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# Design, synthesis and characterization of some new pyrazol-pyrimidine derivatives and evaluation of their biological activities

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**A B S T R A C T**

In this work, we have reported a synthesis of some novel pyrazol-pyrimidine derivatives **(3a-f)** obtained by the reaction of substituted pyrazole aldehydes and barbituric acid derivatives in presence of L-proline as a catalyst *via* Knoevengal condensation reaction. The structures of the synthesized compounds were characterized by spectral methods. From antibacterial activity, compounds **3d** and **3f** exhibited highest zone of inhibition as compared to standard drug gentamicin. Cytotoxicity results revealed that compound **3e** exhibited a promising $IC_{50}$ value against both the cell lines (A549 and MCF-7) as compared to the standard drug doxorubicin. Docking study discloses that, all the newly synthesized compounds displayed promising binding energies with the protein receptor EGFR kinase domain.



## 1. Introduction

Pyrazole and pyrimidine derivatives are spotted in natural products like vitamins, alkaloids and pigments.[1, 2] These derivatives attracted the attention of researchers due to their biological properties and also core structures to achieve many pharmacologically important compounds. The combination of two or more heterocyclic derivatives contributes a new bioactive structure. Especially the aromatic heterocyclic compounds containing five and six-membered rings are the essential source of efficacious drugs and are also enriched with various biological activities.

Pyrazole is five-membered heterocyclic ring containing pyrrole and pyridine like two adjacent nitrogen atoms at 1st and 2nd position of the ring, nitrogen atom located at first position is unreactive whereas nitrogen present at second position is basic in nature due to presence of lone pair of electrons where the electrophilic substitution takes place. Pyrazoles can act as weak bases or acids depending upon their substitution. The remaining three positions in the ring are responsible for the structural modifications from the appropriate precursors.[3] It appears in different forms *viz.,* pyrazoline, pyrazolidine, pyrazolinone and pyrazolidinedione.The incorporation of any new active moieties in the pyrazole ring results in the enhancement of their chemical and biological properties of pyrazole ring.[4] In pyrazoline, the substitution of electron donor and electron withdrawing groups leads to different characteristic property of the compounds, such as stability and structural diversity. The presence of electron-donor groups establishes efficient charge transfer compared to electron-acceptors, which improves the photovoltaic property of pyrazoline based chromophore.[5] 3-oxo-1,2-dihydropyrazole, 3,5-pyrzolidinediones are the two oxo derivatives of pyrazoles that have been used in medicinal field due to their distinctive properties. Additionally, pyrazolidinedione derivatives with alkyl or aryl substitution at 4th position and 5th are used in pharmaceutical area in platelet aggregation and in particular for thrombosis.[6]

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Pyrazol-pyrimidine[7, 8] derivatives have important applications such as, anti-inflammatory,[9] antiviral,[10] antimicrobial,[11] anti-inflammatory,[12] anticancer,[13-15] analgesic[16] and anti-hyperglycemic activities.[17] They also find applications in dyes, electroluminescence, and many other relative applications.[18-20]

In the past few decades, antibiotics have been exceptionally used due to randomly increase of multi-drug resistance immunity in bacteria.[21, 22] Resistance towards antimicrobials has become a serious global problem. Literature reveals that the introduction of different heterocyclic ring on parent moiety is effective in synthesizing a variety of new compounds with potent pharmacological activity.[23] The structural modification of antimicrobial drugs has been known to reduce the life span of antimicrobials, which results in the synthesis of new derivatives against microbes. Cancer exists as a veritable lethal disease. The main cause of cancer is inconsistency that occurs during cell division in mitotic phase and is a serious trouble to mortal life. The development of new drugs regarding this diseases remains in progress for the forestallment and therapy of a variety of uncontrolled tumour cells. Increased risk of cancer from decades led to inventions of new drugs but still the prevention of cancer remains as a challenge.[24]

L-Proline is a promising organocatalyst which catalyses many organic reactions such as knoevengal condensation,[25] biginelli reaction,[26] Hantzsch reaction[27] and Mannich reaction.[28] Among these, knoevengal condensation is the most important method for the preparation of number of substituted alkenes. Some of the pyrazole and pyrimidine drugs which are available in the market and as shown in **Fig. 1.**[29] The leading objective of the present investigation is to describe the pharmacological activities of the newly obtained compounds. Some of the pyrazole based derivatives have been synthesized by using knoevengal reaction. Previous reports reveal that, 4-isopropylidenepyrazolidine-3,5-dione[30] and 3,4,5-Trisubstituted pyrazoles has been synthesized using tandem knoevengal reaction.[31]

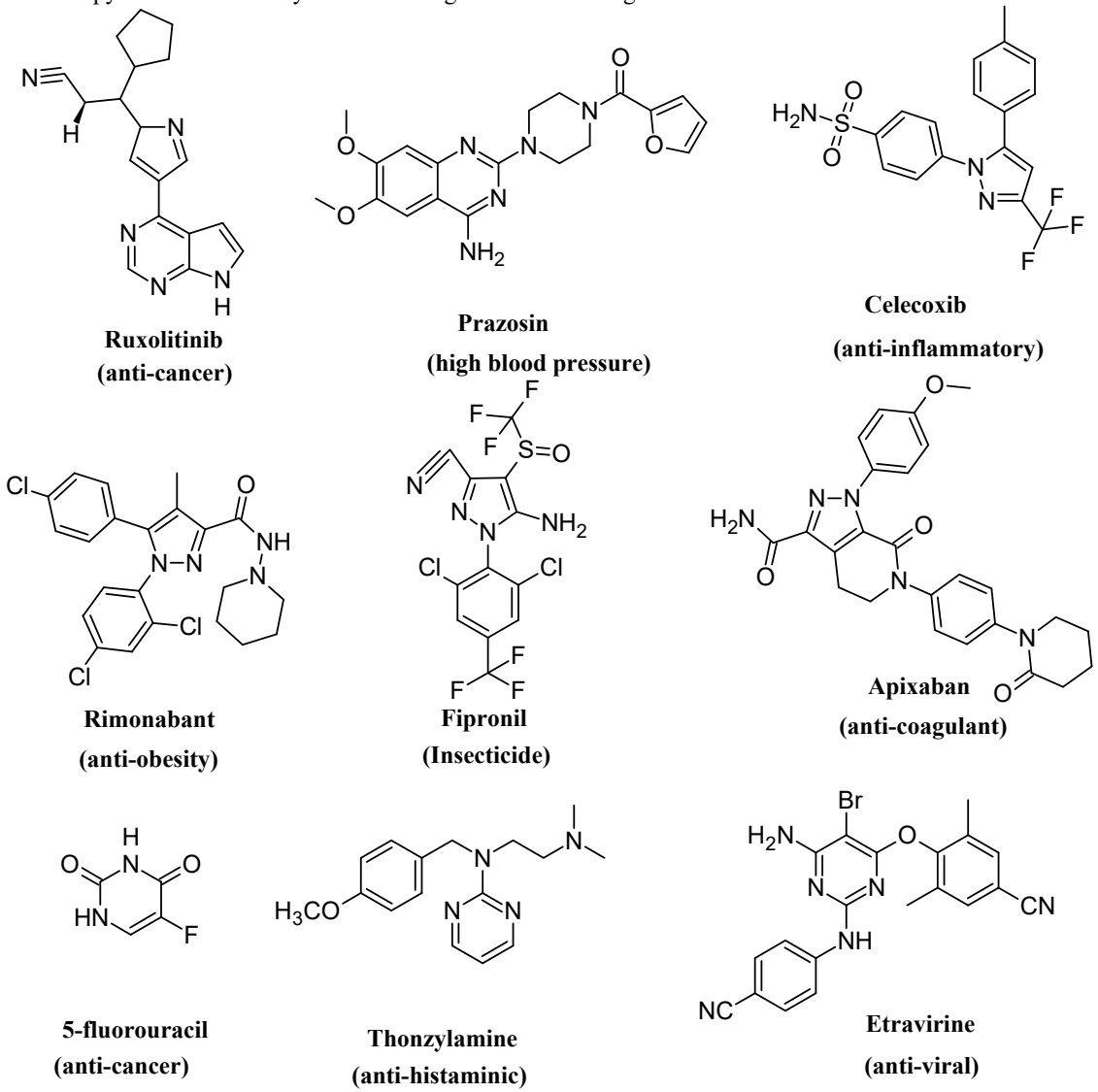


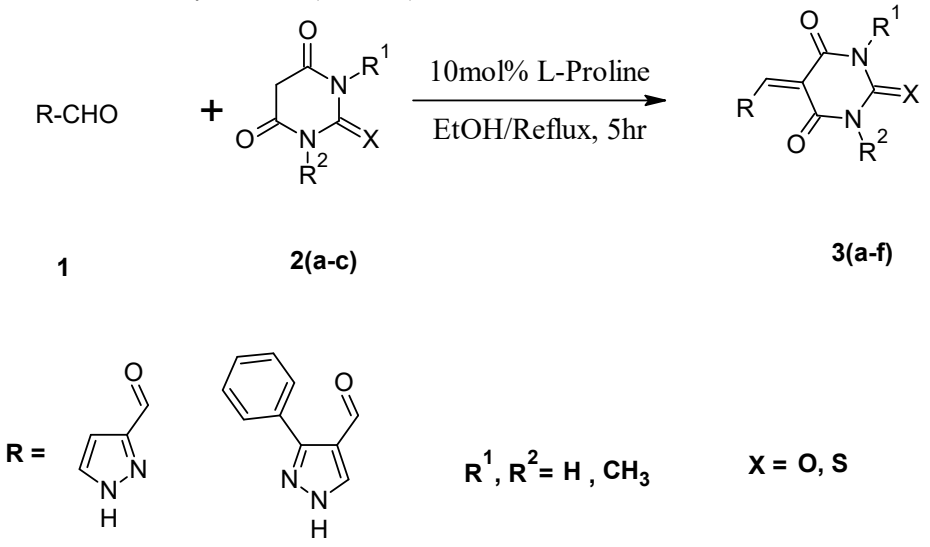

**Fig. 1.** Marketed drugs having pyrazole and pyrimidine derivatives.

Based on the above findings, we have prepared some new pyrazol-pyrimidine derivatives by knoevengal condensation reaction, compounds obtained are in good yield and screening their different biological activities. Further, the synthesized compounds were studied by binding interactions against target protein EGFR kinase domain.

## 2. Results and Discussion

### *2.1. Chemistry*

In the present study, pyrazol-pyrimidine derivatives have been synthesized *via* knoevengal condensation reaction between pyrazole aldehydes and barbituric acid derivatives using L-proline as a catalyst with solvent ethanol according to the reported procedure S.H. Sukanya *et al.,*.[32] **(scheme1).**




**Scheme. 1.** Schematic approach for the synthesis of pyrazol-pyrimidine derivatives **(3a-f)**

The newly synthesized compounds structures **(3a-f)** were characterized by spectra's such as IR, $^{1}H$ &$^{13}C$ NMR and mass. In the case of IR spectrum, compound **3a** exhibited a stretching band at 3259 $cm^{-1}$ due to NH group, an absorption band observed at 1671 $cm^{-1}$ corresponds to C=O group, and the C=N band is detected at 1581 $cm^{-1}$. Again the product formation was confirmed by $^{1}H$ NMR spectra, compound **3a** showed a NH of pyrazole as singlet at δ 13.86 ppm and two singlet resonated at δ 11.34 and δ 11.28 ppm belongs to NH groups of barbituric acid. A singlet appearing at δ 8.24 ppm shows the presence of CH proton. The CH moieties of pyrazole ring appear as two singlet at δ 7.85 ppm and δ 7.66 ppm. $^{13}C$ NMR, a peak at δ 163.50 ppm assigned to carbonyl carbon. The molecular ion peak for compound **3a** observed at m/z 207.0704 $[M+1]^{+}$ confirms the expected molecular weight of the product **3a** and remaining compounds spectra's are appended in the supporting information file (**S1 to S24**). The physical data of compounds from **(3a-f)** is given in **Table 1**.

**Table 1.** Physical data of compounds **(3a-f)**

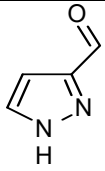
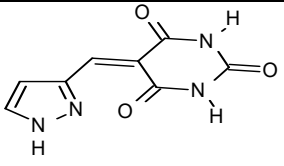
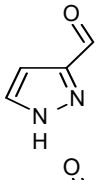
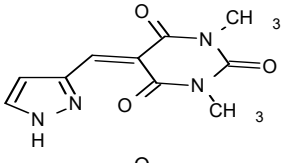
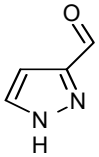
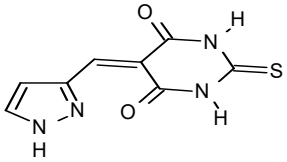
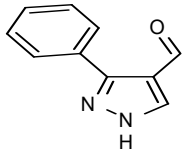
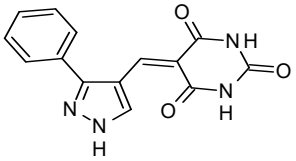
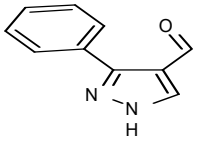
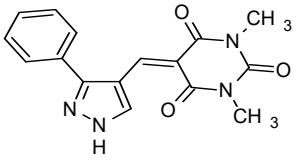
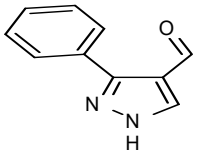
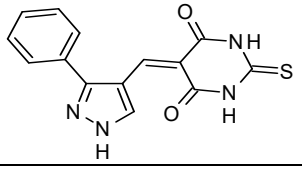
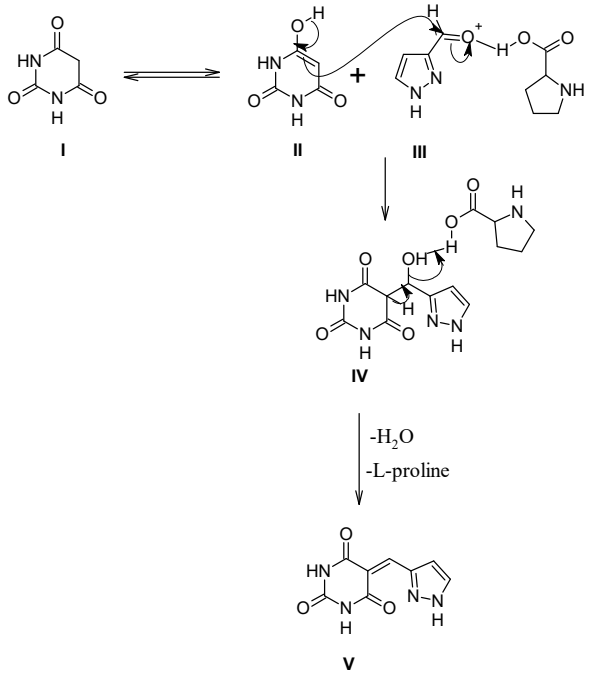
| Comp. | R | $R^1$ | $R^2$ | X | Mol. Structure | Yield (%) |
|---|---|---|---|---|---|---|
| **3a** |  | H | H | O |  | 90.92 |
| **3b** |  | $CH_3$ | $CH_3$ | O |  | 73 |
| **3c** |  | H | H | S |  | 60 |

**Table 1.** Physical data of compounds **(3a-f)** (Continued)

| Comp. | R | $R^1$ | $R^2$ | X | Mol. Structure | Yield (%) |
|---|---|---|---|---|---|---|
| **3d** |  | H | H | O |  | 94.59 |
| **3e** |  | $CH_3$ | $CH_3$ | O |  | 83.62 |
| **3f** |  | H | H | S |  | 98.49 |

The possible mechanism to obtain a series of pyrazol-pyrimidine derivatives has been shown in **scheme 2**. Reaction commence with enol **II** formation from substituted barbituric acid derivatives **I**, then formation of hydrogen bond occurs between L-proline and oxygen atom of pyrazole aldehyde derivatives to form **III** after that, the elimination of $H_2O$ and L-proline takes place from **IV** to give the desired product **V**.



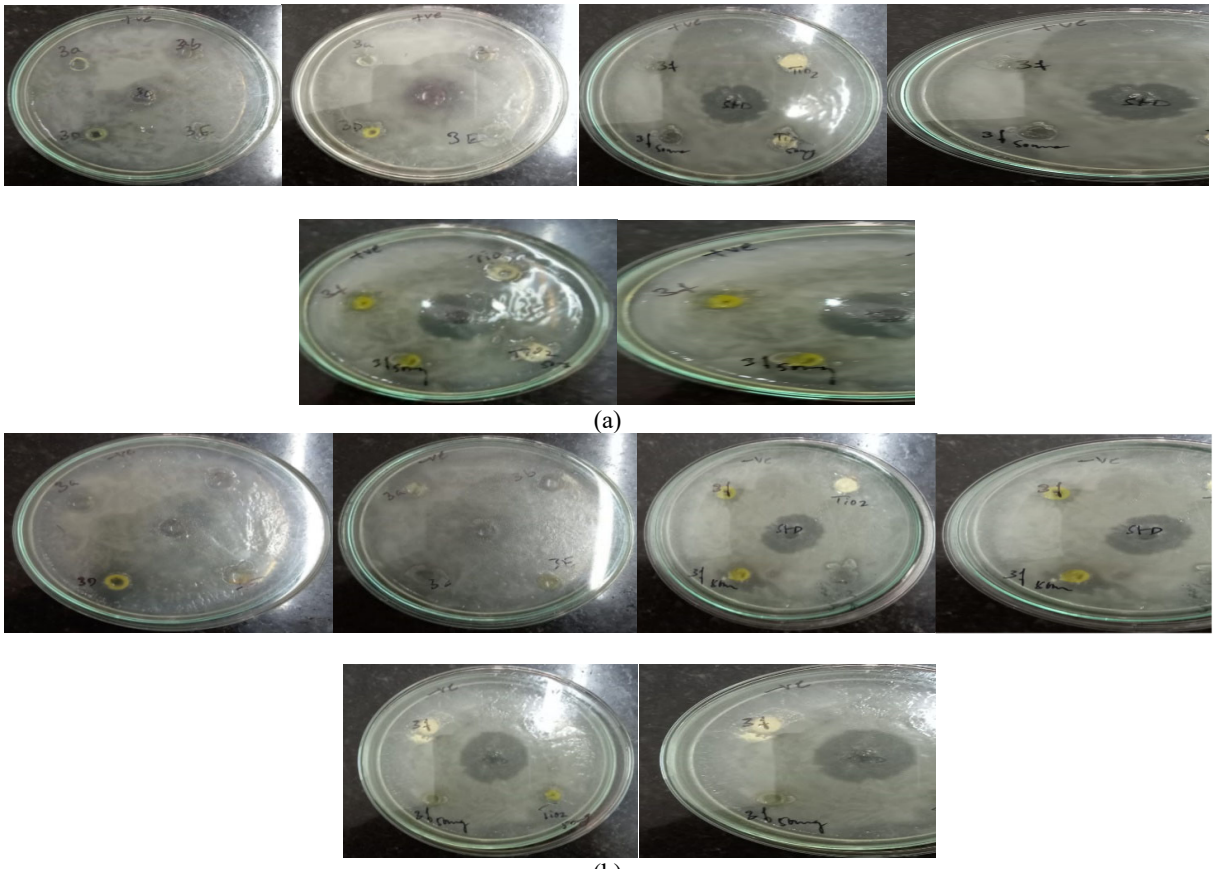

**Scheme. 2.** The possible mechanism of synthesized compounds **(3a-f)**

*2.2. Pharmacological effect*

*2.2.1. Antibacterial activity*

The antibacterial activity of newly synthesized compounds was evaluated against four bacterial strains and standard drug gentamicin using agar well diffusion assay. The activity results reveal that, the screened compounds showed beneficial antibacterial activity with different zone of inhibition. Compounds **3d** and **3f** exhibited highest zone of inhibition **(**10 to12 mm) against *S. aureus*, *B. subtilis, S. Typhi* and *P. aeruginosa* bacterial strains is due to presence of dihydropyrimidinones,

has increased the potent activity of compound **3d** and **3f**,[33] and also compound **3a** exhibited good bacterial zone of inhibition (8 to 12 mm) as compared to reference standard gentamicin **(Table 2** & **Fig. 2.)**.



(a)

(b)

**Fig. 2.** Antibacterial activity of synthesized compounds **(3a-f)** against two Gram positive (**a**) and two Gram negative bacterial strains (**b**)

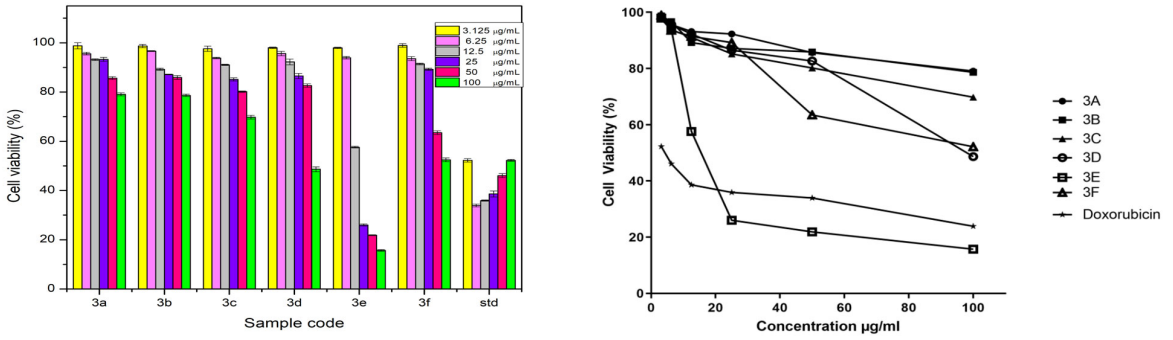
**Table 2.** Zone of inhibition for the compounds **(3a-f)**

| Entry | Zone of inhibition (in mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***P. aeruginosa*** | | | ***B. subtilis*** | | | ***S. aureus*** | | | ***S. Typhi*** | | |
| | Concentration in µg/mL | | | | | | | | | | | |
| | **25** | **50** | **100** | **25** | **50** | **100** | **25** | **50** | **100** | **25** | **50** | **100** |
| **3a** | 8 | 10 | 12 | 8 | 10 | 11 | 9 | 9 | 11 | 9 | 10 | 12 |
| **3b** | 8 | 9 | 10 | 8 | 10 | 10 | 9 | 10 | 10 | 9 | 9 | 11 |
| **3c** | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| **3d** | 10 | 10 | 12 | 10 | 10 | 11 | 12 | 10 | 12 | 10 | 9 | 12 |
| **3e** | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| **3f** | 10 | 10 | 10 | 10 | 10 | 11 | 12 | 10 | 12 | 10 | 10 | 12 |
| **Gentamycin** | 14 | 15 | 16 | 14 | 14 | 14 | 15 | 14 | 15 | 15 | 15 | 15 |

### *2.2.2. Cytotoxicity study*

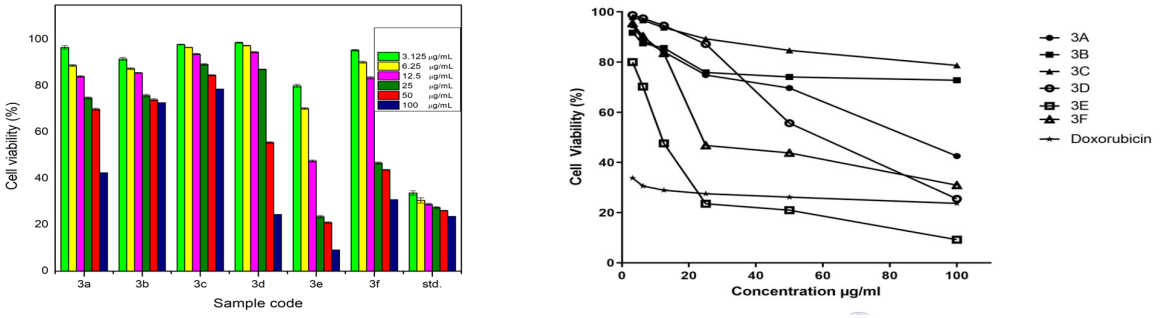
The *in vitro* cytotoxicity of the compounds **(3a-f)** was estimated against two dissimilar cell lines A549 and MCF-7. The cytotoxicity results divulge that, compound **3e** showed a good $IC_{50}$ value of 18.24±0.10 µg/mL against the cell line A549. Similarly, cytotoxicity results against MCF-7 cell line indicated that compound **3e** showed promising $IC_{50}$ value of 11.46±0.23 µg/mL as compared to doxorubicin and rest of the compounds showed moderate cytotoxicity.

The compound **3e** having two electron donating methyl group[34] on barbituric acid nucleus and phenyl group present on the fourth position of pyrazole ring are responsible for increased the cytotoxicity[35] and results as appended in **Table 3 and Table 4**. A graph of percentage of surviving cells of obtained compounds **(3a-f)** against different concentrations as shown in **Fig. 3** and **Fig. 4.**




(a) (b)

**Fig. 3.** A Graph of % of surviving cells of compounds **(3a-f)** at different concentration against A549 cell line (**a** and **b**)




(a) (b)

**Fig. 4.** A Graph of % of surviving cells of compounds **(3a-f)** at different concentration against MCF-7 cell line (**a** and **b**)

**Table 3**. Percentage of cell viability against A549 cell line of the synthesized compounds **(3a-f)**

| **Concentration in µg/mL** | **Mean cell Viability of A549** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **3a** | **3b** | **3c** | **3d** | **3e** | **3f** | **std** |
| **NC** | 100 | | | | | | |
| **3.125** | 98.88±1.27 | 98.70±0.66 | 97.59±1.06 | 98.0±0.26 | 97.94±0.27 | 98.94±0.70 | 52.26±0.67 |
| **6.25** | 95.59±0.53 | 96.65±0.18 | 93.82±0.30 | 95.65±0.90 | 93.94±0.50 | 93.59±0.82 | 33.92±0.54 |
| **12.5** | 93.18±0.36 | 89.24±0.46 | 91.06±0.26 | 92.24±1.15 | 57.61±0.36 | 91.41±0.36 | 35.92±0.26 |
| **25** | 92.29±0.79 | 87.12±0.17 | 85.18±0.63 | 86.53±1.03 | 26.04±0.44 | 89.24±0.46 | 38.62±1.23 |
| **50** | 85.65±0.56 | 85.89±0.76 | 80.18±0.27 | 82.65±0.71 | 21.87±0.18 | 63.49±0.76 | 46.07±0.79 |
| **100** | 79.07±0.62 | 78.66±0.46 | 69.78±0.73 | 48.67±0.98 | 15.69±0.35 | 52.47±0.79 | 52.26±0.36 |
| **IC50 in µg/mL** | 335.27±7.55 | 302.87±2.84 | 202.00±.6.01 | 136.20±0.00 | 18.24±0.10 | 111.00±3.22 | 7.56±0.28 |

**Table 4**. Percentage of cell viability against MCF-7 cell line of the synthesized compounds **(3a-f)**

| **Concentration in µg/mL** | **Mean cell Viability of MCF-7** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **3a** | **3b** | **3c** | **3d** | **3e** | **3f** | **std** |
| **NC** | 100 | | | | | | |
| **3.125** | 96.68±0.71 | 91.69±0.65 | 97.91±0.16 | 98.74±0.22 | 80.1±0.64 | 95.43±0.37 | 33.80±0.93 |
| **6.25** | 88.85±0.40 | 87.48±0.39 | 96.65±0.10 | 97.44±0.16 | 70.26±0.33 | 90.25±0.43 | 30.57±1.14 |
| **12.5** | 84.10±0.33 | 85.65±0.18 | 93.78±0.27 | 94.56±0.24 | 47.60±0.43 | 83.53±0.53 | 28.93±0.42 |
| **25** | 74.86±0.46 | 75.87±0.59 | 89.28±0.40 | 87.2±0.16 | 23.55±0.54 | 46.74±0.40 | 27.53±0.37 |
| **50** | 69.65±0.44 | 74.07±0.48 | 84.64±0.27 | 55.6±0.32 | 20.96±0.38 | 43.79±0.21 | 26.16±0.14 |
| **100** | 42.53±0.44 | 72.78±0.67 | 78.67±0.34 | 24.49±0.33 | 9.20±0.43 | 30.92±0.98 | 23.69±0.53 |
| **IC50 in µg/mL** | 83.00±0.67 | 162.10±2.59 | 312.53±7.75 | 69.90±0.06 | 11.46±0.23 | 37.76±0.39 | 3.16±0.10 |

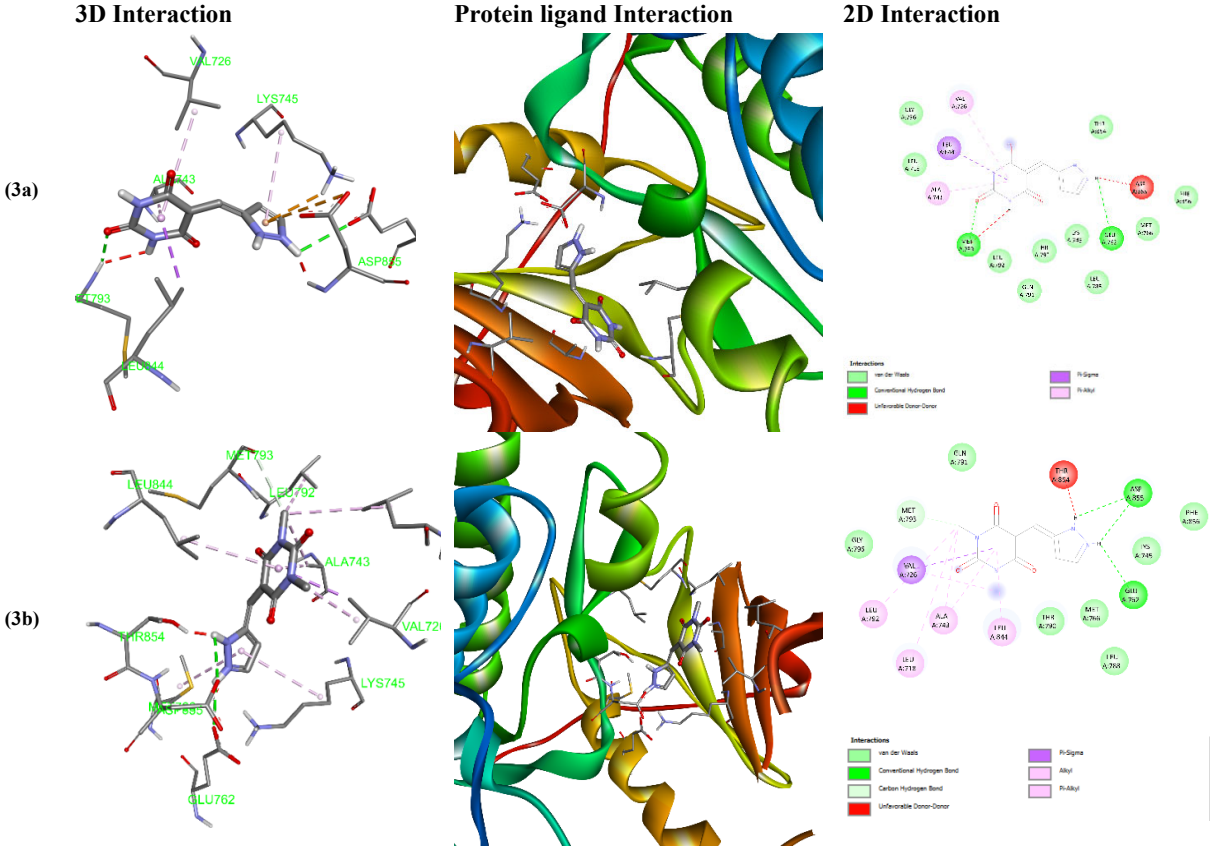
*2.2.3. In silico molecular docking studies*

Molecular docking study is an important tool in pharmaceutical research where interaction between small molecules and protein takes place and is also used in determining the behavior of small molecules at the binding sites of targets. Docking studies is helpful in predicting the orientation and conformation of ligands and is further used in elucidating the fundamental and biological process. EGFR kinase domain (Pdb Id: 4I23) induces tyrosine kinase activity and receptor dimerization, which leads to cell proliferation, differentiation, motility and cell survival. Therefore, EGFR kinase domain is chosen to define binding interaction for *in vitro* cytotoxicity.[36]

The docking result of cytotoxicity confess that, the compounds **(3a-f)** had shown promising docking energies are in between of -8.3 to -7.1 kcal/mol with the reference drug Doxorubicin (-7.9 kcal/mol). On inspecting the results of docked scores of the compounds with protein receptor EGFR kinase domain indicated that, the compound **3f** exhibited lowest docking energy of -8.3 kcal/mol by making four hydrogen bonds with different amino acids such as MET793, GLU762, MET766 and ASP855. The compounds **3e** and **3d** showed same binding energy of -8.0 kcal/mol creating two and four hydrogen bonds with amino acid such as ASP855 & ARG841 and ASP855, THR854, MET793 & LYS745. Leftover compounds are also recognized to exhibit good to moderate docking energies by creating hydrogen bonds with different amino acids, and the outcomes are given in **Table 5** and their docked structures are shown in **Fig. 5.**

**Table 5:** Docking results of designed ligands against 4I23 **(3a-f)**

| Molecule | Binding affinity (kcal/mol) | 'H'- Bonding | 'H'- Bond length (Å) | E-interaction | H-phobic and Other interactions |
|---|---|---|---|---|---|
| **3a** | -7.1 | MET793, GLU762. | 2.19, 2.13. | LEU844, VAL726, ALA743. | GLY796, LEU718, LEU792, GLN791, THR790, LEU788, MET766, PHE856, THR854, ASP855. |
| **3b** | -7.3 | GLU762, ASP855, MET793. | 2.85, 2.22, 2.90. | VAL726, LEU792, LEU718. | ALA743, LEU844, MET766, LYS745, THR854. |
| **3c** | -6.6 | GLU762, ASP855. | 2.81, 2.24. | VAL726, ALA743, LEU844. | GLY796, MET793, LEU792, THR854, PHE856, MET766, LEU788, LYS754, THR790, LEU718. |
| **3d** | -8.0 | ASP855, THR854, MET793, LYS745. | 1.93, 3.46, 1.37, 2.06. | VAL726, ALA743, | GLY719, ILE744, THR790, LEU788, GLU762, MET766, LEU792, LEU844, CYS797, GLY796. |
| **3e** | -8.0 | ASP855, ARG841. | 2.62, 2.44. | LSY745, LEU844. | VAL726, ALA743, LEU718. |
| **3f** | -8.3 | MET793, GLU762, MET766, ASP855. | 2.62, 3.33, 2.98, 2.87. | LYS745, VAL726, ALA743. | GLY719, GLY796, PRO794, LEU792, LEU844, THR790, LEU788, CYS797. |
| **Doxorubicin** | -7.9 | GLY724, ASN842, | 2.02, 2.65. | LEU844, ALA743, VAL726. | LYS745. |



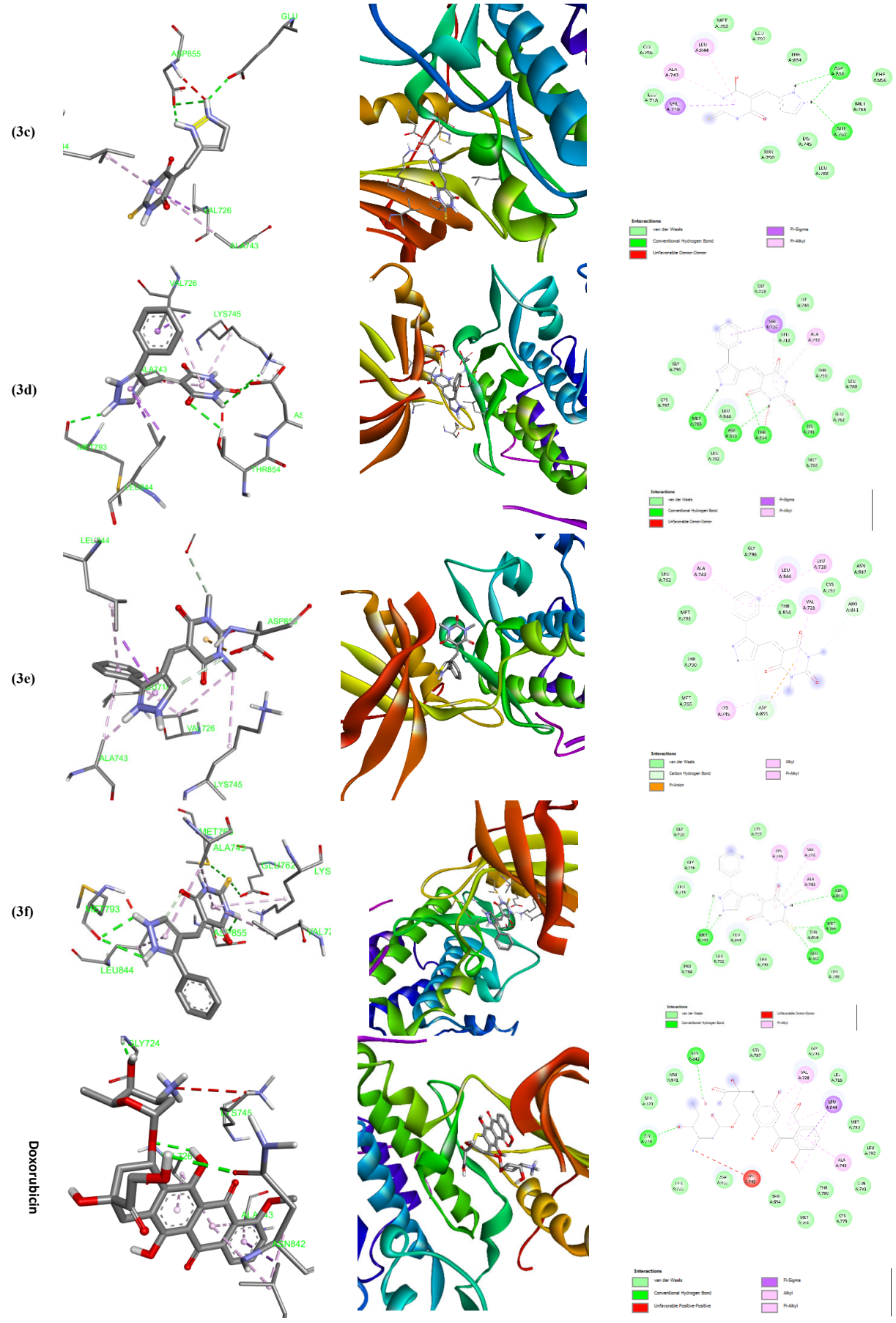

(3c)
(3d)
(3e)
(3f)
Doxorubicin

**Fig. 5.** Docking results of synthesized compounds **(3a-f)** against EGFR kinase domain (pdb Id: 4I23) for cytotoxicity

## 3. Conclusion

In the present research work, a sequence of pyrazol-pyrimidine -pyrimidine derivatives **(3a-f)** were prepared through knoevengal condensation reaction. The biological activity results reveal that, the compound **3d** and **3f** exhibited good zone of inhibition as compared to standard drug gentamicin. The results also revealed that compound **3e** possessed good selectivity for MCF-7 compared to A549 cell line. From the molecular docking results, it was confirmed that compound **3f** exhibited lowest binding energy and residual compounds also established good docking scores. Therefore, in future pyrazol-pyrimidine -pyrimidine derivatives can be used against bacterial activity and cytotoxicity in the pharmaceutical field.

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## 4. Experimental

### *4.1. Materials and Method*

All chemicals purchased from Aldrich Chemical Company were used without any purification. The supervision of the reaction was done by TLC using silica gel GF254 plates. Melting points were determined in open glass capillary methods and were used uncorrected. IR spectra using Shimadzu FTR-IR spectrometer were recorded in KBr pellet method. $^{1}$H and $^{13}$C NMR was recorded on Bruker spectrometer at 400 MHZ for $^{1}$H NMR and 100 MHZ for $^{13}$C NMR using DMSO-$d_6$ as a solvent using TMS as an internal standard and the chemical shifts were measured in ppm. Mass spectra were recorded by the Agilent 1200 series LC & Micro mass Q spectrometer.

### *4.2. General procedure for the synthesis pyrazol-pyrimidine derivatives (3a-f)*

An equimolar mixture of pyrazole aldehydes **1** (1mmol) and barbituric acid derivatives **2(a-c)** (1mmol) was stirred with 10 mol% of L-proline as a catalyst using ethanol (15 mL) as solvent and refluxed at a temperature of 70 $^{o}$C for 5-6 h. After that, the reaction mixture was decanted into cold water along with continuous stirring till the solid separates out. The solid obtained was filtered to afford **(3a-f)**. The product obtained was then recrystallized using hot ethanol.

### *4.3. Spectral Data*

#### *4.3.1. 5-(1H-pyrazol-3-ylmethylene)pyrimidine-2,4,6(1H,3H,5H)-trione (3a)*

Yield: 90.92%, Yellow solid; MP: 230-240 °C. IR (υ cm$^{-1}$): 3259 (NH), 3077 (C-H), 1671 (C=O), (1581) C=N. $^{1}$H NMR (δ ppm): 7.66 (s, 1H, CH-pyrazole), 7.85 (s, 1H, CH-pyrazole), 8.24 (s, 1H, CH), 11.28 (s, 1H, NH-pyrimidine), 11.34 (s, 1H, NH-pyrimidine), 13.86 (s, 1H, NH-pyrazole). $^{13}$C NMR (δ ppm): 115.57, 116.04, 150.19, 162.42 and 163.50 (C=O). LCMS: m/z 207.0704 [M+1]$^{+}$. Anal. Calcd for $C_8H_6N_4O_3$: C 46.61, H 2.93, N 27.18 and O 23.28%. Found: C 46.56, H 2.88, N 27.13 and O 23.23. %

#### *4.3.2 1,3-dimethyl-5-(1H-pyrazol-3-ylmethylene)pyrimidine-2,4,6(1H,3H,5H)-trione (3b)*

Yield: 73%, Black solid; MP: 90-100°C. IR (υ cm$^{-1}$): 3236 (NH), 3123 (C-H), 1673 (C=O), (1519) C=N, 1365 ($CH_3$). $^{1}$H NMR (δ ppm): 3.25 (s, 6H, 2N-$CH_3$), 7.74 (s, 1H, CH, pyrazole), 7.90 (s, 1H, CH-pyrazole), 8.36 (s, 1H, CH), 13.80 (s, 1H, NH-pyrazole). $^{13}$C NMR (δ ppm): 27.94, 28.60, 87.16, 97.51, 115.17, 144.34, 151.06, 156.14, 158.99, 160.84 and 165.90 (C=O). LCMS: m/z 235.0642 [M+1]$^{+}$. Anal. Calcd for $C_{10}H_{10}N_4O_3$: C 51.28, H 4.30, N 23.92 and O 20.49%. Found: C 51.23, H 4.25, N 23.87 and O 0.44%

#### *4.3.3 5-(1H-pyrazol-3-ylmethylene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (3c)*

Yield: 60%, Yellow solid; MP: 270-280°C. IR (υ cm$^{-1}$): 3236 (NH), 3123 (C-H), 1673 (C=O), (1519) C=N, (1141) C=S. $^{1}$H NMR (δ ppm): 7.73-7.74 (d, $J$ = 4Hz, 1H, CH-pyrazole), 7.91-7.90 (d, $J$ = 4Hz, 1H, CH-pyrazole), 8.28 (s, 1H, CH), 11.73 (s, 1H, NH- pyrazole), 12.46 (s, 1H, NH-pyrimidine), 12.40 (s, 1H, NH-pyrimidine). $^{13}$C NMR (δ ppm): 78.67, 113.61, 115.69, 134.05, 160.14, 161.80 (C=O) and 178.47 (C=S). LCMS: m/z 223.0011 [M+1]$^{+}$. Anal. Calcd for $C_8H_6N_4O_2S$: C 43.24, H 2.72, N 25.21, O 14.40 and S 14.43 %. Found: C 43.19, H 2.67, N 25.16, O, 14.35 and S 14.38%

*4.3.4 5-[(4-phenyl-1H-pyrazol-3-yl)methylene]pyrimidine-2,4,6(1H,3H,5H)-trione (3d)*

Yield: 94.59%, Creamy white solid; MP: 272-282°C. IR (υ $cm^{-1}$): 3205 (NH), 1664 (C=O), (1571) C=N. $^1$H NMR (δ ppm): 7.62-7.53 (m, 5H, CH, Ar-H), 8.10 (1H, CH-pyrazole), 8.21 (s, 1H, CH), 11.18 (s, 1H, NH-pyrimidine), 11.22 (s, 1H, NH-pyrimidine), 13.88 (s, 1H, NH-pyrazole). $^{13}$C NMR (δ ppm): 112.66, 112.98, 128.78, 129.18, 129.53, 129.61, 136.90, 144.51, 145.02, 150.26, 156.90, 162.76 and 163.91 (C=O). LCMS: m/z 283.0541 $[M+1]^+$. Anal. Calcd for $C_{14}H_{10}N_4O_3$: C 59.57, H 3.57, N 19.85 and O 17.01% found: C 56.52, H 3.37, N 12.80, O 16.96 %.

*4.3.5 1,3-dimethyl-5-[(4-phenyl-1H-pyrazol-3-yl)methylene]pyrimidine-2,4,6(1H,3H,5H)-trione (3e)*

Yield: 83.62%, Light orange solid; MP: 210-215°C. IR (υ $cm^{-1}$): 3165 (NH), 3023 (C-H), 1664 (C=O), (1574) C=N, 1357 ($CH_3$). $^1$H NMR (δ ppm): 3.18 (s, 1H, $CH_3$), 3.24 (s, 1H, $CH_3$), 7.62-7.53 (m, 5H, CH, Ar-H), 8.19 (s, 1H, CH), 8.31 (1H, CH-pyrazole), 13.93 (s, 1H, NH pyrazole). $^{13}$C NMR (δ ppm): 27.77, 28.37, 112.14, 113.89, 128.74, 129.53, 130.18, 131.59, 136.86, 144.37, 145.42, 146.10, 151.06, 157.11, 161.17 and 162.52 (C=O). LCMS: m/z 311.0903 $[M+1]^+$. Anal. Calcd for $C_{16}H_{14}N_4O_3$: C 61.93, H 4.55, N 18.06 and O 15.47%. Found: C 61.88, H, 4.50, N 18.01 and O 15.44%.

*4.3.6 5-[(4-phenyl-1H-pyrazol-3-yl)methylene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (3f)*

Yield: 98.49%, Orange solid; MP: 310-320 °C. IR (υ $cm^{-1}$): 3165 (NH), 3085 (C-H), 1664 (C=O), (1527) C=N, (1149) C=S. $^1$H NMR (δ ppm): 7.84-7.54 (m, 5H, CH, Ar-H), 8.18 (s, 1H, CH), 9.29 (1H, CH-pyrazole), 12.30 (s, 1H, NH-pyrimidine), 12.32 (s, 1H, NH-pyrimidine), 14.00 (s, 1H, NH-pyrazole). $^{13}$C NMR (δ ppm): 112.87, 113.86, 129.00, 129.63, 145.90, 160.48, 162.22 (C=O) and 178.26 (C=S). LCMS: m/z 299.0335 $[M+1]^+$. Anal. Calcd for $C_{14}H_{10}N_4O_2S$: C 56.37, H 3.38, N 18.78, O 10.73 and S 10.75 %. Found: C 56.32, H 3.33, N 18.73, O 10.68, and S 10.70 %.

*4.4. Pharmacological studies*

*4.4.1. Antibacterial activity*

The newly synthesized compounds **(3a-f)** were screened for antibacterial activity against four bacterial strains *viz.,* Gram positive bacteria *S. aureus, B. subtilis* and Gram-negative bacteria *S. Typhi, P. aeruginosa* by agar well diffusion method, by following the reported procedure T. Venkatesh *et al*.,.[37] The Positive control used for this activity is gentamicin and DMSO is taken as negative control. The antibacterial activity for synthesized compounds was performed with three different concentrations of 25, 50 and 100 µg/mL.

*4.4.2. Cytotoxicity*

*In vitro* cytotoxicity was performed by MTT assay according to the reported procedure S.H. Sukanya *et al.,*[38] against two different cell lines A549 and MCF-7. The synthesized compounds at different concentration (100, 50, 25, 12.5, 6.25, 3.125 µg/ml) were treated. The percentage of cell survival was calculated using the following formula:

$$\%\ \text{of cell servival} = \frac{\text{Mean OD of test compound}}{\text{Mean OD of Negative control}} \times 100$$

*4.4.3. In silico molecular docking*

The energy minimization of given structures was achieved using chemdraw software and USCF chimera software. The ligands energy was minimized using USCF chimera software. The X-ray structure of EGFR kinase domain (pdb Id: 4I23) for cytotoxicity is recovered (https://www.rcsb.org) and further, *In silico* molecular docking was performed according to the reported procedure G.C. Anjan Kumar *et al.,*.[39]

**Supporting information**

The spectra's such as IR, $^1$H NMR and $^{13}$C NMR and Mass spectrometry of the newly prepared compounds are given in supporting information file.

**Conflict of Interest**

The authors state that there is no conflict of interests.

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