Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 3, 2012

Research Article

DOCKING, SYNTHESIS, CHARACTERIZATION AND EVALUATION OF NOVEL CDK2 INHIBITORS: BENZOTHIAZOLE DERIVATIVES

PRIYADHARSINI.R*, JERAD SURESH.A, SATHISH.M, KAVITHA.S, SELVIN THANUJA.C

Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai 600003, Tamil Nadu, India.
Email: rpdharsinimpharm@yahoo.co.in

Received: 03 Feb 2012 , Revised and Accepted: 20 Mar 2012

ABSTRACT

Cyclin-dependent kinases (CDKs) are serine/threonine kinases responsible for regulating progression through the cell division cycle. CDK2, a crucial component of the CDK complex, is responsible for the G_1/S phase transition. It was our effect in this present work to synthesize new compounds incorporating various structurally important moieties like benzothiazole, Schiff bases and were docked against CDK2 kinases protein target when BTZ 6 ligand showed two interactions and found to give an good docking score .All the synthesized compounds were screened to study their antioxidant effect comparing to the known standard drug. and also produced the least IC_{50} values in DPPH 45.67±1.2 and p-NDA 224.89±0.7 exhibiting promising antioxidant activity and hence proving to be in the line of drugs used for treatment for cancer disease in future research.

Keywords: CDK2, Drug Design, Docking, Benzothiazole, Schiff base, Antioxidant activity.

INTRODUCTION

Cyclin-dependent kinases (CDKs) are serine/threonine kinases that require association with a cyclin regulatory protein for activation. They are responsible for regulating progression through the cell division cycle, helping to ensure that the eukaryotic genome is replicated only once per cell cycle[1]. CDKs are required for the correct timing and order of events of cell division. CDK2, a crucial component of the CDK complex, is responsible for the G_1/S phase transition. Cells prepare for division during the G_1 phase and the actual chromosome replication occurs during the synthesis or S phase[2]. CDK levels are low during the G_1 phase but elevated during S, G_2, and M phases. As CDKs play such a huge role in the cell division cycle, they have become important targets in drug-discovery programs for cancer, diabetes, and immune diseases[3]. Certain macrocyclic compounds have been found to be inhibitors to the Cyclin A and E binding sites to CDK2 and therefore show great potential as anti-tumor agents. Due to the potent and significance biological activities, benzothiazole has now attained a great importance in pharmaceutical research[4]. Similarly 2-substituted benzothiazole and their derivatives have been found to be potent biologically active compounds. Literature survey reveals that various benzothiazole and Schiff bases have attracted considerable attention as they are also endowed with wide range of pharmaceutical activities[5, 6]. Therefore, we have been interacted to combine two of the above mentioned biologically active rings together in a molecular framework to see the additive effect of the rings to biological activities.

Design of a novel drug is one of the biggest challenges faced by the pharmaceutical industry. Computational methods are used in various forms of drug discovery like QSAR, virtual screening and structure based drug designing methods.

Computer-aided drug design (CADD) [7]

Computer-aided drug design is known as Rational Drug Design. It is the inventive process of finding new medications based on the knowledge of the biological target.

Types of drug design[8]

There are two major types of drug design

- Ligand based drug design (or indirect drug design) and
- Structure-based drug design (or direct drug design)

Scoring method (docking)

Structure based drug design is that a good ligand molecule should bind tightly to its target i.e., docking score.

Molecular docking is an "lock and key" procedure, interested to finding the correct relative orientation of the "key" which will open up the "lock" (where on the surface of the lock is the key hole, which direction to turn the key after it is inserted, etc). The protein can be thought of as the "lock" and the ligand can be thought of as a "key"[8].

Docking study

Docking procedures aim to identify correct posses of ligands in the binding pocket of the protein and to predict the affinity between the ligands and the protein, in other words docking describe a process by which molecules fit together in three dimensional place[9]. Basic requirements for molecular docking approach require the following components. A target protein structure with or without a bound ligand, the molecules of interest or a data base containing existing or virtual compounds for docking posses and a computational framework that allows the implementation of the desired docking and scoring procedures for which the following steps are involved[10].

- Selection and investigation of protein
- Protein preparation
- Receptor grid generation
- Ligand database creation
- Ligand preparation
- Ligand receptor docking and predicting activity
- Scaffold identification
- Docking of molecules going to be synthesized

Receptor (Protein Target)

Cyclins are a family of proteins that control the progression of cells through the cell cycle by activating cyclin-dependent kinase (Cdk) enzymes[11]. Cyclins function as regulators of CDK kinases. Different cyclins exhibit distinct expression and degradation patterns which contribute to the temporal coordination of each mitotic event. In contrast to cyclin A1, which is present only in germ cells, this cyclin is expressed in all tissues tested. This cyclin binds and activates CDC2 or CDK2 kinases and thus promotes both cell cycle G1/S and G2/M transitions. Cyclin forms a complex with Cdk, which belongs to activate the Cdk, but the complete activation requires phosphorylation, as well. Complex formation results in activation of the Cdk active site. Cyclins themselves have no enzymatic activity but have binding sites for some substrates and target the Cdks to specific sub cellular locations[12]. Cyclins contain two domains of similar all-α fold, the first located at the N-terminus and the second

at the C-terminus. All cyclins are believed to contain a similar tertiary structure of two compact domains of 5 α helices. There are two main groups of cyclins[13].

- G1/S cyclins- essential for the control of the cell cycle at the G1/S transition.
  - Cyclin A/CDK2- active in S phase
  - Cyclin D/CDK4, Cyclin D/CDK 6 and Cyclin E/CDK2- regulates transition from G1 to S phase.
- G2/M cyclins- essential for the control of the cell cycle at the G2/M transition (mitosis). G2/M cyclins accumulate steadily during G2 and are abruptly destroyed as cells exit from mitosis (at the end of the M phase)
  - Cyclin B/CDK1-regulates progression from G2 to M phase.

Inhibitors[14]

Known CDK inhibitors are p21Cip1 (CDKN1A) and p27Kip1 (CDKN1B). Drugs that inhibit Cdk2 and arrest the cell cycle may reduce the sensitivity of the epithelium to many cell cycle-active antitumor agents and, therefore, represent a strategy for prevention of chemotherapy-induced alopecia.

EXPERIMENTATION

Step I: **Preparation of 2-Amino-6-Chloro Benzothiazole**[15]

Chloroaniline (0.06 mol), Ammonium thiocyanate (0.02 mol) are taken in a beaker. To this bromine (0.02 mol in 10ml of Acetic acid) and water (10 ml) are added and stirred well by maintaining temperature below 5ºc until it dissolves. Then it is placed in microwave oven at 40 ºc for 6 ½ minutes for completion of the reaction. Then resulting solid was separated and recrystallized using ethanol. The purity of the product was established by single spot on the T.L.C plate. Solvent system used was Ethanol: Benzene (8:2). The percentage yield was 85%w/w.

Step II: Preparation of 6-Chloro-2-Hydrazinyl Benzothiazole[16]**:**

To the product (0.82 m mol) obtained from the above first step hydrazine hydrate (0.11 m mol) and 50ml of ethylene glycol was added and it is kept in an oven at 40 ºc for 1 minute. The colour of the reaction changes to greenish white indicates completion of the reaction. The obtained solid was filtered and recrystallised by using ethanol. The purity of the product was established by single spot on the T.L.C plate. Solvent system used was Ethanol: Benzene (9:1). The percentage yield was 81%w/w.

Step III: Preparation of 6-Chloro-2-[2-(1-Phenylethylidene) Hydrazinyl]-1, 3-Benzothiazole[17]

Compound (3a-3c)

To the small amount of sample (1.5 m mol) obtained from step II, glacial acetic acid (2-3 drops) and acetophenone (2.2 m mol) is added. And to this 40ml of ethanol is added in a 250 ml beaker. It is then kept in a microwave oven at 40 ºc for10 minutes. The product obtained was recrystallized using ethanol. The purity of the product was established by single spot on the T.L.C plate. Solvent system used was Ethanol: Benzene (8:2). The percentage yield was 73%w/w. Melting point:178ºC; UV λmax: 265nm; IR: 3052(Ar C-H Str), 1567(Ar C-C Str), 759(Ar C-H Bending), 2917, 2958 (Al C-H Str), 1443(C-H Bending), 1361(Hetero C-N), 1602(Hetero C=N), 652(Hetero C-S), 916(Ar C-Cl),3418(C=N-H,b); δ_H :7.9(3H) , 7.5(5H), 2.3(1 H), 1(3H); m/z: 301(M^+ ion), 118(100%).

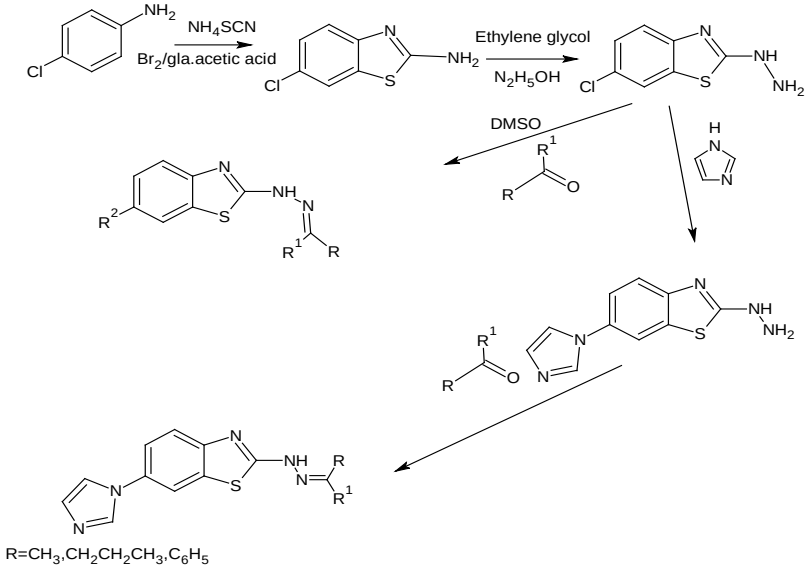
Step IV: Preparation of 6-Imidazolyl-2-Hydrazinyl Benzothiazole[18]

5-chloro-2-hydrazinyl benzothiazole (0.001 mole), imidazole (0.001 mole) in DMSO (25ml) is kept in an oven at 40 ºc for 5 minutes. 5-imidazolyl-2-hydrazinyl benzothiazole obtained was recrystallized using ethanol. The purity of the product was established by single spot on the T.L.C plate. Solvent system used was Ethanol: DMSO (1:2). The percentage of yield was 65%w/w.

Step v: Preparation of 6-Imidazolyl-2-[2-(1-Phenylethylidene) Hydrazinyl]-1, 3-Benzothiazole[19]**:**

Compound 5: (5a-5c)

To the small amount of sample (1.5 m mol) obtained from step IV (imidazole derivative), glacial acetic acid (2-3 drops) and acetophenone (2.2 m mol) is added. Then it is added to 40ml of ethanol taken in a 250 ml beaker. It is then kept in an oven at 40 ºc for 10 minutes. The product obtained was recrystallized using ethanol. The purity of the product was established by T.L.C plate. The solvent system used was Ethanol: Benzene. The percentage yield was 77%w/w. UV λmax: nm; IR: 3052(Ar C-H Str), 1567(Ar C-C Str), 759(Ar C-H Bending), 2917, 2958 (Al C-H Str), 1443(C-H Bending), 1361(Hetero C-N), 1602(Hetero C=N), 652(Hetero C-S), 3418(C=N-H, b); δ_H :7.9(3H) , 7.5(5H), 2.3(1 H), 1(3H), 7.85(1H),7.15(1H), 7.73(1H); m/z: 333(M^+ ion), 118(100%).



Scheme

RESULTS AND DISCUSSION

Free radical damage may lead to cancer. Antioxidant interact with and stabilize free radicals might otherwise cause. The studies of structure-activity relationship interestingly reveal that change of its bioactivity. Hence certain 2-hydrazinyl substituted benzthiazoles are synthesized by microwave assisted synthesis and characterized by UV, IR, NMR, and MASS spectral datas.

Literature survey revealed that these benzothiazole derivatives were found to posses antioxidant and anticancer activities. All the newly synthesized compounds were also screened for antioxidant activity by following two methods.

1. DPPH Method
2. P-NDA Method

The percentage of scavenging activity is determined and found to produce following IC50 values were tabulated for both methods. BTZ_2, BTZ_6, BTZ_1 were found to exhibit more efficient antioxidant activity.

Antioxidant activity

Free radical scavenging activity using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical (2, 2-diphenyl-1-picryl hydroxyl) [20]

The ability of the synthesized compounds to scavenge the free radicals was determined by an in vitro assay method using a stable free radical1, 1-diphenyl-2-picrylhydrazyl (DPPH). The effect of synthesized compounds on DPPH radical was assayed using the method of mensor et al (2001)[17]. A methanolic solution of 0.5ml of DPPH (0.4M) was added to 1ml of the different concentration of all synthesized compounds and allowed to react at room temperature for 30 minutes. Methanol serves as the blank and DPPH in methanol without the sample served as the positive control. After 30 minutes, the absorbance was measured at 517nm and converted into percentage radical scavenging activity as follows

$$\text{Percentage Inhibition} = = \frac{A_{518}\ \text{Control} - A_{518}\ \text{Sample}}{A_{518}\text{Control}} \times 100$$

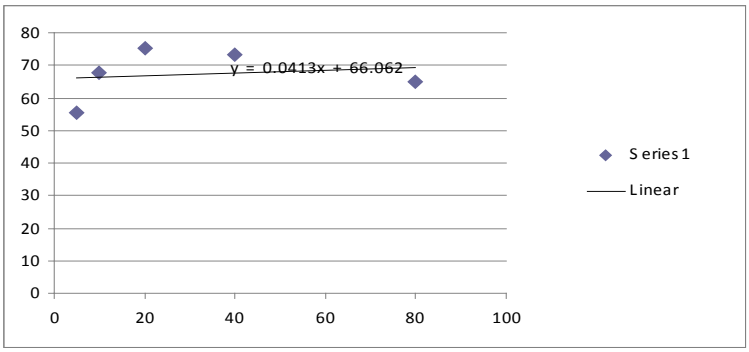
Where, A_{518} Control is the absorbance of DPPH radical + methanol; A_{518} sample extract/standard.

DPPH Method

Table 1:

1. BTZ_1

Conc.	Per. Inhibition
5	55.34
10	67.73
20	75.4
40	73.18
80	65.02

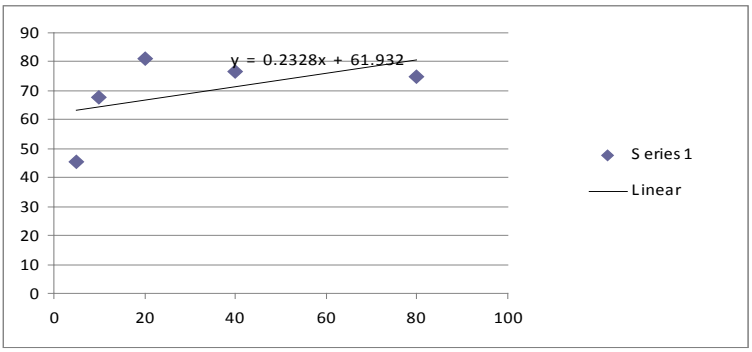


Graph 1

Table 2:

2. BTZ_2

Conc.	Per. Inhibition
5	45.46
10	67.83
20	80.87
40	76.45
80	75.03

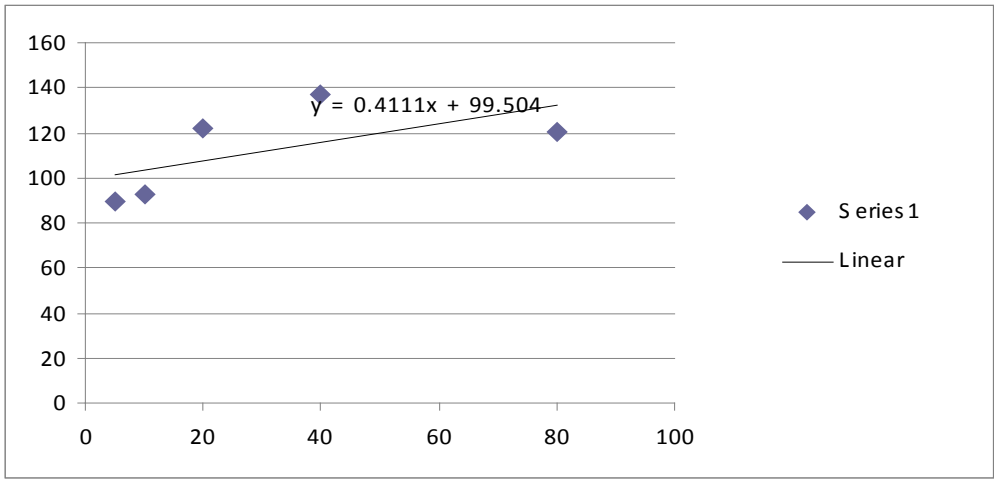


Graph 2

Table3:

3. BTZ_3

Conc.	Per. Inhibition
5	89.45
10	92.67
20	121.67
40	136.89
80	120.56

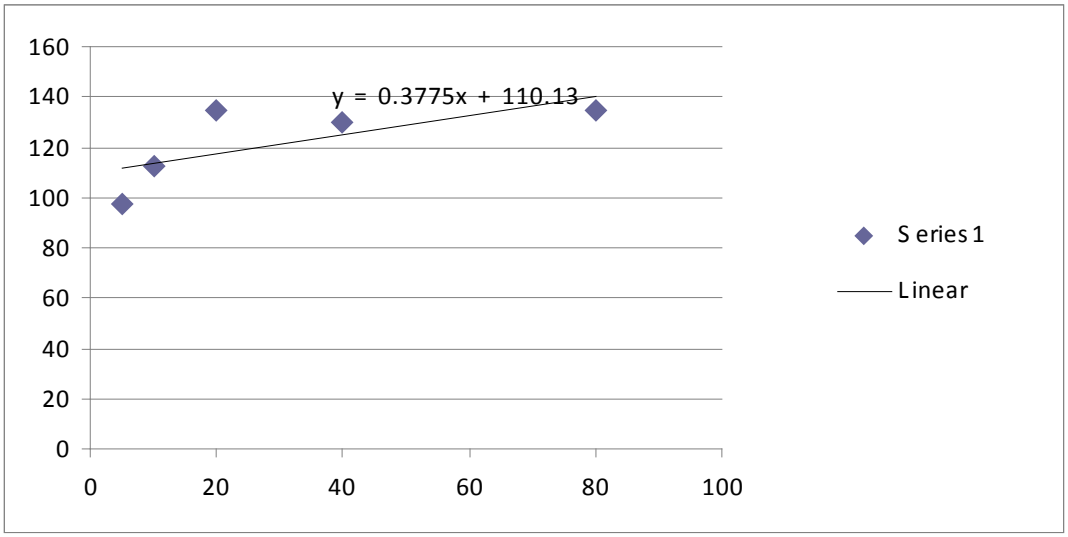


Graph 3

Table 4:

4. BTZ_4

Conc.	Per. Inhibition
5	97.35
10	112.32
20	134.78
40	129.85
80	134.87

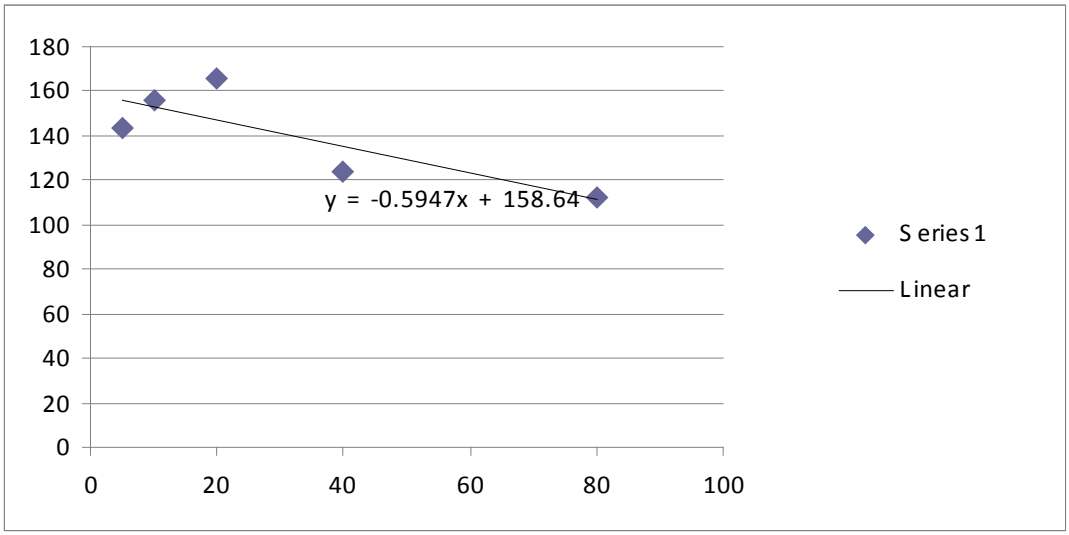


Graph 4

Table 5:

5. BTZ_5

Conc.	Per. Inhibition
5	143.12
10	156.34
20	165.56
40	123.78
80	112.28

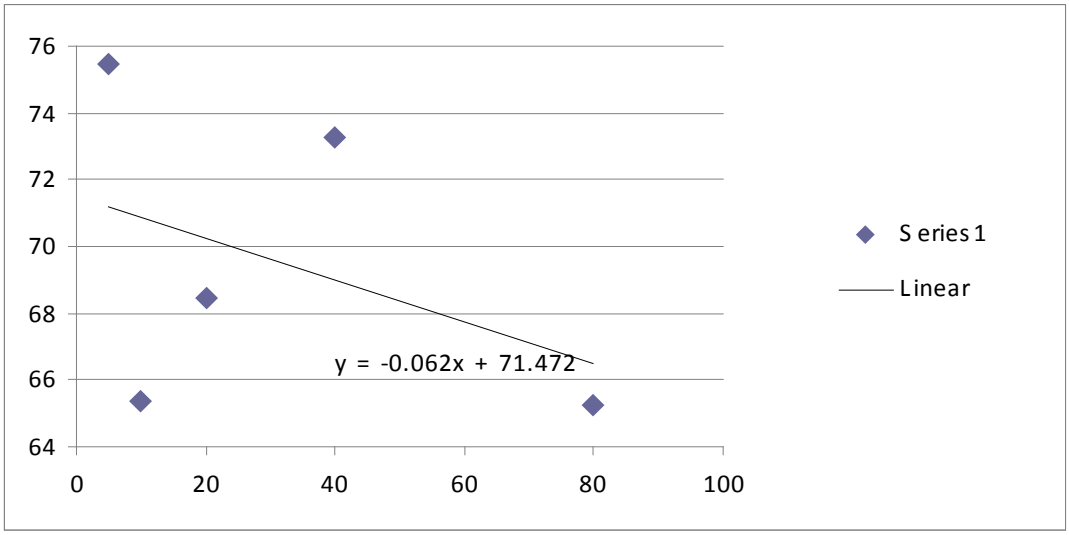


Graph 5

Table 6:

6. BTZ_6

Conc.	Per. Inhibition
5	75.45
10	65.34
20	68.45
40	73.27
80	65.24



Graph 6

Table 7:

S.no.	Tested materials	DPPH method
1	BTZ-1	56.15±0.16
2	BTZ-2	24.78±1.4
3	BTZ-3	64.67±0.56
4	BTZ-4	56.87±0.89
5	BTZ-5	78.34±0.13
6	BTZ-6	45.67±1.2
7	ASCORBIC ACID	2.85±0.6

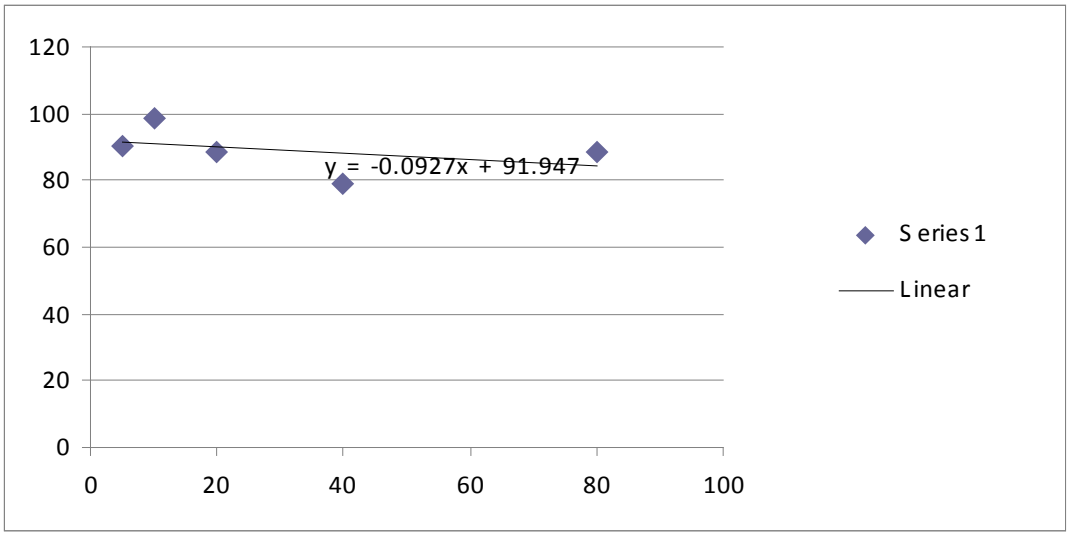
Scavenging of hydroxyl radical in the p-nitro dimethyl amine (p-NDA) method [21]

To a solution containing ferric chloride (0.1mM, 0.5ml), EDTA (0.1mM, 0.5ml), ascorbic acid (0.1mM, 0.5ml), H_2O_2 (2mM, 0.5ml) and p-NDA (0.5mM, 0.5ml) in phosphate buffer (pH of the extract or standard in distilled DMSO) 0.5ml to produce a final volume of 3ml. Absorbance was measured at 440nm.

Table 8:

1. BTZ_1

Conc.	Per. Inhibition
5	90.37
10	98.73
20	88.75
40	78.85
80	88.67

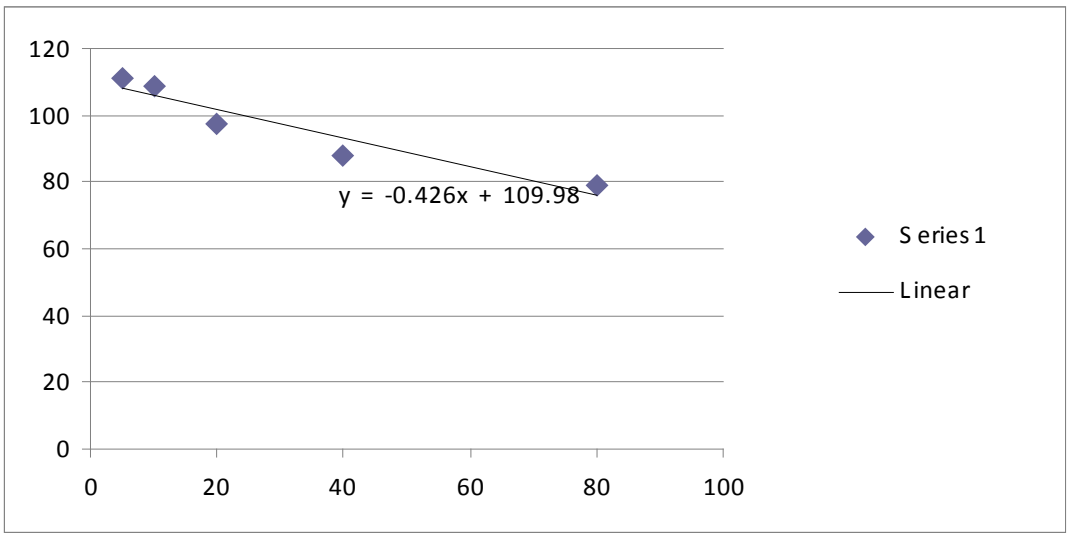


Graph 7

Table 9:

2. BTZ_2

Conc.	Per. Inhibition
5	110.98
10	108.67
20	97.56
40	87.78
80	78.90

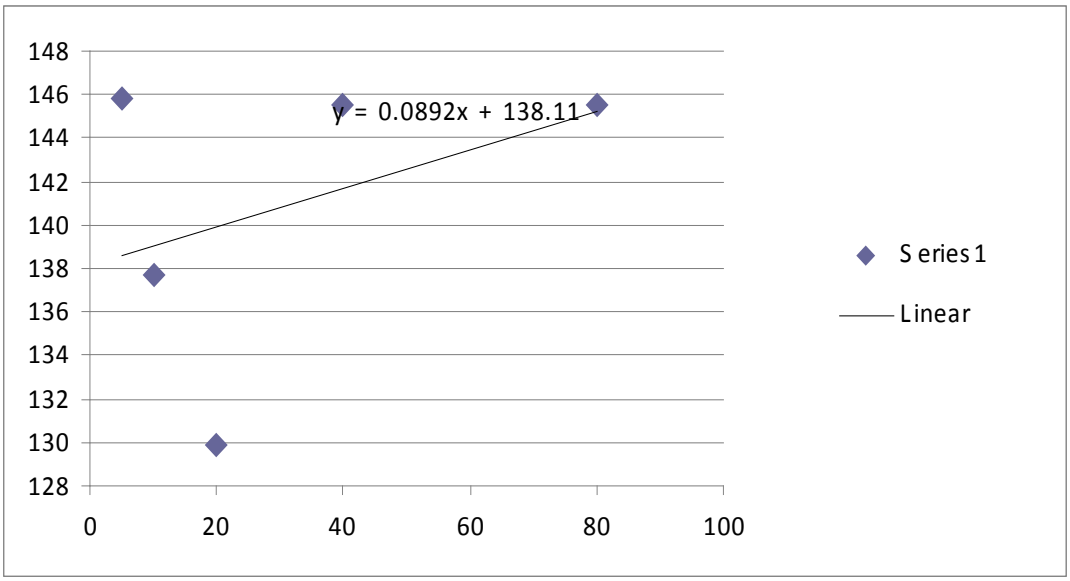


Graph 8

Table 10:

3. BTZ_3

Conc.	Per. Inhibition
5	145.78
10	137.67
20	129.87
40	145.49
80	145.56

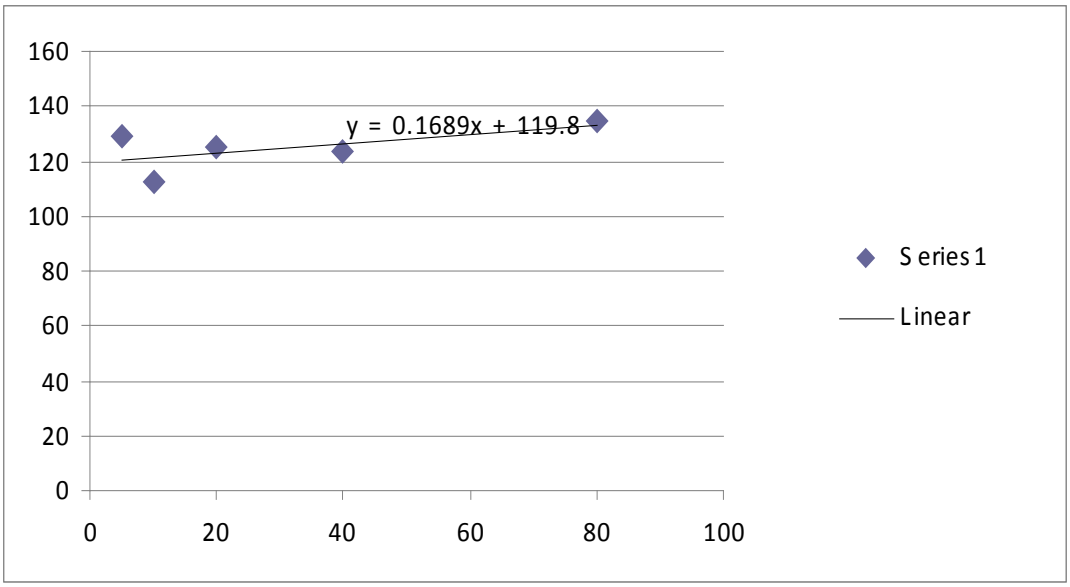


Graph 9

Table 11:

4. BTZ_4

Conc.	Per. Inhibition
5	129.35
10	112.34
20	124.78
40	123.85
80	134.87

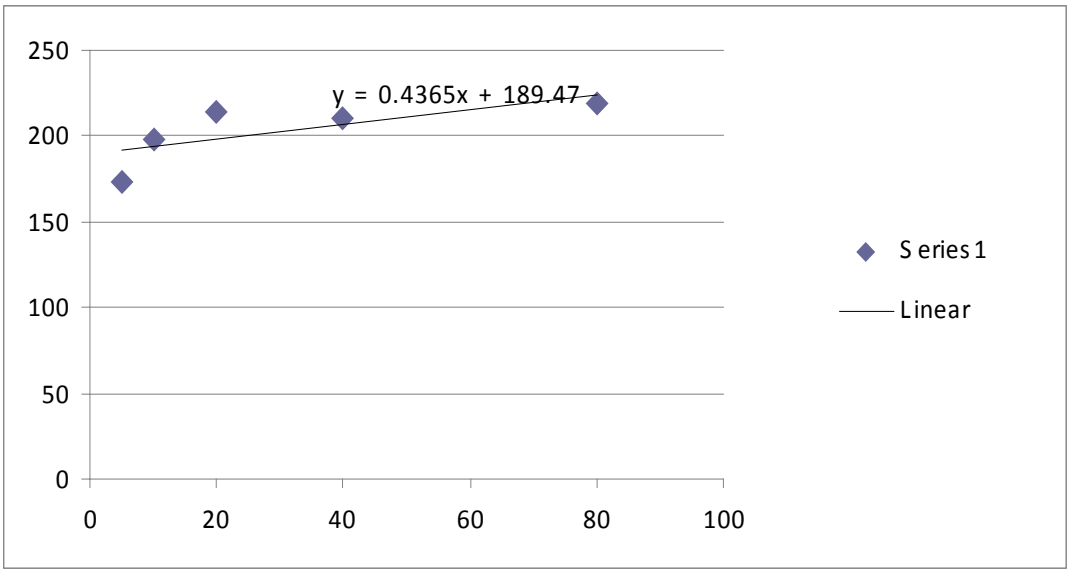


Graph 10

Table 12:

5. BTZ_5

Conc.	Per. Inhibition
5	173.12
10	198.34
20	213.56
40	210.78
80	219.23

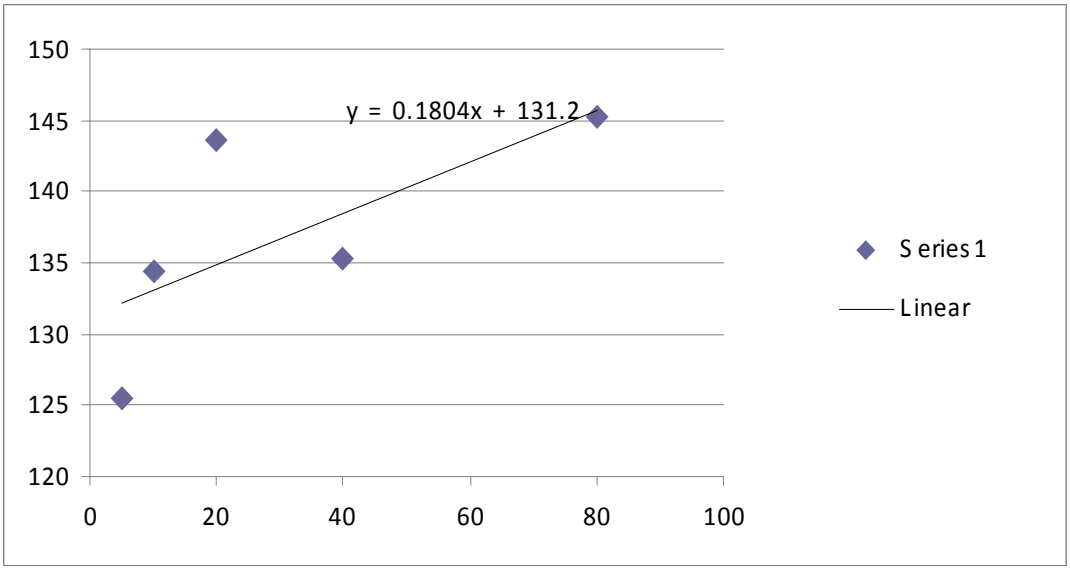


Graph 11

Table 13:

6. BTZ_6

Conc.	Per. Inhibition
5	125.45
10	134.34
20	143.67
40	135.27
80	145.24



Graph 12

Table 15:

S. No.	Tested materials	p-NDA method
1	BTZ-1	267.56±0.15
2	BTZ-2	345.12±1.56
3	BTZ-3	597.23±0.45
4	BTZ-4	475.23±0.56
5	BTZ-5	564.23±0.34
6	BTZ-6	224.89±0.7
7	ASCORBIC ACID	12.78±0.6

CONCLUSION

*Over time, free radical damage may become irreversible and lead to disease including cancer. 2- Substituted benzothiazole has emerged in its usage as a core structure in the diversified therapeutically applications. Totally six compounds like BTZ_1, BTZ_2, BTZ_3, BTZ_4, BTZ_5, BTZ_6 were synthesized from chloroaniline by Microwave assisted synthesis and characterized by UV, IR, NMR, MASS spectral data.

Docking results

Moreover the newly synthesized compounds were docked with CDK cyclin A_2 receptor, an efficacious target for exhibiting antitumor action and BTZ_6ligand proved to produce better binding efficacious exhibiting two of the following interactions.

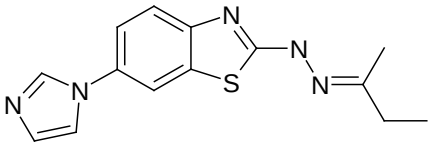
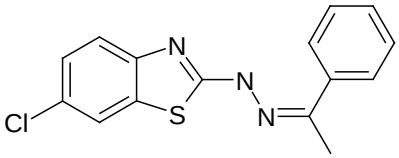
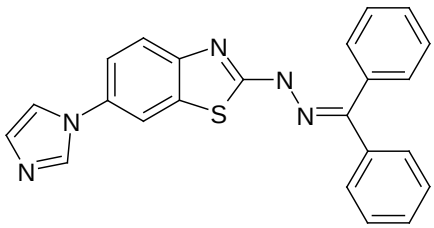
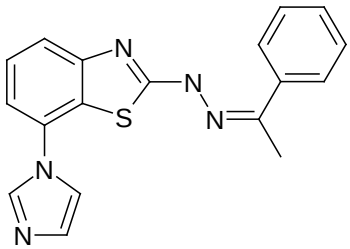
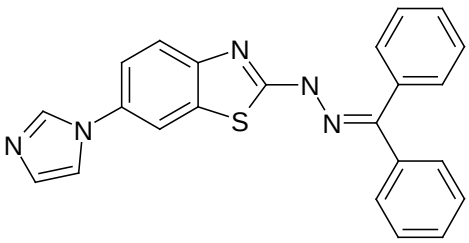
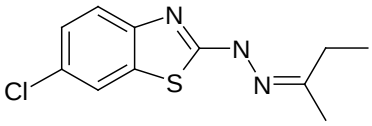
One is between LEU 83 and Nitrogen of IMIDAZOLE (2.120)

Second interaction is between HYDROGEN of IMIDAZOLE with ASP 86 (2.354)

*By adopting DPPH procedure for free radical scavenging activity, BTZ_2 compound found to possess least IC_{50} value of 24.78±1.4 producing more efficient antioxidant activity and BTZ_5 has produced highest IC_{50} value of 78.34±0.13 exhibiting lesser antioxidant activity whereas all other four compound produced moderate action. From the results obtained by performing p-NDA method for free radical scavenging activity, it was found to be BTZ_6 compound found to possess least IC_{50} value of 224.89±0.7 producing more efficient antioxidant activity and BTZ_3 has produced highest IC_{50} value of 597.23±0.45 exhibiting lesser antioxidant activity whereas all other four compound produced moderate action.

* When all the newly synthesized compounds were docked with CDK cyclin A_2 receptor, a efficacious target for exhibiting antitumor action, only BTZ_6 ligand found to give an good docking score and also produced the least IC_{50} values in DPPH 45.67±1.2 and p-NDA 224.89±0.7 exhibiting promising antioxidant activity and hence proving to be in the line of drugs used for treatment for cancer disease in future research.

Table 1:

S. No.	Ligand	Structure	Docking score
1	Ligand no.7		-6.517661
2	Ligand no.1		-6.111412
3	Ligand no.4		-5.774776
4	Ligand no.2		-5.193168
5	Ligand no.5		-5.125003
6	Ligand no.6		-4.794078

Structure of CDK2 CYCLIN –A

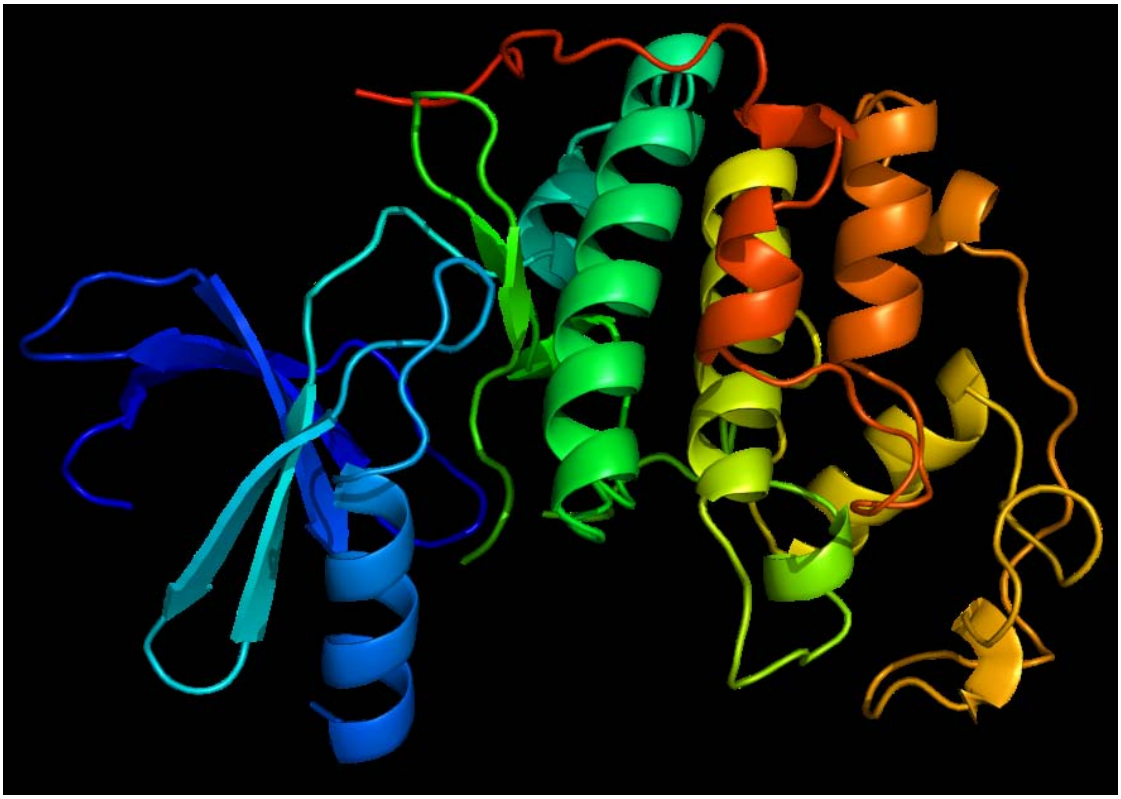


Fig. 1:

Docking results (BTZ WITH CDK CYCLIN A)

Snapshot

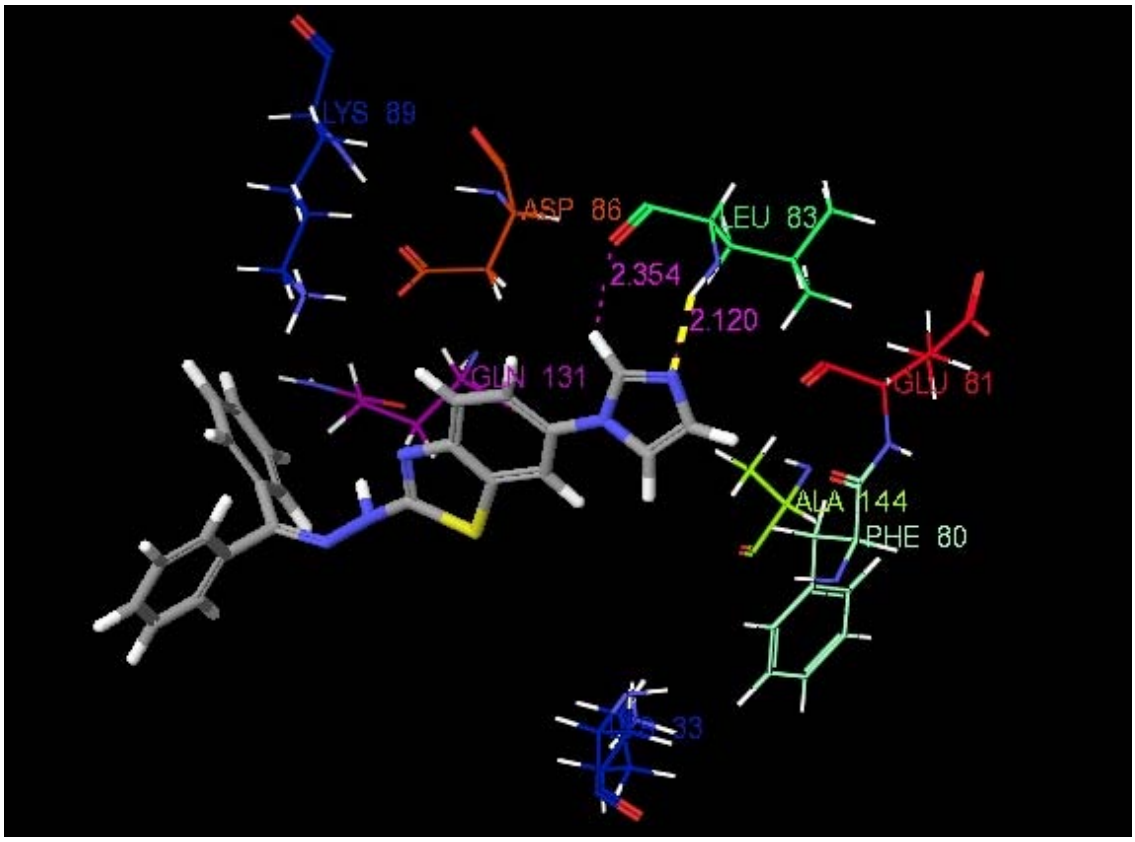


Fig. 2:

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