CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
http://doi.org/10.5281/zenodo.166358

Available online at: http://www.iajps.com Research Article

# SYNTHESIS, *IN VITRO* BIOLOGICAL AND COMPUTATIONAL EVALUATION OF SOME NOVEL PYRAZOLES AS POTENTIAL ANTICANCER AGENTS

**Kenny Chang[1, 2] and VasudevaRao Avupati [3 *]**

[1]Faculty of Pharmacy, Asia Metropolitan University, Selangor Darul Ehsan, 43200, Malaysia.

[2]Faculty of Science, Technology, and Engineering La Trobe University, Bendigo, Australia.

[3]Pharmaceutical Chemistry Department, School of Pharmacy, International Medical University, 126, Jln Jalil Perkasa 19, Bukit Jalil, 57000 Bukit Jalil, Wilayah Persekutuan, Kuala Lumpur, Malaysia.

**Abstract:**

*The major challenge in modern drug discovery has been the design and development of new anticancer drugs with improved efficacy and minimal side effects, especially due to a rapid rise in multidrug resistant tumors. In the recent past, United States Food & Drug Administration (US FDA) newly approved anticancer drug Crizotinib (Xalkori) possess a pyrazole nucleus as core moiety. Therefore in the present investigation, we have synthesised a series of pyrazoles* ***KVP, KVP1-4*** *and evaluated their potential as anticancer agents by using in vitro brine shrimp (Artemia salina) cytotoxicity bioassay. Among the compounds tested, compound* ***KVP2*** *has showed signifcant cytotoxicity at* ***ED50*** *value 5.43 ± 0.16 microgram/mL. Consequently, in silico molecular docking studies have also been performed to evaluate the possible underlying mechanism of action of* ***KVP*** *and* ***KVP1-4*** *against DHFR (Dihydrofolate reductase) anticancer drug target. Molecular docking results revealed that the* ***KVP*** *and* ***KVP1-4*** *is less selective towards inhibition of DHFR.*

**Keywords:** *Cancer, US FDA, Pyrazole, Brine shrimp lethality, Molecular docking, Dihydrofolate reductase (DHFR)*

**Corresponding author:**

**Vasudeva Rao Avupati,**

*Pharmaceutical Chemistry Department,*
*School of Pharmacy, International Medical University,*
*126, Jln Jalil Perkasa 19, Bukit Jalil, 57000 Bukit Jalil,*
*Wilayah Persekutuan, Kuala Lumpur, Malaysia.*

*Please cite this article in press as Kenny Chang and Vasudeva Rao Avupati,* ***Synthesis, In Vitro Biological and Computational Evaluation of Some Novel Pyrazoles as Potential Anticancer Agents****, Indo Am. J. P. Sci, 2016; 3(10).*

## INTRODUCTION:

Cancer is the name given to a collection of related diseases [1](Hennessy et al., 2005). In all types of cancer, some of the body's cells begin to divide without stopping and spread into surrounding tissues [2] (Fesik et al., 2005). Cancer can start almost anywhere in the human body, which is made up of trillions of cells [3] (Shea et al., 2016). Normally, human cells grow and divide to form new cells as the body needs them. When removed, they usually don't grow back, whereas malignant tumors sometimes do. Unlike most benign tumors elsewhere in the body, benign brain tumors can be life threatening[4] (Cheng et al., 2016).

An antimetabolite is a chemical that inhibits the use of a metabolite, which is another chemical that is part of normal metabolism. Such substances are often similar in structure to the metabolite that they interfere with. Antimetabolites can be used in cancer treatment, as they interfere with DNA production and therefore cell division and the growth of tumors. Because cancer cells spend more time dividing than other cells, inhibiting cell division harms tumor cells more than other cells. A target of even greater complexity is the incorporation of triphosphates into DNA or RNA and the subsequent modification of these macromolecules [5] (Renslo et al., 2006).

Dihydrofolate reductase (DHFR) is an essential metabolic enzyme that plays critical rolein one-carbon transfer reactions, including the biosynthetic pathways for deoxythymidine monophosphate (dTMP) [6] (Raoet al., 2000), purines and several amino acids [7] (Banerjee et al., 2002). As such, DHFR has been successfully targeted for both anticancer (eg. methotrexate) and antimicrobial (eg. trimethoprim, pyrimethamine) drug development [8] (Schweitzeret al., 1990). Owing to its essential role in both human and pathogenic cells, the successful development of anticancer DHFR inhibitors requires that the compounds are selective for the carcinogenic cells [9] (Kayeet al., 1998). Hence, it was proposed worthwhile to study the possible protein-ligand interactions using molecular docking studies against cancer targeted DHFR enzyme by using iGemDock software [10] (Hsu et al., 2011).

Pyrazole is a five membered and two-nitrogen containing heterocyclic ring. These structures have been investigated in the development of novel compounds with hypoglycemic, analgesic, anti-inflammatory, antimicrobial, anti convulsant, antidepressant, anti mycobacterial, antioxidant, antiviral, insecticidal and antitumor activities. Therefore, these compounds have been synthesized as target structures by many researchers and were evaluated for their biological activities.

Guniz and Sevil., 2015 [11]**,** have been reviewed the structures of 1H-pyrazoles with their corresponding biological activities for 21st (in 2000-2014 years) century. The pyrazole is a five membered and two-nitrogen containing heterocyclic ring. These structures have been investigated in the development of novel compounds with hypoglycemic, analgesic, anti-inflammatory, antimicrobial, anti convulsant, antidepressant, anti mycobacterial, antioxidant, antiviral, insecticidal and antitumor activities.

Demetrio et al., 2015 [12] , was reviewed the recent advances in bioactive system containing pyrazole fused with a five membered heterocycle, covering the time span of the last decade. All of them are represented around the common structure of the pyrazole ring fused with another five membered heterocycle containing the nitrogen, sulfur and oxygen atoms in all their possible combinations.

Cancer is a primary global cause of mortality, accounting for millions of deaths every year. Though many anticancer agents have been developed to treat different types of cancer effectively, major adverse effects could occur concurrently. Consequently, a huge demand is still there to find some novel molecules to treat this disease in current situation. Hence, we proposed worthwhile to synthesis some novel pyrimidine derivatives (dihydropyrimidones) and studied for their anticancer druggable properties using brine shrimp (Artemia salina) lethality bioassay.

## Materials:

### Instrumentation

- ❖ Melting points were taken in open capillary tubes. Purity of the compounds was checked on silica gel G TLC plates of 2 mm thickness using n-hexane and ethyl acetate as solvent system. The visualization of spot was carried out in an UV-chamber. The spectra IR, NMR and Mass have been recorded by sending the pure sample to the Universiti Putra Malaysia (UPM), Jalan Upm, 43400 Serdang, Selangor, Malaysia.

### Reagents and chemicals

- ❖ Substituted aldehydes and ketones, acetylacetone, acetylhydrazide, sodium hydroxide, potassium hydroxide, pyridine, triethylamine, Brine Shrimp Eggs, hexane, ethyl acetate, acetone, chloroform, methanol, ethanol, iodine, $H_2SO_4$ spraying reagent, silica gel (column & TLC) and other regular laboratory chemical of AR grade were procured from the local chemical suppliers via. Purchase indent from Laboratory Department, Asia Metropolitan University.

### Computational software requirements

- ❖ Computer aided drug discovery softwares along with graphical user interface (GUI) were utilized for molecular modeling, energy minimization, molecular docking and virtual screening protocols.

**Table 1: List of software applications used in the present study**

| S.No | Activity (will be performed) | Software (will be used) | License type (will be obtained) | Source |
|---|---|---|---|---|
| 1). | Molecular modeling (2D-Drawing) | Accelrys Draw | Academic License GPU (General Public User) License | Open Source |
| 2). | Molecular modeling (2D-3D Conversion) | Open Babel | Academic License GPU (General Public User) License | Open Source |
| 3). | Molecular modeling (Molecular Mechanics & Energy Minimization) | ArgusLab v 4.0 | Academic License GPU (General Public User) License | Open Source |

**Table 2: Citations for software applications used in the present investigation**

| S.No | Software | Citation |
|---|---|---|
| 1) | Accelrys Draw | Draw, A. (2011). Accelrys Software Inc. San Diego. |
| 2) | Open Babel | OLBoyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. J Cheminf, 3, 33. |
| 3) | Argus lab | Thompson, M. A. (2004). ArgusLab 4.0. 1. Planaria Software LLC, Seattle, WA. |
| 4) | iGemdock v 2.1 | Hsu, K. C., Chen, Y. F., Lin, S. R., & Yang, J. M. (2011). iGEMDOCK: a graphical environment of enhancing GEMDOCK using pharmacological interactions and post-screening analysis. BMC bioinformatics, 12(1), 1. |
| 5) | Protein Data Bank (PDB) | Bank, P. D. (1971). Protein Data Bank. Nature New Biol, 233, 223. |

**Computational hardware requirements**

- The minimum central hardware system configuration include Intel (R) Core (TM) 2Duo Central Processing Unit (CPU), 2.5 GHz, 1 TB hard disk, 2 KV Power Backup, WinXP or higher operating system was used for running all the selected computer aided drug discovery softwares. All softwares were well compatible with the selected system configuration.

**X-ray crystallographic structure of dihydrofolate reductase (DHFR)**

- X-ray crystallographic data of DHFR Ligand Binding Domain (LBD) was obtained from Brookhaven Protein Data Bank (**http://www.rcsb.org/pdb**). The protein data bank code (**PCB ID: 4KD7**) deposited by **Lamb, et al., 2013 [13]**.

**Methods**

**General procedure for the synthesis of pyrazole derivatives**

- The reaction sequence intended for the preparation of title compounds **(KVP, KVP1-KVP4)** is shown in Scheme 1; we have followed the pre-existing methods for the proposed synthesis.

**Scheme 1: Synthesis of Pyrazoles**

Acetylacetone + Acetylhydrazide

10% NaOH, Reflux, 24 h

Intermediate **(KVP)**

10% KOH, RT, MeOH

Compound **KVP1**

Compound **KVP2**

Compound **KVP3**

Compound **KVP4**

**Table 3: Chemical structure of the titled molecules**

| Intermediate **(KVP)** | |
|---|---|
| Compound **KVP1** | Compound **KVP2** |
| Compound **KVP3** | Compound **KVP4** |

- The reaction as shown in scheme 1 was started by adding equimolar concentration of substituted acetylacetone and acetylhydrazide in q.s. amount of ethanol. To this reaction mixture 10 mole percentage of NaOH was added and stirred for 10 min, followed by reflux for 3 hrs, reaction mixture turns to yellow color liquid. Further the reaction mixture kept aside overnight and examined the TLC profile. After completion of the reaction, the reaction mixture was poured in crushed ice, acidified if necessary with 1:1 dilute hydrochloric acid, and the yellow color liquid which separated

out was isolated by filtration using separating

- The reaction as shown in scheme 1 further proceed by adding substituted benzaldehydes to **KVP** in 1:1 mole concentrations in q.s. amount of ethanol. To this reaction mixture 1.5mL of 10% KOH solution was added in drop-wise until the reaction mixture turns to yellow. Further the reaction mixture kept aside overnight and examined the TLC profile. After completion of the reaction, the reaction mixture was poured in crushed ice, acidified if necessary with 1:1 dilute hydrochloric acid, and the light colorless to yellow color liquids which separated out was isolated by using separating funnel, dried and purified by drying agent. The light colorless or yellow color liquids of pyrazoles **KVP1-KVP4** which separated out from the reaction mixture were collected and used for doing physical characterization and bioassay.

**Identification of pyrazoles (KVP, KVP1-KVP4)**

- The formation of pyrazoles was identified and analyzed by Co-TLC technique with the starting materials. The visualization of the spots was carried out by spraying 5% $H_2SO_4$ in methanol and heating at 110˚C or under UV light or in Iodine chamber.

**Characterization of pyrazoles (KVP, KVP1-KVP4)**

- The chemical structures of the synthetic pyrazoles were established on the basis of their physical, chemical and spectral analytical data.
- Melting points were determined in open capillary tube, and expressed in degree Celsius.
- The synthetic chalcones were characterized by UV, IR, NMR & Mass spectral methods.
- NMR & IR spectral data were obtained by sending the samples to Andhra University, Visakhapatnam, India.

**In vitro Cytotoxicity Evaluation of Pyrazoles (KVP, KVP1-KVP4)**

- **Brine Shrimp Lethality Bioassay (Cytotoxicity Bioassay)**

  The anticancer potential of the synthesized pyrazoles was determined by Brine Shrimp Lethality assay as described by Meyer et al., 1982. Brine Shrimp (Artemia salina) nauplii were hatched in sterile brine solution (prepared using sea water salt 38 g/L and adjusted the pH to 8.5 using 1 N NaOH) under constant aeration for 38 h. After hatching, 10 nauplii were placed in each vial and added various concentrations of drug solutions in a final volume of 5 mL, maintained at 37 $^oC$ for 24 h under light of incandescent lamps and surviving larvae were counted. The $ED_{50}$ values (μg/mL) were determined by comparing mean surviving larvae of test and control tubes. The results of cytotoxicity study are given under results section.

**General procedure for the ligand preparation**

funnel, dried and purified by drying agent.

- The chemical structure of the selected ligand **KVP** and **KVP1-4** was initially modeled as 2D chemical structures using Accelrys Draw software and transformed into 3D chemical structures using Open Babel software and subjected for energy minimization using ArgusLab v 4.0 software. Such energy minimized structures were considered for molecular docking studies using iGemdock v 2.1 software. The corresponding docking engine compatible 'MDL MOL' file format has been adapted to ligand by using integral option (save as /MDL MOL).

**General procedure for the protein target selection, preparation and validation**

- The selection of DHFR Ligand Binding Domain (LBD) for molecular docking studies was carried out based upon several factors such as structure should be determined by X-ray diffraction spectroscopy, and resolution should be between >2.5 $A^o$, it should contain a co-crystallized ligand; the selected protein should not have any protein breaks in their 3D structure. Finally the resultant protein target was prepared for molecular docking simulation in such a way that all heteroatoms (i.e., nonreceptor atoms such as water, ions, etc.) were removed.

**General procedure for the software validation**

- iGEMDOCK v 2.1 software validation was performed by using X-ray structure (**4KD7)** deposited with co-crystallized ligand was obtained from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb). The Root Mean Square Deviation (RMSD) between the X-ray co-crystallized ligand and docked conformation was 1.84 $A^o$ indicated that the parameters for docking simulation was good in reproducing X-ray crystal structure.

**General procedure for the Molecular Docking**

- Molecular docking technique was employed to dock the bioactive pyrazole **KVP** and **KVP1-4** against **4KD7** using iGEMDOCK to locate the interaction between **KVP** and **KVP1-4** and **4KD7**. iGEMDOCK requires the receptor and ligand coordinates in either Mol2 or PDB format. Non polar hydrogen atoms were removed from the receptor file and their partial charges were added to the corresponding carbon atoms. Molecular docking was performed using standard protein-ligand docking protocol. The binding site was defined by crystallographic ligand of **4KD7**. Default settings were used for all the calculations and docking run was performed.

**Statistical analysis**

- The SPSS 2.0 software was used in data analysis. Data was expressed as mean ± SEM.

## RESULTS:

### Results of Chemical Synthesis

**Table 4: Physical characterization data of pyrazole KVP.**

| **Pyrazole (KVP)** | |
|---|---|
| Intermediate **(KVP)** | |
| **Physical state** | Liquid |
| **Color** | Yellowish oil |
| **Nomenclature** | 1-(3,5-dimethyl-1H-pyrazol-1-yl)ethanone |
| **Molecular weight** | 138.17 |
| **Molecular formula** | $C_7H_{10}N_2O$ |
| **Boiling point ($^oC$)** | >300 |
| **Yield (%)** | 67 |
| **Density** | 0.86 |
| **Thin Layer Chromatography (TLC)**<br>**Mobile phase concentration**<br>**$R_f$ value**<br>**UV-254nm observation** | Thin Layer Chromatography (TLC)<br>25% Ethylacetate/Hexane<br>0.22 cm<br>Light Green Fluorescence |
| **UV spectrum data ($\lambda_{max}$)** | 240 |
| **IR spectrum data ($cm^{-1}$)** | 1690C=O), 1620(C=C) |
| **$^1H$ NMR spectrum data (δ ppm)** | 1.9(s, 3H, $CH_3$), 2.3(s, 3H, $CH_3$), 2.5(s, 3H, $CH_3$), 6.1(s, 1H, C4-Pyrazoline) |
| **$^{13}C$ NMR spectrum data (δ ppm)** | Not determined |

**Table 5: Physical characterization data of pyrazole KVP1.**

| **Pyrazole (KVP1)** | |
|---|---|
| Compound **KVP1** | |
| **Physical state** | Liquid |
| **Color** | Yellowish oil |
| **Nomenclature** | (E)-1-(3,5-dimethyl-1H-pyrazol-1-yl)-3-phenylprop-2-en-1-one |
| **Molecular weight** | 226.27 |
| **Molecular formula** | $C_{14}H_{14}N_2O$ |
| **Boiling point ($^oC$)** | >300 |
| **Yield (%)** | 62 |
| **Density** | 1.65 |
| **Thin Layer Chromatography (TLC)**<br>**Mobile phase concentration**<br>**$R_f$ value**<br>**UV-254nm observation** | Thin Layer Chromatography (TLC)<br>25% Ethylacetate/Hexane<br>0.58 cm<br>Light Green Fluorescence |
| **UV spectrum data ($\lambda_{max}$)** | 243 |
| **IR spectrum data ($cm^{-1}$)** | 1684(C=O), 1613(C=C) |
| **$^1H$ NMR spectrum data (δ ppm)** | 2.3(s, 3H, $CH_3$), 2.6(s, 3H, $CH_3$), 6.1(s, 1H, C4-Pyrazoline), 6.7(d, J = 16 Hz, 1H, Hα), 7.7(d, J = 16 Hz, 1H, Hβ) |
| **$^{13}C$ NMR spectrum data (δ ppm)** | Not determined |

**Table 6: Physical characterization data of pyrazole KVP2.**

| **Pyrazole (KVP2)** | |
|---|---|
| Compound **KVP2** | |
| **Physical state** | Liquid |
| **Color** | Dark yellowish oil |
| **Nomenclature** | (E)-3-(2,4-difluorophenyl)-1-(3,5-dimethyl-1H-pyrazol-1-yl)prop-2-en-1-one |
| **Molecular weight** | 262.25 |
| **Molecular formula** | $C_{14}H_{12}F_2N_2O$ |
| **Boiling point ($^oC$)** | >300 |
| **Yield (%)** | 74 |
| **Density** | 1.2 |
| **Thin Layer Chromatography (TLC)**<br>**Mobile phase concentration**<br>**$R_f$ value**<br>**UV-254nm observation** | Thin Layer Chromatography (TLC)<br>25% Ethylacetate/Hexane<br>0.64 cm<br>Light Green Fluorescence |
| **UV spectrum data ($\lambda_{max}$)** | 245 |
| **IR spectrum data ($cm^{-1}$)** | 1671(C=O), 1590(C=C) |
| **$^1H$ NMR spectrum data (δ ppm)** | 2.3(s, 3H, $CH_3$), 2.6(s, 3H, $CH_3$), 6.1(s, 1H, C4-Pyrazoline), 6.7(d, J = 16 Hz, 1H, Hα), 7.7(d, J = 16 Hz, 1H, Hβ) |
| **$^{13}C$ NMR spectrum data (δ ppm)** | Not determined |

**Table 7: Physical characterization data of pyrazole KVP3.**

| **Pyrazole (KVP3)** | |
|---|---|
| Compound **KVP3** | |
| **Color** | White oil |
| **Nomenclature** | (E)-3-(4-chlorophenyl)-1-(3,5-dimethyl-1H-pyrazol-1-yl)prop-2-en-1-one |
| **Molecular weight** | 260.72 |
| **Molecular formula** | $C_{14}H_{13}ClN_2O$ |
| **Boiling point ($^oC$)** | >300 |
| **Yield (%)** | 50 |
| **Density** | 1.5 |
| **Thin Layer Chromatography (TLC)**<br>**Mobile phase concentration**<br>**$R_f$ value**<br>**UV-254nm observation** | Thin Layer Chromatography (TLC)<br>25% Ethylacetate/Hexane<br>0.35 cm<br>Light Green Fluorescence |
| **UV spectrum data ($\lambda_{max}$)** | 250 |
| **IR spectrum data ($cm^{-1}$)** | 1675(C=O), 1640(C=C) |
| **$^1H$ NMR spectrum data (δ ppm)** | 2.3(s, 3H, $CH_3$), 2.6(s, 3H, $CH_3$), 6.1(s, 1H, C4-Pyrazoline), 6.7(d, J = 16 Hz, 1H, Hα), 7.7(d, J = 16 Hz, 1H, Hβ) |
| **$^{13}C$ NMR spectrum data (δ ppm)** | Not determined |

**Table 8: Physical characterization data of pyrazole KVP4.**

| Pyrazole (KVP4) | |
|---|---|
| Compound **KVP4** | |
| **Physical state** | Liquid |
| **Color** | Yellow oil |
| **Nomenclature** | (E)-3-(4-bromophenyl)-1-(3,5-dimethyl-1H-pyrazol-1-yl)prop-2-en-1-one |
| **Molecular weight** | 305.17 |
| **Molecular formula** | $C_{14}H_{13}BrN_2O$ |
| **Boiling point (°C)** | >300 |
| **Yield (%)** | 65 |
| **Density** | 1.0 |
| **Thin Layer Chromatography (TLC)**<br>**Mobile phase concentration**<br>**$R_f$ value**<br>**UV-254nm observation** | Thin Layer Chromatography (TLC)<br>25% Ethylacetate/Hexane<br>0.16 cm<br>Light Green Fluorescence |
| **UV spectrum data ($\lambda_{max}$)** | 258 |
| **IR spectrum data ($cm^{-1}$)** | 1685(C=O), 1615(C=C) |
| **$^1$H NMR spectrum data (δ ppm)** | 2.3(s, 3H, $CH_3$), 2.6(s, 3H, $CH_3$), 6.1(s, 1H, $C_4$-Pyrazoline), 6.7(d, J = 16 Hz, 1H, Hα), 7.7(d, J = 16 Hz, 1H, Hβ) |
| **$^{13}$C NMR spectrum data (δ ppm)** | Not determined |

**Results of Biological Evaluation**

**Table 9: Cytotoxicity data of pyrazoles KVP, KVP1-KVP4.**

| Compound | *Artemia salina lethality* (Brine shrimp) Effective Dose Concentration ($ED_{50}$, μg/mL)* |
|---|---|
| Intermediate **(KVP)**<br>**KVP** | >50 |
| Compound **KVP1**<br>**KVP1** | >50 |
| Compound **KVP2**<br>**KVP2** | 5.43 ± 0.16 |
| Compound **KVP3**<br>**KVP3** | 36.75 ± 0.88 |
| Compound **KVP4**<br>**KVP4** | 12.84 ± 0.31 |

*Mean±SEM, p<0.05

**Results of Computational Evaluation**

**Table 10: Molecular docking information of pyrazole KVP, KVP1-KVP4 against 4KD7**

| Compound | iGemdock score Kcal/mol | No. of Hydrogen bonds/Interacting Amino acid residues |
|---|---|---|
| **KVP** | -76.0298 | 1/Ala 9 |
| **KVP1** | -96.9052 | 1/Ser 59 |
| **KVP2** | -105.193 | 1/Ser 59 |
| **KVP3** | -100.841 | -Nil- |
| **KVP4** | -99.1600 | 1/Tyr 121 |

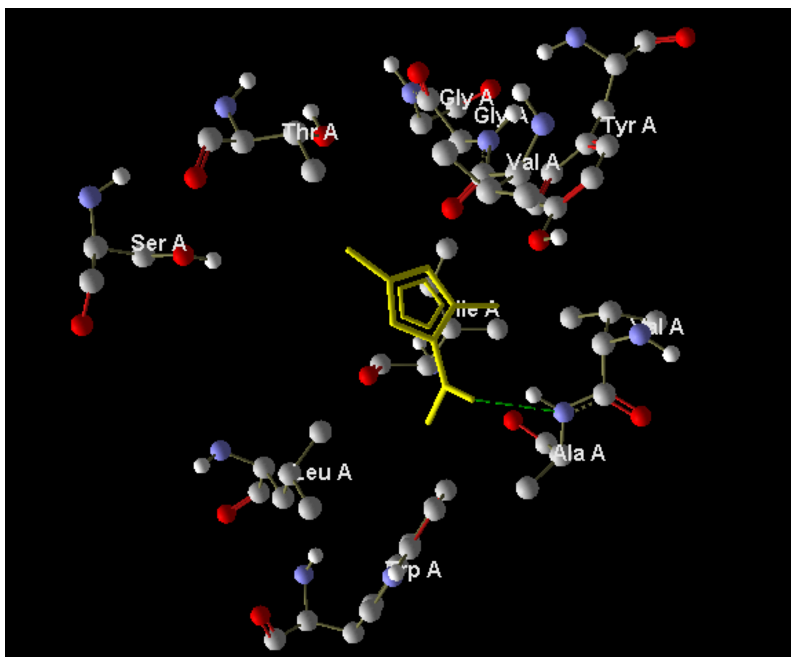


**Fig 1: Binding orientation and H-bond interactions (Green) of KVP (Yellow) within the active binding site region of 4KD7 (Residues)**

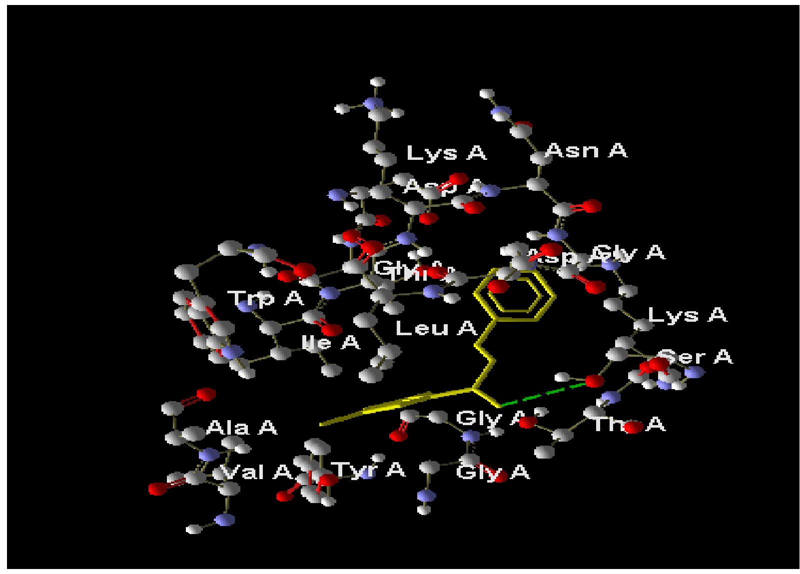


**Fig 2: Binding orientation and H-bond interactions (Green) of KVP1 (Yellow) within the active binding site region of 4KD7 (Residues)**

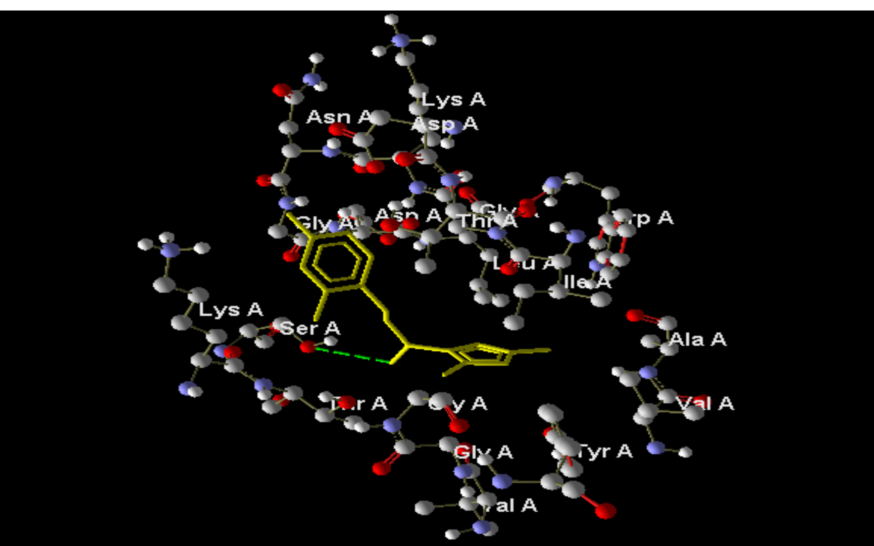


**Fig 3: Binding orientation and H-bond interactions (Green) of KVP2 (Yellow) within the active binding site region of 4KD7 (Residues)**

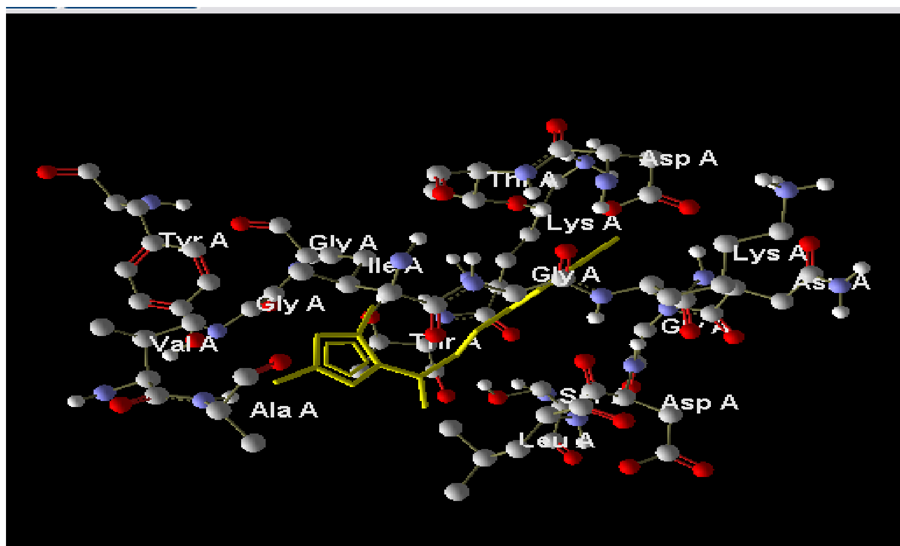


**Fig 4: Binding orientation and H-bond interactions (Green) of KVP3 (Yellow) within the active binding site region of 4KD7 (Residues)**

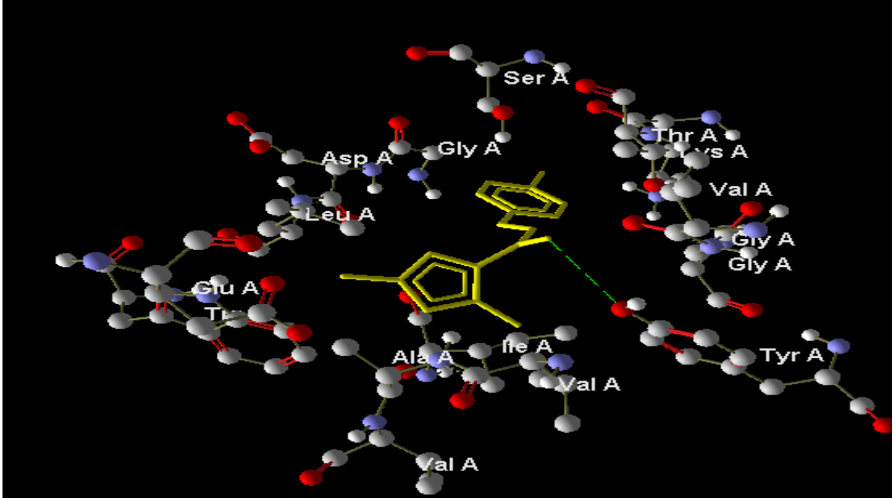


**Fig 5: Binding orientation and H-bond interactions (Green) of KVP4 (Yellow) within the active binding site region of 4KD7 (Residues)**

## DISCUSSION:

**Synthesis**

The compounds synthesized in the present study were keeping with the expected structures by its spectral data as shown in the Tables **4-8**. Following are the representative UV, IR, $^1$H-NMR spectra of the

**Biological Evaluation**

Cancer is the uncontrolled growth of cells that interfere with the growth of healthy cells. The usual treatments of Cancer are surgery, chemotherapy (treatment with anticancer drugs), radiation, or some combination of these methods. Anti-Cancer drugs are targeted to control and treat various Cancer like, Breast cancer, Cervical cancer, Small cell lung cancer, Head and Neck cancer, Ovarian cancer, Hodgkin's and Non-Hodgkin's lymphoma, Oesteo-sarcoma, Seminomas of testis, Myeloblastic leukemia, Lymphoblastic leukemia etc. The use and application of drugs synthesized or procured from natural or synthetic sources for cancer inhibition and cure is known as "chemotherapy" and the drugs are more commonly named as chemotherapeutic drugs.

Brine shrimp (Artemia salina) was used as a simple bioassay tool for cytotoxicity test on new chemicals. The procedure determined $ED_{50}$ value in µg/ mL of active compounds in the brine medium. The activities of known active compounds are manifested as toxicity to shrimps. The advantages of this method are being rapid, reliability, inexpensive and convenient assay [14] (Meyer et al., 1982).

Brine Shrimp Lethality bioassay has an excellent good standard correlation with cytotoxic activity in human solid tumors, and has led to the discovery of new class of natural or synthetic active anticancer agents[15] (McLaughlin et al., 1998).

Brine Shrimp Lethality bioassay has correlation with cytotoxic activity in NCI (National Cancer Institute) cancer cell lines [16,17,18](Mohammed et al., 2009, Mirza et al., 2007, Pimenta et al., 2003).

The investigation of cytotoxicity screening data (Table 9) revealed that the compound **KVP2** demonstrated comparatively the most potent cytotoxicity, with $ED_{50}$ value of 5.43 ± 0.16 µg/mL (Table 9). It is interesting to note that the compound **KVP4** also showed appreciable cytotoxicity with $ED_{50}$ value of 12.84 ± 0.31 µg/mL. The other compounds such as KVP, KVP1 and KVP3 displayed less activity at concentrations >50, >50 and 36.75 ± 0.88 µg/mL, respectively. A close insight into basic chemical structure of the molecules synthesized in the present study clearly displayed the significance of pyrazole and α,β-unsaturated ketone moieties forming part of the core structure as seen in case of most of the natural products such as pyrazole analogs and chalcone flavonoids which are currently in clinical use as potential anticancer agents.

The remarkable observation towards the observed cytotoxicity was substitution and unsubstitution of the phenyl ring B of the chalcone bridge which is covalently attached to the 1H-Nitrogen atom of compounds **KVP, KVP1-4**, respectively. The results of the spectral data of the compounds **KVP, KVP1-4** were in close agreement with those of the structural features of the pyrazoles and the structures of all the compounds were confirmed by their corresponding spectral data.

pyrazole nucleus. On the other hand, there was a clear evidence that further substitution of **KVP** by α,β-unsaturated ketone initially not showed significant activity as seen in case of **KVP1** but strong improvement has been noticed on substitution with halogens on ring B as seen in case of the compounds **KVP2-4**. From the observed activity it was clearly suggested that besides pyrazole the halogen substituent on ring B is evenly necessary to maintain the effectiveness.

**Computational Evaluation**

**Common Mechanism of Action of Anti-Cancer Drugs:**

Cancer drugs have been designed to slowly act on the cancerous cells and halt their progression by suppressing them through various molecular mechanisms:

a) They may act by damaging the DNA of cancerous cells. The anticancer drugs cause single strand (SSB) and double strand (DSB) DNA breaks or may lead to manufacture of nonsense DNA or RNA. Examples of drugs in this category include Cisplatin, Mitomycin C, Daunorubicin, Doxorubicin and Etoposide.
b) They inhibit the synthesis of new DNA to stop the cell from replicating because replication of cells leads to growth of tumor. These agents work in a number of different ways. DNA building blocks are folic acid, heterocyclic bases, and nucleotides, which are made naturally within cells. All of these agents work to block some step in the formation of nucleotides or deoxyribonucleotides (necessary for making DNA). When these steps are blocked, the nucleotides, which are the building blocks of DNA and RNA, cannot be synthesized. Thus the cells cannot replicate because they cannot make DNA without the nucleotides. Examples of drugs in this category include methotrexate, fluorouracil, hydroxyurea and mercaptopurine.
c) They stop mitosis or the actual splitting of the original cells into cell into two new cells. Stopping mitosis stops cell division (replication) of the cancer cells and may ultimately halt the progression of the cancer.

In the present investigation we have selected inhibition of synthesis of new DNA as the potential hypothetical mechanism for the observed cytotoxicity potential of bioactive pyrazole **KVP** and **KVP1-4** (**Table 9**). Hence docking simulation has been performed to determine possible protein-ligand interactions within the active binding site region of the **4KD7** (selected anticancer antimetabolite target protein DHFR) (Table 10, Figure1-5).

In the recent past, the interactions between the selective synthetic DHFR ligands revealed the importance of hydrogen bonding with the amino acids **Asn 64**, **Phe 31** and **Phe34** are important for increased affinity to human DHF[13] R (Lambet al., 2013). The compound **KVP2** revealed one hydrogen bond interactions with the single amino acid **Ser 59** respectively. Since, the bioactive pyrazole **KVP2** not displayed the interactions with amino acids which earlier reported to be most vital towards inhibition of DHFR. Therefore, we hypothesized that the cytotoxicity potential of the **KVP2** might not be due to DHFR inhibition; further target based screening has to be performed to understand the inherent mechanism of action of the series of compounds synthesized in the present study.

## CONCLUSION:

In conclusion, we could synthesis and characterize some novel substituted pyrazoles **KVP, KVP1-KVP4**. These compounds were screened for in-vitro cytotoxicity study by using Brine Shrimp (*Artemia salina*) lethality bioassay and the results revealed the positive and significant contribution of **KVP2** consisting of 2,4-difluoro substitution at position 4 of ring B of chalcone moiety which is covalently attached to the 1H-Nitrogen atom of pyrazole nucleus towards the observed cytotoxicity (*Artemia salina lethality*) at $ED_{50}$ value **5.43 ± 0.16** µg/mL. Likewise, the compound **KVP4** has also been exhibited significant activity not much comparatively with **KVP2** at $ED_{50}$ value **12.84 ± 0.31** µg/mL. The observed remarkable activity of **KVP2** and **KVP4** may be due to the pharmacophores such as pyrazole moiety, α,β-unsaturation moiety, halogen substituents which forming part of basic skeleton of both the molecules. Subsequently, molecular docking studies revealed that the possible underlying mechanism might not be due to the inhibition of DHFR. Further studies have to be processed to understand complete mechanism of cytotoxicity of **KVP2**.

## ACKNOWLEDGEMENTS:

The first author **Kenny Chang** is thankful to the Dean, Faculty of Pharmacy, Asia Metropolitan University, Cheras, Malaysia for providing necessary facilities to carry out research work.

## REFERENCES:

1.Hennessy, B. T., Smith, D. L., Ram, P. T., Lu, Y., & Mills, G. B. Exploiting the PI3K/AKT pathway for cancer drug discovery. Nature reviews Drug discovery, 2005;4(12):988-1004.

2. Fesik, S. W. Promoting apoptosis as a strategy for cancer drug discovery. Nature Reviews Cancer,2005; 5(11): 876-885.

3. Shea, M., Ostermann, L., Hohman, R., Roberts, S., Kozak, M., Dull, R., ...&Sigal, E. Regulatory watch: Impact of breakthrough therapy designation on cancer drug development. Nature Reviews Drug Discovery,2016;15(3), 152-152.

4. Cheng, X. New hopes in the fight against cancer: a special issue on targeted anti-cancer drug discovery and cell signaling. ActabiochimicaetbiophysicaSinica, 2016;48(1): 1-2.

5. Renslo, A. R., &McKerrow, J. H. (2006). Drug discovery and development for neglected parasitic diseases. Nature chemical biology, 2006;2(12): 701-710.

6. Rao, K. N., &Venkatachalam, S. R. Inhibition of dihydrofolate reductase and cell growth activity by the phenanthroindolizidine alkaloids pergularinine and tylophorinidine: the in vitro cytotoxicity of these plant alkaloids and their potential as antimicrobial and anticancer agents.Toxicology in vitro, 2000;14(1): 53-59.

7. Banerjee, D., Mayer-Kuckuk, P., Capiaux, G., Budak-Alpdogan, T., Gorlick, R., &Bertino, J. R. Novel aspects of resistance to drugs targeted to dihydrofolate reductase and thymidylate synthase. BiochimicaetBiophysicaActa (BBA)-Molecular Basis of Disease, 2002;1587(2): 164-173.

8. Schweitzer, B. I., Dicker, A. P., &Bertino, J. R. Dihydrofolate reductase as a therapeutic target. The FASEB Journal,1990; 4(8): 2441-2452.

9. Kaye, S. B. New antimetabolites in cancer chemotherapy and their clinical impact. British journal of cancer, 1998;78(Suppl 3), 1.

10. Hsu, K. C., Chen, Y. F., Lin, S. R., & Yang, J. M. iGEMDOCK: a graphical environment of enhancing GEMDOCK using pharmacological interactions and post-screening analysis. BMC bioinformatics,2011; 12(1): 1.

11. Guniz, K and Sevil, S. "Recent advances in bioactive pyrazoles." European Journal of Medicinal Chemistry,2015; 97: 786–815.

12. Demetrio, R., Benedetta, M., Maria, V., Stella, C., Fabiana, P., Gabriella, C et al.,"Recent advanced in bioactive systems containing pyrazole fused with a five membered heterocycle." European Journal of Medicinal Chemistry,2015; 97: 732–746.

13. Lamb, K. M., G-Dayanandan, N., Wright, D. L., & Anderson, A. C. Elucidating features that drive the design of selective antifolates using crystal structures of human dihydrofolate reductase. Biochemistry, 2013;52(41): 7318-7326.

14. Meyer, BN., Ferrigni, NR., Putnam, JE., Jacobsen, LB., Nichols, DE., McLaughlin JL. “Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents.” Planta Medica,1982; 45: 31-34.

15. McLaughlin, JL.; Rogers, LL., Anderson, JE. “The Use of Biological assays to Evaluate Botanicals.” Drug Information Journal,1998; 32: 513-524.

16. Mohammed, A., Faruqi, F.B., and Mustafa, J. “Edible compounds as antitumor agents. Indian Journal of Science and Technology.” 2009;2(5): 62-74.

17. Mirza, S.H. “Minimising Antibiotic Resistance – Is There a Way Forward? Infectious Diseases.” Journal Pakistan,2007; 16(3): 75-79.

18. Pimenta, LPS., Pinto, JA., Takahashi, Silva LGF., Boaventura, MAD. “Biological screening of Annonaceous Brazilian Medicinal plants using Artemia salina (Brine Shrimp Test).” Phytomedicine,2003; 10: 209-212.

19.Aguilera, A., Alcantara, AR., Marinas, JM., Sinisterra, JV."Ba(OH)2 as the catalyst in organic reactions. Part XIV. Mechanism of Claisen-Schmidt condensation in solid–liquid conditions." J Chem,1987; 65:1165–1171.
20.Ahmed, K., Anver, BS., Nishant, J., Chandan, K., Ananthamurthy, N et al."Design and synthesis of pyrazole-oxindole conjugates targeting tubulin polymerization as new anticancer agents." European Journal of Medicinal Chemistry,2015; 92: 501–513.
21.Chabner, B. A., & Roberts, T. G. Chemotherapy and the war on cancer. Nature Reviews Cancer,2005; 5(1), 65-72.
22.Dwyera, MP., Paruch, K., Labroli, M., Alvarez, C., Keertikar, K., Poker, C et al., "Discovery of pyrazolo[1,5-a]pyrimidine-based CHK1 inhibitors: A template-based approach-Part 1." Bioorg Med Chem Lett ,2011;21: 467-70.
23.Eric, T., Guillaume, L., Natalie, N., Jubrail, R., Anne-Marie, L., Zuomei, L et al,. "Discovery of bicyclic pyrazoles as class III histone deacetylase SIRT1 and SIRT2 inhibitors." Bioorganic & Medicinal Chemistry Letters,2015; 25: 2514–2518
24.Gottesman, M. M., Lavi, O., Hall, M. D., & Gillet, J. P. Toward a better understanding of the complexity of cancer drug resistance. Annual review of pharmacology and toxicology, 2016;56, 85-102.
25.Harish, K., Deepika, S., Sandeep, J., Neelam, J. "Pyrazole scaffold: A remarkable tool in the development of anticancer agents." European Journal of Medicinal Chemistry,2013; 70: 248–258.
26.Hooijberg, J. H., Broxterman, H. J., Kool, M., Assaraf, Y. G., Peters, G. J., Noordhuis, P., ...& Jansen, G. Antifolate resistance mediated by the multidrug resistance proteins MRP1 and MRP2. Cancer Research,1999; 59(11): 2532-2535.
27.Insel, P. A., Amara, S. G., Blaschke, T. F., & Meyer, U. A. Introduction to the Theme "Cancer Pharmacology". Annual review of pharmacology and toxicology,2016; 56, 19-22.
28.Ji, X., Peng, T., Zhang, X., Li, J., Yang, W., Tong, L et al., "Design, synthesis and biological evaluation of novel 6-alkenylamides substituted of 4-anilinothieno[2,3-d]pyrimidines as irreversible epidermal growth factor receptor inhibitors." Bioorg Med Chem2014; 22: 2366-78.
29.Jing, BS., Wen, JT., Xing, B., Rong, L., Xin, HL. "Novel pyrazole-5-carboxamide and pyrazoleepyrimidine derivatives: Synthesis and anticancer activity." European Journal of Medicinal Chemistry,205; 90: 889–896.
30.Kamal, JR., Tamboli, VL., Nayak, SF., Adil, SF., Vishnuvardhan, MV., Ramakrishna, S. "Synthesis of pyrazolo[1,5-a]pyrimidine linked aminobenzothiazole conjugates as potential anticancer agents." Bioorg Med Chem Lett ,2013;23: 3208-15.
31.Kappe, C. O. Biologically active dihydropyrimidones of the Biginelli-type—a literature survey. European journal of medicinal chemistry, 2000;35(12):1043-1052.
32.Kartik, WT., Christina, RR., Gerald, MW., Jan, B., Brian, MC., Katherine, LSR. "Antiproliferative activities of halogenated pyrrolo[3,2-d]pyrimidines." Bioorganic & Medicinal Chemistry,2015; 23: 4354–4363
33.Kassab, E., Gedawy, EM "Synthesis and anticancer activity of novel 2-pyridyl hexahyrocyclooctathieno[2,3-d]pyrimidine derivatives." Eur J Med Chem,2013; 63: 224-30.
Liming, H., Yuyan, Z., Zhipeng, L., Yujie, W., Yongjuan, L., Xuemei, Q., Chengchu, Z. "Design, synthesis, and biological activity of phenyl-pyrazole derivatives as BCR–ABL kinase inhibitors." Bioorganic & Medicinal Chemistry,2015; 23: 3147–3152.
34.Marwa, AI., Sahar, MA., Mona, MH., Mohamed, MA., Nehad, AES."Design, synthesis and biological evaluation of novel condensed pyrrolo[1,2-c]pyrimidines featuring morpholine moiety as PI3Ka inhibitors." European Journal of Medicinal Chemistry,2015; 99: 1–13
35.Marwa, AI., Sahar, MA., Mona, MH., Mohamed, MA., Nehad, AES. "Design, synthesis and biological evaluation of novel condensed pyrrolo[1,2-c]pyrimidines featuring morpholine moiety as PI3Ka inhibitors." European Journal of Medicinal Chemistry,2015; 99: 1–13
36.Meng, L., Bao-Xiang, Zhao. "Progress of the synthesis of condensed pyrazole derivatives (from 2010 to mid-2013)." European Journal of Medicinal Chemistry,2014; 85: 311–340.
37. Meyer, BN., Ferrigni, NR., Putnam, JE., Jacobsen, LB., Nichols, DE., McLaughlin JL. "Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents." Planta Medica,1982; 45: 31-34.
38.Mohammad, S., Abdullah, MM., Bakht, MA., Jaseela, M. "Pyrazoline bearing benzimidazoles: Search for anticancer agent." European Journal of Medicinal Chemistry,2010; 45: 114–119.
39. Mohammed, A., Faruqi, F.B., and Mustafa, J. "Edible compounds as antitumor agents. Indian Journal of Science and Technology." 2009;2(5): 62-74.
40. Mullard, A. Pioneering apoptosis-targeted cancer drug poised for FDA approval. Nature Reviews Drug Discovery,2016; 15(3), 147-149.
41. Nagarapu, L., Vanaparthi, S., Bantu, R., Ganesh Kumar, C. "Synthesis of novel benzo[4,5]thiazolo[1,2-a]pyrimidine-3-carboxylate derivatives and biological evaluation as potential anticancer agents." Eur J Med Chem,2013; 69: 817-22.
42.Navarro-Perán, E., Cabezas-Herrera, J., García-Cánovas, F., Durrant, M. C., Thorneley, R. N., & Rodríguez-López, J. N. The antifolate activity of tea catechins. Cancer research, 2005;65(6): 2059-2064.
43.Nitulescu, G. M., Margina, D., Juzenas, P., Peng, Q., Olaru, O. T., Saloustros, E., ...&Tsatsakis, A. M. Akt inhibitors in cancer treatment: The long journey from drug discovery to clinical use (Review).International journal of oncology, 2016;48(3):869-885.
44. Padmaja, K., Poornima, G., Brahmaiah, B., Pratyusha, C. H., & Nama, S. Indian Journal of Pharmaceutical Science & Research. Indian Journal of

Pharmaceutical Science & Research,2013; 3(2), 69-73.
45.Pimenta, LPS., Pinto, JA., Takahashi, Silva LGF., Boaventura, MAD. “Biological screening of Annonaceous Brazilian Medicinal plants using Artemia salina (Brine Shrimp Test).” Phytomedicine,2003; 10: 209-212.
46.Prasad, S., Gupta, S. C., & Aggarwal, B. B. Serendipity in Cancer Drug Discovery: Rational or Coincidence?. Trends in pharmacological sciences, 2016;37(6), 435-450.
47.Pullela, P. K., Rangappa, P., Alapati, S. R., &Subbarao, P. V. (2010). U.S. Patent No. 7,687,511. Washington, DC: U.S. Patent and Trademark Office.