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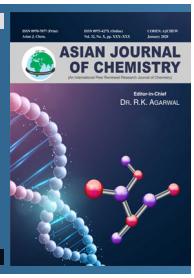

# Synthesis, Characterization, *in silico* and *in vitro* Evaluations of Symmetrical 1,3-Diketones

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1,3-Dicarbonyl compounds have gained significant importance since they are abundantly available in the natural products and possess myriad biological activities. The new symmetrical 1,3-diketones bearing L-proline, 2-methyl-5-iodobenzoic acid, piperidine-3-carboxylic acid and naphthalene-1-acetic acid moieties were synthesized by coupling reaction of appropriate ketone with *N*-acyl triazole in the presence of $MgBr_2{\cdot}Et_2O$ and DIPEA. The chemical structure of the compounds were confirmed from the spectral data such as $^1H$, $^{13}C$ NMR, FT-IR and HRMS. Molecular docking studies were carried out for all the compounds with tumor associated protein tyrosine kinase-6 (PTK6) and inflammatory protein cyclooxygenase-2 (COX2). The *in vitro* evaluation was carried out using breast cancer cell lines (MTT assay) and HRBC stabilization assays. During *in silico* studies, the $k_i$ values obtained against PTK6 and COX2 for (**5a-d**) compounds were in the range (-7.5 to -10.6) and (-7.6 to -9.8) kcal/mol, respectively. The compound **5d** was selected for MTT assay, since it exhibited the highest binding affinity (-10.6 kcal/mol) against PTK6 and gave $IC_{50}$ - 2.4 µg/mL against breast cancer cell lines. The HRBC stabilization of all the compounds (**5a-5d**) were in the range (59.28-93.4) %, with highest stabilization value by **5d**, which also displayed higher binding affinity with -7.6 kcal/mol towards COX2. Thus, the synthesized symmetrical 1,3-diketones with suitable functionality can be both anticancer and anti-inflammatory agents.

**Keywords: 1,3-Diketones, *N*-Acyl triazole, Anticancer activity, Anti-inflammatory activity, Molecular docking.**

## INTRODUCTION

1,3-Dicarbonyl compounds (β-diketones) are important compounds owing to their wide range of applications in organic synthesis and also in pharmaceutical industries [1-4]. They are the building blocks of various heterocyclic compounds such as pyrazole, pyrimidine, oxazole, carbazole, imidazole, thiazole and isoxazole in medicinal chemistry [5]. It is a good bidentate ligand for the complex preparation in coordination field and also a good chelating ligand for different lanthanide and transition metals. 1,3-Diketone derivatives are used widely in medicinal, combinatorial and solid phase chemistry.

There are many reports about the synthesis of 1,3-diketones with different reagent, variable reaction conditions [6-10], molecular structure and biological activity [11,12]. Lim *et al.* [13] proposed an efficient route that involves the coupling reaction of ketones with *N*-acyl benzotriazoles *via* enolization under mild condition to give 1,3-diketones.

The chemical reactivity of β-diketones is mainly based on the keto-enol tautomerism. The equilibrium between keto-enol tautomer can be affected by the solvent, substituents and temperature [14,15]. Nakano *et al.* [16] studied the cytotoxic effect of 1,3-diketones against normal and tumor cells and concluded that it has tumor-specific cytotoxicity and apoptosis. Hu *et al.* [17] studied synthesis and inhibitory activities against HIV-1 of aromatic diketones. The molecules L-proline and naphthalene-1-acetic acid are used in the field of biochemical dietary supplement and as a synthetic plant hormone, respectively [18,19]. Dibenzoylmethane and its derivatives have been found to inhibit tumor promotion and the associated inflammation, proving the synergistic action of 1,3-diketone moieties [20].

The present work is focused on the synthesis of symmetrical diketones bearing L-proline, 2-methyl-5-iodobenzoicacid, piperidine-3-carboxylicacid and naphthalene-1-acetic acid moieties (**5a-d**) by coupling reaction of ketones with *N*-acyl

benzotriazoles *via* enolization under mild condition. *in silico* Studies were carried out by docking the synthesized compounds with breast cancer protein tyrosine kinase-6 (PTK6) and inflammatory protein cyclooxygenase-2 (COX2) to evaluate the anticancer and anti-inflammatory activities. The *in vitro* studies like MTT assay and HRBC stabilization assay were performed and the activities were evaluated using doxorubicin and diclofenac as the standard drug.

## EXPERIMENTAL

All the chemicals and solvents used in this work were purchased from Sigma-Aldrich, Merck and used as purchased without further purification. Reactions were monitored by TLC silica coated plates obtained from Merck. The compounds were purified by column chromatography through silica-gel (60-120 mesh) using hexanes/ethyl acetate as an eluents. The melting point of compounds was determined using open capillary method. $^1$H NMR (400 & 500 MHz) and $^{13}$C NMR (75 & 100 MHz) spectra were recorded on Bruker NMR spectrometer using $CDCl_3$ and DMSO-$d_6$ as solvent. Mass spectra of all the compounds were recorded on TOF and quadrupole mass analyzer types were used for the HRMS measurements and FT-IR spectra were recorded using Bruckner instrument in the range of 4000-400 cm$^{-1}$.

### General procedure for the synthesis of 1,3-diketone compounds 5a-d

**General procedure-A (=NH protection using *p*-toluenesulphonyl chloide):** To a stirred solution of amino acid (**1a/1c**) (1.0 equiv.) in $H_2O$ (10 vol.), $Na_2CO_3$ (2.1 equiv.) at 0 °C and *p*-toluenesulfonyl chloride (2.1 equiv.) were added in portion wise over a period of 45 min. The slurry was warmed to room temperature and allowed to stir for 48 h. The reaction mixture was acidified with 1.5 N HCl to pH 2.0, extracted with ethyl acetate and washed with water and brine. Then organic layer was dried over anhydrous $Na_2SO_4$ and evaporated to dryness to give amine protected carboxylic acid (**2a/2c**) (90-92 %) as a colourless solid.

**General procedure-B (synthesis of weinreb amide):** To a solution of carboxylic acid (**2a-d**) (1.0 equiv.) in dry dichloromethane at 0 °C under nitrogen, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.2 equiv.) and triethyl amine (2.0 equiv.) were added. After 0.5 h, *N,O*-dimethylhydroxylammine hydrochloride (2.1 equiv.) in dichloromethane was added drop wise and the reaction mixture was stirred at room temperature for 8 h. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was extracted with dichloromethane. The organic phase was washed with 10 % aq. $NH_4Cl$, water and brine. The resultant dried over anhydrous $Na_2SO_4$ and evaporated in vacuum. The residue was purified by column chromatography on silica gel using ethylacetate-hexanes to get compounds **2aa-dd** and the yield was obtained in the range 66-72 %.

**General procedure-C (synthesis of methyl ketone):** To a solution of Weinreb amide (**2aa-dd**) (1.0 equiv.) in dry tetrahydrofuran at -78 °C under nitrogen, 3 M $CH_3MgCl$ in THF (1.5 equiv.) was added drop wise, then the reaction mixture was stirred for 0.5 h and it was warmed to room temperature. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was quenched with ammonium chloride solution and then extracted with ethylacetate. The organic phase was washed with water and brine. The resulted dried over anhydrous $Na_2SO_4$ and evaporated in vacuum. The residue was purified by column chromatography on silica gel using ethylacetate-hexanes to get compounds **4a-d** and the yield was obtained in the range 78-83 %.

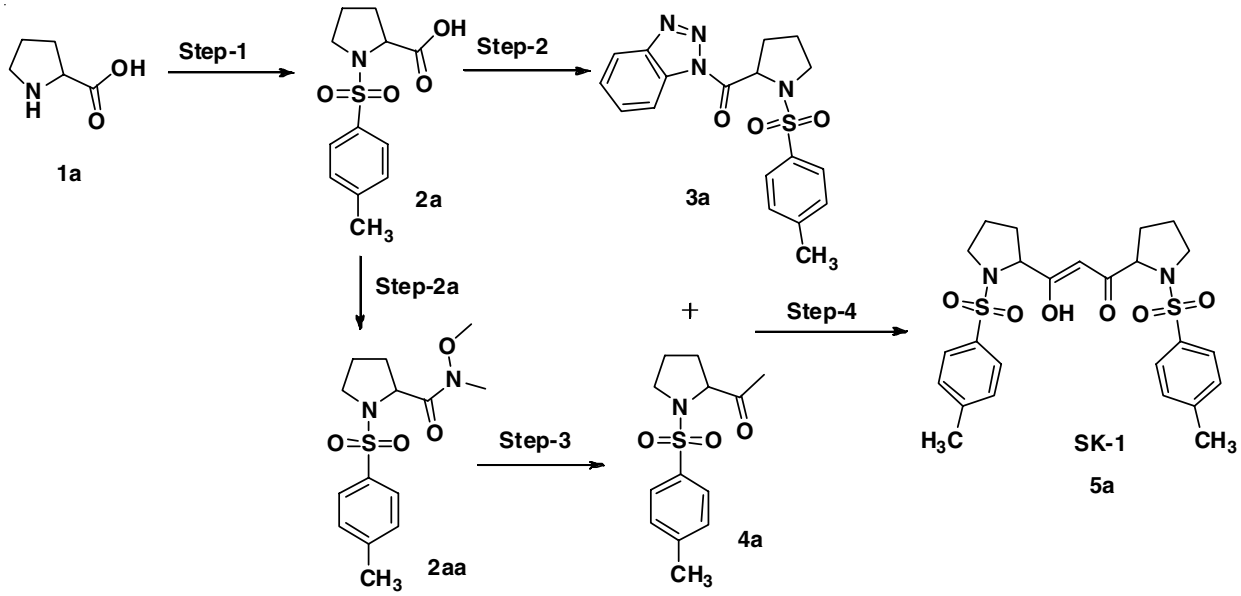
**General procedure-D (benzo-1,2,3-triazole coupling reaction):** To a solution of carboxylic acid (**2a-d**) (1.0 equiv.) in dry dichloromethane at 0 °C under nitrogen, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.6 equiv.) and triethyl amine (1.5 equiv.) were added and followed by the addition of benzo-1,2,3-triazole (1.1 equiv.) in dichloromethane. The reaction mixture was stirred at room temperature for 24 h and the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was extracted with dichloromethane. The organic phase was washed with 10 % aq. HCl, water and brine. The resulted dried over anhydrous $Na_2SO_4$ and evaporated in vacuum. The residue was purified by column chromatography on silica gel using ethylacetate-hexanes to get compounds **3a-d** and the yield was obtained in the range 60-71 %.

**General procedure-E (synthesis of 1,3-diketones):** This reaction was conducted using untreated $CH_2Cl_2$, open to the air. Compounds **4a-d** (1.0 equiv.) was added drop wise *via* a syringe to a stirred suspension of compounds **3a-d** (1.2 equiv.) and $MgBr_2{\cdot}Et_2O$ (2.5 equiv.) in dichloromethane (10 vol.), followed by *N,N*-diisopropylethylamine (1.5 equiv.). The stirring was continued for 3-18 h and the reaction was monitored by TLC. After completion of reaction 10 % aq. HCl (5 vol.) was added. The stirring was continued for 5 min and the aqueous layer was extracted with dichloromethane. The organic phase was washed with brine and the combined organic extracts were dried over anhydrous $Na_2SO_4$, filtered and concentrated under the reduced pressure. The residue was purified by column chromatography on silica gel using ethylacetate-hexanes to get **5a-d** (**Schemes I-IV**) and the yield was obtained in the range 70-82 %.

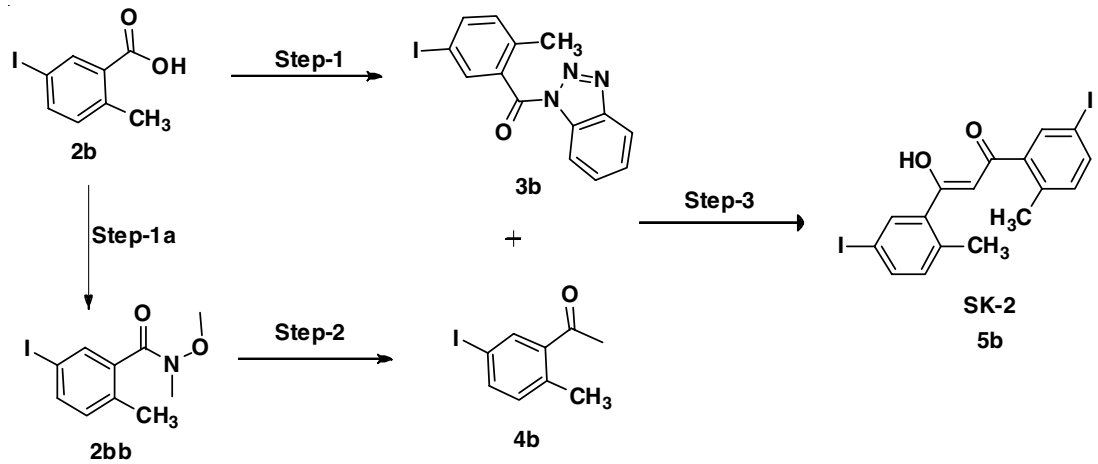
### Spectral data

**1-Tosylpyrrolidine-2-carboxylic acid (2a):** It was obtained according to general procedure-A. Compound **2a** is a colourless solid; Yield: 92 %. $^1$H NMR (400 MHz, $CDCl_3$): δ 7.77 (d, *J* = 8.40 Hz, 2H), 7.35 (d, *J* = 8.00 Hz, 2H), 4.26 (q, *J* = 3.60 Hz, 1H), 3.50-3.52 (m, 1H), 3.22-3.24 (m, 1H), 2.49 (s, 3H), 2.14-2.16 (m, 1H), 1.87-1.90 (m, 2H), 1.72-1.74 (m, 1H); HRMS (ESI-TOF) of $[M+1]^+$ calcd. for $C_{12}H_{15}NO_4S$. 270.0800; found, 270.0801.

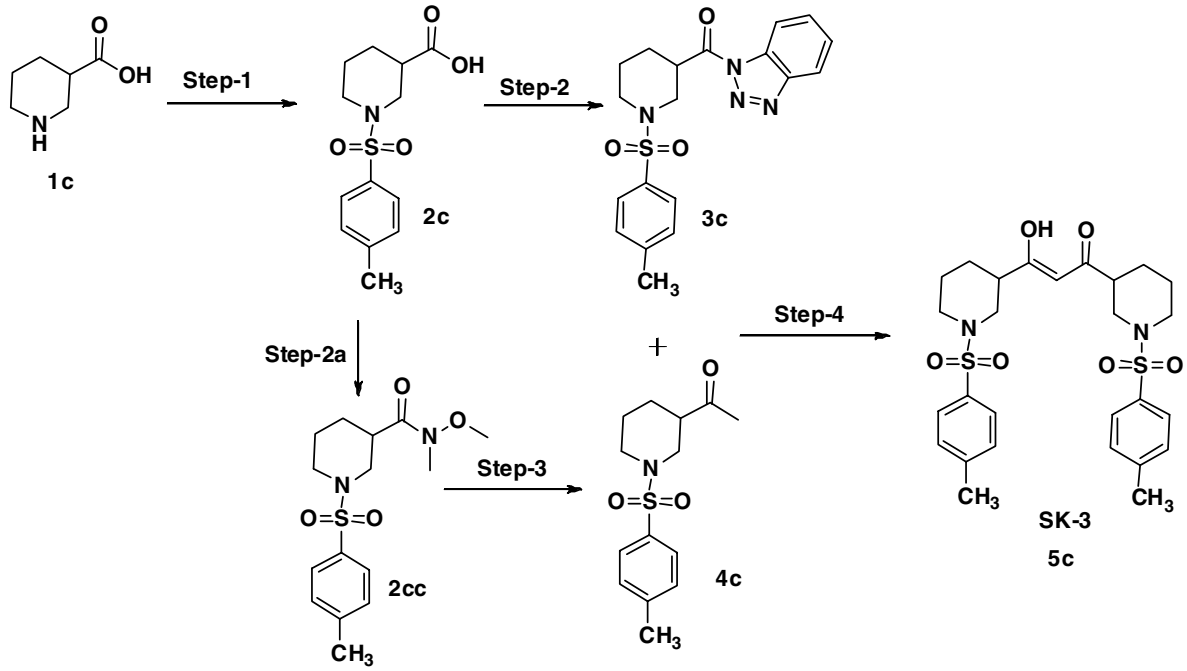
**(1*H*-Benzo[*d*][1,2,3]triazol-1-yl) (1-tosylpyrrolidin-2-yl)methanone (3a):** It was obtained according to general procedure-D. The residue was purified by column chromatography using 15 % of ethylacetate in petroleum ether to afford compound **3a** (60 %) as a white solid. $^1$H NMR (400 MHz, $CDCl_3$): δ 8.31 (d, *J* = 8.40 Hz, 1H), 8.14 (d, *J* = 8.40 Hz, 1H), 7.81 (d, *J* = 8.40 Hz, 2H), 7.69 (t, *J* = 9.20 Hz, 1H), 7.54 (t, *J* = 8.00 Hz, 1H), 7.35 (d, *J* = 8.00 Hz, 2H), 5.80 (q, *J* = 4.40 Hz, 1H), 3.71 (m, 1H), 3.42 (m,1H), 2.50 (s, 3H), 2.36-2.37 (m, 1H), 2.10-2.15 (m, 2H), 1.83-1.84 (m, 1H); HRMS (ESI-TOF) of $[M+1]^+$ calcd. for $C_{18}H_{18}N_4O_3S$. 371.1178; found, 371.1177.



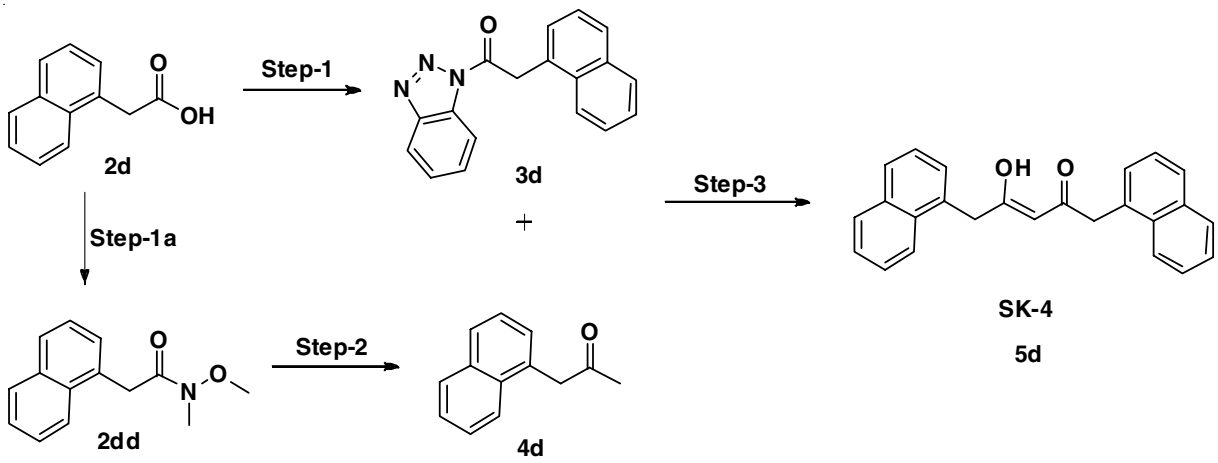

**Scheme-I:** Synthesis of symmetrical 1,3-diketone (**5a**)




**Scheme-II:** Synthesis of symmetrical 1,3-diketone (**5b**)




**Scheme-III:** Synthesis of symmetrical 1,3-diketone (**5c**)




**Scheme-IV:** Synthesis of symmetrical 1,3-diketone (**5d**)

**1-Tosylpiperidine-3-carboxylic acid (2c):** It was synthesized according to general procedure-A. Compound **2c** is a off white solid; Yield: 90 %; $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): δ 12.57 (s, 1H), 7.46-7.47 (m, 4H), 3.52-3.58 (m, 1H), 3.35-3.40 (m, 2H), 2.51-2.57 (m, 2H), 2.42-2.49 (m, 3H), 1.73-1.79 (br m, 2H), 1.51-1.53 (br m, 1H), 1.37-1.39 (br m, 1H); HRMS (ESI-TOF) of $[M+Na]^+$ calcd. for $C_{13}H_{17}NO_4S$ +$Na^+$. 306.0770; found, 306.08318.

***N*-Methoxy-*N*-methyl-1-tosylpyrrolidine-2-carboxamide (2aa):** It was synthesized according to general procedure-B. The residue was purified by column chromatography using 20 % of ethylacetate in hexanes to afford compound **2aa** as a colourless solid; Yield: 67 %; $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): δ 7.80 (d, *J* = 8.40 Hz, 2H), 7.30 (d, *J* = 8.00 Hz, 2H), 4.85 (q, *J* = 4.40 Hz, 1H), 3.80 (s, 3H), 3.39-3.41 (m, 2H), 3.20 (s, 3H), 2.42 (s, 3H), 1.99-2.03 (m, 2H), 1.87-1.88 (m, 1H), 1.70-1.80 (m, 1H); HRMS (ESI-TOF) of $[M+Na]^+$ calcd. for $C_{14}H_{20}N_2O_4S$ +$Na^+$. 335.1036; found, 335.1042.

**5-Iodo-*N*-methoxy-N,2-dimethylbenzamide (2bb):** It was synthesized according to general procedure-B. The residue was purified by column chromatography using 3 % of ethylacetate in hexanes to afford compound **2bb** as a colourless liquid; Yield: 72 %; $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): δ 7.67 (d, *J* = 8.00 Hz, 1H), 7.61 (s, 1H), 7.08 (d, *J* = 8.00 Hz, 1H), 3.46 (s, 3H), 3.24 (s, 3H), 2.18 (s, 3H); HRMS (ESI-TOF) of $[M+Na]^+$ calcd. for $C_{10}H_{12}NO_2I$ +$Na^+$. 327.9805; found, 327.97739.

***N*-Methoxy-*N*-methyl-1-tosylpiperidine-3-carboxamide (2cc):** It was synthesized according to general procedure-B. The residue was purified by column chromatography using 15 % of ethylacetate in hexanes to afford compound **2cc** as a colourless solid; Yield: 66 %; $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): δ 7.64 (d, *J* = 8.00 Hz, 2H), 7.47 (d, *J* = 8.00 Hz, 2H), 3.67-3.70 (m, 3H), 3.62-3.64 (m, 2H), 3.09 (s, 3H), 2.86-2.86 (m, 1H), 2.42 (s, 3H), 2.18-2.20 (m, 2H), 1.72-1.73 (m, 2H), 1.47-1.50 (m, 1H), 1.24-1.26 (m, 1H); HRMS (ESI-TOF) of $[M+Na]^+$ calcd. for $C_{15}H_{22}N_2O_4S$ +$Na^+$. 349.1192; found, 349.11514.

***N*-Methoxy-*N*-methyl-2-(naphthalene-1-yl)acetamide (2dd):** It was obtained according to general procedure-B. The residue was purified by column chromatography using 5 % of ethylacetate in hexanes to afford compound **2d** as a colourless liquid; Yield: 70 %; $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): δ 7.97-7.99 (m, 1H), 7.94-7.95 (m, 1H), 7.84-7.86 (m, 1H), 7.53-7.54 (m, 2H), 7.46-7.47 (m, 1H), 7.41-7.43 (m, 1H), 4.22 (s, 2H), 3.75 (s, 3H), 3.16 (s, 3H); HRMS (ESI-TOF) of $[M+Na]^+$ calcd. for $C_{14}H_{15}NO_2$ +$Na^+$. 252.0995; found, 252.10011.

**1-(1-Tosylpyrrolidin-2-yl)ethanone (4a):** It was synthesized according to general procedure-C. The residue was purified by column chromatography using 5 % of ethylacetate in hexanes to afford compound **4a** as a colourless liquid; Yield: 78 %; $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): δ 7.74 (d, *J* = 7.60 Hz, 2H), 7.36 (d, *J* = 8.00 Hz, 2H), 4.00 (q, *J* = 6.00 Hz, 1H), 3.52-3.53 (m, 1H), 3.27-3.28 (m, 1H), 2.46 (s, 3H), 2.37 (s, 3H), 1.86-1.87 (m, 4H); IR (KBr, $\nu_{max}$, $cm^{-1}$): 3422, 2973, 1707, 1584, 1452, 1157, 1084, 1014, 830; HRMS (ESI-TOF) of $[M+H]^+$ calcd. for $C_{13}H_{17}NO_3S$+ $H^+$. 268.1002; found, 268.1004.

**1-(5-Iodo-2-methylphenyl)ethanone (4b):** It was synthesized according to general procedure-C. The residue was purified by column chromatography using 0.5 % of ethylacetate in hexanes to afford compound **4b** as a colourless liquid; Yield: 83 %; $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): δ 8.07 (s, 1H), 7.76 (d, *J* = 8.00 Hz, 1H), 7.10 (d, *J* = 8.00 Hz, 1H), 2.55 (s, 3H), 2.35 (s, 3H); IR (KBr, $\nu_{max}$, $cm^{-1}$): 3451, 2958, 1589, 1430, 1280, 1091, 796, 628; HRMS (ESI-TOF) of $[M]^+$ calcd. for $C_{10}H_9IO$. 259.9698; found, 259.16322.

**1-(1-Tosylpiperidin-3-yl)ethanone (4c):** It was synthesized according to general procedure-C. The residue was purified by column chromatography using 10 % of ethylacetate in hexanes to afford compound **4c** as a colourless liquid; Yield: 81 %; $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): δ 7.64 (d, *J* = 8.00 Hz, 2H), 7.46 (d, *J* = 8.00 Hz, 2H), 3.52 (d, *J* = 10.50 Hz, 1H), 3.36 (d, *J* = 9.00 Hz, 1H), 2.65 (t, *J* = 10.00 Hz, 1H), 2.41-2.43 (m, 4H), 2.31 (t, *J* = 10.50 Hz, 1H), 2.10 (s, 3H), 1.83-1.84 (m, 1H), 1.69-1.70 (m, 1H), 1.50-1.50 (m, 1H), 1.24-1.26 (m, 1H); IR (KBr, $\nu_{max}$, $cm^{-1}$): 3357, 3026, 2926, 2856, 1691, 1591, 1430, 1169, 1089, 948, 867, 798; HRMS (ESI-TOF) of $[M+Na]^+$ calcd. for $C_{14}H_{19}NO_3S$ +$Na^+$. 304.0978; found, 304.10244.

**1-(Naphthalene-1-yl)propan-2-one (4d):** It was synthesized according to general procedure-C. The residue was purified by column chromatography using 1 % of ethylacetate in hexanes to afford compound **4d** as a colourless liquid; Yield: 80 %; $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): δ 7.93-7.94 (m, 1H), 7.85-7.86 (m, 2H), 7.52-7.52 (m, 2H), 7.46-7.48 (m, 1H), 7.40-

7.41 (m, 1H), 4.26 (s, 2H), 2.18 (s, 3H); IR (KBr, $\nu_{max}$, $cm^{-1}$): 3441, 3052, 2932, 1704, 1603, 1514, 1425, 1155, 1015, 796; HRMS (ESI-TOF) of $[M+Na]^+$ calcd. for $C_{13}H_{12}O+Na^+$. 207.0780; found, 207.07601.

**(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(5-iodo-2-methylphenyl)-methanone (3b):** It was synthesized according to general procedure-D. The residue was purified by column chromatography using 2 % of ethylacetate in hexanes to afford compound **3b** as a colourless solid; Yield: 70 %; $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): δ 8.34 (d, *J* = 8.50 Hz, 1H), 8.30 (d, *J* = 8.00 Hz, 1H), 8.12 (s, 1H), 7.92 (d, *J* = 8.00 Hz, 1H), 7.80 (t, *J* = 8.00 Hz, 1H), 7.68 (t, *J* = 8.00 Hz, 1H), 7.26 (d, *J* = 8.00 Hz, 1H), 2.30 (s, 3H); HRMS (ESI-TOF) of $[M+Na]^+$ calcd. for $C_{14}H_{10}IN_3O$ $+Na^+$. 385.9761; found, 385.97557.

**(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(1-tosylpiperidin-3-yl) methanone (3c):** It was synthesized according to general procedure-D. The residue was purified by column chromatography using 25 % of ethylacetate in hexanes to afford compound **3c** as a colourless solid; Yield: 61 %; $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): δ 8.27 (d, *J* = 8.00 Hz, 1H), 8.20 (d, *J* = 8.50 Hz, 1H), 7.79 (t, *J* = 7.50 Hz, 1H), 7.66 (d, *J* = 8.00 Hz, 2H), 7.63 (t, *J* = 7.00 Hz, 1H), 7.46 (d, *J* = 8.00 Hz, 2H), 4.02 (d, *J* = 10.50 Hz, 2H), 3.57 (d, *J* = 12.00 Hz, 1H), 2.70 (t, *J* = 11.00 Hz, 1H), 2.48-2.51 (m, 2H), 2.42-2.46 (m, 3H), 2.10-2.12 (m, 1H), 1.85-1.86 (m, 1H), 1.62-1.64 (m, 1H); HRMS (ESI-TOF) of $[M+Na]^+$ calcd. for $C_{19}H_{20}N_4O_3S$ $+Na^+$. 407.1148; found, 407.11413.

**(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(1-tosylpiperidin-3-yl)methanone (3d):** It was synthesized according to general procedure-D. The residue was purified by column chromatography using 1.5 % of ethylacetate in hexanes to afford compound **3d** as a colourless solid; Yield: 71 %; $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): δ 8.21-8.25 (m, 1H), 8.09-8.11 (m, 2H), 7.83-7.85 (m, 2H), 7.60-7.61 (m, 2H), 7.42-7.42 (m, 4H), 5.20 (s, 2H); HRMS (ESI-TOF) of $[M+Na]^+$ calcd. for $C_{18}H_{13}N_3O$ $+Na^+$. 310.0951; found, 310.09570.

**3-Hydroxy-1,3-*bis*(1-tosylpyrrolidin-2-yl)prop-2-en-1-one (5a):** It was synthesized according to general procedure-E. The residue was purified by column chromatography using 25 % of ethylacetate in hexanes to afford compound **5a** as a pale yellow solid; Yield 70 %; reaction time: 16 h, m.p.: 157 °C; $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): δ 7.79 (d, *J* = 8.00 Hz, 4H), 7.38 (d, *J* = 8.00 Hz, 4H), 6.36 (s, 1H), 4.19 (q, *J* = 13.20 Hz, 2H), 3.60-3.62 (m, 2H), 3.27-3.29 (m, 2H), 2.48 (s, 6H), 2.01-2.02 (m, 2H), 1.91-1.92 (m, 4H), 1.69-1.70 (m, 2H); IR (KBr, $\nu_{max}$, $cm^{-1}$): 3440, 3051, 2981, 2871, 1923, 1603, 1433, 1343, 1153, 1083, 1013, 804; HRMS (ESI-TOF) of $[M+Na]^+$ calcd. for $C_{25}H_{30}N_2O_6S_2$ $+Na^+$. 541.1437; found, 541.4308.

**3-Hydroxy-1,3-*bis*(5-iodo-2-methylphenyl)prop-2-en-1-one (5b):** It was synthesized according to general procedure-E. The residue was purified by column chromatography using 0.1 % of ethylacetate in hexanes to afford compound **5b** as colourless solid; Yield 82 %; reaction time: 3 h, m.p.: 145 °C; $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): δ 7.98 (s, 2H), 7.79 (d, *J* = 7.50 Hz, 2H), 7.15 (d, *J* = 8.00 Hz, 2H), 6.53 (s, 1H), 2.52 (s, 6H); $^{13}$C NMR (125 MHz, DMSO-$d_6$, δ ppm): 200.6, 187.4, 151.1, 140.1, 139.5, 137.4, 137.1, 136.5, 136.3, 133.7, 133.4, 101.2, 91.1, 57.8, 19.9; IR (KBr, $\nu_{max}$, $cm^{-1}$): 3452, 3055, 2952, 1575, 1513, 1411, 1350, 1197, 1146, 1075, 891, 784, 626; HRMS (ESI-TOF) of $[M]^+$ calcd. for $C_{17}H_{14}I_2O_2$. 503.9083; found, 503.9101.

**3-Hydroxy-1,3-*bis*(1-tosylpiperidin-3-yl)prop-2-en-1-one (5c):** It was synthesized according to general procedure-E. The residue was purified by column chromatography using 24 % of ethylacetate in hexanes to afford compound **5c** as colourless solid; Yield 72 %; reaction time: 18 h, m.p.: 126 °C; $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): δ 7.63 (d, *J* = 8.00 Hz, 4H), 7.32 (d, *J* = 8.00 Hz, 4H), 5.58 (s, 1H), 3.64-3.67 (m, 4H), 2.51-2.53 (m, 2H), 2.39 (s, 6H), 2.26-2.29 (m, 2H), 1.70-1.76 (m, 4H), 1.64-1.61 (m, 3H), 1.38-1.40 (m, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$, δ ppm): 193.9, 193.2, 143.5, 132.8, 132.8, 129.6, 127.5, 97.7, 48.06, 48.01, 46.3, 44.2, 44.1, 26.99, 26.97, 24.1, 21.6; IR (KBr, $\nu_{max}$, $cm^{-1}$): 3474, 3067, 2954, 2852, 1596, 1442, 1331, 1147, 1086, 841; HRMS (ESI-TOF) of $[M+Na]^+$ calcd. for $C_{27}H_{34}N_2O_6S_2$+$Na^+$. 569.1750; found, 569.17539.

**4-Hydroxy-1,5-di(naphthalene-1-yl)pent-3-en-2-one (5d):** This compound was already reported [21]. It was synthesized according to general procedure-E. The residue was purified by column chromatography using 0.5 % of ethylacetate in hexanes to afford compound **5d** as a pale yellow solid; Yield 75 %; reaction time: 3 h, m.p.: 80 °C; $^1$H NMR (500 MHz, DMSO-$d_6$, δ ppm): δ 7.93-7.94 (m, 2H), 7.85-7.86 (m, 4H), 7.52-7.52 (m, 4H), 7.46-7.48 (m, 2H), 7.40-7.41 (m, 2H), 4.26 (s, 4H), 3.36 (s, 2H); IR (KBr, $\nu_{max}$, $cm^{-1}$): 3464, 3046, 2924, 1586, 1504, 1403, 954, 780; HRMS (ESI-TOF) of $[M+H]^+$ calcd. for $C_{25}H_{20}O_2$ + $H^+$. 353.1536; found, 353.15420.

**Molecular docking studies:** The docking studies have been carried out using docking program Auto dock tool (ADT) version 1.5.6 and Auto dock version 4.2.6 [22]. The 3D X-ray crystal structure of PTK6 otherwise known as BrK (PDB ID: 6CZ3) and COX2 (PDB ID: 3LN1) were retrieved from PDB (protein data bank) (www. rcsb.org). The 3D X-ray crystallographic PDB receptors were initially processed in Discovery studio visualizer to remove the water molecules, heteroatoms, miscellaneous molecules and ligands attached to it. All the structures of compounds were saved as PDB file format for input to ADT. Using ADT, a grid box was constructed around the binding site of receptor protein and pdbqt files of ligands. The Auto dock calculation such as binding score (kcal/mol) and inhibition constant ($k_i$) values were calculated from the best docked poses of ligands (**5a-d**) at the active sites of PTK6 and COX2.Binding mode and interaction of the compounds with amino acid residues in the active sites of the protein was analyzed using Discovery studio visualizer.

**Cytotoxicity studies on breast cancer cell line-MTT assay:** The MDAMB231 cells were plated separately using 96 well plates with the concentration of $1 \times 10^4$ cells/well in DMEM media with 1X antibiotic antimycotic solution and 10 % fetal bovine serum (Himedia, India) in $CO_2$ incubator at 37 °C with 5 % $CO_2$. The cells were washed with 200 µL of 1X PBS and incubated for 24 h with various concentrations (0.01-15 µg/mL) of the compound **5d**, which exhibited the higher binding affinity with PTK6, *in silico* studies and the standard, Doxorubicin (in serum free media). The medium was aspirated from cells at the end of the treatment period. 0.5 mg/mL MTT prepared in 1X PBS was added and incubated at

37 °C for 4 h using $CO_2$ incubator. After incubation period, the medium containing MTT was discarded from the cells and washed using 200 µL of PBS. The formed crystals was dissolved with 100 µL of DMSO and thoroughly mixed. The development of colour intensity was evaluated at 570 nm. The formazan dye turns to purple blue colour. The absorbance was measured at 570 nm using microplate reader [23].

$$\text{Cell viability (\%)} = \left(\frac{\text{Mean (OD)}}{\text{Control (OD)}}\right)\times 100$$

***in vitro* Anti-inflammatory activity of 1,3-diketones (5a-d):** The anti-inflammatory activity of synthesized 1,3-diketones **(5a-d)** were evaluated through HRBC membrane stabilization assays [24]. Blood from healthy volunteer was collected with his consent, which was mixed with equal volume of Alsever solution. The blood was centrifuged at 3000 rpm, the packed cells were washed with isosaline and a suspension was made with isosaline (10 % v/v). The assay mixture was prepared by adding 1 mL of phosphate buffer, 2 mL of hypo saline, 0.5 mL of HRBC suspension with 100 µL of 1 mg/mL of synthesized 1,3-diketones, diclofenac was used as the reference drug. For control, 2 mL of distilled water was used instead of hypo saline. The assay mixtures were incubated at 37 °C for 30 min and centrifuged. The supernatant was collected to estimate the haemoglobin content by using spectrophotometer at 560 nm. The percentage haemolysis was calculated by assuming the hemolysis produced in the presence of distilled water as 100 %. The percentage of HRBC membrane stabilization was calcu-lated using the formula:

$$\text{Inhibition of haemolysis (\%)} = \left[100 - \left(\frac{\text{OD of test}}{\text{OD of control}}\right)\right]\times 100$$

## RESULTS AND DISCUSSION

The stability, reactivity and wide range of biological properties of 1,3-diketone, inspired researchers to synthesize new 1,3-dicarbonyl compounds with desirable properties. Numerous syntheses were reported for 1,3-diketones in literature involving different synthetic routes depending upon the nature of the substrate. Reported bioactivities of molecules containing L-proline, naphthalene-1-acetic acid, 2-methyl-5-iodo benzoic acid and piperidine-3-carboxylicacid were the important deciding factor for synthesizing new symmetrical-1,3-diketones with the above given functionalities. The synthesis of compounds **5a-d** was carried out according to the reported procedure [13].

The final step of all the scheme proposed, involved the reaction between appropriate acetophenone (**4a-d**) with *N*-acyl triazole (**3a-d**), which was carried out in the presence of $MgBr_2{\cdot}Et_2O$, *i*-$Pr_2NEt$ in untreated dichloromethane under open atmosphere that led to the corresponding β-diketone (**5a-d**) in good yields (70-82 %). All the compounds were purified by column chromatography on silica gel using ethylacetate-hexanes as eluents. In this reaction, magnesium bromide diethyl etherate is used to polarize the carbonyl carbon of the methyl ketone **4a-d**, thereby increasing the acidity of the α-proton, So that α-proton can be removed easily by a weak base like diisopropylethylamine resulting enol react with electrophilic species (*N*-acyl benzotriazoles) directly, resulting in the formation of enol form of β-diketone [13]. The structures of the synthesized compounds were elucidated by IR, $^1$H NMR, $^{13}$C NMR spectra and mass spectrometry.

The synthesized compounds **5a-d** showed the characteristic FT-IR peaks at 3440, 3452, 3474 and 3464 $cm^{-1}$ corresponding to –OH stretching; peaks at 1603, 1575, 1596 and 1596 $cm^{-1}$ for carbonyl group; peaks at 1433, 1411, 1442 and 1403 $cm^{-1}$ for –C=C– stretching.

The $^1$H NMR spectrum of compound **5a** showed a singlet peak at δ 6.36 and 2.48 ppm corresponding to ene proton of enol and methyl proton of tosyl group. Compound **5c** showed the same pattern at δ 5.58 and 2.39 ppm and so compound **5b** showed at two peaks at δ 6.53 and 2.52 ppm corresponds to ene proton of enol and methyl proton apart from their respective aromatic, aliphatic and alicyclic protons.

In $^{13}$C NMR spectrum of compounds **5b** and **5c**, the peaks at δ 200.6 and 193.9 ppm corresponds to 2 carbonyl carbon atoms and peaks at δ 187.4 and 193.2 ppm are due to the carbon which is attached to -OH group of enol. The peaks at δ 140.1 and 148.5 ppm are due to the ene carbon which is flanked between the carbonyl carbon and enol carbon. Thus, $^{13}$C NMR spectral data support and confirm the structure of 1,3-diketone, apart from their respective aromatic, aliphatic and alicyclic carbon. The *m/z* values confirm the molecular weight of the respective 1,3-diketones (**5a-d**).

***in silico* Activity:** All the synthesized compounds (**5a-d**) and the standard compounds were individually, docked with PTK6 and COX2. The results were interpreted based on the docking parameter such as binding score, which means higher the binding affinity score, better will be the interaction between ligands and proteins. The binding affinity score, hydrogen bonding interaction, hydrophobic interaction and other interactions are shown in Tables 1 and 2. Thus, it is clear that all the synthesized compounds (**5a-d**) exhibited high docking score than the standard compound.

When the synthesized compounds were docked with PTK6, compound **5d** exhibited higher binding affinity (-10.6 kcal/mol) than other compounds (**5a-c**) including doxorubicin, the anticancer drug used as standard. Compound **5d** did not exhibit any hydrogen bond interaction with protein but it exhibited three hydrophobic interactions with the amino acid residue like ALA A:217 and VAL A:205.

When the synthesized compounds were docked with COX2, compound **5c** exhibited higher binding affinity (-9.8 kcal/mol) than other compounds (**5a,b,d**) including diclofenac, the anti-inflammatory drug used as standard. Compound **5c**, exhibited two hydrogen bond interaction with amino acid residues like CYS A:21 and ASN A:19. It also exhibited five hydrophobic interactions with the amino acid residues like ARG A:29; ALA A:142; CYS A:32; LEU A:138 and PRO A:139. Even though **5d** exhibited -7.6 kcal/mol, there were two π-π stacking interactions with His:372, in addition to two hydrogen bonding and π-alkyl interactions, making the bond between **5d** and the enzyme more stronger. The molecular docking 3D and 2D images of synthesized compound and standard compounds are shown in Figs. 1 and 2.

**MTT assay for 4-hydroxy-1,5-di(naphthalene-1-yl)pent-3-en-2-one (5d) and doxorubicin:** The compound **5d** exhibited higher binding affinity towards PTK6; hence it was evaluated *in vitro* for its anticancer activity using different concentration

TABLE-1
MOLECULAR DOCKING PARAMETER OF COMPOUNDS (**5a-d**) AND STANDARD DRUG AGAINST PROTEIN PTK6 (PDB:6CZ3)

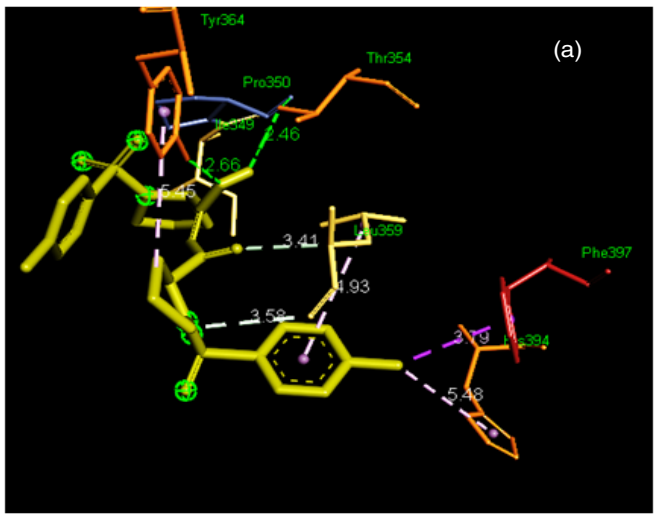
| Ligand | B.E. (kcal/mol) | $k_i$ (μM) | H-bond interaction | Hydrophobic interaction | Other interaction* |
|---|---|---|---|---|---|
| **5a** | -7.5 | 3.16 | THR A:354; TYR A:364 | ILE A:349; PROA:350; HIS A:394; LEU A:359; TYR A:364 | PHE A:397; LEU A:359 |
| **5b** | -8.7 | 0.42 | SER A:271 | ALA A:217; ARG A:316; LEU A:197; 248; 319; LYS A:219; VAL A:205; PHE A:202 | SER A:199; GLY A:198; 200 |
| **5c** | -9.5 | 0.11 | GLU A:274 | ALA A:217; HIS A:345; LEU A:319; 343; LYS A:219; TYR A:342; VAL A:205 | SER A:199; VAL A:205 |
| **5d** | -10.6 | 0.02 | - | ALA A:217; VAL A:205 | GLU A:274; LEU A:319; VAL A:205 |
| Doxorubicin | -6.9 | 8.72 | ARG A:311; ALA A:334; TYR A:201; 364; LEU A:359 | ARG A:335 | GLU A:339 |

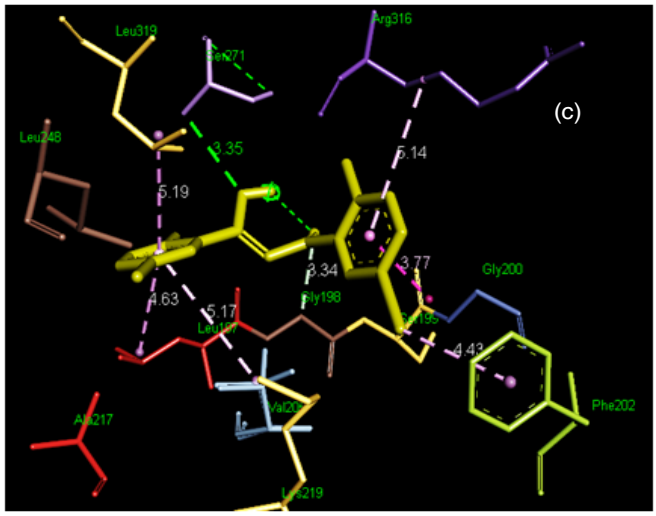
*van der Waals, π-π stacked, unfavourable interaction.

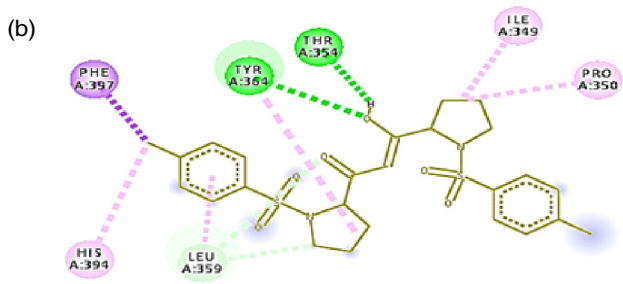
TABLE-2
MOLECULAR DOCKING PARAMETER OF COMPOUNDS (**5a-d**) AND STANDARD DRUG AGAINST COX2 (PDB:3LN1)

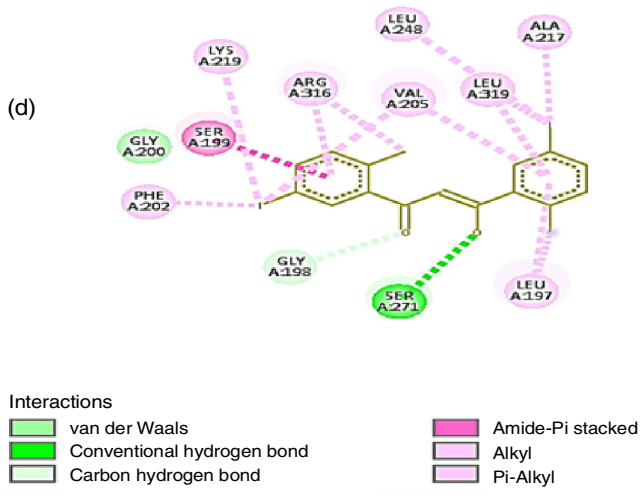
| Ligand | B.E. (kcal/mol) | $k_i$ (μM) | H-bond interaction | Hydrophobic interaction | Other interaction* |
|---|---|---|---|---|---|
| **5a** | -8.4 | 0.69 | HIS A:374; THR A:198 | HIS A:193; 200; 372; LEU A:377; 394; PHE A:384; 390; VAL A:277;430 | – |
| **5b** | -6.5 | 17.09 | SER A:34; ASN A:19 | CYS A:21;32; PRO A:139; TYR A:122 | TYR A:122 |
| **5c** | -9.8 | 0.06 | CYS A:21; ASN A:19 | ARG A:29; ALA A:142; CYS A:32; LEU A:138; PRO A:139 | ASN A:24; GLY A:30; TYR A:122 |
| **5d** | -7.6 | 2.66 | HIS A:200; THR A:198 | VAL A:277 | HIS A:372 |
| Diclofenac | -6.1 | 33.6 | ASN A:28 | ARG A:29; LEU A:65; LYS A:454 | LEU A:458; SER A:457 |

*van der Waals, π-π stacked, unfavourable interaction.



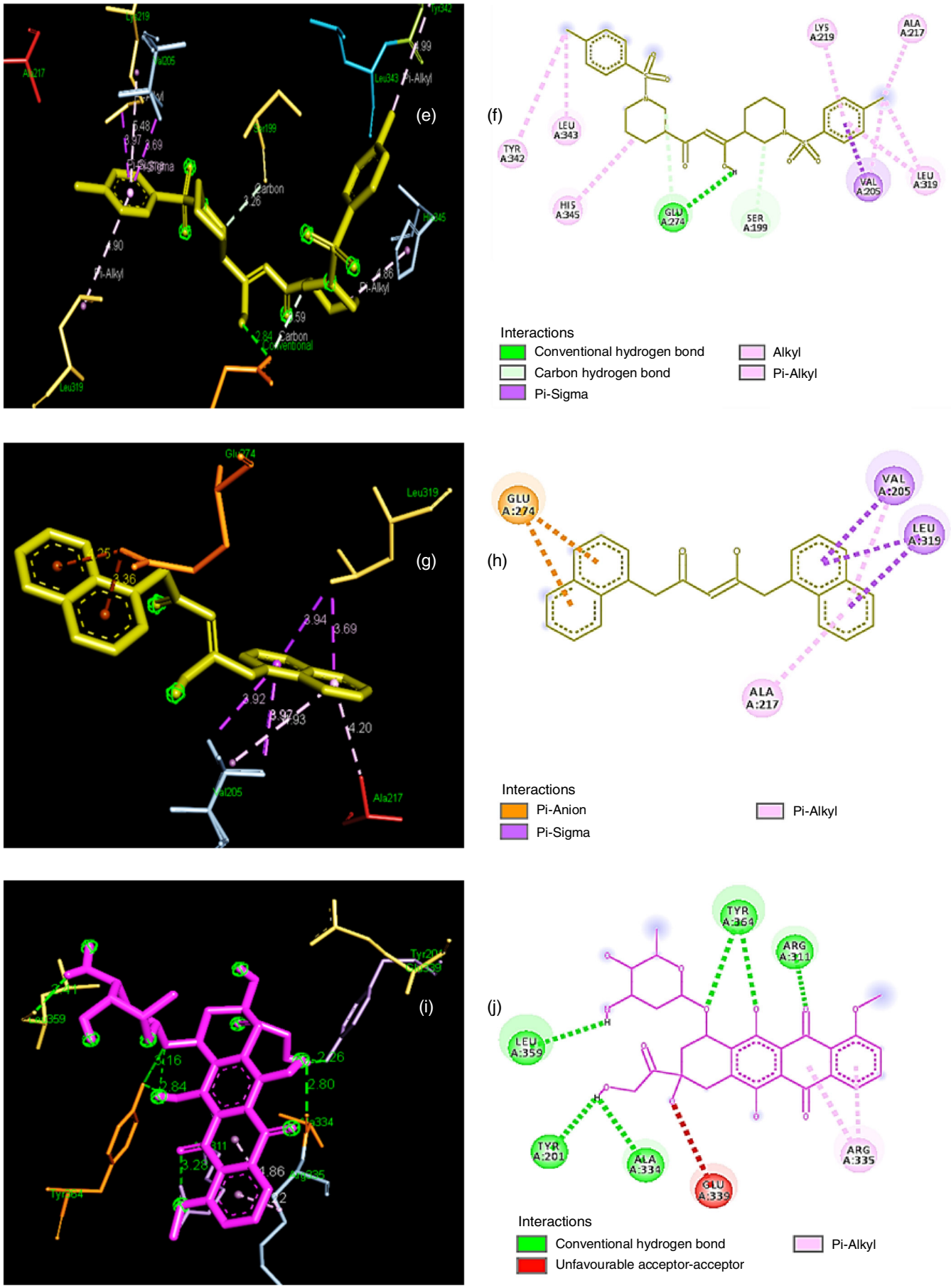
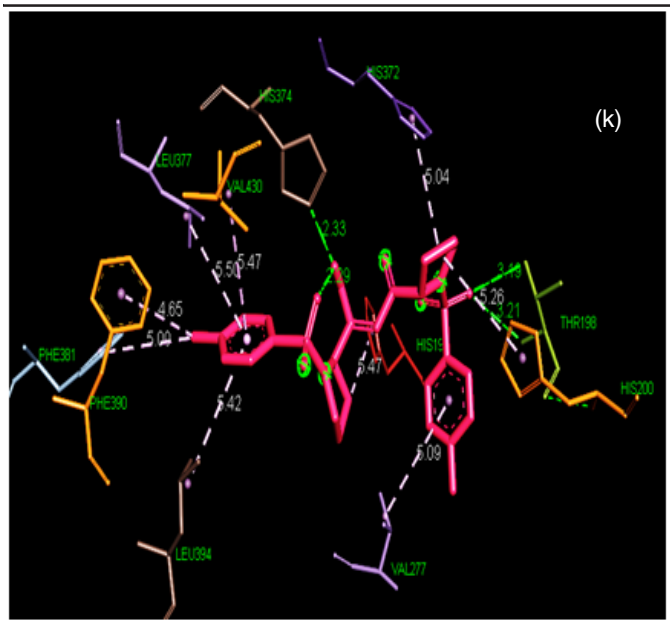



Fig. 1. Molecular docking 3D and 2D images for compounds **5a-d**, doxorubicin with breast cancer protein PTK6 [compound **5a** (a,b), compound **5b** (c,d), compound **5c** (e,f), compound **5d** (g,h), doxorubicin (i,j)]


(k)
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HIS374
LEU377
VAL430
5.04
2.33
5.47
5.50
4.65
5.00
PHE381
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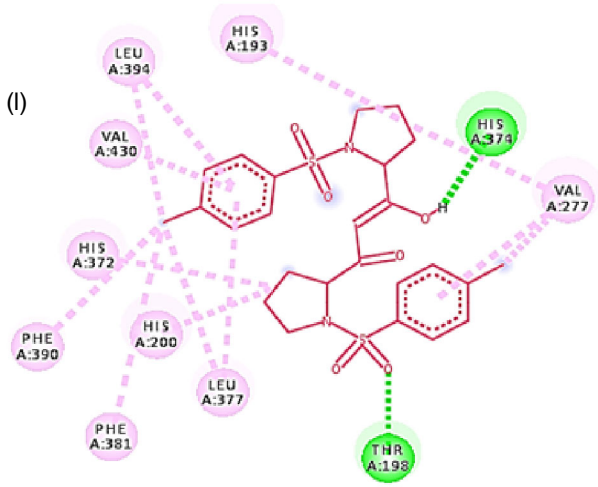
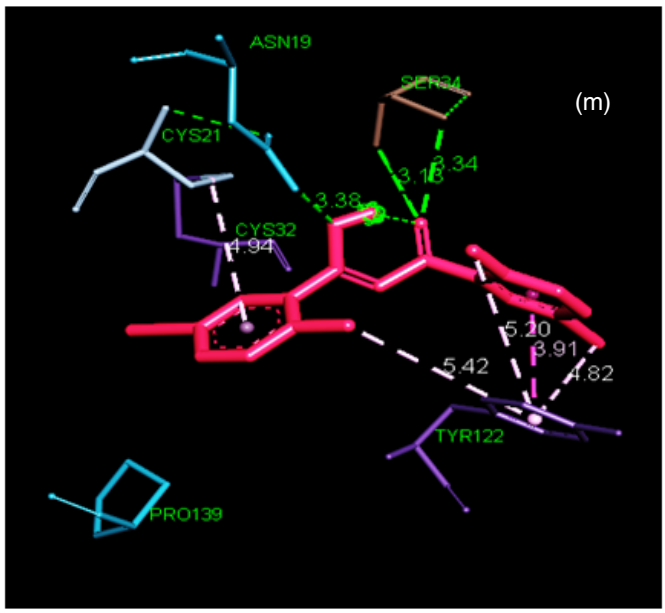
HIS19
5.47
5.26
3.21
THR198
HIS200
5.09
LEU394
VAL277


(l)
HIS A:193
LEU A:394
VAL A:430
HIS A:374
VAL A:277
HIS A:372
PHE A:390
HIS A:200
LEU A:377
PHE A:381
THR A:198
Interactions
Conventional hydrogen bond
Alkyl
Pi-Alkyl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CYS21
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CYS32
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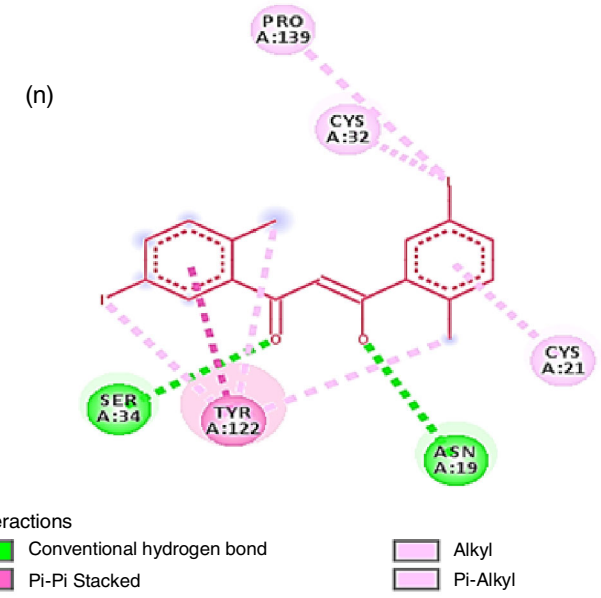
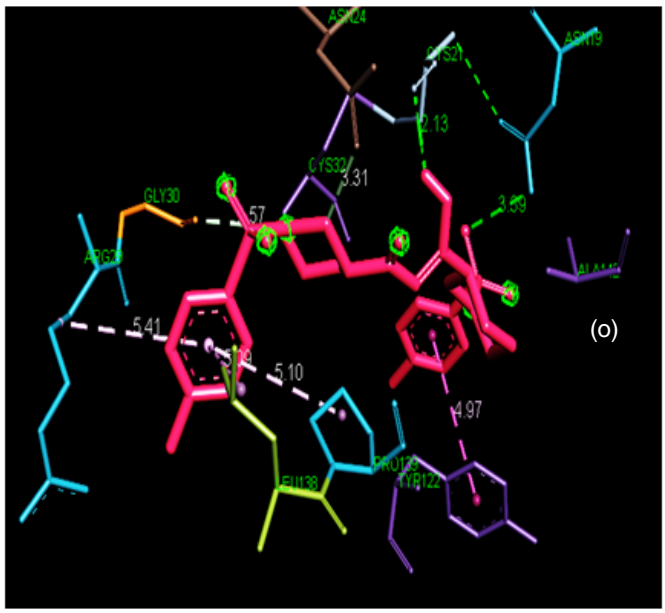
4.82
TYR122
PRO139


(n)
PRO A:139
CYS A:32
CYS A:21
SER A:34
TYR A:122
ASN A:19
Interactions
Conventional hydrogen bond
Pi-Pi Stacked
Alkyl
Pi-Alkyl


(o)
ASN24
CYS21
ASN19
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CYS32
3.31
GLY30
3.59
ARG29
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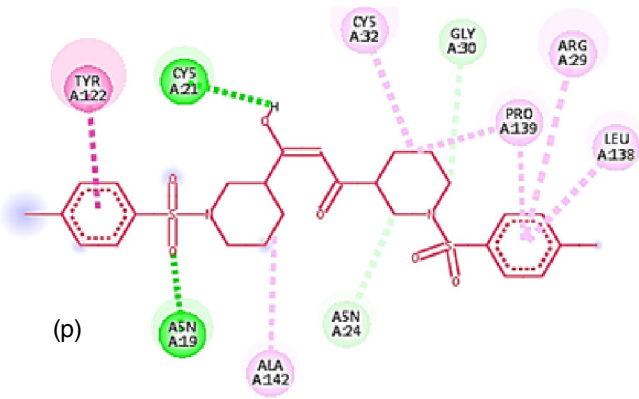
5.10
4.97
LEU138
PRO139
TYR122


(p)
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GLY A:30
ARG A:29
TYR A:122
CYS A:21
PRO A:139
LEU A:138
ASN A:19
ASN A:24
ALA A:142
Interactions
Conventional hydrogen bond
Carbon hydrogen bond
Pi-Pi Stacked
Alkyl
Pi-Alkyl

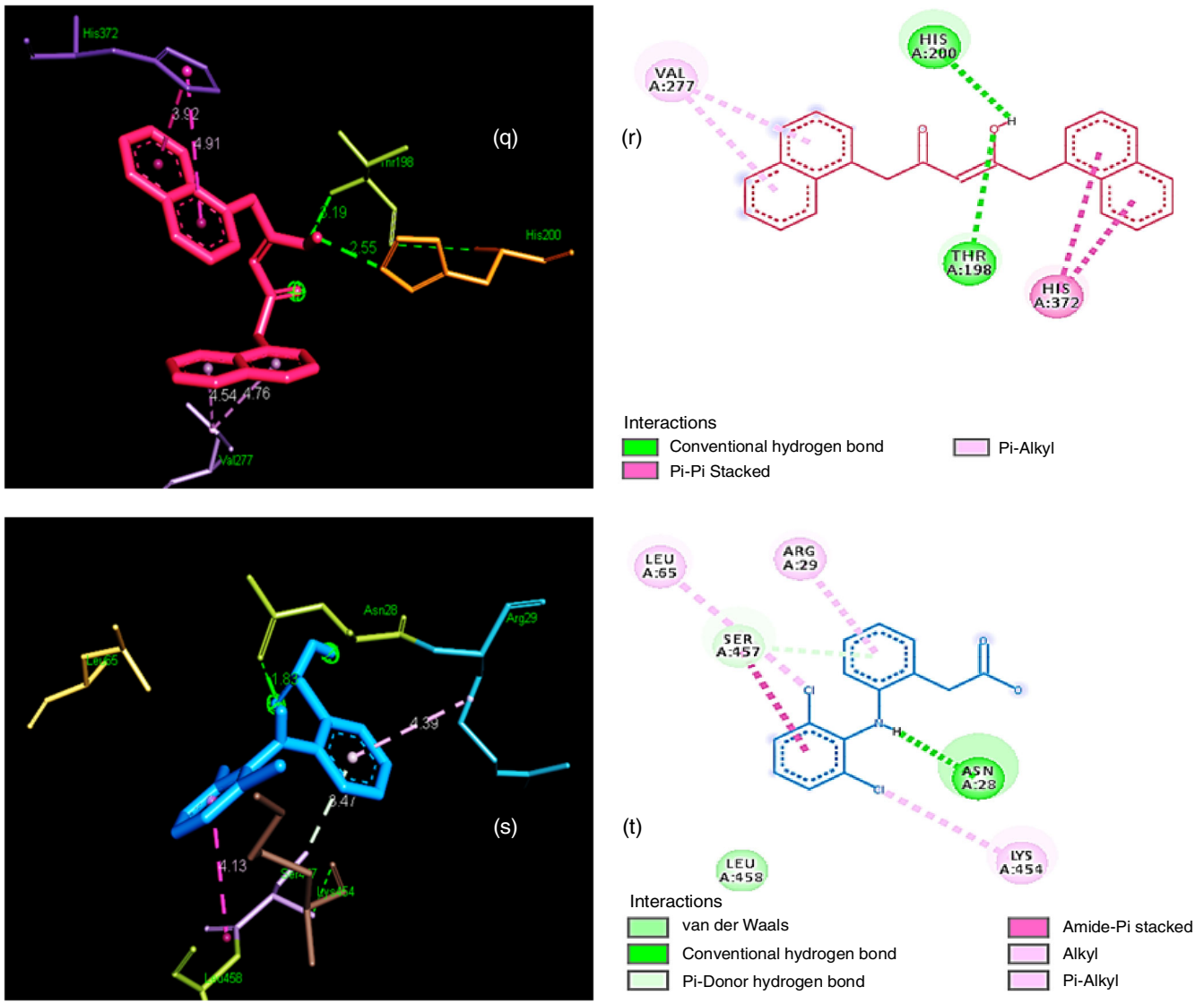


Fig. 2. Molecular docking 3D and 2D images for compounds **5a-d**, diclofenac with COX2 enzyme [compound **5a** (k,l), compound **5b** (m,n), compound **5c** (o,p), compound **5d** (q,r), diclofenac (s,t)]

(0.01-15 µg/mL). The cytotoxic effect of the most effective compound **5d** on the breast cancer MDAMB231 cells was determined by 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.

The $IC_{50}$ ± SEM values obtained for compound **5d** against MDA-MB-231 cancer cell line, along with the standard drug Doxorubicin is represented in Table-3. The $IC_{50}$ value of symmetrical diketone, **5d** was 2.4 µg/mL and this may be attributed to the presence of naphthyl moiety, which shows more inhibition against breast cancer cell line (Fig. 3a-b).

***in vitro* HRBC stabilization assay:** As the HRBC has similar membrane structure to that of lysosomal membrane, this assay was carried out to evaluate the nature of the synthesized compound to maintain the stability of HRBC membrane thereby stopping the release of lysosomal enzymes, which are responsible for inflammation progression. The HRBC membrane stabilizing activities of synthesized compounds are given in the Table-4, from the values it can be inferred that all the compounds exhibited significant anti-inflammatory activity in HRBC membrane stabilization method. Diclofenac, the standard used exhibited 91.67 % membrane stability. Among symmetrical 1,3-diketones (**5a-d**), the compound with two naphthyl groups (5d) exhibited the maximum value of 93.4 %, which is higher than that of diclofenac (Fig. 4).

TABLE-3
*in vitro* CYTOTOXICITY EVALUATION OF THE COMPOUND **5d** AND DOXORUBICIN AGAINST MDA-MD-231 CANCER CELL LINE-MTT ASSAY

| Compound | $IC_{50}$ ± SEM* |
|---|---|
| **5d** | 2.4 ± 0.22 |
| Doxorubicin | 0.93 ± 0.08 |

*Data are the mean of three independent experiments

TABLE-4
HRBC MEMBRANE STABILIZING ACTIVITIES OF SYNTHESIZED COMPOUNDS **5a-d** AND DICLOFENAC

| Compound | Stabilization (%) |
|---|---|
| **5a** | 88.36 ± 0.19 |
| **5b** | 59.28 ± 0.23 |
| **5c** | 80.7 ± 0.08 |
| **5d** | 93.4 ± 0.21 |
| Diclofenac | 91.67 ± 0.19 |

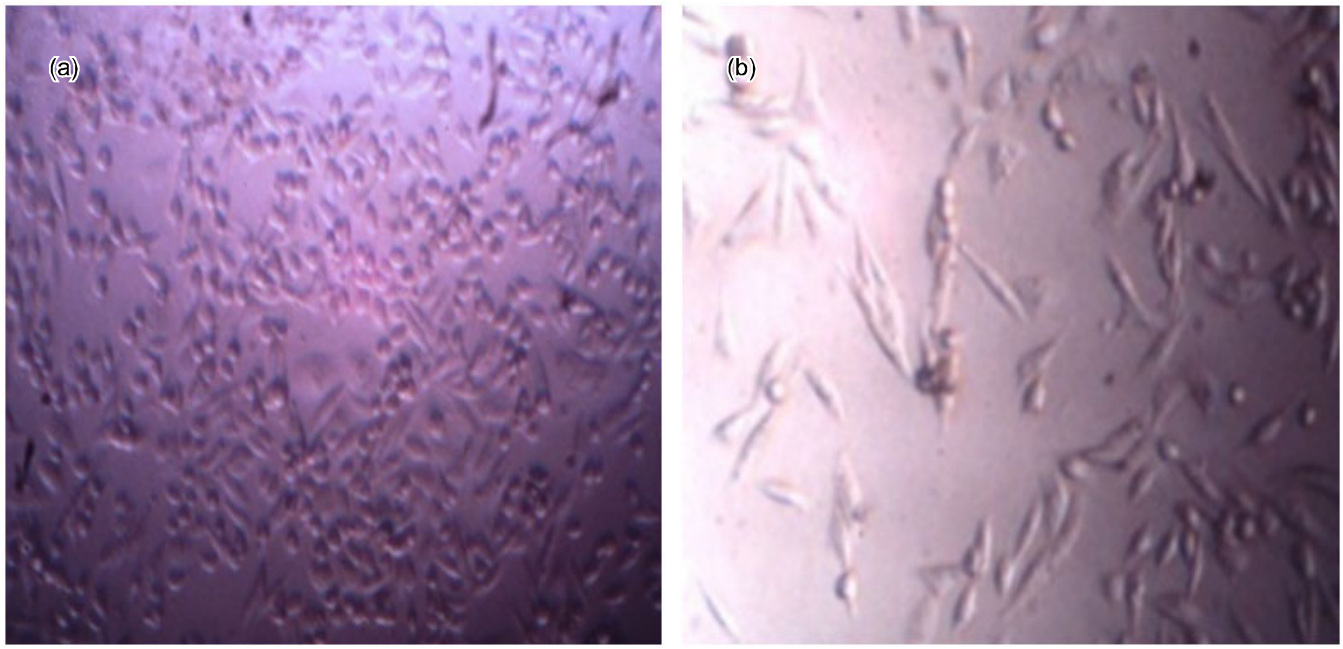


Fig. 3. *in vitro* Anticancer activity images of (a) Control (b) compound **5d** against MDA-MB-231cell line (MTT assay)

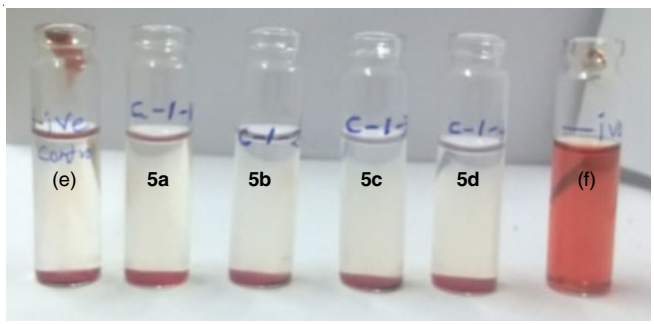


Fig. 4. Photographs of HRBC membrane stabilizing activities of compounds (**5a-d**), (e) diclofenac and (f) control

## Conclusion

In summary, new symmetrical-1,3-diketones (**5a-d**) were synthesized by coupling reaction of appropriate ketone with *N*-acyl triazole in the presence of $MgBr_2 \cdot Et_2O$ and DIPEA. Chemical structures were confirmed by $^1H$, $^{13}C$ NMR, IR and HRMS spectral data. The molecular docking study of compounds (**5a-d**) was carried using breast cancer protein (PTK6) and inflammatory protein (COX2). It revealed that compounds have greater hydrophobic, hydrogen bonding interaction with receptor in binding sites. All the compounds showed high binding affinity value than standard drugs with higher values by compounds **5c** and **5d**. The $IC_{50}$ value of the compound **5d** was found to be 2.4 µg/mL against breast cancer cell line (MDA-MB-231 cell line) and also the percentage HRBC stabilization value was found to be 93.4 %. Thus, the synthesized symmetrical 1,3-diketones can be evaluated, *in vivo,* for both anticancer and anti-inflammatory activities.

## ACKNOWLEDGEMENTS

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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