Journal of Molecular Science

Journal of Molecular Science
Volume 36 Issue 1, Year of Publication 2026, Page 339-348
DoI-10.004687/1000-9035.2026.043

Journal of Molecular Science
www.jmolecularsci.com ISSN:1000-9035

Synthesis, Characterization and Evaluation of Pyrazole based Schiff Bases for Anticancer Activities

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Article Information
Received: 09-03-2026
Revised: 21-03-2026
Accepted: 12-04-2026
Published: 07-05-2026

Keywords
Pyrazole Schiff bases, ADME properties, anticancer activity, Structural characterization, Cytotoxicity study.

ABSTRACT
Pyrazole is an important nitrogen-containing heterocyclic compound that has attracted significant attention in medicinal chemistry due to its diverse biological and pharmacological properties. Among its many therapeutic applications, pyrazole derivatives have emerged as promising candidates for anticancer drug development because of their ability to regulate multiple molecular mechanisms involved in cancer initiation and progression. The present study is aimed at the synthesis and biological assessment of novel Schiff base derivatives containing the pyrazole nucleus for potential anticancer activity. 5-amino-1-(2,6-dichloro-4-(trifluoromethyl) phenyl)-1H-pyrazole-3-carbonitrile were synthesized by the reaction of 2,6-dichloro-4-trifluoromethyl aniline with the ethyl 2,3-dicyanopropionate, then the synthesized intermediate were reacted with the various aromatic aldehydes to prepare Schiff bases of pyrazole derivatives (MPY1-MPY10). The synthesized derivatives were characterized by spectroscopic techniques such as Fourier Transform Infrared (FT-IR) spectroscopy, Proton Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy, and Mass Spectrometry to confirm their structures. Their anticancer potential was evaluated through in vitro cytotoxicity studies against selected human cancer cell lines. Compound MPY2 demonstrated strong growth inhibitory activity against most of the cell lines. In addition, computational investigations will be conducted to assess drug-likeness, ADME properties, and molecular interactions with cancer-related biological targets. The findings of this study may contribute to the discovery of novel pyrazole-based lead compounds with improved anticancer efficacy and favorable pharmacological profiles for future therapeutic development.

1. INTRODUCTION:

Pyrazole ranks as one of the crucial nitrogen-containing heterocyclic compounds utilized in medicinal chemistry. It consists of a five-membered aromatic ring containing three carbon atoms and two neighboring nitrogen atoms. Due to its unique chemical structure, pyrazole has attracted considerable attention in pharmaceutical research and drug development. Pyrazole derivatives are widely studied for their various biological activities, such as anti-inflammatory, analgesic, antimicrobial, antifungal, antiviral, antipyretic, antidiabetic, anticonvulsant, antioxidant, and anticancer properties. Owing to these notable characteristics, pyrazole has become an important structural base in the development of various therapeutic compounds [(1)].

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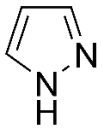

Figure 1: Structure of Pyrazole

The pyrazole core is found in various drugs available on the market. Numerous scientists persist in creating novel pyrazole derivatives to enhance effectiveness, specificity, safety, and pharmacological effects. Owing to its adaptability and capacity to connect with diverse biological targets, pyrazole is regarded as one of the most potential heterocyclic frameworks in medicinal chemistry. Pyrazole derivatives can engage with enzymes, receptors, proteins, and nucleic acids. Their action relies on the type of substitution on the pyrazole ring. Minor structural changes in pyrazole derivatives can result in significant alterations in biological activity [(2)].

Pyrazole is an aromatic heterocyclic ring composed of five members, featuring three carbon atoms and two neighbouring nitrogen atoms. Due to its distinctive electronic characteristics, structural adaptability, and capacity to engage with different biological targets, the pyrazole framework has become a significant pharmacophore in medicinal chemistry. Various pyrazole derivatives have been created and examined for a broad spectrum of pharmacological activities, such as anti-inflammatory, antimicrobial, analgesic, antidiabetic, and anticancer effects.[(3)]

In cancer chemotherapy, compounds based on pyrazole have garnered considerable interest for their capacity to impede cancer cell growth via various mechanisms. The two nitrogen atoms in the pyrazole ring enable these molecules to create robust interactions with biological macromolecules like enzymes, receptors, and nucleic acids. As a result, pyrazole derivatives can specifically aim at pathways related to tumor development and advancement. Numerous compounds with pyrazole structures demonstrate anticancer effects by blocking protein kinases, cyclooxygenase-2 (COX-2), tubulin polymerization, angiogenesis, and various signaling pathways vital for the survival of cancer cells. Certain pyrazole derivatives trigger apoptosis (programmed cell death), halt the cell cycle, restrain metastasis, and reduce tumor angiogenesis. Their capacity to influence various molecular targets renders them potential candidates for creating new anticancer treatments.[(4)]

The pyrazole core acts as an excellent foundation for structural alterations. Substituents may be added at various locations on the ring to improve potency, selectivity, metabolic stability, and pharmacokinetic characteristics. These alterations have led to the identification of many pyrazole-derived lead compounds exhibiting potential anticancer effects. The pyrazole structure is also found in various clinically significant medications and lead candidates being studied for cancer therapy. Changes made to the pyrazole ring have allowed medicinal chemists to enhance potency, selectivity, pharmacokinetic characteristics, and safety profile.[(5,6)] Researchers have turned to pyrazole derivatives in their search for new anticancer agents, attracted by their strong pharmacological properties and ease of synthesis. Compounds containing pyrazole have shown strong cytotoxic effects against multiple human cancer cell lines, such as those from breast, lung, colon, prostate, liver, ovarian, cervical cancers, and leukemia. [(7)]

Cancer consists of a complex set of diseases defined by the unchecked growth, multiplication, and dissemination of abnormal cells. In typical physiological conditions, cells grow, divide, and die in a controlled way to sustain tissue homeostasis. However, genetic and epigenetic changes can interfere with these regulatory processes, resulting in the development of cancerous tumors. Cancer continues to be one of the primary causes of illness and death globally, placing a major strain on healthcare systems and society. Cancer is a complex disease marked by the unchecked growth of cells, their spread into surrounding tissues, and their ability to metastasize. Even with major progress in diagnosis and treatment, it continues to be a major global health challenge. Grasping the molecular mechanisms, risk factors, and treatment methods is crucial for creating more effective strategies in cancer prevention, diagnosis, and management. Ongoing research into targeted therapies, immunotherapy, and personalized medicine presents encouraging prospects for enhancing patient outcomes and quality of life.[(8)]

Cancer is a severe illness characterized by uncontrolled cell growth that spreads to various parts of the body, making it one of the top causes of death globally. For this reason, scientists are continually striving to create safer and more effective anticancer drugs. Among the numerous chemical compounds researched, pyrazole derivatives have attracted considerable attention because of their potent medicinal properties. Pyrazole is a compact chemical structure consisting of a five-membered ring with two nitrogen atoms, conferring it distinctive properties. These properties enable it to interact readily with key biological targets like enzymes, proteins, and DNA, which makes it valuable in drug development. A key benefit of pyrazole is that its structure is readily adaptable, enabling scientists to enhance its efficacy

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and minimize adverse effects. Studies indicate that pyrazole derivatives are effective against several cancers, such as breast, lung, colon, liver cancer, and leukemia. They inhibit cancer cell proliferation, interfere with tumor-promoting signals, and induce cancer cell death. Because of their varied actions and adaptability, pyrazole derivatives are seen as promising candidates for developing new anticancer drugs. [9,10]

2. Experimental Section:

2.1 Materials and Methods: Commonly employed materials in the synthesis of pyrazole derivatives encompass hydrazine hydrate, phenyl hydrazine, substituted hydrazines, acetylacetone, ethyl acetoacetate, chalcones, diketones, beta-keto esters, aldehydes, ketones, active methylene compounds, substituted anilines, thiourea, sulfonyl chlorides, carboxylic acids, isocyanates, hydrazides, and heterocyclic amines. The choice of solvent varies based on the reaction type and the characteristics of the starting materials. Typical solvents are ethanol, methanol, acetic acid, dimethylformamide, dimethyl sulfoxide, tetrahydrofuran, chloroform, dichloromethane, toluene, and ethyl acetate. Commonly employed catalysts and reagents in synthesis include piperidine, triethylamine, potassium carbonate, sulfuric acid, hydrochloric acid, acetic anhydride, phosphorus oxychloride, sodium hydroxide, and sodium acetate. [11]

In biological screening, various cancer cell lines are frequently employed. These encompass breast cancer cell lines like MCF-7, lung cancer cell lines like A549, liver cancer cell lines like HepG2, colon cancer cell lines such as HCT-116, cervical cancer cell lines including HeLa, and leukemia cell lines such as K562. Normal cell lines are also employed to assess the toxicity and selectivity of the synthesized compounds. [12]

Commonly used instruments in the preparation and characterization of pyrazole derivatives include reflux apparatus, microwave synthesizer, ultrasonic bath, hot air oven, magnetic stirrer, thin-layer chromatography plates, melting point apparatus, ultraviolet-visible spectrophotometer, infrared spectrophotometer, proton nuclear magnetic resonance spectroscopy, carbon-13 nuclear magnetic resonance spectroscopy, mass spectrometry, and elemental analysis. [13]

2.2 Synthesis of Pyrazole Scaffold and Schiff Bases

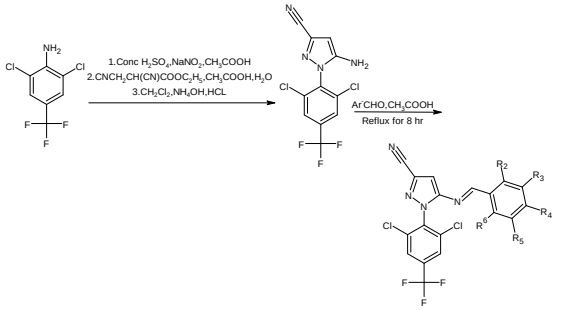

Figure 2: Scheme for synthesis of Pyrazole Scaffold and Schiff Bases (MPY1-MPY10)

Step: I Synthesis of Pyrazole Scaffold and Schiff Bases

a. Procedure for the Synthesis of 5-amino-1-(2,6-dichloro-4-(trifluoromethyl) phenyl)-1H-pyrazole-3-carbonitrile:

In a reflux setup, a round-bottom flask containing conc. 3ml of sulfuric acid were added dropwise to a mixture containing 0.75 grams of sodium nitrate dissolved in 2.5 ml of acetic acid. The flask was then continuously shaken for 15 minutes using a mechanical stirrer. To the above solution, 2,6-dichloro-4-trifluoromethyl aniline (2.12 g), prepared in 10 ml of acetic acid, was added; the resulting reaction mixture was then left to stand at room temperature. The above-prepared reaction mixture is then heated at 60 °C for 45 minutes, after which it is poured into a mixture containing 1.4 g of ethyl 2,3-dicyanopropionate, 7 ml of acetic acid, and 10 ml of water, which is kept in an ice bath. The prepared reaction mixture is stirred on a mechanical shaker for 2.30 hours at room temperature. Following the addition to the reaction mixture, water and dichloromethane were used. The aq. dichloromethane was used to extract the layer of the reaction mixture. The remaining organic layer of the reaction mixture is shaken at high speed for 12.15 hours in 100 ml of 30% ammonium hydroxide solution. Finally, the organic layer of the reaction mixture is washed with a mixture of water (60 ml) and 1 M HCl (30 ml), then dried over sodium sulfate and concentrated. The crude product is further washed with CH_2Cl_2 and pet. ether to obtain 5-amino-1-(2,6 -dichloro-4-(trifluoromethyl) phenyl)-1H -pyrazole.[14]

b. Procedure for the Synthesis of Pyrazole based Schiff Bases:

An equivalent amount of 5-amino-1-(2,6 -dichloro-4-(trifluoromethyl) phenyl)-1H -pyrazole-3-carbonitrile (1.0 mmol) and various aromatic aldehydes (1.0 mmol) were combined in 10 mL of absolute ethanol, with 3.0 mmol of acetic acid added as catalyst, and the mixture was heated under reflux for 7 to 10 hours. The progress of the reaction was monitored using thin-layer chromatography plates. Once the reaction was complete, the reaction mixture was poured into a petri dish, and the solvent

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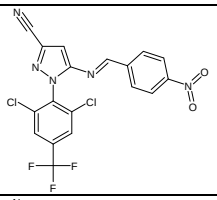
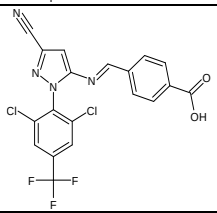
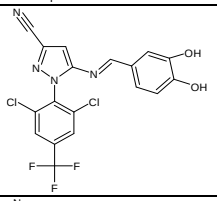
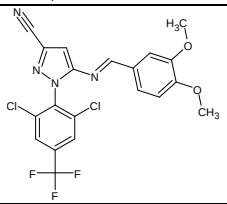
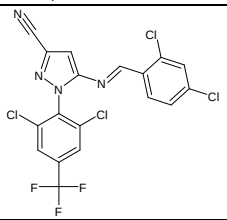
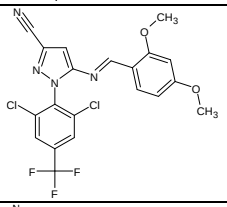
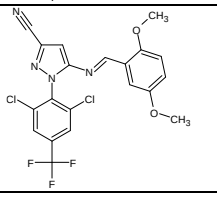
was evaporated. After the reaction was complete, the reaction mixture was decanted into a petri dish and allowed to evaporate the solvent. The crude product is then recrystallized using ethanol as the solvent. An attempt was made to synthesize the pyrazole-based Schiff bases for all the top hits identified from molecular docking studies; however, we were only able to successfully synthesize the top ten hits (MPY1-10). The physical constants of all the synthesized compounds were mentioned [15].

Table 1: Physical data of synthesized compounds

Comp Code	R	Structure	Mol. Formula	Mol Wt	% Yield	R_f Value	M P (°C)
MPY1	4-NO_2		$C_{18}H_8Cl_2F_3N_5O_2$	454	74	0.74	172-176
MPY2	4-COOH		$C_{19}H_9Cl_2F_3N_4O_2$	453	76	0.67	300-308
MPY3	3-OH, 4-OH		$C_{18}H_9Cl_2F_3N_4O_2$	441	73	0.70	218-220
MPY4	2-OCH_3, 4-OCH_3		$C_{20}H_{13}Cl_2F_3N_4O_2$	469	70	0.72	292-298
MPY5	2-Cl,4-Cl		$C_{18}H_7Cl_4F_3N_4$	478	77	0.71	164-168
MPY6	3-OCH_3, 4-OCH_3		$C_{20}H_{13}Cl_2F_3N_4O_2$	469	71	0.70	272-276
MPY7	2-OCH_3, 5-OCH_3		$C_{20}H_{13}Cl_2F_3N_4O_2$	469	74	0.71	158-162

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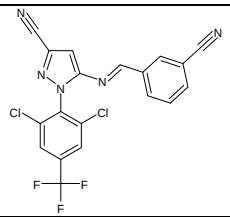
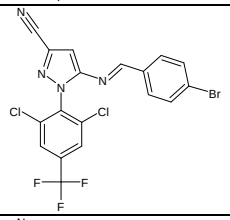
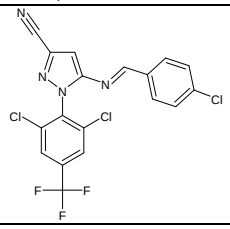
MPY8	3-CN		$C_{19}H_8Cl_2F_3N_5$	434	75	0.70	310-318
MPY09	4-Br		$C_{18}H_8BrCl_2F_3N_4$	488	76	0.68	182-184
MPY10	4-Cl		$C_{18}H_8Cl_3F_3N_4$	443	78	0.69	298-304

2.3 Characterization of Synthesized compounds:

5-amino-1-(2,6-dichloro-4-(trifluoromethyl) phenyl)-1H-pyrazole-3-carbonitrile (MPY)

^{1}HNMR (DMSO, 500MHz) δ ppm: 4.0 (s, 2H of –NH2), 6.5 (s, 1H of pyrazole), 7.3 (s, 2H, (2,6-dichloro-4-(trifluoromethyl) phenyl)); ES-MS m/z (%): 320.99 (M+H).

5-(4-nitrobenzylideneamino)-1-(2,6-dichloro-4-(trifluoromethyl) phenyl)-1H-pyrazole-3 carbonitrile (MPY1)

FT-IR (cm^{-1}): 3388.24 (N–H stretching), 3088.00 and 3008.00 (aromatic C–H stretching), 2924.00 and 2861.00 (aliphatic C–H stretching), 1712.10 (C=O stretching), 1528.00 (NO_2 asymmetric stretching), 1506.20 (Ar–C=C stretching), 1342.00 (NO_2 symmetric stretching), 1175.00 and 1033.00 (C–F stretching of CF_3), 1032.00 (C–N stretching), 970.00 and 911.00 (aromatic C–H bending), and 687.13 (C–Cl stretching).

^{1}HNMR (DMSO, 500MHz) δ ppm: 6.5 (s, 1H of pyrazole), 7.3 (s, 2H, (2,6-dichloro-4-(trifluoromethyl) phenyl)), 7.9 (m, 2H,-CH of phenyl), 8.1 (s, 1H,-CH=N), 8.2 (m, 2H, - CH of phenyl); ES-MS m/z (%): 454.00 (M+H).

4-((1-(2,6-dichloro-4-(trifluoromethyl) phenyl)-3-cyano-1H-pyrazol-5-limino) methyl) benzoic acid (MPY2)

^{1}HNMR (DMSO, 500MHz) δ ppm: 6.5 (s, 1H of pyrazole), 7.3 (s, 2H, (2,6-dichloro-4-(trifluoromethyl) phenyl)), 7.8 (m, 2H,-CH of phenyl), 8.1 (s, 1H,-CH=N), 8.2 (m, 2H, - CH of phenyl),11(s,1H-COOH); ES-MS m/z (%): 453.01 (M+H).

4-(2-Ethoxy-5-(4-methylpiperazin-1-ylsulfonyl) benzylideneamino) benzoic acid (MPY4)

FT-IR (cm^{-1}): 3390.30 (N–H stretching), 3062.30 and 3040.20 (aromatic C–H stretching), 2916.15 and 2828.45 (aliphatic C–H stretching of OCH_3), 1731.30 (C=O stretching), 1631.45 (azomethine CH=N stretching), 1581.00 (Ar–C=C stretching), 1459.00 and 1369.35 (C–N stretching), 1280.40 (C–O stretching), 1158.40 (C–F stretching of CF_3), 1032.00 (C–O stretching of methoxy group), 970.25 and 911.00 (aromatic C–H bending), and 687.13 (C–Cl stretching).

^{1}HNMR (DMSO, 500MHz) δ ppm: 3.73 (s, 6H of Ar–OCH3) 6.3 (s, 1H, -CH of Phenyl), 6.4 (s, 1H, -CH of Phenyl), 6.5 (s, 1H of pyrazole), 7.3 (s, 2H, (2,6-dichloro-4-(trifluoromethyl) phenyl)), 7.4 (s, 1H,-CH of phenyl), 8.1 (s, 1H,-CH=N).

5-[(3-cyanophenyl) methylidene] amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-1H-pyrazole-3-carbonitrile (MPY8)

FT-IR (cm^{-1}): 3300.06 (N–H stretching), 3058.20 (aromatic C–H stretching), 2226.12, 2190.18 and 2160.47 (C≡N stretching), 1621.03 (azomethine CH=N stretching), 1588.35 (Ar–C=C stretching), 1459.75 and 1369.60 (C–N stretching), 1369.75 (aromatic ring vibration), 1138.55 and 1032.56 (C–F stretching of CF_3), 911.74 (C–Cl stretching), and 970.28 (aromatic C–H bending).

^{1}HNMR (DMSO, 500MHz) δ ppm: 6.5 (s, 1H of pyrazole), 7.3 (s, 2H, (2,6-dichloro-4-(trifluoromethyl)phenyl)), 7.5 (m, 2H,-CH of phenyl) 7.9 (m, 2H,-CH of phenyl), 8.1(s, 1H,-CH=N); ES-MS m/z (%): 469.04 (M+H).

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5-(4-bromobenzylideneamino)-1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carbonitrile (MPY10)

FT-IR (cm^{-1}): 3590.11 (N–H stretching), 3070.22 and 3010.10 (aromatic C–H stretching), 2924.17 and 2854.35 (aliphatic C–H stretching), 1621.03 (azomethine CH=N stretching), 1512.38 (Ar–C=C stretching), 1462.65 and 1438.55 (C–N stretching), 1339.60 (aromatic ring vibration), 1280.28, 1176.20 and 1030.28 (C–F stretching of CF_3), 911.44 (C–Cl stretching), and 970.28 (aromatic C–H bending).

^{1}HNMR (DMSO, 500MHz) δ ppm: 6.5 (s, 1H of pyrazole), 7.3 (s, 2H, (2,6-dichloro-4-(trifluoromethyl)phenyl)), 7.5 (m, 4H, -CH of phenyl) 8.1(s, 1H,-CH=N); ES-MS m/z (%): 486.93 (M+H)

2.4 Insilico Screening for Drug likeness

The physicochemical properties play a crucial role in predicting the pharmacokinetics and toxicity of drug-like molecules. It primarily impacts the absorption, distribution, metabolism, excretion, and toxicity of molecules. The prediction of drug likeness was carried out using the FAFDrug4 tool. All the designed Schiff bases adhere to Lipinski's rule of five (RO5) and its modifications for orally active drugs, including criteria such as MW≤ 500 Da, LogP value ≤5.5, tPSA≤140 Å², n-HBD≥5, and n-HBA≥10. They are also further evaluated for additional pharmacokinetic properties and toxicity assessments. Analysis of Lipinski's Rule of Five (RO5) and its modified parameters indicates that certain Schiff bases exceed the specified limits for molecular weight and Log P, and these compounds were excluded from further study. The remaining compounds were then subjected to molecular docking analysis. The pyrazole-based Schiff bases that comply with Lipinski's rules (RO5) were further evaluated for additional pharmacokinetic properties, including Log SW, solubility in mL/L, oral bioavailability, phospholipidosis, and undesirable moieties or substructures associated with toxicity. The results were revealed in **Table 2** and **Table 3**. All of them exhibited parameter values within the accepted ranges specified. [16-19]

Table 2: ADMET profiling of compounds

Comp Code	Molecular Weight	Log P	Log Sw	tPSA	Rota B	Rigid B
MPY1	454	5	-6	99	5	21
MPY2	453	5	-5	94	5	20
MPY3	441	5	-5	94	4	19
MPY4	469	5	-6	72	6	19
MPY5	478	6	-7	53	4	19
MPY6	469	5	-6	83	6	19
MPY7	469	5	-6	72	6	19
MPY8	434	5	-6	77	4	20
MPY9	488	5	-6	57	5	19
MPY10	443	6	-6	53	4	19

Table 3: Computed properties of compounds

Comp Code	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Oral Bioavailability	Phospholipidosis	Lipinski Rule/ Violations
MPY1	0	7	Good	Non Inducer	Acceptable/0
MPY2	1	6	Good	Non Inducer	Acceptable/0
MPY3	2	6	Good	Non Inducer	Acceptable/0
MPY4	0	6	Good	Non Inducer	Acceptable/0
MPY5	0	4	Good	Non Inducer	Acceptable/0
MPY6	1	6	Good	Non Inducer	Acceptable/0
MPY7	0	6	Good	Non Inducer	Acceptable/0
MPY8	0	5	Good	Non Inducer	Acceptable/0
MPY9	0	5	Good	Non Inducer	Acceptable/0
MPY10	0	4	Good	Non Inducer	Acceptable/0

2.5 NCI-60 DTP Human Tumor Cell Line Screen:

The DTP at the NCI operates an anti-cancer screening program that features a cell-based assay designed to assess compounds for their capacity to suppress the growth of human tumor cells in culture. The assay is known as the NCI 60 Cell screen

In order to replace the use of transplantable animal tumours in anticancer drug screening, the US National Cancer Institute (NCI60) human tumour cell line anticancer drug screen was created in the late 1980s as an in vitro drug-discovery tool. In April 1990, the in vitro cell line screen was fully operationalised. The development took about five years (1985–1990), and the effort's perseverance was a reflection of discontent with the results of earlier in vivo primary screens. This screening model quickly gained recognition as a valuable resource for learning about the mechanisms of tumor-cell death and growth inhibition. Its function has recently shifted to that of a service screen that aids the cancer research community [20]. Out of the ten synthesized compounds four compounds were chosen by NCI for in vitro testing against a panel of

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about 60 tumor cell lines. The compounds were evaluated at a single dose concentration of 10 µl. The mean growth percent of 60 cell lines; representing nine different cancer types was displayed in **Table 4**.

Table 4: Mean growth percent of 60 cell lines at 10µl of compound

Panel /cell line	Growth % of tested compound			
	D-771881 (MPY1)	D-771850 (MPY4)	D-771846 (MPY3)	D-773068 (MPY2)
Leukemia				
CCRF-CCM	98.22	92.22	93.10	74.69
HL60 (TB)	99.34	94.53	96.47	42.05
K-562	99.39	96.80	90.28	73.02
MOLT-4	94.07	89.79	92.19	76.55
RPMI-8226	96.88	99.19	97.98	82.89
SR	86.47	92.23	78.97	16.11
Non Small cell lung cancer				
A549/ATCC	97.38	105.07	99.04	44.07
HOP-62	102.70	104.05	87.00	62.26
HOP-92	64.93	82.95	NA	50.34
NCI-H226	101.87	100.22	90.57	90.87
NCI-H23	98.23	107.45	99.50	101.23
NCI-H322M	91.42	93.31	80.23	97.84
NCI-H460	107.50	105.31	110.00	92.78
NCI-H522	94.01	93.73	93.60	-52.20
Colon Cancer				
COLO-205	114.67	116.49	99.86	109.57
HCC-2998	104.31	111.01	96.66	116.57
HCT-116	102.95	103.45	87.05	92.79
HCT-15	103.03	93.84	96.65	84.68
HT-29	112.24	102.10	99.64	98.46
KM-12	106.45	107.36	98.97	27.31
SW-620	109.30	99.92	101.60	109.12
CNS Cancer				
SF-2668	108.26	104.73	102.51	104.98
SF-295	91.68	94.00	113.35	76.41
SF-539	99.95	93.48	108.97	108.61
SNB-19	100.26	109.08	100.95	81.57
SNB-75	86.47	98.56	76.20	95.09
U251	99.76	98.54	97.86	NA
Melanoma				
LOX-IMVI	96.30	97.94	91.96	NA
MALME-3M	96.47	91.97	94.58	103.28
M14	96.20	98.68	104.11	106.99
MDA-MB-435	91.13	96.72	94.74	100.08
SK-MEL2	100.32	93.19	105.33	NA
SK-MEL28	104.55	112.17	97.42	104.43
SK-MEL-5	100.83	100.37	98.44	NA
UACC-257	97.23	95.86	105.43	103.93
UACC-62	97.93	105.78	98.34	84.32
Ovarian Cancer				
IGROV1	106.67	99.03	95.61	100.65
OVCAR-3	111.33	108.89	107.19	115.92
OVCAR-4	99.41	108.30	100.37	93.22
OVCAR-5	95.96	96.05	102.80	110.16
OVCAR-8	101.52	101.02	101.47	94.78
NCI/ADR-RES	107.21	110.88	102.22	103.95
SK-OV-3	108.70	114.09	95.66	118.65
Renal Cancer				
786-0	100.96	97.15	99.88	96.72
A498	102.96	100.72	92.77	85.80
ACHN	95.55	98.25	100.12	105.60
CAKI	1 95.44	97.18	88.98	98.76
RXF 393	105.43	111.24	96.71	NA
SN12C	97.95	100.14	98.53	80.64
TK-10	111.61	104.67	97.19	109.78
UO-31	77.02	82.91	74.74	52.46
Prostate Cancer				
PC-3	91.88	95.61	93.38	32.28
DU-145	114.37	117.39	105.17	113.41
Breast Cancer				
MCF7	109.10	100.83	95.64	92.56

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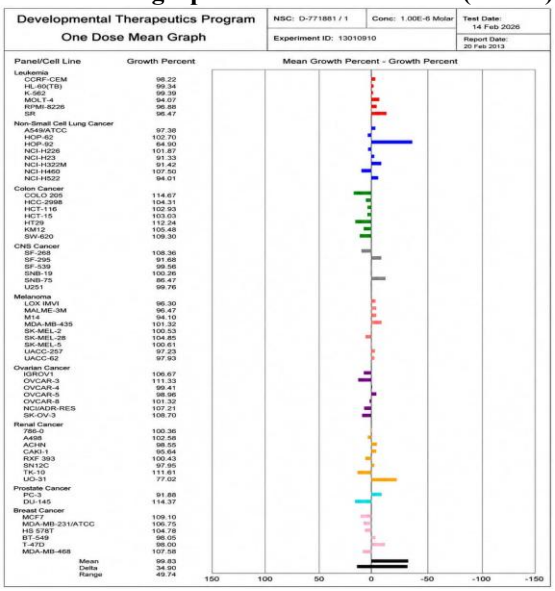
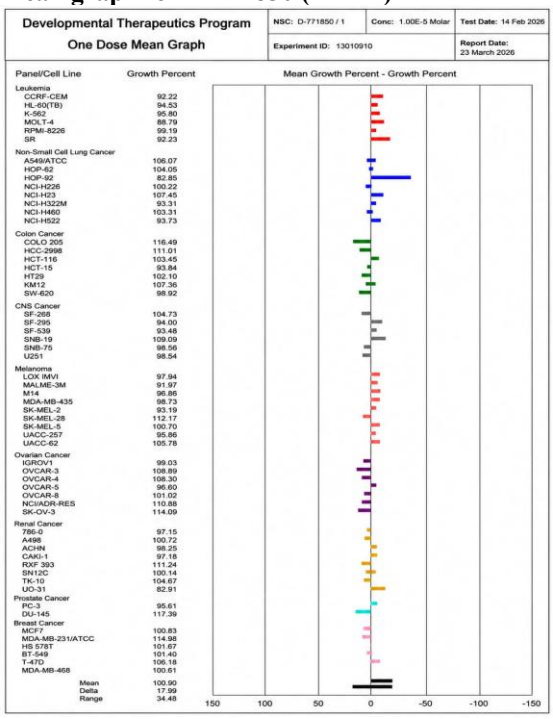
MDA-MB-231/ATCC	106.75	114.98	110.44	97.82
HS 578T	105.62	101.67	99.60	104.77
BT-549	96.05	101.40	95.95	90.50
T-47D	88.00	108.18	84.14	85.84
MDA-MB-468	107.58	100.61	90.33	89.08

2. Mean graph for D-771881 (MPY1)



Mean graph for D-771850 (MPY4)



3. RESULT & DISCUSSION:

5-amino-1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carbonitrile were synthesized by the reaction of 2,6-dichloro-4-trifluoromethyl aniline with the ethyl 2,3-dicyanopropionate, then the synthesized intermediate were reacted with the various aromatic aldehydes to prepare Schiff bases of pyrazole derivatives. All the synthesized compounds were recrysallized with ethanol as the solvents. The synthesized compounds were characterized by FT-IR, ^{1}H NMR and mass spectroscopy technique. The FT-IR spectrum of compounds shows diagnostic absorption bands corresponding to Aromatic (N-H), Aromatic (C-H) and Aromatic carbon (C=C) at wave number between 3300.06-3590.11, 3088.00-3010.10 and 1588.35-1506.20 cm^{-1} respectively. Similarly, in ^{1}H NMR spectrum, Schiff bases of pyrazole derivatives displayed distinctive aromatic proton multiplet peaks with chemical shift values ranging between 6.7-7.9 ppm. The Aromatic NH proton showed a singlet peak with chemical shift values 6.5 ppm. The spectral characterization of synthesized pyrazole derivatives was displayed under the general procedure.

The structures of ten newly designed derivatives were submitted online to the US National Cancer Institute (NCI; Bethesda, MD), and from these, four compounds were chosen by NCI for in vitro testing against a panel of about 60 tumor cell lines, representing nine different cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast. The compounds were evaluated at a single dose concentration of 10 µM illustrates the mean growth %, range of growth %, Delta, % growth, and % GI relative to the most sensitive cell lines. The tested compounds demonstrated growth inhibitory activity primarily against human tumor cells from renal cancer and non-small cell lung cancer. Compound (MPY4) demonstrated strong growth inhibitory effects against non-small cell lung cancer (NCI-H522) with the most sensitive cell line showing a growth reduction of -52.20%. It also exhibited significant inhibitory activity against leukemia (SR), with a growth inhibition of 16.11% in the most sensitive line; against colon cancer (KM 12), at 27.31%; and against prostate cancer (PC-3), at 32.28%. Additionally, Compounds (MPY1), (MPY2), and (MPY3) demonstrated selectivity for non-small cell lung cancer (HOP-92) and renal cancer (UO-31), with the growth percentages of the most sensitive cell lines being 99.83, 82.91, and 74.74, respectively. Every compound tested demonstrated selectivity for leukemia cell lines. The growth inhibition percentages across sixty cell lines ranged from 49.74% for compound MPY1, 34.48% for compound MPY4, 38.61% for compound MPY3. Compound MPY2 demonstrated strong growth inhibitory activity against most of the cell lines. Therefore, it appears that the methoxy substitution at the 2nd and 4th positions on the

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pyrazole-based Schiff base ring is responsible for its good anticancer activity. All tested compounds exhibited activity due to the presence of Cl and CF3 substitutions on the ring. The results of most sensitive cancer cell lines screening data of tested compounds were shown in **Table 5**.

Table 5-Most sensitive cancer cell lines screening data of tested compounds

NSC code (Co. No)	Mean growth %	Range	Delta	Most sensitive cell lines	% Growth	% GI
D-771881 (MPY1)	99.83	49.74	34.90	Non small cell lung cancer (HOP-92)	64.93	35.07
				Renal cancer (UO-31)	77.02	22.81
D-771850 (MPY4)	100.90	34.48	17.99	Renal cancer (UO-31)	82.91	17.09
				Non small cell lung cancer (HOP-92)	82.95	17.95
D-771846 (MPY3)	96.70	38.61	21.96	Renal cancer (UO-31)	74.74	25.26
				CNS (SNB 75)	76.20	20.05
D-773068 (MPY2)	85.82	170.85	138.02	Non small cell lung cancer (NCIH522)	-52.20	138.02
				Leukemia (SR)	16.11	69.71
				Colon cancer (KM12)	27.31	58.51
				Prostate cancer (PC-3)	32.28	53.54

4. CONCLUSION:

This study demonstrates that pyrazole derivatives hold significant importance in medicinal chemistry, particularly in the development of novel cancer treatments. Schiff bases of pyrazole derivatives were synthesized and evaluated for their anticancer activity by using standard protocol. Following synthesis, the compounds were characterized and analyzed using various scientific methods to verify their structure. The results indicated that the compounds had been successfully synthesized and were of high purity. When the synthesized compounds were tested for biological activity, these compounds demonstrated potent effects against various cancer cell lines; represents nine different cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast, including those from breast, lung, liver, and blood cancers. A key discovery is that minor alterations to the chemical structure such as introducing hydroxy, chloro, or nitro groups can significantly enhance the compounds anticancer potency. These pyrazole derivatives function through various mechanisms, including inhibiting cancer cell proliferation, blocking key enzymes, and triggering cancer cell death. The study also indicated that these compounds can tightly attach to key targets in the body, making them valuable for precision cancer therapy. This implies they can operate with greater accuracy and potentially result in fewer side effects. Overall, pyrazole derivatives show great potential for developing new anticancer drugs. They are simple to prepare, adaptable for modification, and demonstrate significant biological activity. Nevertheless, further research particularly trials in humans is still required before these can be considered actual medicines.

ACKNOWLEDGEMENTS:

The authors are thankful to the Management, Samarth Rural Educational Institute, Rajuri, Pune for providing research facilities. Authors are also thankful to Teaching and Non-teaching Staff of Samarth Institute of Pharmacy, Belhe, Pune for constant support during research work.

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