

## DESIGN AND SYNTHESIS OF TRIPHENYL-1H-PYRAZOLE DERIVATIVES AS ANTICANCER AGENTS

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## ABSTRACT

Some novel triphenyl-1H-pyrazole derivatives have been designed (4a-f) based on the *in silico* docking studies using the crystal structure of interleukin-1beta convertase (caspase-1) (PDB ID: 1BMQ) employing GLIDE v5 XP docking program (Schrodinger Inc.). All the designed compounds showed binding affinities as well as interactions with all the crucial amino acid residues on par with the reference standard. Compounds which were predicted to have good binding affinity (docking score) were considered for synthesis following the appropriate scheme.

**Keywords:** Triphenyl-1H-pyrazole, Docking study, Interleukin-1beta convertase, 1BMQ.

## INTRODUCTION

Pyrazole is a unique template that is associated with wide variety of biological functions such as antimicrobial, antiviral, anticancer, angiotensin converting enzyme inhibitor and anti-inflammatory activities<sup>1</sup>. Interestingly, a number of modifications on pyrazole chromophore have been reported with promising anticancer activity. In particular, 3-imidazolyl-4-pyridylvinylpyrazole derivatives (I, II) showed potent and selective cyclin dependent kinase inhibitory activities and inhibited *in vitro* cellular proliferation in various human cell lines<sup>2</sup>. Several trisubstituted pyrazole derivatives have also been reported as potent

antiangiogenic agents. Moreover compounds containing the fused pyrazole [4,3-*c*] quinololine nucleus emerged as potent antiangiogenic compounds with *in vitro* inhibitory effect on the growth of human breast (MCF-7) and cervical (Hela) carcinoma cell lines. Compound (III) found to be active, eliciting 64% of inhibition ( $p < 0.01$ ) by chicken chorioallantoic membrane (CAM) assay [3]. Pyrazole-1-carbothioic acid derivatives (IV) found to possess high antiproliferative activity against MCF-7 with  $IC_{50}$  0.08  $\mu$ M [4]. Bis (Pyrazolylmethyl) aniline derivative (V) showed cytotoxic activity against P815 (murin mastocytoma) and Hep (human laryngeal carcinoma) with  $IC_{50}$  of 3.25 and 17.82  $\mu$ M respectively [5].

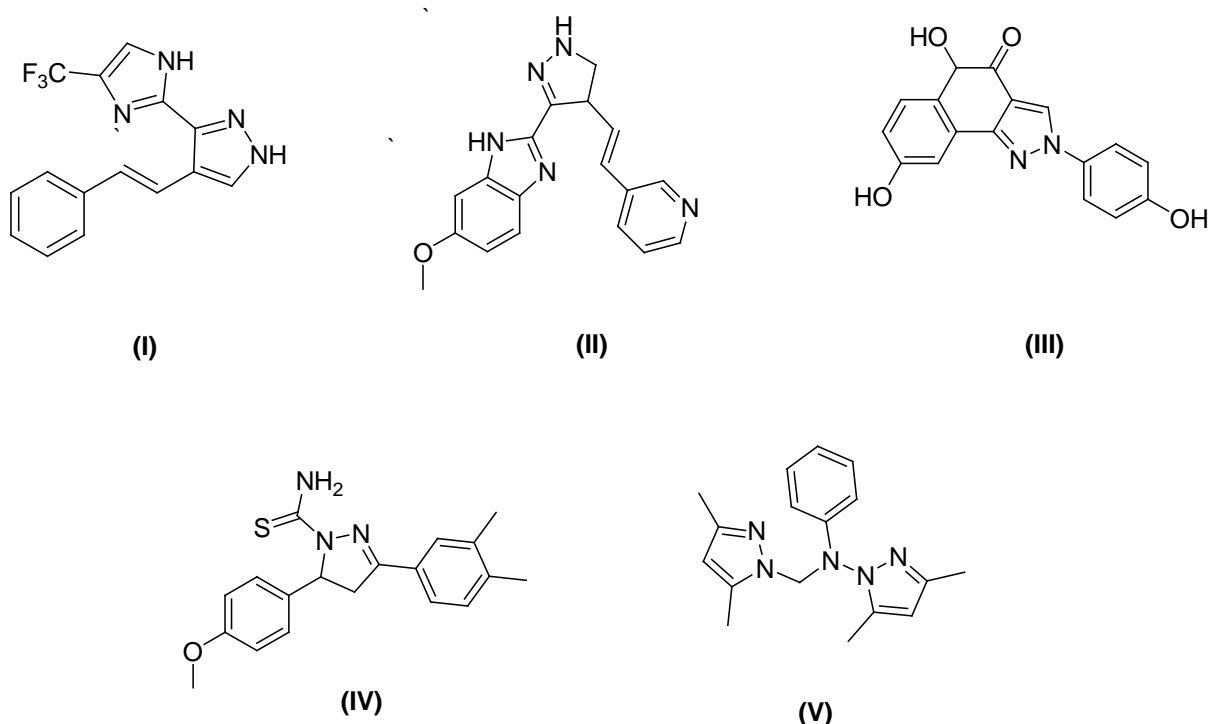


Fig. 1: Reported potent antiangiogenic agents

The above observations prompted us to design and synthesize some novel pyrazole derivatives as potent anticancer agents. In the present study we report the design and synthesis of a series of novel triphenyl-1H-pyrazole derivatives. Further, we propose the molecular interactions and the binding mode of the synthesized compounds using the caspase-1 protein (PDB ID: 1BMQ) based on *in silico* docking studies.

## MATERIAL AND METHODS

### Molecular Modeling Study

Molecular modeling investigations were carried out using Dell Precision work station T3400 running Intel Core2 Duo Processor, 4GB RAM, 250 GB hard disk, and NVidia Quadro FX 4500 graphics card. GLIDE v5 XP docking program (Schrodinger Inc.)<sup>67</sup> was employed for the docking studies.

### Preparation of Protein

PDB structure (www.rcsb.org) 1BMQ (Crystal structure of caspase-1) was downloaded, refined, and prepared using Schrodinger protein preparation wizard tool (Glide), which performs the following steps: assigning of bond orders, addition of hydrogen, optimization of hydrogen bonds by flipping amino side chains, correction of charges, and minimization of the protein complex. All the bound water molecules, ligands and cofactors were removed (preprocess) from the proteins which were taken in .mae format. The tool neutralized the side chains that are not close to the binding cavity and do not participate in salt bridges. This step is then followed by restrained minimization of co-crystallized complex, which reorients side chain hydroxyl groups and alleviates potential steric clashes. The complex obtained was minimized using OPLS\_2005 force field<sup>9</sup> with Polack-Ribiere Conjugate Gradient (PRCG) algorithm. The minimization was terminated either completion of 5,000 steps (or) after the energy gradient converged below 0.05 kcal/mol.

### Preparation of Ligands

Structures of the ligands (4a-f) were sketched using built panel of Maestro and taken in .mae format. LigPrep is a utility of Schrodinger software suit that combines tools for generating 3D structures from 1D (Smiles) and 2D (SDF) representation, searching for tautomers, steric isomers and perform a geometry minimization of the ligands. Molecular Mechanics Force Fields (OPLS\_2005) with default settings were employed for the ligand minimization.

### Docking Studies

Docking studies were carried out using the above mentioned prepared protein (1BMQ) and ligands (4a-f), by employing Glide XP docking program (Schrodinger Inc) following the reported procedure<sup>67</sup>.

### Chemistry

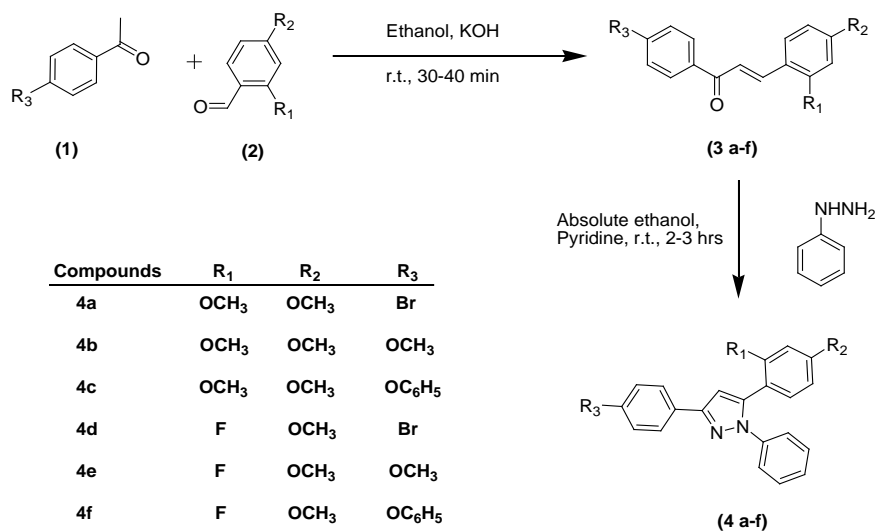
Starting materials, reagents and solvents were purchased from commercial suppliers and purified/distilled/crystallized before use. All solvents employed were of commercial grade (LR) and used without further purification. Reactions were routinely monitored by thin layer chromatography (TLC) on silica gel (pre-coated F254 Merck plates) and visualized the products under UV light (254 nm). <sup>1</sup>H NMR spectra were determined in CDCl<sub>3</sub> or DMSO-d<sub>6</sub> solutions with a Bruker Avance II 400 MHz spectrometer and signals recorded in parts per million ( $\delta$ ) downfield from tetramethylsilane as internal standard, and *J* values are given in Hz. IR spectra were recorded on Perkin Elmer FT-IR Spectrometer (Spectrum RX I) using KBr pellet technique. The elemental analyses were performed using Thermo EA 2110 series. Melting points were recorded in open capillaries on LABINDIA melting point apparatus and were uncorrected. Mass spectra (ESI) were recorded on Waters Micromass Q-TOF Micro.

### General procedure for the Synthesis of Chalcone Derivatives (3a-f)

To ethanolic solution of Acetophenones (1a-f, 1.1 mmol) at 0°C, ethanolic KOH (6 mol) was with stirring followed by addition of appropriate aldehydes (2a-f, 1 mmol) and allowed to stir for 10-30 minutes at room temperature. The completion of the reaction was monitored by TLC (CHCl<sub>3</sub>:CH<sub>3</sub>OH:: 95:5) and resulted precipitate was collected by filtration, dried and purified using column chromatography (10% ethyl acetate : hexane as mobile phase) to afford pure product (3a-f). (Scheme-1) The formation of the chalcone derivatives were characterized based on their reported physical and spectral data<sup>9</sup>.

### General procedure for the Synthesis of Triphenyl-1H-Pyrazole Derivatives (4a-f)

The synthesized chalcone derivatives (3a-f) in absolute alcohol on treatment with phenyl hydrazine in presence of pyridine resulted in the formation of final pyrazole derivatives (4a-f) in quantitative yields (Scheme-1). These derivatives (4a-f) were further purified using column chromatography (5-15% ethyl acetate-hexane as mobile phase) to afford pure product and characterized based on their physical and spectral analysis<sup>10,11</sup>.



Scheme 1: Synthetic pathway for the preparation of triphenyl-1H-pyrazole derivatives (4a-f)

3-(4-Bromo-phenyl)-5-(2,4-dimethoxy-phenyl)-1-phenyl-1H-pyrazole (4a)

Yield: 64%, Color: Light yellow, m.p.: 176 °C, IR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 3043, 2840, 1558, 1497, 732, 553;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz,  $\delta$  ppm): 3.71 (3H, s,  $\text{OCH}_3$ ), 3.72 (3H, s,  $\text{OCH}_3$ ), 6.40 (2H, m, Ar), 7.08 (1H, d, =CH), 7.28 (1H, dd, Ar), 7.32(7H, m, Ar), 7.51(2H, d, Ar); ESI-MS ( $m/z$ , %) = 436, 437 ( $\text{M}^{+2}$ ; 72%, 64%).

5-(2,4-Dimethoxy-phenyl)-3-(4-methoxy-phenyl)-1-phenyl-1H-pyrazole (4b)

Yield: 71%, Color: Yellow solid, m.p.: 131 °C, IR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 3031, 2846, 1554, 1493, 729;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz,  $\delta$  ppm): 3.70 (3H, s,  $\text{OCH}_3$ ), 3.71 (3H, s,  $\text{OCH}_3$ ), 3.72 (3H, s,  $\text{OCH}_3$ ), 6.37 (2H, m, Ar), 6.84 (2H, d, Ar), 7.00 (1H, d, =CH), 7.28 (1H, dd, Ar), 7.33(7H, m, Ar); ESI-MS ( $m/z$ , %) = 388 ( $\text{M}^{+1}$ , 77%).

5-(2,4-Dimethoxy-phenyl)-3-(4-phenoxy-phenyl)-1-phenyl-1H-pyrazole (4c)

Yield: 74%, Color: Yellow solid, m.p.: 207 °C, IR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 3033, 2846, 1553, 1492, 729;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz,  $\delta$  ppm): 3.71 (3H, s,  $\text{OCH}_3$ ), 3.72 (3H, s,  $\text{OCH}_3$ ), 6.35 (2H, dd, Ar), 7.00 (6H, m, =CH, Ar), 7.31 (8H, m, Ar), 7.45(2H, dd, Ar); ESI-MS ( $m/z$ , %) = 450 ( $\text{M}^{+1}$ , 92%).

3-(4-Bromo-phenyl)-5-(2-fluoro-4-methoxy-phenyl)-1-phenyl-1H-pyrazole (4d)

Yield: 65%, Color: Light yellow, m.p.: 241 °C, IR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 3042, 2837, 1560, 1499, 1301, 734, 553;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz,  $\delta$  ppm): 3.72 (3H, s,  $\text{OCH}_3$ ), 6.62 (2H, m, Ar), 7.07 (1H, d, =CH), 7.32 (8H, m, Ar), 7.51 (2H, d, Ar); ESI-MS ( $m/z$ , %) = 424, 426 ( $\text{M}^{+2}$ , 73%, 69%).

5-(2-Fluoro-4-methoxy-phenyl)-3-(4-methoxy-phenyl)-1-phenyl-1H-pyrazole (4e)

Yield: 76%, Color: Yellow solid, m.p.: 284 °C, IR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 3041, 2839, 1562, 1501, 1302, 734;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz,  $\delta$

ppm): 3.70 (3H, s,  $\text{OCH}_3$ ), 3.72 (3H, s,  $\text{OCH}_3$ ), 6.55 (2H, m, Ar), 6.83 (2H, d, Ar), 7.02 (1H, d, =CH), 7.30 (1H, dd, Ar), 7.33(7H, m, Ar); ESI-MS ( $m/z$ , %) = 375.5 ( $\text{M}^{+1}$ , 78%).

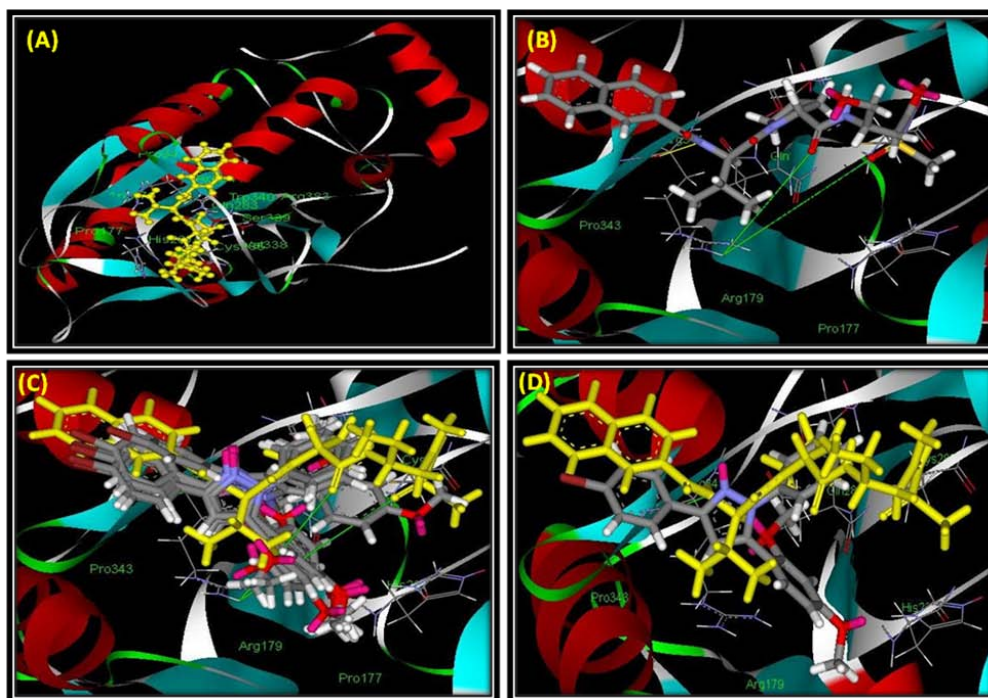
5-(2-Fluoro-4-methoxy-phenyl)-3-(4-phenoxy-phenyl)-1-phenyl-1H-pyrazole (4f)

Yield: 79%, Color: Yellow solid, m.p.: 284 °C, IR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 3031, 2846, 1553, 1491, 1302, 728;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz,  $\delta$  ppm): 3.72 (3H, s,  $\text{OCH}_3$ ), 6.35 (2H, dd, Ar), 7.00 (6H, m, =CH, Ar), 7.3 (8H, m, Ar), 7.44(2H, dd, Ar); ESI-MS ( $m/z$ , %) = 437 ( $\text{M}^{+1}$ , 89%).

## RESULTS AND DISCUSSION

In recent years, much effort has been directed toward searching for potent anticancer agents but their undesirable side effects delineates the urgent need for the development of NCEs as effective anticancer agents. In addition, the design, development, and commercialization of a drug is a tedious, time consuming and cost-intensive process. For these reasons, any tool or technique that increases the efficiency of any stage of the drug discovery process is highly desirable. As the main paradigm of medicinal chemistry is that the biological activity, as well as physical, physicochemical, and chemical properties of organic compounds depends on their molecular structure, computer-aided drug design based on target based docking studies is one such tool that can be used to increase the efficiency of the drug discovery process<sup>12</sup>.

In the present investigation, *in silico* docking studies were performed using the crystal structure of interleukin-1beta convertase (caspase-1) (PDB ID: 1BMQ)<sup>13,14</sup> to recognize the hypothetical binding mode of the ligands with the receptor in order to design a series of new triphenyl-1H-pyrazole derivatives (4a-f) as possible anticancer agents. To investigate the ability of molecular docking to reproduce an experimentally observed ligand binding mode, the co-crystallized ligand MNO 601 (3s)-n-methanesulfonyl-3-({1-[n-(2-naphthoyl)-l-valyl]-l-prolyl}amino)-4-oxobutanamide has been used as reference ligand ( $\text{IC}_{50}$  = 38 nM) and docked back into its binding site (Fig. 2A-B) of the crystal structure of the caspase-1 using Glide XP docking program (Schrodinger Inc)<sup>6</sup>.



**Fig. 2** (A) crystal structure of interleukin-1beta convertase (caspase-1) (PDB ID: 1BMQ) and location of binding site (MNO 601 is shown). (B) Superimposition of redocked MNO 601 ( $\text{IC}_{50}$  = 38nM) with its original position as co-crystal in the crystal structure of interleukin-1beta convertase (rmsd-0.71 Å) showing H-bond interactions with the amino acid residues Arg341, Ser339, His237 and  $\pi$ -stacking interaction with His342 and Arg383 respectively. (C) Superimposition of designed compounds 4a-f and MNO 601 original position (yellow colour) and orientation in crystal structure of interleukin-1beta convertase. (D) Hypothetical binding motif of 4a (superimposed with the MNO 601) at the binding site of the crystal structure of interleukin-1beta convertase.

The docking pose closely resembled the co-crystallized conformation with a heavy atom root-mean-square deviation (RMSD) 0.71 Å. As shown in Fig. 2B, the naphthyl group showed significant  $\pi$ -stacking interaction with His342 and Arg383 which was proposed to be crucial for having the enhanced binding affinity of the co-crystal ligand<sup>13</sup>. The carbonyl oxygen atom formed a strong hydrogen bond with the side chains of Arg341 at a distance 2.70 Å. Moreover, the nitrogen atom of the amide group seems to be deprotonated and to have a salt bridge with Arg341. The oxygen atom of methanesulfonamino carbonyl group was found to form hydrogen bond with the nitrogen atom of guanidine moiety of Arg341 and pyrazole ring of His237 at a distance of 2.47 and 3.10 Å respectively. The aldehyde group of the ligand formed strong covalent bond with the sulphur atom of Cys285. All the designed

compounds were docked following the same docking protocol and analyzed for their interactions (Fig. 2C & 2D) with the binding site (active site) amino acids of caspase-1.

The designed compounds were found to accommodate the binding pocket of the receptor showing the important interactions with all the crucial amino acid residues. In particular, the bromophenyl moiety was found to form a significant  $\pi$ -stacking interaction with His342 and Arg383 respectively. Interestingly, no salt bridge formation was observed with the designed compounds. The methoxy group of the phenyl ring formed crucial hydrogen bond with Arg341. All the compounds were predicted to have good binding affinities (docking score) as compared to the reference standard (MNO 601) (Table-1).

**Table 1: Binding affinities (GLIDE XP\_Score) of the designed compounds 4a-f**

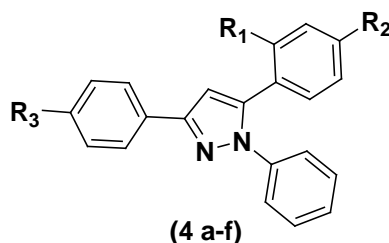
S. No.	Compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	GLIDE XP_Score
1	4a	OCH <sub>3</sub>	OCH <sub>3</sub>	Br	-9.08
2	4b	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	-9.47
3	4c	OCH <sub>3</sub>	OCH <sub>3</sub>	OPh	-9.53
4	4d	F	OCH <sub>3</sub>	Br	-9.61
5	4e	F	OCH <sub>3</sub>	OCH <sub>3</sub>	-9.35
6	4f	F	OCH <sub>3</sub>	OPh	-9.50
7	MNO601 (IC <sub>50</sub> = 38nM)	--	--	--	-11.49

Compounds which were predicted to have good binding affinity (docking score) were considered for synthesis following the scheme-1. The synthesis of key intermediates - chalcone derivatives (3a-f) was carried out by Claisen-Schmidt condensation of acetophenones (1a-f) with appropriate aldehydes (2a-f) under basic conditions following the well reported methods and characterized based on their physical and spectral data.<sup>9</sup> The chalcone derivatives (3a-f) on further treatment with phenyl hydrazine under basic conditions resulted in the formation of designed compounds (4a-f) in quantitative yields. All the synthesized compounds were purified

using column chromatography (Table-2). The formation of the target compounds (4a-f) were confirmed based on the disappearance of carbonyl peak at IR spectra and alkene protons at  $\delta$  6.5-6.7 ppm along with the additional appearance of pyrazole peak as singlets at  $\delta$  7.0-7.08 ppm of <sup>1</sup>H NMR spectra. Finally, the presence of molecular ion peak at mass spectra confirms the formation of compounds 4a-f respectively. Further efforts are currently being taken up to evaluate and optimize the lead structure as possible interleukin-1beta convertase inhibitors and the results of which will be the basis of our future publication.

**Table 2: Reaction time, Melting point and Percentage yield of products 4a-f**

S. No.	Compound			Reaction time (hrs)	M. P. (°C)	Yield (%)	
1	4a	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>			
2	4b	OCH <sub>3</sub>	OCH <sub>3</sub>	Br	3.0	176	64
3	4c	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	2.5	131	71
4	4d	OCH <sub>3</sub>	OCH <sub>3</sub>	OPh	2.7	207	74
5	4e	F	OCH <sub>3</sub>	Br	2.9	241	65
6	4f	F	OCH <sub>3</sub>	OCH <sub>3</sub>	2.3	284	76



## CONCLUSION

In the present study, a structure based docking studies were carried out using the crystal structure of interleukin-1beta convertase (caspase-1) (PDB ID: 1BMQ) to gain insight into the structural requirement for effective binding with the receptor to design a series of novel triphenyl-1H-pyrazole derivatives (4a-f) as possible anticancer agents. All the designed compounds showed binding affinities as well as interactions with all the crucial amino acid residues on par with the reference standard. Compounds which were predicted to have good binding affinity (docking score) were

considered for synthesis following the scheme-1 and purified using column chromatography and characterized based on their physical and spectral data. Efforts are under progress to evaluate and optimize the lead structure as possible interleukin-1beta convertase inhibitors.

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