.27 δ ppm, PYS-4 as multiplet at the range of 6.57-7.96 δ ppm. PYS-2 showed the presence of –CH protons as two singlets at 2.51 and 3.40 δ ppm respectively and PYS-4 revealed –CH protons as two singlets at 2.53 and 3.40 δ ppm respectively. PYS-2 showed the presence of phenolic –OH protons as two singlets at 9.70 and 10.15 δ ppm. PYS-4 revealed the presence of phenolic –OH proton as singlet at 10.06 δ ppm.

 The 13CNMR spectrum of the compound, PYS-2 revealed the presence of aromatic carbon as multiplet at the range of 112.34-146.31 δ ppm, PYS-4 as multiplet at the range of 115.06-129.50 δ ppm. PYS-2 showed the presence of imine protons at 158.29 and 160.75 δ ppm respectively and PYS-4 revealed imine protons at 146.51 and 152.76 δ ppm respectively.

 The molecular ion peaks was in correlation with the molecular weight of the synthesized compounds.

INVITRO TYROSINASE INHIBITION ASSAY

The *invitro* growth inhibitory activity of the newly synthesized compounds PYS-1, PYS- 2, PYS-3, PYS-4 and PYS-5 was investigated by tyrosinase inhibition assay, in comparison with a well-known standard drug (Kojic acid) under the same conditions using a colorimetric Mushroom tyrosinase assay. Data generated were used to plot a dose response curve of which the concentration of test compounds required to kill 50% of cell population (IC50) was determined. The result reveals that descending order of activity of the newly synthesized compounds towards the Tyrosinase inhibition assay.

All the synthesized compounds were tested *invitro* cytotoxicity activity against tyrosinase enzyme inhibition assay and their **percentage inhibition and IC50values** were obtained. Among the tested compounds PYS-1 and PYS-4 showed the most cytotoxic effects on tyrosinase enzyme inhibition assay and the other compounds PYS-2, PYS-3, PYS-5 showed the lowest toxicity on the tyrosinase enzyme inhibition assay.

Among the synthesized compounds only two compounds PYS-1 and PYS-4 offered good percentage inhibition in comparison to the standard Kojic acid (19.17µg/ml). This proves the extreme activity of the synthesized compounds.

Cytotoxic activity of the synthesized compounds

Compound code	Concentrations(µg/ml)						IC_{50}
	3.125	6.25	12.5	25	50	100	
PYS-1	7.991	10.101	16.267	30.020	48.113	71.115	**62.21**
PYS-2	8.113	11.967	21.541	25.070	32.941	39.432	**<100**
PYS-3	6.044	13.062	21.784	26.531	31.075	43.569	**<100**
PYS-4	7.545	20.040	31.440	45.922	54.523	73.265	**32.51**
PYS-5	8.194	8.924	12.576	17.849	31.075	44.259	**<100**
KOJIC ACID	12.535	20.811	37.444	58.215	73.387	86.572	**19.17**

All the determinations were carried out in triplicate and the values are expressed as the mean±SD

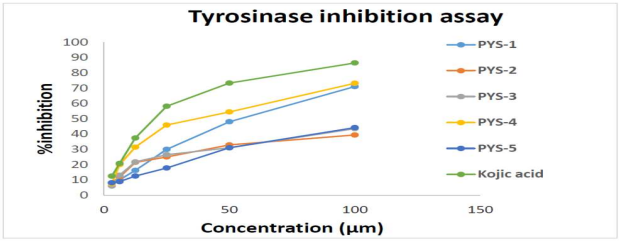
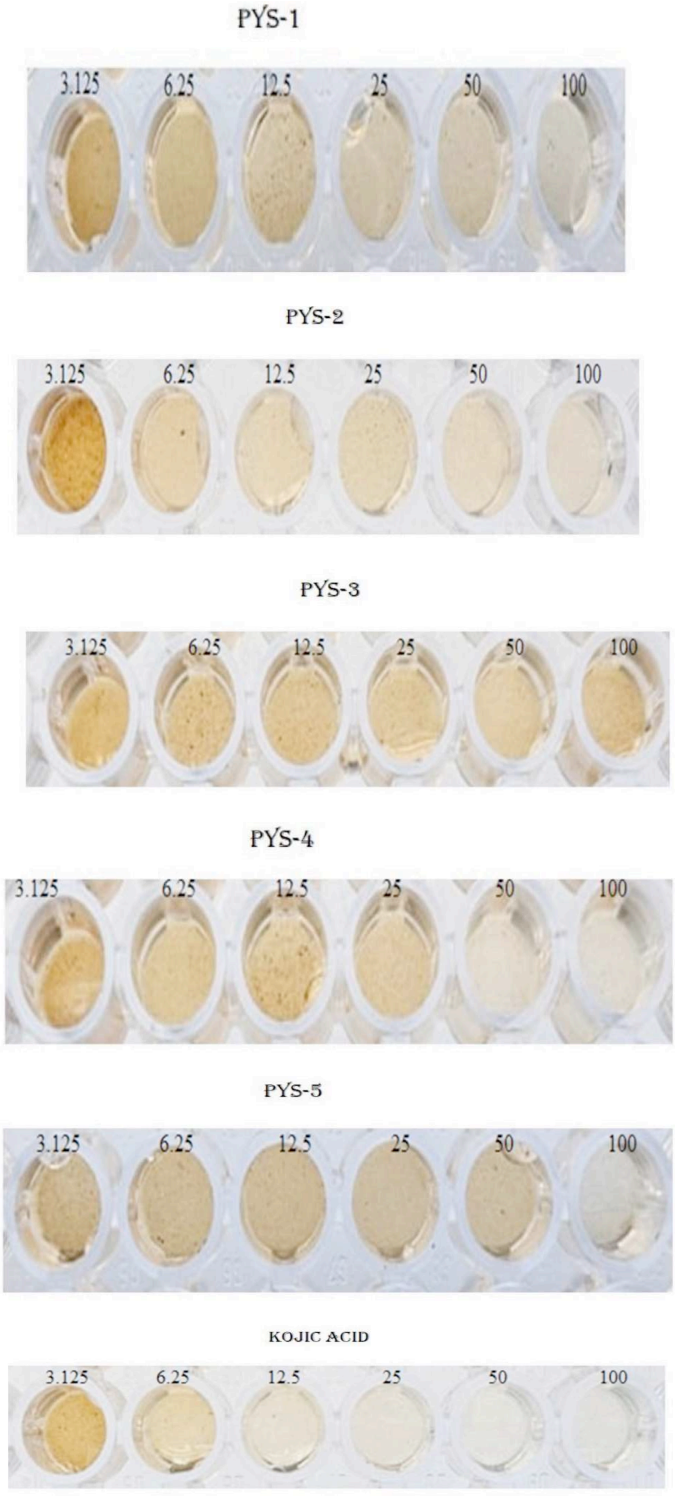


Fig. No.: 28 Dose Response Curve



A375 cell line activity of synthesized compounds PYS-4 at different concentrati

COMPOUNDCODE	Conc(µg/ml)	Mean	SD
PYS-4	20	83.15	0.89
	40	73.32	1.37
	80	66.98	1.51
	160	50.61	0.24
	320	44.48	0.97
CISPLATIN	20	52.60	1.49
	40	45.26	1.46
	80	34.87	0.88
	160	26.89	0.90
	320	19.70	0.66

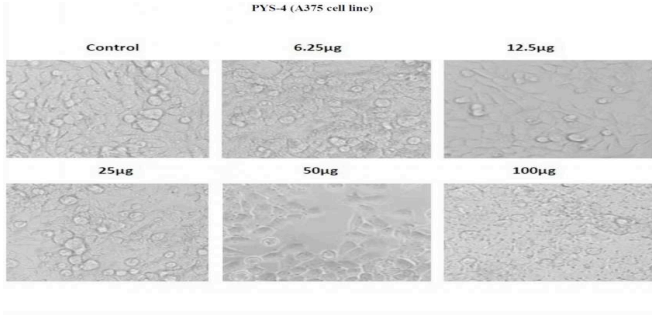


Fig. No.: 44 PYS-4 A375 cell line

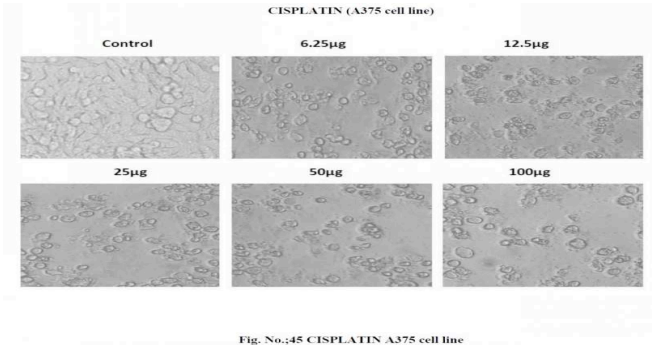


Fig. No.;45 CISPLATIN A375 cell line

SUMMARY AND CONCLUSION

Sixty compounds were docked against target protein. Based upon the glide score and feasibility of synthesis, five compounds from Schiff base linked pyrazole series were synthesized. The synthesized compounds were characterized based on its physicochemical properties and spectral data. The synthesis of Schiff base linked pyrazoles involves a four step process, where **4-[(1*E*)-1-(2-phenylhydrazinylidene)ethyl]aniline** was synthesised by reaction between *p*-aminoacetophenone and phenylhydrazine. The product obtained was converted into **3-(4-aminophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde** by Vilsmeier Haack reaction The product so obtained was cyclised into **4-(1-phenyl-4-{(*E*)-[(pyridin-4-yl)imino]methyl}-1*H*-pyrazol-3-yl)aniline** by treatment with 2-aminopyridine. The formed schiff bases linked pyrazole derivatives were further refluxed with various substituted aromatic aldehydes to afford the target compounds. The purity of the synthesized compounds was assessed by melting point and thin layer chromatography analysis. Other physicochemical parameters like percentage yield, molecular weight, molecular formula of synthesised compounds were determined. The final compounds were obtained in moderate to high yield. The percentage yield of the synthesised compounds was found to be in the range of 53-58 %.

A library of 60 compounds containing schiff base linked pyrazole derivatives were designed, optimized and docked into the active site of the **Tyrosinase Receptor** (**PDB ID:6QXD**). The docking protocol included four steps, Protein preparation wizard, Ligand Preparation, Receptor Grid Generation, Ligand Docking and evaluation of the docked orientations. According to the docking scores and their interaction with receptor, the compounds were selected for synthesis. All the synthesized compounds were evaluated for their physicochemical properties including molecular formula, molecular weight, percentage yield, melting Point, and thin Layer chromatography analysis. The synthesized compounds were interpreted for spectral analysis including Ultraviolet Spectroscopy, Infrared Spectroscopy, 1H & 13C NMR spectroscopy and Mass Spectroscopy. The synthesized compounds were screened for their anti-melanogenic activity and anti-skin cancer using MTT assay and Mushroom tyrosinase assay.

A new series of schiff base linked pyrazole derivatives have been synthesized and evaluated for their potency and efficacy as **Tyrosinase Receptor (J**KB inhibitors). These results suggest that the synthesized compounds can be good nominees for future investigation of anti-melanogenic anti-skin cancer agents..

REFERENCE

1. Richard C. Mohs, Nigel H. Grieg. Drug discovery and development: Role of basic biological research. Alzheimer's & Dementia. Translational Research & Clinical Interventions .2017. 651-665
2. Nutan Prakash, Patel Devangi. Drug Discovery. Journal of Antivirals & Antiretrovirals. 2010. Volume 2(4). 063-068
3. Giersiefen H, Hilgenfeld R, Hillisch A. Modern Methods of Drug Discovery. Institute of Molecular Biotechnology, Beutenbergster. Germany 2003. 109-155
4. Shu-Feng Zhou, Wei-Zhu Zhong. Drug Design and Discovery: Principles and Applications. Molecules. 2017, 222-279.
5. Garyfallia I Makrynitsa, Michail Lykouras, Georgios A Spyroulias, Minos-Timotheos Matsoukas, John Wiley & Sons. 2018.1-5.
6. Arup K. Ghose, Vellarkad N, Viswanadhan , John J. Wendoloski. A Knowledge-Based Approach in Designing Combinatorial or Medicinal Chemistry Libraries for Drug Discovery. A Qualitative and Quantitative Characterization of Known Drug Databases. American Chemical Society. J. Comb. Chem. 1999, Volume1, 55–68.
7. Terry P Lybrand, Ligand-protein docking and rational drug design. Elsevier. Current Opinion in Structural Biology 1995. Volume 5(2):224-228.
8. Antunes DA, Devaurs D, Kavraki LE (December 2015). "Understanding the challenges of protein flexibility in drug design". Expert Opinion on Drug Discovery.2018 Volume 10 : 1301–13.
9. Dipesh Tamrakar.phenylalanine and tyrosine amino acid metabolism, Tyrosine biosynthesis metabolic products functions disorders of tyrosine metabolism. Dec 25, 2017, 7 – 45.
10. An overview of skin biology is found in RUTH K. FREINKEL and DAVID T. WOODLEY (eds.), The Biology of the Skin (2001).
11. ARTHUR ROOK et al. (eds.), Rook's Textbook of Dermatology, 7th ed., 4 vol. (2006), is a comprehensive work on normal and pathological human skin. Special aspects of the subject are discussed in HOWARDI. MAIBACH and EDWARD K. BOISITS (eds.), Neonatal Skin: Structure and Function, 2nd ed. (2003); and JOACHIM.
12. Nordlund JJ, Boissy RE, Hearing VJ, King RA, Oetting WS, Ortonne JP. The Pigmentary System: Physiology and Pathophysiology. 2nd ed. Oxford: Blackwell Publishing Ltd (2006). .
13. Abdel-Malek Z, Kadekaro AL (2006) Human pigmentation: its regulation by ultraviolet light and by endocrine, paracrine, and autocrine factors. In: The pigmentary system (Nordlund JJ, Boissy RE, Hearing VJ, King RA, Oetting WS, Ortonne JP. eds), 2nd ed.

Malden, MA: Blackwell Publishing, 410–20
14. Pawelek JM, Lerner AB. 5,6-Dihydroxyindole Is a Melanin Precursor Showing Potent Cytotoxicity. Nature (1978) 276:626–8.
15. W. FLUHR and PETER ELSNER (eds.), Tissue Engineering in Dermatology (2009). Current research in the field is covered in The Journal of Investigative Dermatology (monthly).
16. Buist, N.R.M. et al.: Disorders of tyrosine metabolism: In " Heritable Disorders of Amino Acid Metabolism" ed. W. L. Nyhan, John Wiley & Sons, New York , p. 160–176. 1974.
17. Kumar CM, Sathisha UV, Dharmesh S, Rao AG, Singh SA (Mar 2011). "Interaction of sesamol (3,4-methylenedioxyphenol) with tyrosinase and its effect on melanin synthesis". Biochimie. 93 (3): 562–9
18. J. P. Greenstain Chemistry of the amino acids, 1961
19. S. Eller, K. Ostrom, and B. A. Gilchrest, "DNA damage enhances melanogenesis," Proceedings of the National Academy of Sciences of the United States of America, vol. 93, no. 3, pp. 1087–1092, 1996
20. Hunter T (2012-09-19). "Why nature chose phosphate to modify proteins". Philosophical Transactions of the Royal Society B: Biological Sciences. 367 (1602): 2513–2516.
21. Lu Z, Jiang F, Wu Y (2021-12-11). "Phosphate binding sites prediction in phosphorylation-dependent protein-protein interactions". Bioinformatics (Oxford, England). 37 (24): 4712–4718
22. Xu YG, Aylward JL, Swanson AM, Niederhuber JE, Armitages JO, Doroshow JH, kastan MB, Tepper JE, eds. Abeloff's Clinical Oncology. 6th ed. Philadelphia, P: Elsevier; 2020. Beélik, A. in Advances in carbohydrate chemistry Vol. 11 145-183.
23. Shi, W.; Hu, J.; Bao, N.; Li, D.; Chen, L.; Sun, J. Design, synthesis and cytotoxic activities of scopoletin-isoxazole and scopoletin-pyrazole hybrids. Bioorg. Med. Chem. Lett. 2017, 27, 147–151.
24. Abd El-Karim, S.S.; Anwar, M.M.; Mohamed, N.A.; Nasr, T.; Elseginy, S.A. Design, synthesis, biological evaluation and molecular docking studies of novel benzofuran–pyrazole derivatives as anticancer agents. Bioorg. Chem. 2015, 63, 1–12.
25. Yamamoto, S.; Tomita, N.; Suzuki, Y.; Suzaki, T.; Kaku, T.; Hara, T.; Yamaokaa, M.; Kanzakia, N.; Hasuokaa, A.; Babaa, A.; et al. Design, synthesis, and biological evaluation of 4-arylmethyl-1-phenylpyrazole and 4-aryloxy-1-phenylpyrazole derivatives as novel androgen receptor antagonists. Bioorg. Med. Chem. 2012, 20, 2338–2352.
26. Miamoto, N.; Sakai, N.; Hirayama, T.; Miwa, K.; Oguro, Y.; Oki, H.; Okada, K.; Takagi, T.; Iwata, H.; Awazu, Y.; et al. Discovery of N-[5-({2-[(cyclopropylcarbonyl) amino]imidazo[1,2-b]pyridazin-6-yl}oxy)-2- methylphenyl]-1,3-dimethyl-1H-pyrazole-5-carboxamide (TAK-593), a highly potent VEGFR2 kinase inhibitor. Bioorg. Med. Chem. 2013, 21, 2333–2345.
27. Darshan Joshi et. al., developed a novel structural optimizations for Bruton's Tyrosine Kinase (BTK) inhibitors, with compound 14b being found to be potent, selective, and irreversible, anti-cancer activity, 2014.
28. Muttaqin et. al., investigates the interaction of quinoline alkaloids with Bruton's Tyrosine Kinase (BTK) in leukemia treatment. Molecular docking simulations reveal ten compounds have the best affinity, anyi-cancer activity, 2018
29. Fan, C.-D.; Zhao, B.-X.; Wei, F.; Zhang, G.-H.; Dong, W.-L.; Miao, J.-Y. Synthesis and discovery of autophagy inducers for A549 and H460 lung cancer cells, novel 1-(20 -hydroxy-30 -aroxypropyl)-3-aryl1H-pyrazole-5-carbohydrazide derivatives. Bioorg. Med. Chem. Lett. 2008, 18, 3860–3864.
30. Mohamed Salah K. Youssef, Mohamed S. Abbady, Ragaa A. Ahmed, Ahmed A. Omar Journal of Heterocyclic Chemistry, 2013, Vol 50(2):179–187
31. Bonesi, M, Loizzo, M.R, Statti, G.A, Michel, S, Tillequin F. and Menichini F. Angiotensin Converting Enzyme (ACE) inhibitory activity of chalcones and their pyrazole derivatives. Bioorganic & Medicinal Chemistry Letters. 2010, 20 (6); 1990-1993
32. A. Kamal, A. B. Shaik, N. Jain, C. Kishor, A. Nagabhushhana, B. Supriya, G. B. Kumar, S. S. Chourasiya, Y. Suresh, R. K. Mishra and A. Addlagatta, Eur. J. Med. Chem. 92, 501, (2015).
33. F. Q. Wang, H. Yang, B. He, Y. K. Jia, S. Y. Meng, C. Zhang, H. M. Liu and F. W. Liu, Tetrahedron Lett. 72, 5769, (2016).
34.J. Wen, Q. Niu, J. Liu, Y. Bao, J. Yang, S. Luan, Y. Fan, D. Liu and L. Zhao, Bioorg. Med. Chem. Lett., 26, 375, (2016).
35. Y. J. Esvan, F. Giraud, E. Pereira, E. Pereira, V. Schuaud, L. Nauton, V. Thery, L. G. Dezhenkova, D. N. Kaluzhny, V. N. Mazov, A. A. Shtil, F. Anizon and P. Moreau, Bioorg. Med. Chem. 24, 3116, (2016).
36. K. Vaarla, R. K. Kesharwani, K. Santosh, R. R. Vedula, S. Kotamraju, M. K. Toopurani, Bioorg. Med. Chem. Lett. 25, 5797, (2015).
37. V. G. Reddy, T. S. Reddy, V. L. Nayak, B. Prasad, A. P. Reddy, A. Ravikumar, S. Taj and A. Kamal, Eur. J. Med. Chem. 122, 164, (2016).
38. Sakya, S.M.; DeMello, K.M.L.; Minich, M.L.; Rast, B.; Shavnya, A.; Rafka, R.J. 5-Heteroatom substituted pyrazoles as canine COX-2 inhibitors. Part 1: Structure-activity relationship studies of 5-alkylamino pyrazoles and discovery of a potent, selective, and orally active analog. Bioorg. Med. Chem. Lett. 2006, 16, 288–292
39. Vijesh, A.M.; Isloor, A.M.; Shetty, P.; Sundershan, S.; Fun, H.K. New pyrazole derivatives containing 1,2,4-triazoles and benzoxazoles as potent antimicrobial and analgesic agents. Eur. J. Med. Chem. 2013, 62, 410–415.
40. Bekhit, A.A.; Ashour, H.M.; Bekhit, A.E.D.A.; Abdel-Rahman, H.M.; Bekhit, S.A. Synthesis of some pyrazolyl benzenesulfonamide derivatives as dual anti-inflammatory antimicrobial agents. J. Enzym. Inhib. Med. Chem. 2009, 24, 296–309
41. Rashad, A.E, Hegab, M.I, Abdel-Megeid, R.E., Micky, J.A and Abdel-Megeid F.M. Synthesis and antiviral evaluation of some new pyrazole and fused pyrazolopyrimidine derivatives. Bioorganic & Medicinal Chemistry. 2008, 16 (15); 7102-7106
42. Lin, R, Chiu, G, Yu, Y, Connolly, P.J, Li, S, Lu, Y, Adams, M, Fuentes Pesquera, A.R, Emanuel, S.L. and Greenberger L.M. Design, synthesis and evaluation of 3, 4-disubstituted pyrazole analogues as anti- tumor CDK inhibitors. Bioorganic & medicinal chemistry letters. 2007, 17(16); 4557-4561
43. Ragavan, R.V.; Vijayakumar, V.; Kumari, N.S. Synthesis and antimicrobial activities of novel 1,5-diaryl pyrazoles. Eur. J. Med. Chem. 2010, 45, 1173–1180.
44. Kalaria, P.N.; Satasia, S.P.; Avalani, J.R.; Raval, D.K. Ultrasound-assisted one-pot four-component synthesis of novel 2-amino-3-cyanopyridine derivatives bearing 5-imidazopyrazole scaffold and their biological broadcast. Eur. J. Med. Chem. 2014, 83, 655–664
45. Akbas.; Kumar, S.; Kumar, P.; Kaushik, P.; Kaushik, D.; Dhingra, Y. Synthesis and biological evaluation of some 1H pyrazole -3-carboxylic acid as antimicrobial agents. Eur. J. Med. Chem. 2010, 45, 2650–2655..