Original Research Article

Microwave assisted synthesis, biological evaluation and In-Silico Molecular docking and pharmacokinetic studies of novel heterocyclic hybrid dihydropyrazol-1-yl-2-(quinolin-8-yloxy) derivatives

Abstract:

A novel heterocyclic hybrid dihydropyrazol-1-yl-2-(quinolin-8-yloxy) derivatives were synthesized. By reacting 8-hydroxyquinoline **1** in absolute ethanol with potassium carbonate followed ethyl chloroacetate by conventional and microwave irradiation methods, precursor **2** were synthesized, further treated with hydrazine hydrates to achieve precursors **3** and by Claisen-Schmidt condensation methods, different chalcone derivatives **6(a-g)** were prepared and to these precursors **3** is then cyclized to give the target compounds **7(a-g).** The structures of the target compounds were evaluated by the spectral and elemental method of analysis. Among the synthesized ccompounds, **7a** and **7g** exhibited to be the most active. Furthermore molecular docking studies have been carried out against PDB codes: 5MTX (p38 MAPK) using Biovia Discovery Studio 2021. The compounds **7a** (-8.947 Kcal/mol) **a**nd **7g** (-8.994 Kcal/mol) had shown remarkable binding affinities which were greater than the binding affinity of recommended drugs; diclofenac sodium (-6.8 kcal/mol), and nimesulide (-7.8 kcal/mol).

Keywords: dihydropyrazol-1-yl-2-(quinolin-8-yloxy) derivatives; molecular docking; pharmacokinetics; *in-vitro* anti-inflammatory; *in-vitro* antioxidant, p38 MAPK.

Introduction:

Quinoline or 1-aza-naphthalene or benzo[b]pyridine is a nitrogen-containing heterocyclic aromatic compound. [1] The quinolone nucleus possesses an extensive range of biological

activities such as antimalarial, [2] anti-inflammatory, [3] antibacterial, [4] and anticancer agents, [5] and this brings it to intense interest and essential aspect in the field of synthetic and medicinal chemistry.[6] Molecular docking studies have been widely explored to understand and predict the protein/enzyme interaction with the synthesized moieties to visualize the probable interaction with amino acids of the target site. This will give insight advantage in the clinical stage for finding potential moieties, and it has been the core of heterocyclic chemistry by reducing the time and costs.[7,8] It exhibited free energy of binding value in Kcal/mole. Investigation of drug binding to mutated receptors and interpretation of conformation of drug-receptor complexes is nowadays regulating the development of safer drugs. Novel drug/drug candidates are needed urgently, and *in silico* simulation/virtual screening, can be vigorous and fast predictions to scrutinize to yield individualized drugs conferring to pharmacogenomics desirable.[9,10] Mitogen-activated protein kinase (MAPK) is the crucial intermediaries of signal transduction in mammalian cells and is a group of profoundly conserved intracellular signaling molecules involving serine-threonine kinases that are consecutively phosphorylated by upstream kinases, mitogen-activated protein kinase kinase kinases (MAPKKK), and mitogen-activated protein kinase kinase (MAPKK). The MAPK signally peers extracellular signals to the intracellular progression that regulates growth, reproduction, relocation, and program cell death.[11-13] Inflammation is a serious constituent of cancer evolution. Various cancers originate from local infection, prolonged aggravation, and inflammation. The tumor ambient, fundamentally organized by inflammatory cells, is a requisite associate in the neoplastic proceeding, promoting proliferation, endurance, and voyage.[14] The activation p38 MAPK signalling pathway plays a vital character in the progress of tumours incursion, voyaging, and the outbreak of cancer cells. It has been associated with intricate living processes like cell reproduction, differentiation, mortality, voyage, and incursion. The p38 MAPK works a binary nature as it modulates cell death and intercedes continuances of a cell to live or die not only on the activation type although also in the cell nature distinct mode. In summation to accentuating of the cell to live, an imperative task of p38 MAPK in cell movement and incursion management gives another possibility to target this pathway regarding cancer metastasis.[15-18]

Synthesis using microwave-assisted development is a safe, non-toxic, environmental remediation, eco-friendly, cost-effective, and green chemistry method. The result of green synthesis by this route/ method is increasing in recent decades because of their various applications in the fields of bio-analytical or medicals, impurity detections, novel drug

delivery systems, cancer therapy, water remediation, biomembranes, nanocapsules, gene modification, targeted therapy, biochips-diagnosis systems, regenerative medicine, and molecular devices. [19-22] Exploring new drug discovery and development approaches in medicinal chemistry, heterocyclic hybridization gains more interest due to improved efficiency and affinity for the new targets. This new approach is based on the synthesis of two or more active compounds with known bioactive moieties. The present paper deals with the study protocol for the design and synthesis of some novel heterocyclic hybrid dihydropyrazol-1-yl-2-(quinolin-8-yloxy) scaffold.

MATERIALS AND METHODS:

All the chemicals, solvents, and reagents used in the synthesis were acquired from Sisco Research Laboratories Pvt. Ltd. (SRL)-India, Fisher Scientific (India), ACROS ORGANICS manufacturers, SD-FINE Chem Limited (India) and Merck (India) and were used without further purification. The purity of the compounds was determined by using Silica Gel coated aluminium-backed TLC sheets.

***Procedure for the synthesis of Ethyl 2-(quinolin-8-yloxy) acetate* 2 (Scheme 1)** [23-26]

Method A: The Ethyl 2-(quinolin-8-yloxy) acetate (**2**) was prepared by heating 0.1mol. of 8-hydroxyquinoline **1** with an equivalent amount of potassium carbonate in the presence of absolute alcohol and 0.1mol. of ethyl chloroacetate (10.7 ml) under anhydrous conditions for 3 hrs in a 250ml RBF. The reaction mixture was allowed to cool and filtered, and the filtrate was placed into 100 ml of ice-cold water. The separated ester was extracted with ether, and dried. The excess ether was evaporated under water bath and crude ester was purified by column chromatography with methanol (1): dichloromethane (40) as a solvent. The reaction was checked by using pre-coated TLC (Mobile phase: methanol: acetone (6:4).

***Method B: Microwave assisted synthesis of Ethyl 2-(quinolin-8-yloxy) acetate* 2:** 8-hydroxyquinoline (0.01mol.) **1** was dissolved in required amount of absolute alcohol and then added equivalent amount of potassium carbonate in the presence of 0.01mol. of ethyl chloroacetate (8.5 ml) under anhydrous conditions into the 10 mL of glass reaction vial equipped with micro magnetic stirrer and caped tightly. The reaction vial was then placed into closed-vessel Anton Paar Monowave microwave synthesizer and set temperature at 105^{o}C and reaction was carried out for 7 min. After completion of the reaction, the reaction mixture was poured into ice-cold water. The product was extracted with ether and purified by column chromatography using methanol: dichloromethane (1:40) as a solvent. The yields of compound **2** were obtained 48%.

***Synthesis of 8-Quinolinoxyacetic acid hydrazide,* 3 (Scheme 1)** [27,28]

Method A: A solution of 0.1mol. of compound **2** in absolute ethanol (50ml) was taken in a 100 ml RBF, 7 g of hydrazine hydrate was added, and heated for 2 h. The crude hydrazide, which splitted on cooling was collected by filtration and recrystallized from ethanol to obtained compound 3. White solid, m.p.: 164-166 ^{o}C, λ_{max}: 556 nm, Yield: 77%, R_f : 0.73 (mobile phase: n-hexane: ethyl acetate; 3:7).

Method B: Microwave assisted synthesis of 8-Quinolinoxyacetic acid hydrazide, 2: Ethyl 2-(quinolin-8-yloxy) acetate (0.01mol.) **2** was dissolved in required amount of absolute alcohol and then added 0.04mol. of hydrazine hydrate into the 10 mL of glass reaction vial equipped with micro magnetic stirrer and caped tightly. The reaction vial was then placed into closed-vessel Anton Paar Monowave microwave synthesizer and set temperature at 95^{o}C and reaction was carried out for 4.5 min. After completion of the reaction, the reaction mixture was poured into ice-cold water. The product was collected by filtration and recrystallized from ethanol. The yields of compound **3** were 88%.

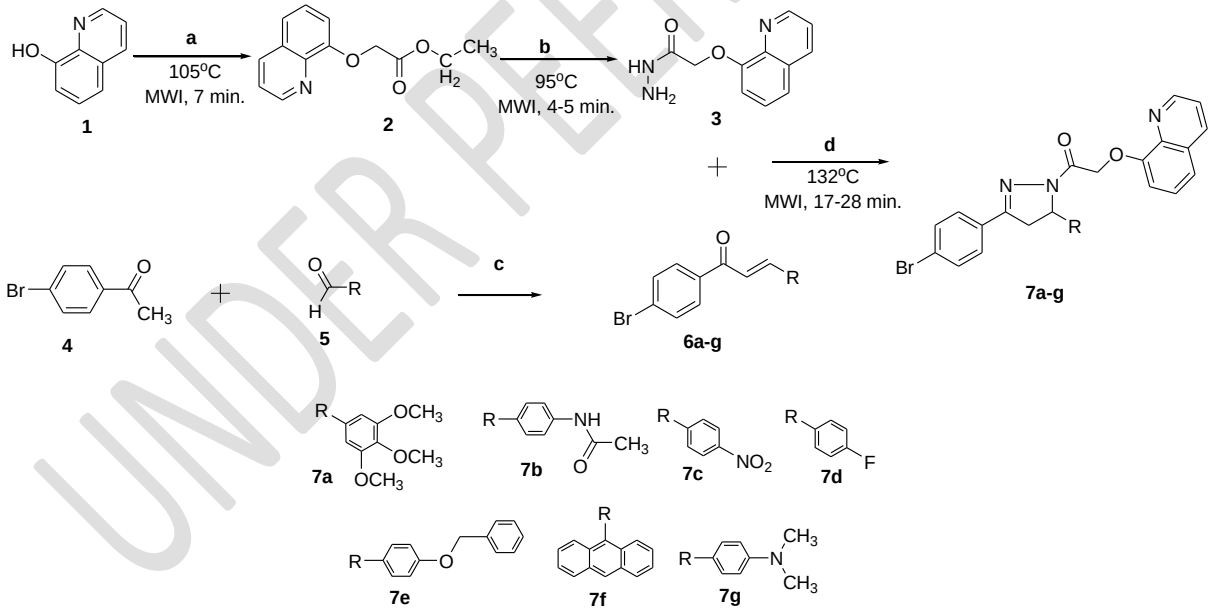
***Procedure for the synthesis of chalcone derivatives, 6a-g* (Scheme 1)** [29]

A solution of 2.19 g (0.055 mol.) of sodium hydroxide in 20ml of distilled water and 10ml of alcohol are taken into a 250ml bottle, lightly shielded with a cardboard disk, furnished by an adequate mechanical stirrer, and keep in a large container to allow cooling with broken ice. In the basic solution, 8.5 g (0.043 mol.) of *p*-bromoacetophenone **4** was decanted; the bottle was rapidly surrounded with cracked ice and constant stirring. To these 0.043 mol. of substituted aromatic aldehydes **5** was then added to prepared different chalcone derivatives. The temperature of the mixture was maintained between 15°C to 30°C during the reaction. After two to three hours, the mixture becomes so thick that no more in effect against stirring. The stirrer was later stopped, and the mixture was kept in an icebox for nearly ten hours. The mixture composed a thick paste of small shot-like grains suspended in an almost transparent liquid. It was cooled in a chilled mixture, separated on a Buchner funnel, rinsed with water continuously until neutral to litmus, and ultimately washed with alcohol, which had earlier been reduced to 0°C. Subsequent air drying, the crude product was purified by recrystallization from alcohol. By this process, different chalcone derivatives, **6a-g**, were prepared with yield of 57-87%.

***Synthesis of pyrazoline quinolone derivatives, 7a-g* (Scheme 1)** [30,31]

Method A: A solution of compound 3 (0.01 mol.) in 7ml acetic acid and with substituted chalcone derivatives, **6a-g** (0.01mol.), was added with continuous stirring. The reaction mixture was then heated for 7 h. along with a mechanical stirrer. The reaction rate was examined by TLC (Mobile phase: methanol: acetone; 6:4). After completing the reaction, the subsequent reaction mixture was transferred toward smashed ice. The crude product obtained was filtrated, rinsed with water, dried, and recrystallized from alcohol to get the compounds **7a-g**.

Method B: Microwave assisted synthesis of pyrazoline quinolone derivatives, 7a-g: Equimolar quantities (0.01 mol) of substituted chalcone derivatives, **6a-g** was taken into a 10 mL of glass reaction vial equipped with micro magnetic stirrer and then added equimolar quantities (0.01 mol) of acetic acid then caped properly. The reaction vial was then placed into closed-vessel Anton Paar Monowave microwave synthesizer and set temperature at 132^{o}C and reaction was carried out for 17-28 min. After the end of the reaction, the reaction mixture was quenched in cracked ice, and solid was collected by filtration and dried at room temperature, recrystallized from ethanol to obtained pyrazoline quinoline derivatives **7a-g.** The yields of pyrazoline quinolone derivatives **7a-g** were obtained more than 53%.



Scheme 1: Reagents and reaction condition: MWI: Microwave irradiation, (a) K_2CO_3, $ClCH_2COOC_2H_5$, ethanol, reflux, 3 h., (b) ethanol, N_2H_4, reflux, 2h., (c) EtOH, aq. NaOH, stirrer, 15-30 ^{o}C, 2-3 hr., (d) CH_3COOH, reflux, 7hr.

Biological Screening

In vitro study

Anti-oxidant activity [32,33]

The synthesized compounds **7a-g** was evaluated for *in vitro* antioxidant activities against 2,2-diphenyl-1-picrylhydrazyl (DPPH). 1ml of 0.1mM of DPPH (2, 2 diphenyl-2-picrylhydrazyl) in methanol was added in 1ml of references standard (10µg/ml, 50µg/ml, 100µg/ml, 250µg/ml, and 500µg/ml) and test solution of different concentration (10µg/ml, 50µg/ml, 100µg/ml, 250µg/ml, and 500µg/ml). It was kept in dark for 30 minutes to protect from light and the absorbance was measured at λ-max 517nm in UV Visible spectrophotometer (SHIMADZU). The assay was performed in triplicate. Ascorbic acid was used as a reference standard. All solution was freshly prepared. The test solution was prepared by dilution with methanol from stock solution of 10mg/ml. The DPPH scavenging was measured by the following equation:

$$\textbf{\% inhibition} = A_0\text{-}A_t/A_0\times 100.$$

Where, A_0 = absorbance of control (containing all measurement except test).

A_t = absorbance of test solution. The percentage inhibition after 30 min was plotted against concentration; calculated data for antioxidant are presented as means ± SD of triplicate.

Anti-inflammatory activity [34,35]

All the target compounds were studied for *in vitro* anti-inflammatory activity by albumin denaturation method. The standard and targeted compounds were dissolved in the least quantity of DMF and diluted to phosphate buffer saline (pH 7.4).

The target compounds were studied for anti-inflammatory activity by using the inhibition of the albumin denaturation method. The standard drug and targeted compounds were dissolved in the least quantity of DMF and diluted with phosphate buffer saline (pH 7.4) so that the concentration of DMF in all solutions was less than 2.5%. Targeted compounds solution (1 ml, 100 mg/ml) was blended with 1 ml of 1% albumin solution in phosphate buffer saline and incubated at 27 ± 1^{o}C for 15 min. Denaturation was influenced by retaining the reaction mixture at 60 ± 1 ^{o}C in a water bath for 10 min. After cooling, the turbidity was measured at 660 nm with a UV visible spectrophotometer. The percentage of inhibition of denaturation was calculated from control, where no drug was added. Each experiment was done in triplicate, and the average was taken. The diclofenac sodium was used as a standard drug. The percentage of inhibition was calculated using the following equation:

% Inhibition of denaturation = [(A_t/A_c) - 1] x 100

Where, A_t = absorption of test. A_c= absorption of control.

p38 MAPK assay [36,37]

Nonradioactive immunosorbent p38 kinase activity assay method has been followed which is applicable for routine screening of small molecule p38 kinase inhibitors. In the method activating transcription factor-2 (ATF-2) was used as a substrate for phosphorylation, which showed linearity in 6-24 ng/well range, based on this 12 ng/well and 60 minute incubation period was optimized. Microtiter plates were coated with 50 µL/well of the p38 kinase substrate ATF-2 (10 µg/mL in TBS) for 1.5 h at 37 ˚C. After washing three times with distilled water, remaining open binding sites were blocked with blocking buffer (BB; 0.05% Tween 20, 0.25% BSA, 0.02% NaN_3 in TBS) for 30 min at room temperature. Plates were washed again, 50 µL of the respective test solution was filled into the wells and the plates were incubated for 1 h at 37 ˚C. Test solutions containing 12 ng/well p38 MAPK were diluted in kinase buffer (50 mM Tris, pH 7.5, 10 mM $MgCl_2$, 10 mM β-glycerophosphate, 100 µg/mL BSA, 1mM dithiothretol, 0.1 mM Na_3VO_4, 100 µM rATP) with or without test substance (10^{-4} to 10^{-8} M). Test substances were dissolved in dimethyl sulfoxide to form stock solution of 10^{-2} M, all further dilutions were carried out in kinase buffer. After subsequent washing, plates were blocked again with BB for 15 min followed by a fourth washing step. Wells were filled with 50 µL of the specific anti-bis-($Thr^{69/71}$)-phospho-ATF-2 (1. AB, 1:500 in BB) and incubated for 1 h at 37 ˚C followed by washing and consecutive incubation with 50 µL of the secondary antibody (2. AB (alkaline phosphatase conjugated), 1:1400 in BB). Then 100 µL of 4-NPP (N-Phenethyl-4-piperidinone is a derivative of 4-piperidinone) was pipetted in each well after a final washing step and colour development was measured 1.5-2 h later with an enzyme linked immunosorbent assay reader at 405 nm.

In Silico study

Molecular Docking studies [38-42]

Molecular docking studies were performed using Biovia Discovery Studio 2021 (v21.1.0.20298) software to determine the possible interaction modes of the target compounds in the p38 mitogen activated protein kinase (p38 MAPK). The X-ray crystallographic structure of the enzymes p38 MAPK, was downloaded from the protein data bank, (https://www.rcsb.org/structure/5MTX), PDB codes: 5MTX respectively. While the

bound substances (ligand and cofactors) and solvent molecule associated with the receptor were removed. The chemical structure of the molecules were drawn with Chemdraw ultra Version 8.0 and further pre-optimized for docking. Furthermore the prepared ligands were docked with the prepared structure of p38 MAPK inhibitors using Biovia Discovery Studio 2021 (v 21.1.0.20298).

Pharmacokinetics and drug likeness studies [43,44]

The targeted compounds were study for pharmacokinetics and drug-likeness in silico using swissADME software web-based (http://www.swissadme.ch/index.php). Chemical structures of the compounds (2D) were drawn in ChemDraw ultra 8.0 versions (Cambridge Software), and relevant SMILES of each compound were converted. Virtual SMILES and structure generator initiate in online tool SwissADME transformed each compound SMILE into molfiles. The prediction was made to investigate that the synthesized compounds were inhibitors of CYP450 isoforms (CYP1A2, CYP2C19, and CYP2C9). To predict pharmacokinetics like gastrointestinal absorption, P-glycoprotein (P-gp), and blood-brain barrier (BBB), and drug-likeness like Lipinski, Ghose, and Veber rules and bioavailability score. The molecular weight, LogP, number of hydrogen bond acceptors (HBA), and number of hydrogen bond donors (HBD) predicted according to Lipinski, Ghose, and Veber rules to measure the drug-likeness properties whether the compounds has to be bioactive.

Statistical Analysis

All statistical analyses were performed with the help of SPSS 17.0 software. The level of statistical significance was expressed by *p*-value. The *in-vitro* antioxidant, anti-inflammatory, and p38 MAPK assay were expressed by mean ± S.E.M.

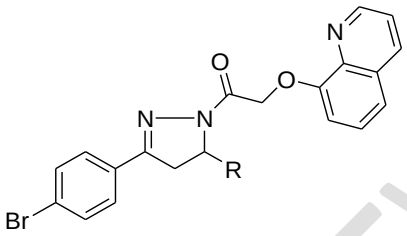
RESULTS AND DISCUSSION:

Chemistry 7(a-g):

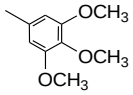
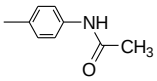
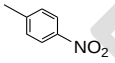
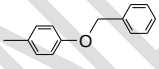
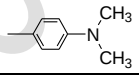
The reaction sequences leading to the different chalcone substituted quinoline are outlined in the **Scheme 2.** The synthesis of Ethyl 2-(quinoline-8-yloxy) acetate **2** was carried out by reaction of 8-hydroxyquinoline (0.1mole) **1** in 60 ml of absolute ethanol with potassium carbonate (0.1 moles) in 20 ml of absolute ethanol followed ethyl chloroacetate (0.1 moles) by conventional and microwave irradiation methods and filtered. The filtrate was poured into ice-cold water, and separated ester was extracted with ether and dried. Ethyl 2-(quinoline-8-

yloxy) acetate **2** was formed, and the product was converted into their hydrazine derivatives **3** by treatment with hydrazines hydrates and then cyclized with different chalcone derivatives **6(a-g)** to give the chalcone substituted quinolines analogs **7(a-g)**. The physical data, reaction time, yields and melting point of the target compounds **7(a-g)** via both methods are presented in Table 1.

Table 1. Physical data, comparison of the time and yields of the target compounds **7(a-g)** using conventional and microwave irradiation methods



7a-g

Comp. codes	R	Mol. formula	m.p. ^{o}C	Conventional method		Microwave irradiation	
				Time (hr)	% Yields	Time (min)	% Yields
7a		$C_{28}H_{22}BrN_3O_5$	182-184	1	67%	12	81%
7b		$C_{27}H_{19}BrN_4O_3$	169-171	1	73%	5	85%
7c		$C_{25}H_{15}BrN_4O_4$	173-175	1	83%	3	90%
7d		$C_{25}H_{15}BrFN_3O_2$	110-112	1	80%	8	89%
7e		$C_{32}H_{22}BrN_3O_3$	114-116	1	77%	10	85%
7f		$C_{33}H_{20}BrN_3O_2$	158-160	1	65%	7	78%
7g		$C_{27}H_{21}BrN_4O_2$	229-231	1	86%	8	94%

R= substituents at C5 position of pyrazole nucleus, Mol. Formula: Molecular formula, m.p: melting point, hr: hour, % percentage

The elucidated structure of compound 7a: Quinolin-8-yl 3-(4-bromophenyl)-5-(3,4,5-trimethoxyphenyl)-1H-pyrazole-1-carboxylate: light greyish solid, M.P. : 120-122 ^{o}C, λ_{max}: 342nm, Yield: 45%, R_f :0.89, solvent: methanol: acetone (6:4), IR (ATR) : 1660.25 cm^{-1}(C=O, ketone), 1573.41 cm^{-1}, 1495.82 cm^{-1} (C=N), 1401.36 cm-1 (C=C), 1117.28 cm^{-1}(C-O-

C), 696.72 cm^{-1} (C-Br). ^{1}H NMR ($CDCl_3$): δ 8.63 (dd, 1H, *J*=4Hz), 7.85 (dd, 1H, *J*=8Hz), 7.71 (m, 3H, *J*=4Hz), 7.50 (dd, 1H, *J*= 4Hz), 7.39 (m, 2H, *J*=8, 4Hz), 7.13 (m, 2H, *J*=8, 4Hz), 6.69 (d, 2H), 5.47 (m, 1H), 3.85 (d, 9H, *J*=4Hz), 3.74 (dd, 1H, *J*= 4Hz), 3.49 (dd, 1H, *J*= 4Hz). ^{13}C NMR ($CDCl_3$): δ 165.10, 163.13, 152.41, 149.28, 147.44, 147.08, 141.02, 138.58, 135.27, 129.86, 128.43, 125.17, 123.23, 122.75, 116.01, 115.83, 103.10, 62.67, 61.04, 56.17, 43.57. ESI-MS: 562.09 ($[M+H]^+$). Anal. calcd for $C_{28}H_{24}BrN_3O_5$: C, 59.80; H, 4.30; Br, 14.21; N, 7.47; O, 14.22; found: C, 60.01; H, 3.96; Br, 14.26; N, 7.50; O, 14.27.

***The elucidated structure of compound 7b: quinolin-8-yl 5-(4-acetamidophenyl)-3-(4-bromophenyl)-1H-pyrazole-1-carboxylate*:** light brown solid**,** M.P. : 162-164 **°**C, λ_{max}: 268 nm, Yield: 34 %, R_f : 0.68, solvent: methanol: acetone (6:4), IR (ATR) : 3229.87 cm^{-1}(N-H), 1650.43 cm^{-1}(C=O, ketone), 1584.33, 1505.44 (C=N), 1256.78 cm^{-1}(C-O), ;696.44 cm^{-1} (C-Br). ^{1}H NMR ($CDCl_3$): δ 9.13 (s, 1H), 8.62 (dd, 1H, *J*=4Hz), 7.85 (dd, 1H, *J*=8Hz), 7.74-7.66 (m, 3H, *J*=4Hz), 7.54 (m, 3H, *J*=4Hz), 7.36 (m, 4H, *J*= 8, 4Hz), 7.13 (m, 2H, *J*= 8, 4Hz), 5.43 (m, 1H, *J*=4Hz), 3.68 (dd, 1H, *J*=4Hz), 3.43(dd, 1H). ^{13}C NMR ($CDCl_3$): δ 169.51, 165.10, 163.13, 153.83, 149.22, 139.01, 135.24, 134.64, 129.86, 128.43, 126.46, 125.17, 123.46, 120.82, 119.61, 116.01, 115.83, 61.46, 43.72, 24.00. ESI-MS: 539.08 ($[M+H]^+$). Anal. calcd for $C_{27}H_{21}BrN_4O_3$: C, 61.26; H, 4.00; Br, 15.09; N, 10.58; O, 9.07; found: C, 61.49; H, 3.63; Br, 15.15; N, 10.62; O, 9.10.

***The elucidated structure of compound 7c: quinolin-8-yl 3-(4-bromophenyl)-5-(4-nitrophenyl)-1H-pyrazole-1-carboxylate*:** brown solid, M.P. : 98-100 **°**C, λ_{max}: 328 nm, Yield: 32%, R_f : 0.92, solvent : methanol: acetone (6:4) IR (ATR) : 1659.92 cm^{-1}(C=O, ketone), 1589.91 (C=N), 1514.31 cm^{-1}(NO_2, str), 1335.85 cm^{-1}(NO_2, sym), 745.31 cm^{-1} (C-Br). ^{1}H NMR ($CDCl_3$): δ 8.63 (dd, 1H, *J*=4Hz), 8.13 (m, 2H, *J*=4Hz), 7.85 (dd, 1H, *J*=8Hz), 7.71 (m, 3H, *J*= 4Hz), 7.58 (d, 2H, *J*=4Hz), 7.50 (dd, 1H, *J*= 4Hz), 7.39 (m, 2H, *J*= 8, 4Hz), 7.13 (m, 2H, *J*= 8, 4Hz), 5.43 (dd, 1H, *J*= 8, 4Hz), 3.68 (dd, 1H, *J*= 8, 4Hz), 3.43 (dd, 1H, *J*= 8, 4Hz). ^{13}C NMR ($CDCl_3$): δ 165.10, 163.13, 152.39, 149.24, 147.44, 147.08, 146.80, 144.23, 141.02, 135.24, 129.86, 128.43, 126.53, 125.17, 123.58, 123.23, 122.75, 119.61, 116.01, 115.83, 62.22, 48.72. ESI-MS: 517.04 ($[M+H]^+$). Anal. calcd for $C_{25}H_{17}BrN_4O_4$: C, 58.04; H, 3.31; Br, 15.45; N, 10.83; O, 12.37; found: C, 58.27.01; H, 2.93; Br, 15.51; N, 10.87; O, 12.42.

***The elucidated structure of compound 7d: quinolin-8-yl 3-(4-bromophenyl)-5-(4-fluorophenyl)-1H-pyrazole-1-carboxylate*:** brown solid, M.P.: 118-120 **°**C, λ_{max}: 312 nm, Yield: 41%, R_f : 0.73, solvent : methanol: acetone (6:4), IR (ATR) : 1655.15 cm^{-1}(C=O,

ketone), 1580.16 cm-1 (C=N), 1496.28 (C=C, aromatic), 1202.98 cm^{-1}(C-F), 695.96 cm^{-1} (C-Br). ^{1}H NMR ($CDCl_3$): δ 8.62 (dd, 1H, *J*=4Hz), 7.85 (dd, 1H, *J*=8Hz), 7.71 (m, 3H, *J*=8, 4Hz), 7.50 (dd, 1H, *J*=8, 4Hz),7.39 (m, 4H, *J*=4Hz), 7.14 (m, 4H, *J*=4Hz), 5.43 (m, 1H, *J*= 4Hz), 3.68 (dd,1H, *J*=4Hz), 3.43 (dd, 1H, *J*=8,4Hz). ^{13}C NMR ($CDCl_3$): δ 165.10, 163.99, 163.13, 162.02, 152.39, 149.24, 147.44, 147.08, 141.02, 136.21, 135.24, 129.86, 128.43, 127.01, 125.17, 123.23, 122.75, 119.61, 116.01, 115.76, 115.50, 62.07, 43.72. ESI-MS: 490.05 ($[M+H]^+$). Anal. calcd for $C_{25}H_{17}BrFN_3O_2$: C, 61.24; H, 3.49; Br, 16.30; F, 3.87; N, 8.57; O, 6.53; found: C, 61.49; H, 3.10; Br, 16.36; F, 3.89; N, 8.61; O, 6.55.

***The elucidated structure of compound 7e: quinolin-8-yl 5-(4-(benzyloxy)phenyl)-3-(4-bromophenyl)-1H-pyrazole-1-carboxylate*:** light brown solid, M.P.: 122-124 ^{o}C, λ_{max}: 422 nm, Yield: 34%, R_f :0.94, solvent: methanol: acetone (6:4), IR (ATR) : 1644.37cm^{-1}(C=O, ketone), 1574.10 cm^{-1}(C=N), 1328.79 cm^{-1}(C-N), 1169.49 cm^{-1} (C-O), 506.40 cm^{-1} (C-Br).). ^{1}H NMR ($CDCl_3$): δ 8.62 (dd, 1H, *J*=4Hz), 7.85 (dd, 1H, *J*=8Hz), 7.71 (m, 3H, *J*=8, 4Hz), 7.50 (dd, 1H, *J*=4Hz), 7.39 (m, 6H, *J*=8, 4Hz), 7.26 (m, 3H, *J*= 8, 4Hz), 7.13 (m, 2H, *J*= 8, 4Hz), 6.90 (m, 2H, *J*=4Hz), 5.43(m, 1H, *J*=4Hz), 5.06 (t, 1H), 3.68 (dd, 1H, *J*=4Hz), 3.43 (dd, 1H, *J*=8,4Hz). ^{13}C NMR ($CDCl_3$): δ 165.10, 163.13, 158.07, 152.39, 149.24, 147.44, 147.04, 141.02, 136.78, 135.24, 133.29, 131.50, 129.86, 128.49, 128.74, 126.56, 125.17, 123.23, 122.75, 119.61, 116.01, 115.83, 115.40, 69.57, 62.17, 43.72. ESI-MS: 578.10 ($[M+H]^+$). Anal. calcd for $C_{32}H_{24}BrN_3O_3$: C, 66.44; H, 4.18; Br, 13.81; N, 7.26; O, 8.30; found: C, 66.68; H, 3.85; Br, 13.86; N, 7.29; O, 8.33.

***The elucidated structure of compound 7f: quinolin-8-yl 5-(anthracen-9-yl)-3-(4-bromophenyl)-1H-pyrazole-1-carboxylate*:** brown solid, M.P. : 84-86 ^{o}C, λ_{max}: 404 nm, Yield: 37%, R_f : 0.91, solvent: methanol: acetone (6:4), IR (ATR) : 3233.62 cm^{-1}(CH), 1661.61 cm^{-1} (C=O, ketone), 1496.87 (C=C, aromatic), 1366.95 cm^{-1} (C-N), 1156.75, 1043.82 cm^{-1}(C-O), 723.68 cm^{-1} (C-Br).). ^{1}H NMR ($CDCl_3$): δ 8.62 (dd, 1H, *J*=4Hz), 8.38 (t, 1H, *J*=4Hz), 8.32 (m, 2H, *J*=4Hz), 7.95 (m, 2H, *J*=4Hz), 7.85 (dd, 1H, *J*= 4Hz), 7.71 (m, 3H, *J*=8,4Hz), 7.51 (m, 5H, *J*=4Hz), 7.39 (m, 2H, *J*= 8, 4Hz), 7.13 (m, 2H, *J*= 8, 4Hz), 6.01 (t, 1H, *J*=8, 4Hz), 3.75 (dd,1H, *J*=4Hz), 3.50 (dd, 1H, *J*=8,4Hz). ^{13}C NMR ($CDCl_3$): δ 165.10, 163.13, 152.02, 148.95, 147.44, 147.08, 141.02, 135.49, 135.24, 133.53, 129.89, 128.82, 128.43, 128.01, 126.98, 125.17, 124.31, 123.23, 122.75, 119.61, 116.01, 115.83, 58.77, 41.80. ESI-MS: 572.09 ($[M+H]^+$). Anal. calcd for $C_{33}H_{22}BrN_3O_2$: C, 69.24; H, 3.87; Br, 13.96; N, 7.34; O, 5.59; found: C, 69.48; H, 3.53; Br, 14.01; N, 7.37; O, 5.61.

The elucidated structure of compound 7g: quinolin-8-yl 3-(4-bromophenyl)-5-(4-(dimethylamino)phenyl)-1H-pyrazole-1-carboxylate: brown solid, M.P. : 149-151^{o}C, λ_{max}: 418 nm, Yield: 68%, R_f : 0.88, solvent: methanol: acetone (6:4), IR (ATR) : 3170.87 cm^{-1}(CH), 1655.15 cm^{-1}(C=O, ketone), 1582.93cm^{-1}(C=N), 721.54 cm^{-1} (C-Br). ^{1}H NMR ($CDCl_3$): δ 8.62 (dd, 1H, *J*=4Hz), 7.85 (dd, 1H, *J*=4Hz), 7.74-7.66 (m, 3H, *J*=8,4Hz), 7.50 (dd, 1H, *J*=4Hz), 7.39 (m, 2H, *J*= 8, 4Hz), 7.27 (m, 5H, *J*= 8, 4Hz), 7.13 (m, 2H, *J*=4Hz), 6.73 (m, 1H, *J*= 4Hz), 5.43 (m, 1H, *J*= 4Hz), 3.68 (dd,1H, *J*=4Hz), 3.43 (dd, 1H, *J*=8,4Hz), 2.97 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 165.10, 163.13, 152.20, 149.21, 148.45, 147.44, 147.08, 141.02, 135.24, 132.08, 130.12, 129.86, 128.43, 126.72, 126.42, 125.17, 123.23, 122.75, 119.61, 116.01, 115.83, 114.36, 59.73, 45.34, 41.93. ESI-MS: 515.10 ($[M+H]^+$). Anal. calcd for $C_{27}H_{23}BrN_4O_2$: C, 62.92; H, 4.50; Br, 15.50; N, 10.87; O, 6.21; found: C, 63.17; H, 4.12; Br, 15.56; N, 10.91; O, 6.23.

Biological assay

All the synthesized compounds **7(a-g)** were evaluated for in-vitro antioxidant, anti-inflammatory, and p38 MAKP inhibitory activities. The screened compounds **7a** and **7g** produced better action than other compounds but somewhat lesser than the standard drugs.

In vitro study

Anti-oxidant activity

The *in vitro* antioxidant activities of the target compounds **7(a-g)** were determined by using DPPH radical scavenging assay with Ascorbic acid as standard drugs. The IC_{50} values ranges from 2.76 µg /ml to 3.30 µg /ml compared with that of standard Ascorbic acid (IC_{50} value 2.09 µg /ml). Compounds **7a (**R=3, 4, 5-$(OCH_3)_3C_6H_5$**)** (IC_{50} value 2.76 µg /ml), **7b** (R=4-$NHCOCH_3)C_6H_5$) (IC_{50} value 2.97 µg /ml), and **7g** (R=4-$N(CH_3)_2C_6H_5$) (IC_{50} value 2.97 µg /ml) exhibit good activity because of the presence of methoxy and methyl act as electron releasing groups maintaining the stability and improved the activity. Substitution of electron withdrawing groups like nitro, fluorine slightly decreases the activity **7c** (R=4-$NO_2C_6H_4$) (IC_{50} value 3.30 µg /ml), **7d** (R=4-FC_6H_5) (IC_{50} value 3.01 µg /ml), **7e** (R= C_6H_5-4-$OCHC_6H_5$) (IC_{50} value 3.20 µg /ml), and **7f** (R=10-anthracene) (IC_{50} value 3.16 µg /ml). The antioxidant activity values are shown in Table 2.

Table 2. Antioxidant activities of the synthesized compounds were recorded in terms of % scavenging shown by each compounds at different concentrations.

Comps. code	10µg/ml	20µg/ml	40µg/ml	50µg/ml	100µg/ml	IC_{50} (µg /ml)
7a	53.54±0.005*	57.24±0.005*	62.43±0.008*	67.63±0.011*	72.18±0.011*	2.76
7b	42.64±0.008*	53.53±0.005*	57.56±0.005*	63.43±0.008*	68.67±0.008*	2.97
7c	37.82±0.008*	45.68±0.005*	51.21±0.005*	57.81±0.008*	63.21±0.008*	3.30
7d	43.93±0.008*	50.94±0.008*	54.61±0.005*	61.72±0.005*	66.15±0.005*	3.01
7e	40.24±0.020*	48.53±0.017*	53.74±0.031*	58.30±0.011*	65.15±0.017*	3.20
7f	42.93±0.027*	49.33±0.020*	55.74±0.017*	59.84±0.029*	63.03±0.018*	3.16
7g	42.55±0.011*	47.62±0.014*	51.29±0.025*	63.22±0.008*	75.05±0.020*	2.97
control	0	0	0	0	0	
Ascorbic acid	81.43±0.04*	83.46±0.003*	84.85±0.037*	85.98±0.034*	91.51±0.020*	2.09

Each value represents the mean ± SEM (n = 3); *p < 0.05 significant.

In-vitro anti-inflammatory activity

The protein denaturation method was employed to determine the *in-vitro* anti-inflammatory activities of the target compounds **7(a-g)**. In this methodology, protein's tertiary and secondary structure would vanish when an external molecule or pressure was implemented. When denatured, the biological role of most biological proteins would be disappeared. The strength of the title targeted compounds was measured to inhibit protein denaturation (in triplicate). Diclofenac sodium was used as a standard drug. In this examination, **7a** provided 67.26% inhibition and **7g** provided 75.35% inhibition and diclofenac sodium exhibited 91.89% inhibition of protein denaturation. The *in-vitro* anti-inflammatory activity targeted compounds are provided in Table 3.

Table 3. *In vitro* anti-inflammatory activity of target compounds **7(a-g)**

Compd. codes	% inhibition
7a	67.26±0.05*
7b	61.92±0.02*
7c	54.21±0.06*
7d	56.07±0.02*
7e	48.16±0.02*
7f	51.14±0.04*
7g	75.35±0.02*
Diclofenac sodium	91.15± 0.01*

Each value represents the mean ± SEM (n = 3); *p < 0.05 significant.

In vitro p38 MAPK assay

To gain more discernment into the interactions of the target compounds molecular docking studies were carried out using Biovia Discovery Studio 2021, using *p*38 MAPK proteins to correlate the binding mode. The targeted compound's inhibitory strengths on p38α MAPK were assessed in a non-radioactive immunosorbent p38R MAPK enzyme assay. ATP competed with the prohibited for the equivalent binding pocket catalytic site of p38 α MAPK and associated with that exerted by the well-known p38 MAPK inhibitor SB203580. According to the efficacy of the inhibitor and the resulting p38 α kinase activity, the phosphorylation reaction of the activating transcription factor 2 (ATF-2), the endogenous protein substrate of p38 MAPK, is prevented to a more immense or minor amount so that the amount of phosphorylated ATF-2 contrarily correlates with the inhibitory action of the p38 α MAPK inhibitor. Compounds with **7a** (IC_{50} value 22.93 mM) and **7g** (IC_{50} value 30.36 mM) shown to be endowed with moderate inhibitory activity as compared to other synthesized compounds, with an IC_{50} in the range of 22.93 to 67.46 mM. The reference compound SB203580 showed IC_{50} value 0.66 mM against p38 kinase. The *in-vitro* p38 MAPK activity of the targeted compounds is provided in Table 4.

Table 4. *In vitro* p38 MAPK activity of target compounds **7(a-g)**

Compd. codes	IC_{50} (mM)
7a	22.93±0.028*
7b	43.66±0.008*
7c	46.36±0.008*
7d	50.40±0.081*
7e	67.46±0.032*
7f	54.65±0.057*
7g	30.36±0.032*
SB2035807	0.66±0.008*

Each value represents the mean ± SEM (n = 3); *p < 0.05 significant.

Structure-activity relationship

From the *in-vitro* antioxidant studies, it was found that target compounds exhibit good antioxidant activities which might be due to the presence of electron withdrawing substituents like 3,4,5-trimethoxybenzene (**7a**), phenylacetaminde (**7b**), 4-nitrobenzene (**7c**), 4-flourobenzene (**7d**) on the phenyl ring attached at the C-5 of the pyrazole nucleus. Compounds consisting electron-donating substituents like *N*,*N*-dimethylbenzenamine ($R=N(CH_3)_2C_6H_5$) (**7g)**, possesses good antioxidant and anti-inflammatory activity at phenyl

ring attached at the C-5 of the pyrazole nucleus. Compounds **7e** and **7f** also possess good antioxidant activity but have lessen anti-inflammatory activity as compared to other target compounds due to the presence of 4-phenoxymethylbenzen (**7e)** and 10-anthracene (**7f)** at the phenyl ring attached at the C-5 of the pyrazole nucleus. The structural activity relationship of the target compound is shown in Fig. 1.

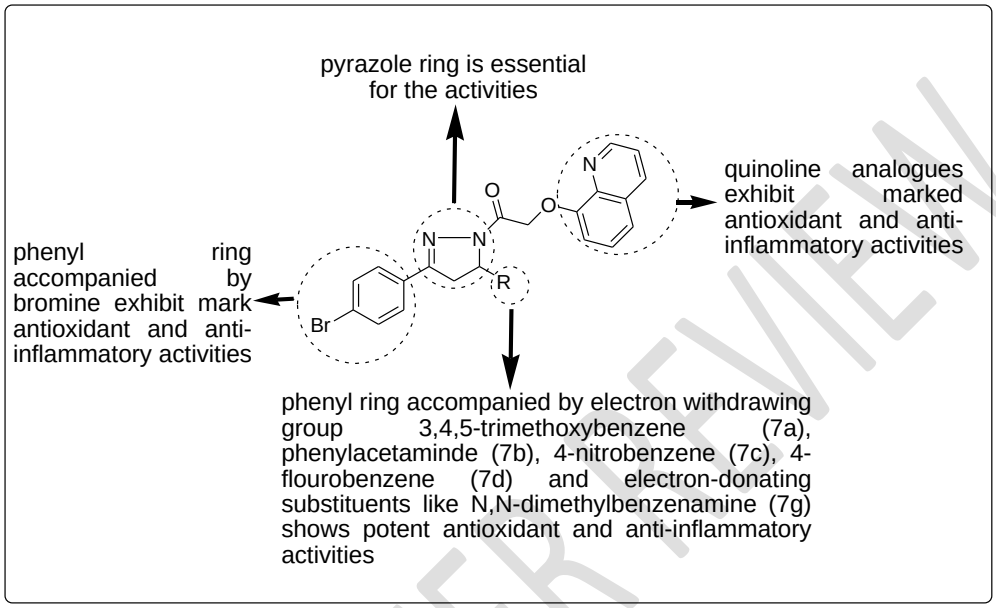


Fig. 1. Structure-activity relationships of the target compounds **7a-g**.

In Silico study

Molecular Docking studies

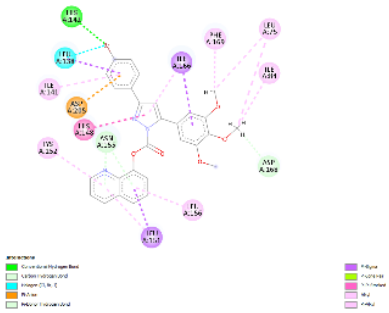
Molecular docking study of the target compounds was carried out against p38 MAP kinase enzyme and is displayed in **Table. 5**. Due to the flexibility of (linker) connecting pyrazole and quinoline moieties, the target compounds can be housed more favourably and effectively in p38 MAP kinase binding site. Compounds **7a-g**, with best binding were visualized and analysed using Discovery Studio. The binding affinity values for all the target compounds range from -8.081 Kcal/mol to -8.994 Kcal/mol with the target site against p38 MAP kinase enzyme. The target compounds **7a** (-8.947 Kcal/mol), **7b** (-8.200 Kcal/mol), and **7g** (-8.994 Kcal/mol) had shown remarkable binding affinities which were greater than the binding affinity of recommended drugs; diclofenac sodium (-6.8 kcal/mol), and nimesulide (-7.8 kcal/mol).

Table 5. Molecular docking study of the target compounds **7(a-g)** with p38 MAPK.

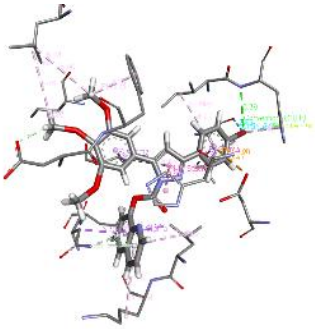
Comp. codes	Binding Affinity (Kcal/mol)	Hydrogen bond		Hydrophobic bond Amino acid	Hydrophilic Interaction
		Amino acid	Bond length (A˚)		
7a	-8.947	HIS142	3.39	LEU138, LEU151, LEU156, LEU75, ILE141, ILE84, ILE166, PHE169	ASP205, LYS152 ASP168 ASN155
7b	-8.200	PHE169	2.70 2.72	LEU138, LEU156, LEU151, ILE141, ILE166	ASN155 LYS152
7c	-8.157	-	-	LEU167, LEU157, LEU151, ILE141, ILE166	ASN155 LYS152
7d	-8.105	ASN155	2.73	LEU156, LEU167, ILE141, ILE166	ASP150 LYS152
7e	-8.081	-	-	LEU151, LEU156, ILE206, ILE141, ILE166, LEU138	ASN155 ASP168 ASP205 ALA299 LYS152
7f	-8.053	LEU167	3.33	LEU138, ILE141, ILE166, LEU151, ILE206	ASN155 ASP205
7g	-8.994	LEU167	3.05	LEU138, LEU74, LEU75, LEU151, ILE206, ILE84, ILE166, ILE141, PHE169	ASP150 ASN155 ASP168 ASP205 LYS152
Std-1	-6.8	LYS152 ASN155	3.07 3.17	ILE141,ILE166, LEU151	-
Std-2	-7.8	GLY170 LEU171	2.16 2.20	ILE166,ILE141, LEU151	-

"-" indicates no interactions

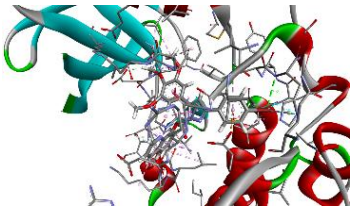
Compound **7a** (Fig. 2) accounts for one hydrogen bond (HIS142) with the target site. Compound **7a** formed a hydrophobic bond with ILE84, ILE166, ILE141, LEU151, LEU138, LEU167, LEU75, PHE169, MET78, VAL83, and ALA299 of the target site. Compound **7a** formed hydrophilic interaction with the target site ASP205, ASP150, and LYS152. Compound **7b** formed one hydrogen bond with the target site. The C=O group phenylacetamide of compound **7b** acts as a hydrogen acceptor and forms one hydrogen bond with PHE169 of the target. Compound **7g** (Fig. 2) formed one hydrogen bond with the target site. The –C=O group pyrazole of the compound acted as a hydrogen bond acceptor and formed one hydrogen bond with ASN155 of the target. Compound **7g** formed hydrophobic bonds with ILE206, ILE166, LEU138, LEU151, LEU156, LEU75, PHE169, VAL83, and MET78 of the target site. Compound **7g** formed hydrophilic interactions with ASP205 and LYS152 of the target site.



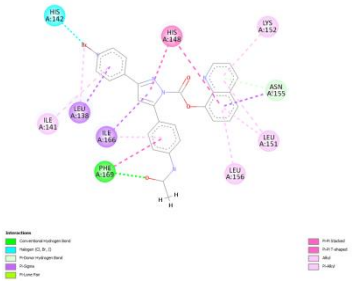
7a 2D interactions



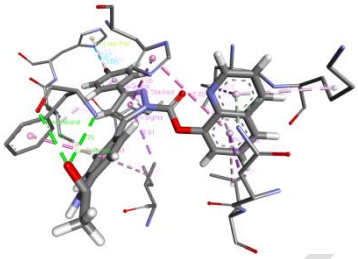
7a 3D interactions



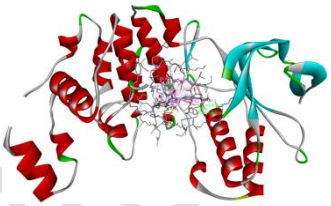
7a Interaction with p38 MAP kinase



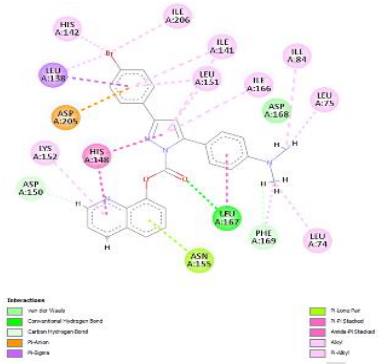
7b 2D interactions



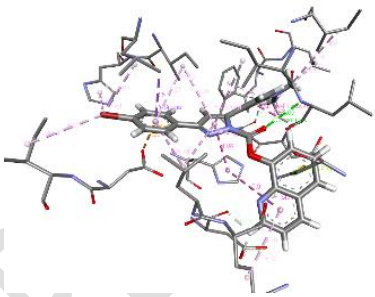
7b 3D interactions



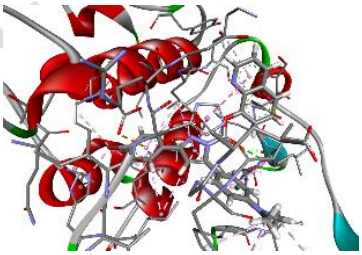
7b Interaction with p38 MAP kinase



7g 2D interactions



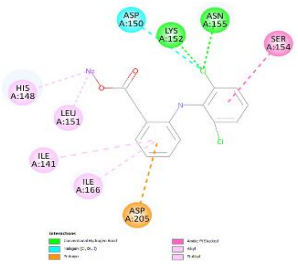
7g 2D interactions



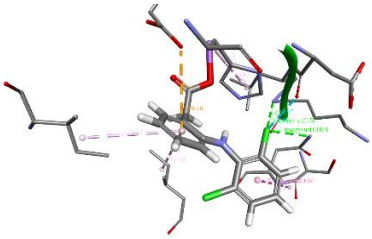
7g Interaction with p38 MAP kinase

Fig. 2: Show the investigated 3D and 2D Interactions between synthesized compounds **7a, 7b,** and **7g** with the target site of the p38 MAP kinase enzyme.

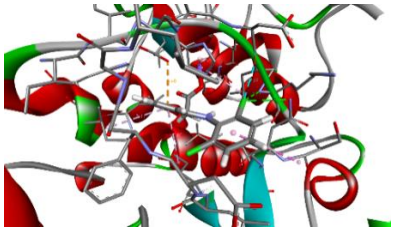
The recommended drugs, diclofenac sodium (std-1) (**Fig. 3**), account for two hydrogen bonds (LYS152, ASN155) while hydrophobic bonds were observed with ILE141, ILE166, LEU138, LEU151, VAL209, and VAL83 and account hydrophilic interaction with ASP168 of the target site of p38 MAPK. Nimesulide (std-2) (**Fig. 3**) accounts for two hydrogen bonds (GLY170, LEU171), while hydrophobic bonds were observed with ILE166, LEU51, and LEU138, while hydrophilic interaction accounts with ASP205 of the target site.



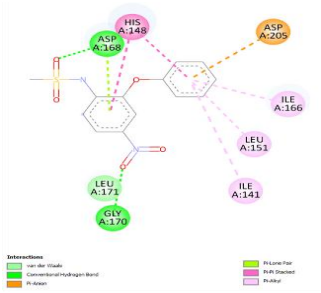
Std-1 2D interactions



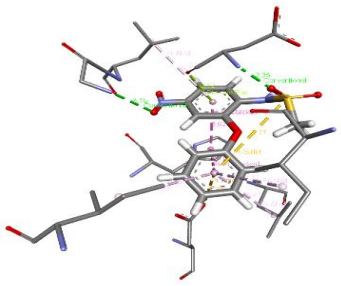
Std-1 2D interactions



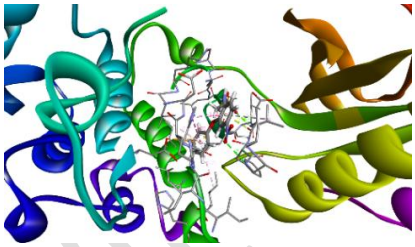
Std-1 Interaction with p38 MAP kinase



Std-2 2D interactions



Std-2 2D interactions



Std-2 Interaction with p38 MAP kinase

Fig. 3: Show the investigated 3D and 2D Interactions between std-1 and std-2 with the target site of the p38 MAP kinase enzyme.

Pharmacokinetics (ADME) and drug likeness's studies

The synthesized compounds were performed *in silico* SWISS ADME for pharmacokinetics studies and the results are shown in **Table. 6**. Predicted logP values, reveals that out of 7 synthesized compounds, 6 compounds namely **7a,7b, 7c, 7d, 7e,** & **7g** logP values were above 3.6, whereas **7f** logP values lies within the range of 1.6 to 3.6. This parameter could be the probability to depict the compounds as a drug like candidates those showing the logP values ranges within 1.6 to 3.6. Among the synthesized compounds some of them possess favourable ADME properties. **7g** predicted to have a good GIT absorption based on WlogP to tPSA. Among the target compounds none of them penetrate blood-brain-barrier (BBB). Among the target compounds, none of them predicted to act on P-gp substrate. Most of the compounds inhibits to CYP450 isoforms. The target compounds interact with the isoform CYP2C19 and CYP2C9 are found to be the major isoform. [45-50]

Table. 6. Pharmacokinetics parameter of the synthesized compounds

Comp. codes	GI absorption	BBB permeat	P-gp	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	LogKp (Skin permeation), cm/s

7a	Low	No	No	No	Yes	Yes	-5.27
7b	Low	No	No	No	Yes	Yes	-5.58
7c	Low	No	No	No	Yes	No	-5.05
7d	Low	No	No	No	Yes	Yes	-4.69
7e	Low	No	No	No	Yes	Yes	-4.26
7f	Low	No	No	No	Yes	No	-3.49
7g	High	No	No	No	Yes	Yes	-4.83
Std-1	High	Yes	Yes	No	No	No	-5.12
Std-2	High	No	No	No	Yes	Yes	-6.33

"Comp. codes: compound codes; "GI absorption": gastro-intestinal absorption; "BBB permeat": blood brain barrier permeation; "P-gp": permeation-glycoprotein; Std-1: diclofenac sodium; Std-2: nimesulide.

At early research phase of drug discovery and development, drug-likeness is the main principles in screening for the drug candidates. This unveil the physicochemical parameter of the compound i.e., bioavailability of per oral route along with their pharmaceutical characteristics in human body. Also the prediction for drug-likeness conducted on the bioavailability score, Veber rules, Lipinski, and ghose. The Lipinski's Rule of five states that the molecular weight under 500 g/mol, will be more likely to be absorb and permeate, logP value must be lower than 5, number of Hydrogen bond donor has utmost 5 and 10 hydrogen bond acceptor atoms. Drug-likeness constraints define by Ghose filter (Amgen) are calculated logP values should be lie between -0.4to 5.6, MW of the molecule is in between 160-480, molar refractivity between 40-130, and the total number of atoms 20 to 70. Veber, rules for drug-likeness defines that the rotatable bond count should be less than or equal to 10 and polar surface area (PSA) should be less than or equal to 140.[45-50] According to Lipinski Rule of Five, screening process of the compounds reveals that out of total 7 compounds, only 3 compounds **7a**, **7b** and **7d** were meet the criteria of druglikeness with one violation (**Table. 7**) i.e. MW>500. Whereas 4 compounds (**7c, 7e, & 7g**) were rejected with two violations. According to Ghose rules all 7 compounds were rejected with two. According to Veber rules, all the 7compounds meet the criteria of drug likeness (**Table. 7**). For good bioavailability TPSA value should be <140. Another significance parameter for good oral bioavailability is total polar surface area (TPSA) and those compound passively absorbed with TPSA<140 have low oral bioavailability.[45-50]

Table. 7. Physical properties and drug-likeness of synthesized compounds

Comp.	**Physical properties**	**Drug-Likeness**

codes	MW	RB	HBA	HBD	MR	TPSA ($^0A^2$)	Lipinski	Ghose	Veber	BA score
7a	560.40	8	7	0	143.44	84.70	Yes; 1 violation: MW>500	No; 2 violations: MW>480, WLOGP>5.6, MR>130	Yes	0.55
7b	527.37	7	5	1	148.28	86.11	Yes; 1 violation: MW>500	No; 3 violations: MW>480, WLOGP>5.6, MR>130	Yes	0.55
7c	515.31	6	6	0	132.79	102.83	No; 2 violation: MW>500, MLOGP>4.15	No; 3 violations: MW>480, WLOGP>5.6, MR>130	Yes	0.17
7d	488.31	5	5	0	123.92	57.01	Yes; 1 violation: MLOGP>4.15	No; 2 violations: MW>480, WLOGP>5.6	Yes	0.55
7e	576.44	8	5	0	154.94	66.24	No; 2 violation: MW>500, MLOGP>4.15	No; 3 violations: MW>480, WLOGP>5.6, MR>130	Yes	0.17
7f	570.43	5	4	0	158.98	57.01	No; 2 violation: MW>500, MLOGP>4.15	No; 3 violations: MW>480, WLOGP>5.6, MR>130	Yes	0.17
7g	513.39	6	4	0	138.17	60.25	No; 2 violation: MW>500, MLOGP>4.15	No; 3 violations: MW>480, WLOGP>5.6, MR>130	Yes	0.17
Std-1	318.13	4	2	1	75.61	52.16	Yes, 0 violation	Yes	Yes	0.55
Std-2	308.31	5	5	1	80.05	109.60	Yes, 0 violation	Yes	Yes	0.55

"RB": rotatable bonds; "HBD": hydrogen bond acceptors; "HBD": hydrogen bond donors; "MR": molar refractivity; "Std-1": standard drug -1: sodium diclofenac; "Std-2": standard; drug-2: nimesulide; "MW": molecular weight; "TPSA": topological polar surface area; "BA score": Bioavailability score.

Conclusion

The present paper reported that the novel heterocyclic hybrid scaffolds **7(a-g)** was synthesized using microwave irradiation techniques, showing better yield and lesser time than the conventional synthesis method. The *in-silico* and *in-vitro* biological screening revealed that the compounds **7a** and **7g** showed better activity than other target compounds and had shown remarkable binding affinities greater than the recommended drugs. However, further extensive research may lead to the generation of ideal pharmaceuticals to treat inflammation shortly.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

1. Grutzkau A, Kruger-Krasagakes S, Baumeister H, Schwarz C, et al. *Mol Biol Cell*. 1998; 9(4):875-84.
2. Shacter E, Weitzman SA. *Oncology (Williston Park)*. 2002; 16(2):217-26.
3. Mackenzie I, Rous P. *J Exp Med*. 1941; 73(3):391-416.
4. Kuper H, Adami HO, Trichopoulos D. *J Intern Med*. 2000; 248(3):171-83.
5. Maeda H, Akaike T. *Biochemistry (Mosc)*. 1998; 63(7):854-65.
6. Yamanishi Y, Boyle DL, Rosengren S, Green DR, Zvaifler NJ, Firestein GS. *Proc Natl Acad Sci U S A*. 2002; 99(15):10025-30.
7. Huang G, Shi L Z, Chi H. *Cytokine*, 2009, 48(3): 161-169.
8. Cuadrado A, Nebreda A R. *Biochemical Journal*. 2010; 429(3): 403-417.
9. Yang Y, Kim SC, Yu T, Yi YS, Rhee MH, Sung GH, Yoo BC, Cho JY. *Mediators of inflammation*. 2014; 13 pages.
10. InformedHealth. org [Internet]. Cologne, Germany: Institute for Quality and Efficiency in Health Care (IQWiG). Prostate cancer: Overview. 2006.
11. Norman J. Kennedy, Roger J. Davis, Handbook of Cell Signaling (Second Edition), *Elsevier*. 2010.
12. Hastings M, Farah CA, Sossin WS. Handbook of Behavioral Neuroscience. *Elsevier*. 2013: l (22); 221-235.
13. Alugoju P, Chaitanya NS, Swamy VK, Kancharla PK. *Academic Press*. 2021; 129-163.
14. Balkwill F, Mantovani. *A Lancet*. 2001; 357(9255):539-45.
15. Koul HK, Pal M, Koul S. *Genes & cancer*. 2013; 4(9-10):342-59.
16. Greenberg AK, Basu S, Hu J, Yie TA, Tchou-Wong KM, Rom WN, Lee TC. *American journal of respiratory cell and molecular biology*. 2002;26(5):558-64.
17. Corre I, Paris F, Huot J. *Oncotarget*. 2017; 8(33):55684.
18. Wang B, Jiang H, Ma N, Wang Y. *Springer plus*. 2016; 5(1):1-9.
19. Siby Joseph and Beena Methew. *Journal of Nanoparticle*. 2014; (**2014**): 9 Pages. http://dx.doi.org/10.1155/2014/967802.
20. Deene Manikprabhu and K. Lingappa. *Bioinorganic Chemistry and Applications*. **2013**; (2013: 5 pages. http://dx.doi.org/10.1155/2013/341798
21. Agrawal N, Mishra P. *Med Chem Res*. **2018**; 27(5):1309-44.

22. Leonard K, Ahmmad B, Okamura H, Kurawaki J. *Colloids and Surfaces B: Biointerfaces*. **2011;** 82(2): 391-6.
23. Deshmukh N, Das PK, Karma K. *Pharm. Biol. Eval*. **2016**; 3: 135-139.
24. Chan SH, Chui CH, Chan SW, *et al*. *ACS Med. Chem. Lett*. **2012;** 4(2): 170-174.
25. Vasudevan A, Wodka D, Verzal MK. *Bioorg Med Chem Lett*. **2004**; 14(19): 4879-4882.
26. Soliman R, Hammouda NA. *J Pharm Sci.* **1979**; 68(11): 1377-1381.
27. Abdellatif KR, Labib MB, El-Badry OM, Roshdy SM, El-Enany MM. *Beni Suef Univ J Basic Appl Sci*. **2016;** 5(2): 147-155.
28. Kohler EP, Chadwell HM. *Org Synth*, **2003**; 2: 1-1.
29. Manna K, Agrawal YK. *Bioorg Med Chem Lett*. **2009**; 19(10): 2688-2692.
30. Kalirajan R, Rathore L, Jubie S, Gowramma B, Gomathy S, Sankar, S. *Indian J Chem.* **2011;** 1794-1799.
31. Amr AGE, Abdulla MM. *Archiv der Pharmazie: An International Journal Pharmaceutical and Medicinal Chemistry*. **2006**; 339(2): 88-95.
32. Grosdidier, A., Zoete, V. and Michielin, O., *Nucleic Acids Res*. **2011**; 39: W270-W277.
33. Wang J, Xia F, Jin WB, Guan JY, Zhao H. *Bioorg. Chem.* **2016**; 68: 214-218.
34. Ghule SG, Deshmukh VK, Chaudhari SR. *J Pharm Res*. **2013**; 7(7):600-5.
35. Mizushima Y, Kobayashi M. *J Pharm and Pharmacol*. **1968**; 20(3):169-73.
36. G Kulkarni R, A Laufer S, V M C, Garlapati A. *Med Chem*. **2013**; 9(1):91-9.
37. Tariq S, Alam O, Amir M. *Archiv der Pharmazie*. **2018**; 351(3-4): 1700304.
38. Syahri J, Yuanita E, Nurohmah BA, Armunanto, R. and Purwono, B. *Asian Pac. J. Trop. Biomed*. **2017**; 7(8): 675-679.
39. Walter, N.M., Wentsch, H.K., Bührmann, M., et al. *J. Med. Chem.* **2017**; 60(19): 8027-8054.
40. Niu, M.M., Qin, J.Y., Tian, C.P., *Acta Pharmacol. Sin.* **2014**; 35(7): 967-979.
41. Dyrager, C., Möllers, L.N., Kjäll, L.K., et al. *J. Med. Chem*. **2011**; 54(20): 7427-7431.
42. Li, J., Li, D., Xu, Y., Guo, Z., Liu, X., Yang, H., Wu, L. and Wang, L., *Bioorg. Med. Chem. Lett.* **2017**; 27(3): 602-606.
43. Daina, A., Michielin, O. and Zoete, V., *Sci. Rep.* **2017**; 7(1): 1-13.
44. Grosdidier, A., Zoete, V. and Michielin, O., *Nucleic Acids Res*. **2011**; 39: W270-W277.
45. Gurjar, V.K. and Pal, D. *RSC Advances*. **2020**; 10(23): 13907-13921.

46. Suhud, F., Tjahjono, D.H., Yuniarta, T.A., Putra, G.S. and Setiawan, J. *In IOP Conference Series: Earth and Environmental Science*. **2019**; 293(1); 012018. Publishing.
47. Lipinski, C.A., Lombardo, F., Dominy, B.W. and Feeney, P.J., *Adv. Drug Deliv. Rev.* **1997**; 23(1-3): 3-25.
48. Ghose, A.K., Viswanadhan, V.N. and Wendoloski, J.J. *J. Comb. Chem*. **1999**; 1(1): 55-68.
49. Veber, D.F., Johnson, S.R., Cheng, H.Y., Smith, B.R., Ward, K.W. and Kopple, K.D. *J. Med. Chem*. **2002**; 45(12): 2615-2623.
50. Lipinski, C.A., *Drug Discov. Today Technol*. **2004**; 1(4): 337-341.