# Microwave Assisted Synthesis, Biological evaluation and Computational Studies of Novel 3,5- Disubstituted Pyrazole Derivatives as Anti-Inflammatory and Antineoplastic Agents.

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## ABSTRACT

**Objective:** Objective of this research was to do synthesis and anti-inflammatory and antiproliferative evaluation of new synthetic analogues. **Method:** Synthesis of novel pyrazole derivatives from chalcone intermediates carried out using microwave. Compounds were characterized using IR, NMR, LCMS spectra. Compounds with dimethyl-amino, chloro ring substitution shown good yield whereas those with pyridine ring substitution and involving diphenyl ring shown optimum yield. Microwave assisted synthesis is preferred as green method for final step of reaction. Derivatives screened for in vitro anti-inflammatory potential. As from some literature it is clear that compounds having anti- swelling property

further may result in effective anticancer agent so we have checked series for anticancer potential against MCF-7 cell line **Result**: Compound 1a,1f shown remarkable in vitro anti-inflammatory score. These compounds carry -OCH3, -OCF3, ring substitution respectively. As from literature review it was revealed that Cox inhibition is one of the mechanism for anti-inflammatory activity; hence to predict probable mechanism for anti-inflammatory activity docking interactions of synthesized compounds against COX I enzyme was studied. For comparative analysis anti-inflammatory drug Ibuprofen used as a standard. Compound 1a, 1f ,1g exhibited a good degree of binding interactions in the selected binding site region with binding score of -7.8, -8.8. -8.0, kcal/mol which is equal to and better than Ibuprofen (-7.8 kcal/mol**)**. 1f shows moderate cytotoxic activity against MCF7 cell line. **Conclusion**: Compound 1f shown promising anti-inflammatory potential than Ibuprofen. Further moderate anticancer activity is obtained for it (GI50- 78.4µg/ml) against MCF-7 cell line. Binding interactions were checked by docking against EGFR.

**Keywords**: EGFR, Anti-inflammatory, Anticancer, Pyrazole.

## Introduction

Pyrazole derivatives are pharmacologically popular active scaffold that flourished with number of pharmacological activities (1-3). The presence of this moiety in various marketed drugs of diverse therapeutic categories such as celecoxib, a potent anti-inflammatory (4), the antipsychotic CDPPB (5), Rimonabant-anti obesity agent, Difenamizole-an analgesic, Betazole-H2-receptor agonist and the antidepressant agent proved its pharmacological importance (7-10). Owing to this diversity of pyrazole moiety led us to synthesize novel pyrazole analogues from substituted chalcones and screened them for in vitro anti-inflammatory activity and anti-neoplastic potential.

## Methodology

### Materials

Synthetic reagents and solvents were of analytical grade or of the highest quality commercially available. The chemicals were purchased from Aldrich Chemical.

### Instrumentation

Microwave: Catalyst
Solvent systems used were a- $CHCl_3$–MeOH (7:3), b- Hexane: Ethyl Acetate (7.5:2.5),
c - Acetone: Pet ether (9:1).
IR- Brooker, NMR- Brooker 500 MHz, LCMS: Waters TOF US.

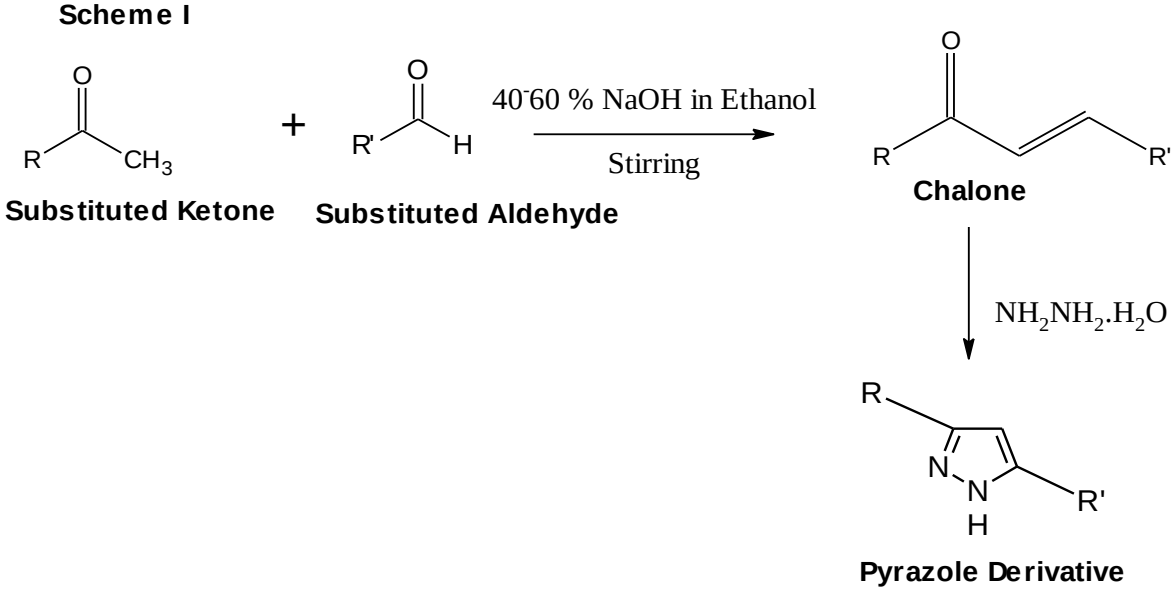
### Procedure

**Step 1-**Chalcone derivatives were prepared by simple stirring of equimolecular quantity of aldehyde and ketone derivatives in 40 to 60 % of basic alcohol under cold conditions.

**Step 2-**Chalcone derivatives (0.01Mol) and hydrazine hydrate (0.02Mol) with 2-3 drops of HCl were kept in microwave at 250 to 350 W at 30s intervals periodically until product formation confirmed. The reaction mixture was allowed to cool and poured in cold water.

Mixture then filtered, dried and purified by recrystallization using suitable solvent to obtain final compounds. Completion of reaction were checked with TLC. Yield reported and Melting point detection was carried out. [10-15]

**Scheme I**




**Anti-inflammatory Activity**

In vitro anti-inflammatory activity testing was carried out by inhibition of albumin denaturation. To the known concentration (10 µg/ml) of the synthesized compounds having volume 2 ml, we have added 0.2ml of eggs albumin and 2.8ml of phosphate buffered saline (pH 6.4). Control was prepared by taking same quantity of double distilled water. Above matrix was incubated at temperature 37 ± 1 °C for 15 min. and then allowed to heat at 70°C for 5 mins. Absorbances of matrixes were recorded after cooling at 660 nm wavelength by using blank. Diclofenac sodium (standard drug) was used as reference drug and treated as it is for determination of absorbance. Following formula is used for same [16-21].

$$\%\ Inhibition = \frac{Absorbance\ of\ sample - Absorbance\ of\ control}{Absorbance\ of\ sample} * 100$$

**Molecular Docking Studies**

The protein preparation is carried out using Autodock 4.0 tool. Docking is carried using Autodock Vina tool. The 3D crystallized COX1 in complex with NSAID downloaded from RCSB Protein data bank. Desired binding site region is selected and saved in .pdbqt format as a default format. Ligand prepararion is carried out and geometrically optimized ligands were further energy minimized. Finally, ligands were allowed to bind with binding site of protein, multiple conformations generated. Conformation with the lowest energy and RMSD is considered as an optimum outcome [22-27].

**Anticancer activity**

Cells were seeded in 96 well plate and temperature, Pressure conditions were maintained as per standard protocol. i.e. body temperature (37 $^0$ C), 95 % humidity and 5 % CO2 provided overnight to microplate. Stock solution of experimental drug were diluted to 1mg/ml and stored frozen. Aliquots of frozen concentrates were further utilized to prepare drug dilutions

of 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml. 10 µl of these solutions were further added to 90 µl of media already present in micrometer well so that final drug concentration were achieved as 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml. further plate incubation done for 48 hrs. In situ cell fixation is done by 10 % TCA addition and incubated at 4 $^0$C for 60 min. In next step supernatant discarded followed by 5 times wash with tap water and air dried. After that cells were added with 50 µl sulphorhodamine B solution (0.4 % w/v in 1 % acetic acid) and incubated for 20 min at room temp. After staining with SRB solution again washing given with 1 % acetic acid to remove excess dye, plates air dried and bound stain eluted with 10mM Trizma base and absorbance was read at wavelength of 540 nm with reference wavelenth 690 nm. The ratio of average absorbance of test solution to the average absorbance of control *100 give percent growth inhibition [28-31].

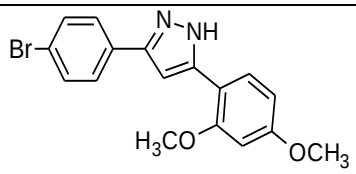
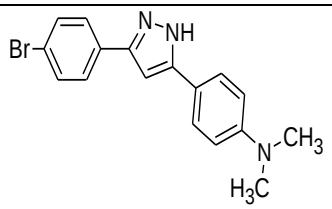
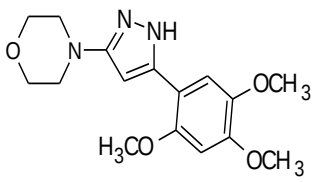
**Molecular Docking for EGFR**

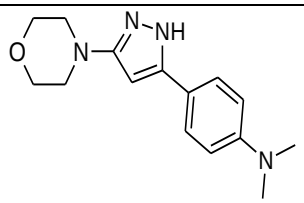
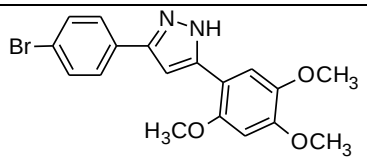
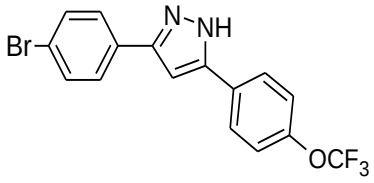
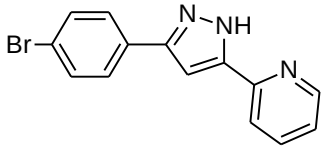
Molecular docking was carried out using autodock vina tool by taking EGFR target [32-33]

## RESULT

Synthesis of 7 pyrazole derivatives was carried out from substituted 1, 3-diphenylpropenone in moderate to good yield using microwave. Reaction time, yield, melting point, TLC solvent as given in table 1.

**Table 1:** Physical data of compounds

| Sr. No. | Comp. Code | Structure | Molecular Formula | Molecular Weight | Reaction Time in microwave (min) | Melting Point ($^0$C) | Yield (%) | TLC Mobile Phase |
|---|---|---|---|---|---|---|---|---|
| 1. | **1a** |  | $C_{17}H_{15}BrN_2O_2$ | 359.2172 | 15 | 180 | 68 | a |
| 2. | **1b** |  | $C_{17}H_{16}BrN_3$ | 342.23304 | 10 | 200 | 75 | a |
| 3. | **1c** |  | $C_{16}H_{21}N_3O_4$ | 319.35564 | 17 | 285 | 57 | a |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 4. | **1d** |  | $C_{15}H_{20}N_4O$ | 272.3455 | 12 | 280 | 75 | b |
| 5. | **1e** |  | $C_{18}H_{17}BrN_2O_3$ | 389.24318 | 14 | 290 | 52 | b |
| 6. | **1f** |  | $C_{16}H_{10}BrF_3N_2O$ | 383.16260 | 18 | 292 | 57 | a |
| 7. | **1g** |  | $C_{14}H_{10}BrN_3$ | 300.1533 | 20 | 270 | 52 | c |

a- $CHCl_3$–MeOH (7:3), b- Hexane: Ethyl Acetate (7.5:2.5), c - Acetone: Pet ether (9:1)

Spectroscopy:

**1.** *(4-bromophenyl)-5-(2,4-dimethoxyphenyl)-1H-pyrazole* (1a)

Yield 65%, MP 180 $^0$C, $^1$H NMR (ppm, $CDCl_3$): 7.80 -7.79 (d, 2H), 7.54 - 7.49 (d, 2H), 7.09 (s, 1H), 6.69 (s, 1H), 6.47 (s, 1H), 6. 41 (s, 1H), 6.63 (s, 1H), 3.30-3.80 (s, 6H)
IR (cm$^{-1}$) 3338.67 (-NH, str, pyrazol), 3073.79(CH str arom), 2844-2944 (CH aliphatic) 1229 (N-N str, pyrazol), 1590(C=C str pyrazol) 1062(C-O str methoxy) 901,807. Mass: $C_{17}H_{15}BrN_2O_2$ m/z: 358

**2.** *4-[3-(4-bromophenyl)-1H-pyrazol-5-yl]-N, N-dimethylaniline* (1b)
Yield 75 %, MP 200 $^0$C, $^1$H NMR (ppm, DMSO-d6):7.82-7.80(2H, d), 6.81-6.79 (2H, d), 7.64-6.63 (4H, d), 7.02(s 1H, CH-pyrazol), 13.13(s, NH-pyrazol), 2.96 (S,6H).$^{13}$C NMR (DMSO-d6):64.03, 98.66, 112.78, 113.07, 120.86, 126.54,127.52,127.78, 131.92, 132.07, 151.28. IR (cm$^{-1}$) 3377 NH,str,pyrazol), 1228(N-N str, pyrazol), 1592( C=C str pyrazol), 2875 (CH str, CH3), 666 (C- Br str).

**3.** 4-*[5-(2,4,5-trimethoxyphenyl)-1H-pyrazol-3-yl] morpholine* (1c)
Yield :57 % MP 285 $^0$C, NMR (ppm): 6.68-6.66 (s, 1H), 6.35-6.32 (s, 1H), 6.62 (s, 1H),2.89-2.86 (m,4H), 2.50-2.48 (m, 4H), 3.33-3.90 (s, 9H). Mass: $C_{16}H_{21}N_3O_4$ m/z: 319
IR (cm$^{-1}$): 3408 (-NH-, *str*, secondary amine), 3073 (=C-H str arom.), 2944 (-CH str, aliph), 2190 (ring =C-N, *str*, imine), 1630 (C=C str, arom), 1022(C-O, str, ether)

**4.** *N, N-dimethyl-4-(3-morpholin-4-yl-1H-pyrazol-5-yl) aniline* (1d)

Yield: 75 %, MP 280 $^0$C, NMR (ppm): 9.99 (1H, s, -NH) 8.57- 6.67 (9H, m, aromatic), 3.02--2.86 (m,4H), 3.05 (6H, s, -$CH_3$). IR (cm$^{-1}$): 3108 (NH- Str, Sec. amine) 3073(=CH - str aromatic) 2944 (CH str aliphatic), 2190 (ring =C-N str imine), 1630(C=C str aromatic), 1010 (C-F str.)

**5.** 3-*(4-bromophenyl)-5-(2,4,5-trimethoxyphenyl)-1H-pyrazole* (1e)

Yield: 52%, MP 290 $^0$C, NMR (ppm): 10.57 (1H, s, -NH) 7.70- 6.71 (4H, m, aromatic), 8.5-7.68 (2H, s, aromatic), 3.0 (9H, s, three -$OCH_3$).

IR (cm$^{-1}$): 3330 (-NH-, *str*, sec amine), 2830 (-CH str, aliphatic), 2262 (ring =C-N, *str*, imine), 1505,1454 (C=C str, aromatic), 1028(C-O, str)

**6.** *3-(4-bromophenyl)-5-[4-(trifluoromethoxy) phenyl]-1H-pyrazole* (1f)

Yield 57 %, MP 292 $^0$C,$^1$H NMR: (ppm, DMSO-d6):7.98- 7.48(8H, aromatic), 7.30 (1H, s, olefinic =CH- pyrazole),13.53(1H, s, -NHPyrazole).$^{13}$CNMR(400MHzDMSO-d6):100.97,121.24,121.94,127.43,127.62, 128.34, 129.17, 130.79, 132.01, 132.31. $C_{16}H_{10}BrF_3N_2O^{\bullet+}$ m/z:383 $C_{16}H_{10}BrF_3N_2O^{3\bullet+}$Exact Mass: 381.

IR (cm$^{-1}$) 3105(NH- Str, sec amine), 2890 (=CH - str aromatic), 2150(ring =C-N str imine) ,1049 (C-O str ether), 1010 (C-F str.)

**7.** 2-[3-(4-bromophenyl)-1*H*-pyrazol-5-yl] pyridine (1g)

Yield: 52 %, MP 270 $^0$C, NMR: (ppm):8.5- 6.67 (8H, m, aromatic),6.706 (1H, s, olefinic =CH- pyrazole ) 3.05(1H, s, -NH Pyrazole), Mass: $C_{14}H_{10}BrN_3$ m/z: 300. IR (cm$^{-1}$): 3332 (-NH-, *str*, secondary amine), 3030 (=C-H str aromatic), 2232 (ring =C-N, *str*, imine), 1588 (C=C, str aromatic), 748 (C-Br str). $C_{14}H_{10}BrN_3$ m/z: 300

## DISCUSSION

Compound **1a** shows sharp band at 3310-3330 corresponds to C=N and NH bond respectively. other bands are (N-N str, pyrazol) at 1229 cm$^{-1}$, (C=C str pyrazol) at 1590 cm$^{-1}$, C-O str. (methoxy group) at 1062 cm$^{-1}$ it supports formation of pyrazole ring. Absence of Signal at 1670 cm$^{-1}$ in final compound indicates absence of α-β unsaturated ketone of chalone which further confirms formation of pyrazole ring. NMR spectra shows signal at 7.7 to 6.7 δ for 4 hydrogens of phenyl group at 5 position. Whereas at δ 7.2(1H, S, olefinic =CH-pyrazol), similarly for aromatic ring at third position of pyrazole signal is obtain for two protons, 7.69 (d, =CH,aromatic). $C_{17}H_{15}BrN_2O_2$ M/z: 358. Similarly, other compounds give singlet for the pyrazolyl proton at 8.57 to 10.99 ppm range. Further mass data of compounds supports formation of newer derivatives of pyrazole.

## PHARMACOLOGICAL ACTIVITY

### Anti-inflammatory activity

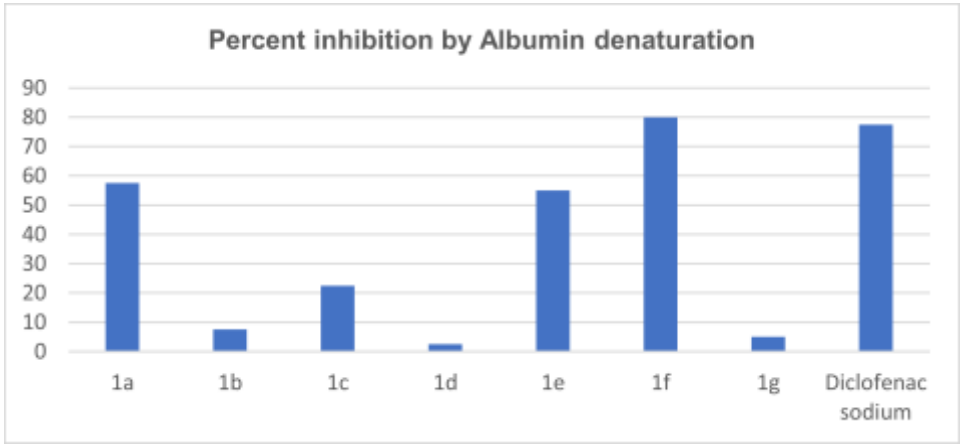
Compound **1f** shows promising percent inhibition when compared with diclofenac sodium. Inhibition score given in table 2.

**Table 2 Anti-inflammatory data**

| Compound | Concentration (µg/ml) | Absorbance of control | Absorbance of sample | Percent inhibition |
|---|---|---|---|---|
| 1a | 10 | 0.4 | 0.63 | 57.5 |
| 1b | 10 | 0.4 | 0.43 | 7.5 |

| | | | | |
|---|---|---|---|---|
| 1c | 10 | 0.4 | 0.49 | 22.5 |
| 1d | 10 | 0.4 | 0.41 | 2.5 |
| 1e | 10 | 0.4 | 0.62 | 55 |
| 1f | 10 | 0.4 | 0.72 | **80** |
| 1g | 10 | 0.4 | 0.42 | 05 |
| **Diclofenac sodium** | 10 | 0.4 | 0.71 | **77.5** |

**Fig 1: Graph of percent inhibition by 1a-1g with standard**




**Molecular Docking**

Docking interaction of standard drug with target are shown in fig2. ARG 120 of target is forming hydrogen bonding with oxygen of Ibuprofen. Similarly, carbonyl oxygen of Ibuprofen involved in H-bonding with TYR355 of target. Phenyl ring and two methyl groups of Ibuprofen involved in pi bond interactions with target. Further drug covering majority of amino acids of target by Van der Waals interaction. In case of 1f it shows binding energy of -8.8 kcal/mol and is showing hydrogen bonding interactions by amino acid residues like TYR355, TYR385, pi bond interactions through ILE89, VAL116, ARG120, VAL349, ILE523, ALA527 and majority of amino acid residues involved in Van der Waals interaction e.g ARG83, LEU93, LEU115, VAL119, VAL344, TYR348, LEU352, SER353, LEU357, LEU359, PHE381, TRP387 as shown in fig.3

**Table 3 % control growth against MCF-7 cancer cell line**

**Human Breast Cancer Cell Line MCF-7**

**% Control Growth**

**Drug Concentrations (µg/ml)**

| | Experiment 1 | | | | Experiment 2 | | | | Experiment 3 | | | | Average Values | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 | 20 | 40 | 80 | 10 | 20 | 40 | 80 | 10 | 20 | 40 | 80 | 10 | 20 | 40 | 80 |
| 1a | 124.8 | 127.9 | 110.2 | 126.0 | 100.2 | 99.4 | 85.2 | 84.7 | 86.8 | 101.1 | 96.1 | 115.2 | 103.9 | 109.5 | 97.2 | 108.6 |
| 1b | 107.4 | 95.8 | 95.9 | 104.0 | 119.8 | 81.8 | 99.8 | 84.8 | 129.7 | 102.0 | 95.3 | 110.4 | 119.0 | 93.2 | 97.0 | 99.8 |
| 1c | 113.7 | 118.0 | 120.6 | 131.4 | 108.8 | 94.2 | 90.9 | 103.2 | 84.5 | 112.7 | 108.0 | 132.6 | 102.3 | 108.3 | 106.5 | 122.4 |
| 1d | 116.6 | 83.7 | 87.2 | 43.1 | 105.6 | 105.5 | 65.2 | 41.5 | 113.5 | 97.3 | 93.8 | 73.5 | 111.9 | 95.5 | 82.0 | 52.7 |
| 1e | 109.0 | 82.4 | 105.5 | 61.0 | 91.3 | 104.3 | 67.0 | 83.8 | 110.0 | 100.6 | 79.1 | 81.4 | 103.4 | 95.8 | 83.9 | 75.4 |
| 1f | 70.6 | 59.5 | 95.3 | 40.7 | 87.2 | 73.3 | 81.1 | 34.3 | 74.1 | 77.3 | 68.7 | 56.7 | 77.3 | 70.0 | 81.7 | 43.9 |
| 1g | 66.6 | 76.3 | 125.6 | 116.0 | 102.1 | 140.1 | 105.1 | 84.0 | 88.3 | 86.8 | 108.0 | 116.8 | 85.7 | 101.1 | 112.9 | 105.6 |
| ADR | -55.7 | -57.1 | -59.2 | -20.2 | -55.7 | -57.1 | -59.2 | -20.2 | -55.7 | -57.1 | -59.2 | -20.2 | -55.7 | -57.1 | -59.2 | -20.2 |

**Fig 2- 2D and 3D plot of interaction of Ibuprofen with Target Protein**

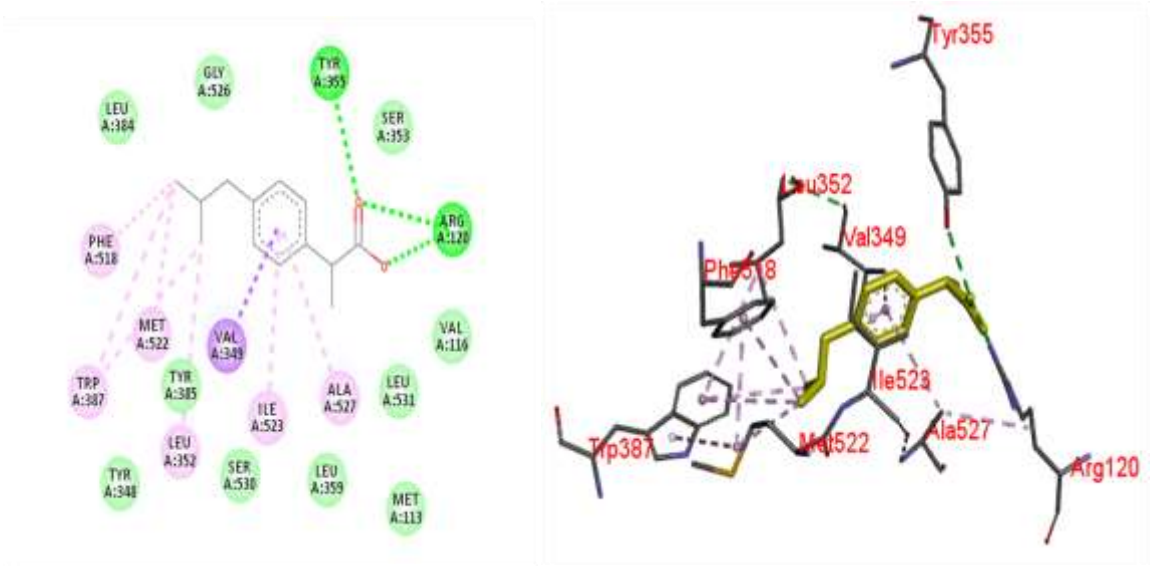
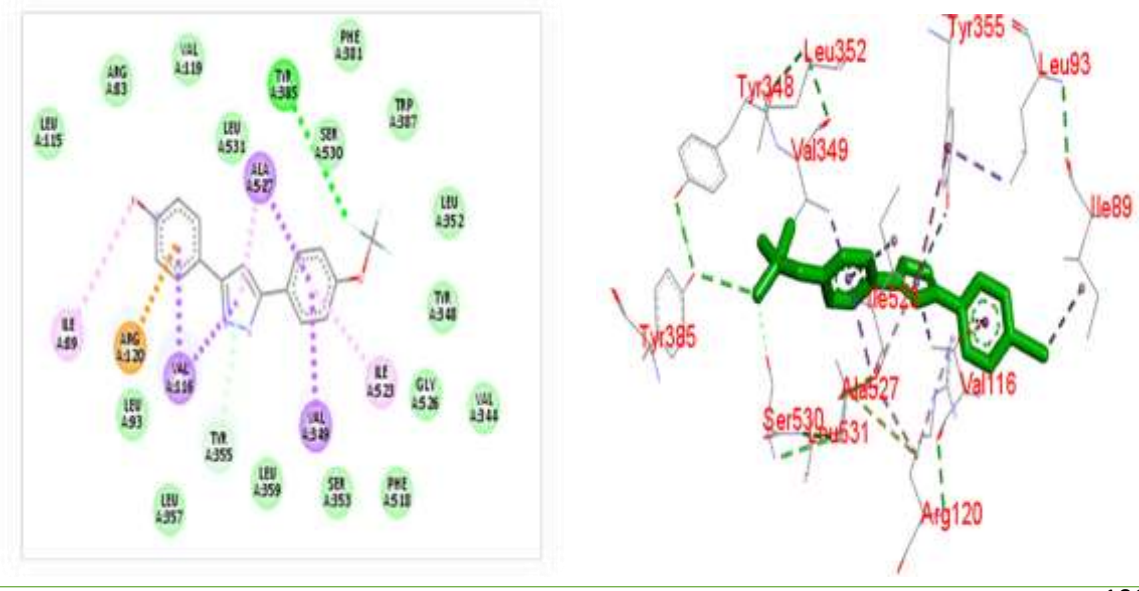



**Fig 3- 2D and 3D plot of interaction of If with Target Protein (COX I)**



**Anticancer activity:** Compound 1f shows lowest IC50 value in this series of compound. It has para -OCF3 –phenyl substitution at 3rd position of pyrazole ring. Followed by 1f, 1e shows 52.7 % control growth inhibition. However, IC50 value is >80 µg/ml. Result reveal that derivatives with morphonyl, Trimethoxy substituted phenyl ring at 5th position of pyrazole ring not shown significant effect on growth of MCF 7 cancer cell (fig5).

**ADR –** Adriyamycin

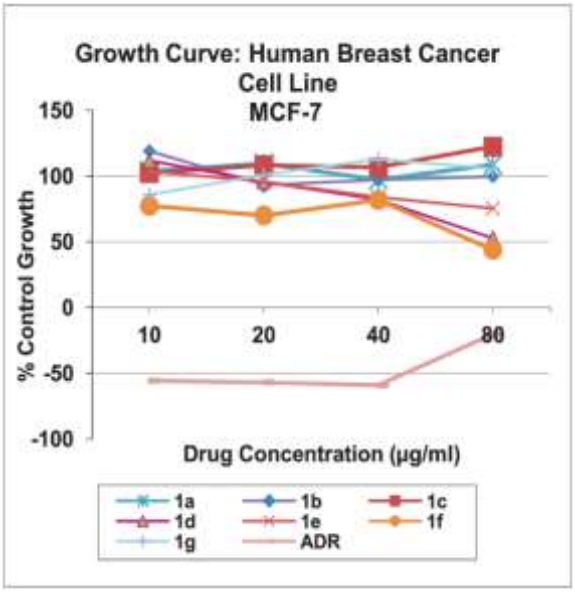


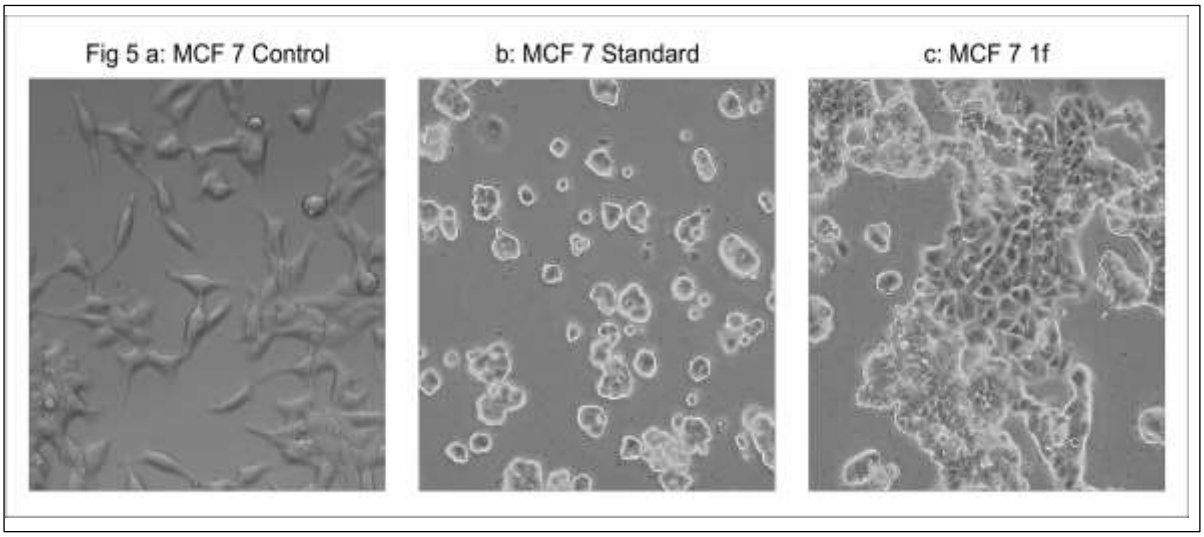

**Fig 4: Growth Curve of 1a to 1f**

**Table 4 Activity data**

| MCF 7 cell line | Drug concentrations (µg/ml) from graph | | |
|---|---|---|---|
| | LC50 | TGI | GI50* |
| 1a | NE | NE | >80 |
| 1b | NE | NE | >80 |
| 1c | NE | NE | >80 |
| 1d | NE | NE | >80 |
| 1e | NE | NE | >80 |
| 1f | NE | NE | **78.4** |
| 1g | NE | NE | >80 |
| ADR | **<10** | **<10** | **<10** |

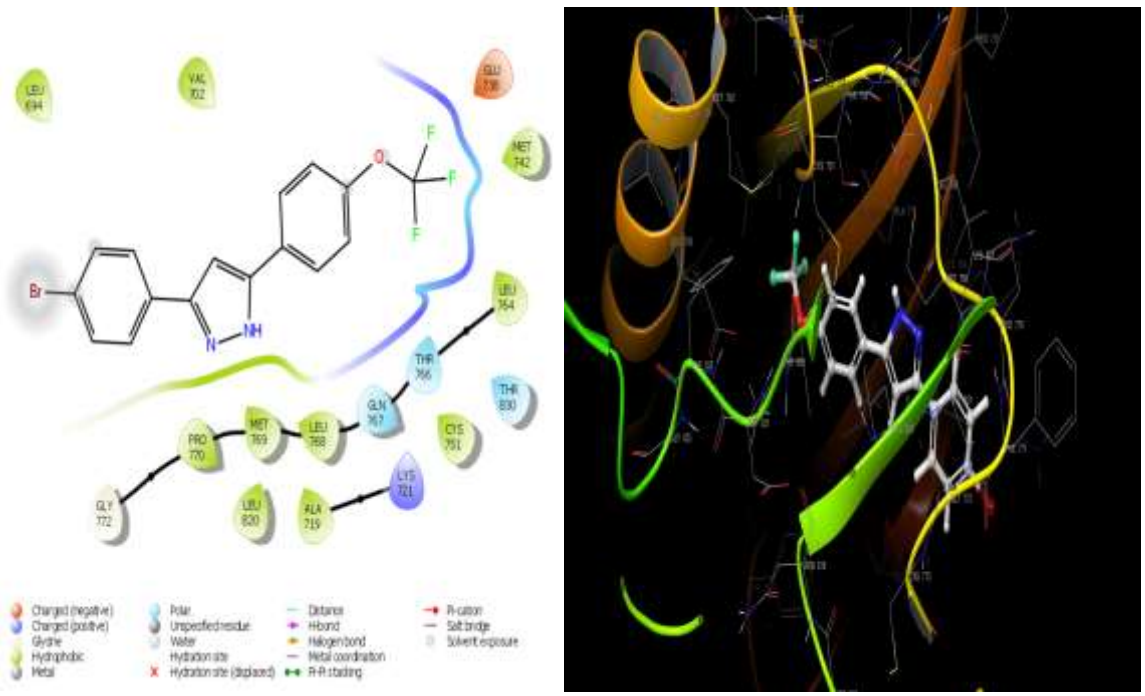
**NE**- Non evaluable data. **ADR**- Adriamycin,

MCF 7 treated with control, standard, and pyrazole derivatives are shown below pictures (fig 5)



**Molecular docking against EGFR:** To understand the binding of compound 1f with target, it was docked against EGFR target (PDB id-1M17) and docking interactions were compared with Doxorubicin Standard. Common amino acid involved in hydrophobic interaction with EGFR are LEU694A and ALA719A other amino acids are LEU768A, LEU820A at distance of 3.76, 3.47 $A^0$ respectively. 1f is involved in hydrogen bond interactions through GLN767A at a distance of 3.92 and 336 respectively. Docked poses of 1f are seen as given below in fig 6.

**Fig 6. 2D and 3D docking interaction of 1f with 1M17 protien**




**CONCLUSION:** Some Pyrazol analogues were successfully synthesized from 1,3-diphenyl propenones. Compound 1f have found to shows good anti-inflammatory potential and same was confirmed by observing good binding of compound with COX I target. Compound 1f shows comparable in vitro anti-inflammatory potential which is 80 and 77.5 for 1f and Diclofenac Sodium respectively. Docking interactions supported the practical findings and therefore activity of compound may be because of inhibition of COX1 enzyme. Hence it encouraged to check anticancer activity of series. Anticancer potential was checked against MCF 7 cell line using SRB assay method. Further compound 1f shows lowest IC50 value (78.4µg/ml) against MCF 7 cell line in this series. In docking 1f shows some common amino acid involved in binding interactions with target protein as that of Doxorubicin. Saturated substituents not shown any significant effect on anticancer activity of derivatives. Compound have p-trifluoromethoxy phenyl group on 5 th position of pyrazole moiety hence such kind of substitution will result in potentially active therapeutic agents and serve as a good template for potent anticancer compounds.

**Author's contribution:** SAW planned and did synthesis of novel analogues and sent to ACTREC for screening cytotoxicity. Manuscript prepared by SAW and checked by RLS. All authors approved manuscript.

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**REFERENCE:**

1. Ignatius Ou S.H, et al. Crizotinib: a novel and first-in-class multitargeted tyrosine kinase inhibitor for the treatment of anaplastic lymphoma kinase rearranged nonsmall cell lung cancer and beyond. Drug. Des. Devel. Ther. 2011; 5:471–485. https://doi.org/10.2147/dddt.s19045.
2. Kim, H.C. & Oh, SM. Noncommunicable diseases: current status of major modifiable risk factors in Korea. J. Prev. Med. Public. Health. 2013;46(4):165–172. https://doi.org/10.3961%2Fjpmph.2013.46.4.165.
3. Selvam, T.P, Kumar P.V, Saravanan, G, & Prakash, C.R. Microwave-assisted synthesis, characterization and biological activity of novel pyrazole derivatives. J. Saudi Chem. Soc. 2014; 18:1015-1021. http://dx.doi.org/10.1016%2Fj.jscs.2011.12.006.
4. Poeggeler, B, Pappolla, M.A, Hardeland, R, Rassoulpour, A, Hodgkins, P.S, Guidetti, P. & Schwarcz, R. Indole-3-propionate: a potent hydroxyl radical scavenger in rat brain. Brain. Res.1999; 815(4):382-388.https://doi.org/10.1016/s0006-8993(98)01027-0.
5. Liu J.J, Zhao M.Y, Zhang X, Zhao X. & Zhu H.L. Pyrazole Derivatives as Antitumor, Anti-Inflammatory and Antibacterial Agent. Mini. Rev. Med. Chem., 2013; 3(13):1957-1966. https://doi.org/10.2174/13895575113139990078.
6. Sayed A. R, Gomha S.M, Abdelrazek F.M, Farghaly M.S, Hassan, S.A. & Metz P. Design, efficient synthesis and molecular docking of some novel thiazolyl-pyrazole derivatives as anticancer agents. BMC Chemistry.2019;13(116):1-13.https://doi.org/10.1186/s13065-019-0632-5.
7. Alsayari A, Asiri Y.I, Muhsinah A.B. & Hassan, M. Z. Anticolon Cancer Properties of Pyrazole Derivatives Acting through Xanthine Oxidase Inhibition J. Oncol., 2021;1-5. https://doi.org/10.1155/2021/5691982.
8. Karrouchi K, Radi S, Ramli Y, Taoufik, J, Mabkhot Y.N, Al-aizari, F.A. & Ansar, M. Synthesis and Pharmacological Activities of Pyrazole Derivatives: A Review. Molecules. 2018;23(1):134. https://doi.org/10.3390%2Fmolecules23010134.
9. Barcelo M, Ravina E, Masaguer C.F, Domınguez E, Areias F.M, Brea, J. & Loza, M.I. Synthesis and binding affinity of new pyrazole and isoxazole derivatives as

potential atypical antipsychotics. Bioorg. Med. Chem. Lett. 2007;17(17):4873-4877. https://doi.org/10.1016/j.bmcl.2007.06.045.

10. EI Shery M.F, Ewies E.F. & Zayed, E.M. Synthesis of new pyrazol derivatives, their antiinflammatory and analgesic activity, and molecular docking studies. Russ. J. Gen. Chem., 2019;89(3):492-498. http://dx.doi.org/10.1134/S1070363219030216.
11. Farook, S. & Nagaini, Z. Microwave-assisted synthesis, antimicrobial activities and molecular docking of methoxycarboxylated chalcone derived pyrazoline and pyrazole derivatives. chemistry Select. 2022; 7:1-7. http://dx.doi.org/10.1002/slct.202103984.
12. Dhivare R.S. & Rajput, S.S. Microwave Assisted Synthesis and Antimicrobial activities of 3,4-bis-(4-hydroxy-3- methoxybenzylidene)-7-(N-phenyl)-3,3a,3b,4,5,7-hexahydro-2H-pyrrolo[2,3-c,5,4-c] di pyrazole. Int. J. Curr. Pharm.2015;3(6):1106–1109.
13. Rashad A.E, Hegab M.I., Abdel-Megeid, R.E, Micky, J.A. & Abdel-Megeid, F.M. Synthesis and antiviral evaluation of some new pyrazole and fused pyrazolopyrimidine derivatives. Bioorg. Med. Chem 2008; 16:7102–7106. https://doi.org/10.1016/j.bmc.2008.06.054.
14. Kumara P, Sood S, Kumar A. & Singh, K. Microwave- assisted Vilsmeier- Haack synthesis of Pyrazole- 4- carbaldehydes. J. Heterocycl. Chem. 2019; 57(2):796–804. http://dx.doi.org/10.1002/jhet.3824.
15. Gul, H.I, Yamali, Cem., Yesilyurt, F., Sakagami, H., Kucukoglu, K., Gulcin, I., Gule, M. & Supuran, C.T. Microwave-assisted synthesis and bioevaluation of new sulfonamides, J. Enzyme. Inhib. Med. Chem.2017;32(1):369–374. https://doi.org/10.1080%2F14756366.2016.1254207.
16. El-Moghazy S.M, Barsoum, F.F, Abdel-RahmanH.M. & Marzouk, A.A. Synthesis and anti-inflammatory activity of some pyrazole derivatives, Med. Chem. Res. 2012; 21:1722–1733. http://dx.doi.org/10.1007/s00044-011-9691-4.
17. Barsoum, F.F., Hosni, H.M. & Girgis, A.S. Novel bis (1-acyl-2- pyrazolines) of potential anti-inflammatory and molluscicidal properties. Bioorg. Med. Chem.2006; 14:3929–3937. doi: 10.1016/j.bmc.2006.01.042.
18. Achutha D.K., Kameshwar, V.H., Ningappa, M.B. & Kariyappa, A.K. Synthesis and in vitro biological evaluation for antioxidant, anti-inflammatory activities and molecular docking studies of novel pyrazole derivatives. Heliyon.2017;7(3):2040 – 2047.
19. Tewari A. K, Singh, V.P, Yadav P, Gupta G, Singh A, Goel R, Shinde P.& Mohan G. Synthesis, biological evaluation and molecular modeling study of pyrazole derivatives as selective COX-2 inhibitors and anti-inflammatory agents. Bioorg. Chem.2014; 56:8–15. https://doi.org/10.1016/j.bioorg.2014.05.004
20. Tahera A.T, Mostafa Sargc, M.T., El-Sayed Alid, N.R. & Elnagdie, N.H. Design, synthesis, modeling studies and biological screening of novel pyrazole derivatives as

potential analgesic and anti-inflammatory agents. Bioorg. Chem.2019; 89:1-13. doi: 10.1016/j.bioorg.2019.103023

21. Ketha, A, Vedula G.S. & Sastry V.S. In vitro antioxidant, anti-inflammatory, and anticancer activities of methanolic extract and its metabolites of whole plant Cardiospermum canescens. Wall. Future J. Pharm. Sci. 2020;6(11):1-10. https://doi.org/10.1186/s43094-020-00028-y.
22. Selinsky BS, Gupta K, Sharkey C.T. & Loll P.J. Structural Analysis of NSAID Binding by Prostaglandin H 2 Synthase: Time-Dependent and Time-Independent Inhibitors Elicit Identical Enzyme Conformations. Biochemistry. 2001;40(17):5172–5180. doi: 10.1021/bi010045s.
23. Morris G.M, Huey R, Lindstrom W, Sanner M.F, Belew R.K. & Goodsell, D.S. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J. Comput. Chem 2009;30(16):2785–2791. doi: 10.1002/jcc.21256.
24. Chemsketch [Internet]. 2018. Available from: https://www.acdlabs.com
25. Rappe A.K., Casewit, C.J, Colwell, K.S., Goddard, W.A. & Skiff, W.M. A full periodic table force field for molecular mechanics and molecular dynamics simulations. J. Am. Chem. Soc.1992;114(25):10024–10035. https://doi.org/10.1021/ja00051a040
26. Thompson, M.A. Molecular docking using ArgusLab, an efficient shape-based search algorithm and A Score scoring function. In: Proceedings of the ACS Meeting. Philadelphia. 2004
27. Trott, O. & Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput. Chem.2010;31(2):455–461.
28. Vichai, V. & Kirtikara, K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nature Protocols.2006;1:1112–1116. https://doi.org/10.1038/nprot.2006.179
29. Skehan P, Storeng R, Scudiero D., Monks A., Mc Mahon J, Vistica D, Warren J.T, Bokesch H, Kenney S. & Boyd, M.R. New colorimetric cytotoxicity assay for anticancer-drug screening. J. Nat. Cancer. Inst. 1990; 82:1107-1112. https://doi.org/10.1093/jnci/82.13.1107
30. Abdelgawad M.A., Abdellatif Khaled, R.A. & Ahmed O.M. Design, Synthesis and Anticancer Screening of Novel Pyrazole Derivatives Linking to Benzimidazole, Benzoxazole and Benzothiazole. Medicinal chemistry., 2014; S1:1-7 doi : 10.4172/2161-0444.S1-00
31. Guerriero E, Sorice A. Capone, F. Storti, G. Colonna, G. Ciliberto, G. & Costantini, S. Combining doxorubicin with a phenolic extract from flaxseed oil: Evaluation of the effect on two breast cancer cell lines. Int. J. Oncol. 2017; 50:468-476. https://doi.org/10.3892/ijo.2017.3835
32. Liaoa Q-H., Gaoa, Q-Z, Weia, J. & Chou, K-C. Docking and Molecular Dynamics Study on the Inhibitory Activity of Novel Inhibitors on Epidermal Growth Factor

Receptor (EGFR). Med. Chem ,2011;7:24-31. https://doi.org/10.2174/157340611794072698

33. Abd El-Karim, S.S Manal MA, 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. doi.org/10.1016/j.bioorg.2015.08.006.