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Biological Evaluation of Some Novel Sulphur Substituted Chalcones as New Scaffold

Mr. Mejo Joseph[1*], Dr.S. Alaxander[2], Dr. J. Banurekha[3] and Dr. Anandkumar[4]

[1*] *Nehru College of Pharmacy, Thiruwilvamala, Thrissur Dist.Kerala. India.680588.*

[2,3.] *Vinayaka Mission College of Pharmacy, Vinayaka Mission Research Foundation (Deemed to Be University), Salem, Tamil Nadu. India.636308*

[4] *Swamy Vivekanandha College of Pharmacy, Namakkal. Tamilnadu, India*

Abstract: In the past few decades, developing antifungal and anticancer agents has become essential in drug research. Cancer is one of the most challenging diseases in the world. Most reviews on chalcones suggested that substituted chalcones have numerous biological activities. This study aim includes synthesizing different Sulphur containing chalcones in the presence of para-amino acetophenone linked to various aldehydes in the presence of aqueous alcoholic alkali. The structural analysis of synthesized compounds was also carried out by IR, 1HNMR 13CNMR and MASS spectra. The docking study was performed by Biovia discovery studio 20202. Invitro cytotoxic activity was carried out by human breast cancer cell line MCF-7 using MTT assay. Antifungal activity was conducted by diffusion method against *Candida albicans* and *Aspergillus niger*. Novel synthesized compounds, especially BT-IV-A, BT-IV-B, BT-IV-C, BT-IV-E and BT-IV-I showed significant antifungal activity as BT-IV-H, BT-IV-E, and T-IV-F revealed excellent growth-inhibitory impact for the development of new anticancer agents.

Keywords: Thiophene, Benzothiophene, Chalcones, Antifungal, *Aspergillus niger* Cytotoxic and MCF-7

***Corresponding Author**

Mr. Mejo Joseph , Nehru College of Pharmacy, Thiruwilvamala, Thrissur Dist.Kerala. India.680588.

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1. INTRODUCTION

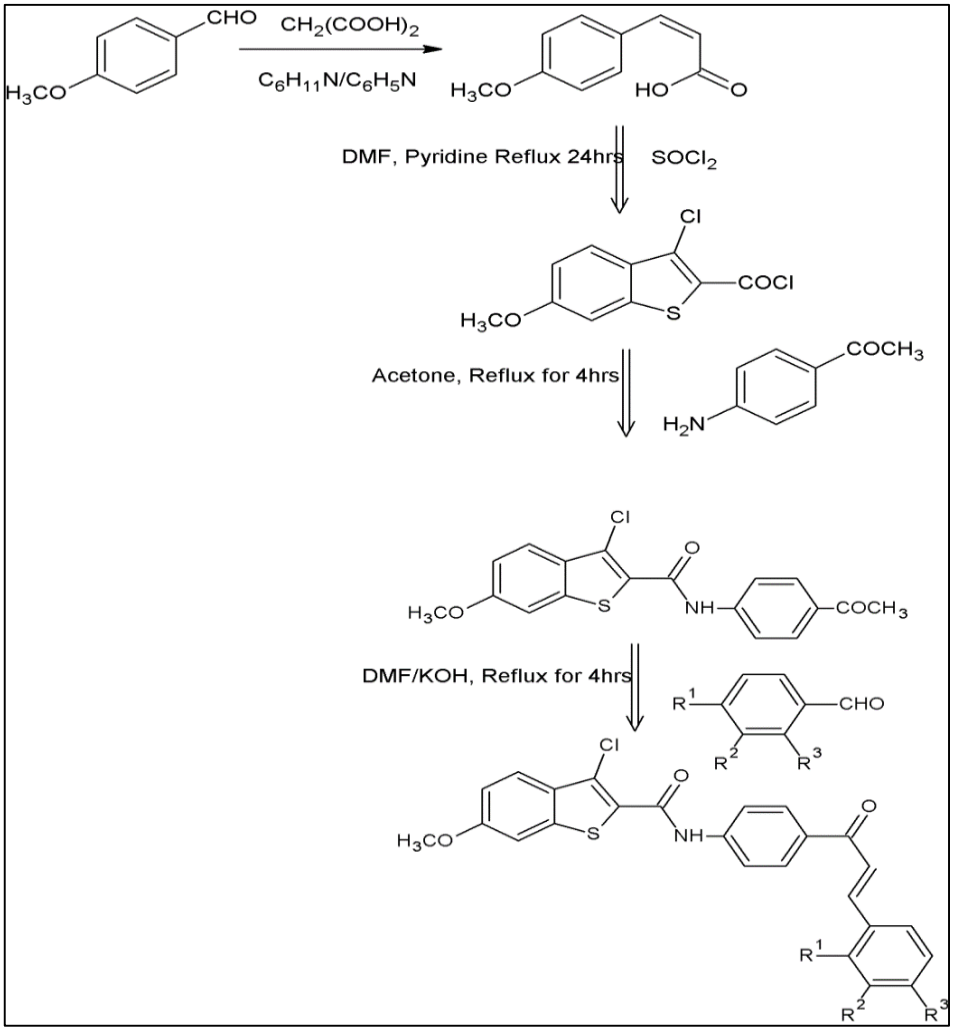
Drugs are the chemical entity that prevents disease or assists in restoring health to diseased individuals. As such, they play an indispensable role in the modern system of medicine[1]. Medicinal chemistry is the branch of science, that provides these drugs through discovery or design. Classical drugs were primarily discovered in the last century by altering natural substances or entirely by laboratory synthesis[2]. In recent years, an ever-increasing understanding of diseases' pathophysiology has led to novel opportunities for deliberate design, synthesis, and evaluation of candidate drug molecules[3]. Pharmaceutical chemistry is the branch of science that studies the molecular and mechanical aspects of pharmaceuticals. The discipline emphasizes the chemistry of drug design and development, drug action, drug transport, drug delivery, and targeting[4]. The development of new pharmaceuticals is critically dependent on a molecular-level understanding of biological processes and mechanisms of drug action. Progress in the field now depends on designing and synthesizing new molecules using structure-activity relationships, combinatorial chemistry, and computer-aided drug design[5]. In recent years, the rational design of drugs tuned to specific target sites is becoming a reality due to advances in chemistry and biology, including elucidating the human genome[6]. Chemists remain at the forefront of drug design, synthesis, testing, and development[7]. Molecular biology and genetic engineering have produced a deluge of potential new drug design targets and have unraveled traditional targets' structures and mechanisms. In contrast, advances in computers and computer-aided design have allowed medicinal chemists to take full advantage of this newly earned knowledge[8]. The first successful attempts at designing a drug to work at a particular target happened nearly simultaneously in 1976 with the discovery of cimetidine, a selective H_2-antagonist, and Captopril, an angiotensin-converting enzyme inhibitor. Since then, the art of rational drug design has undergone an explosive evolution, using the sophisticated computational and structural methodology to help in the effort. A literature survey reveals that when one biologically active heterocyclic system is coupled with another, there will be an increase in the biological activity of the resultant molecule. In the present study, Sulphur-containing heterocyclic compounds have been synthesized in which benzothiophene and thiophene are linked with methyl ketone and different aldehydes to form chalcones. In sulfur-containing heterocycles, thiophene and benzothiophene substituted chalcone derivatives are at the focus as these candidates have structural similarities with active compounds to develop new potent lead molecules in drug design[9]. Thiophene and benzothiophene scaffold is one of the privileged structures in drug discovery as this core exhibit various biological activities allowing them to act as antimicrobial, anticancer, anti-inflammatory, antioxidant, anti-tubercular, antidiabetic, anticonvulsant agents, and many more[10]. Further, numerous thiophene and benzothiophene-based compounds as clinical drugs have been extensively used to treat various diseases with high therapeutic potency, leading to their extensive developments[11]. Due to the wide range of biological activities of substituted benzothiophene and thiophenes, their structure-activity relationships (SAR) have generated interest among medicinal chemists, culminating in the discovery of several lead molecules against numerous diseases[12]. The present work aims to highlight the progress in the various pharmacological activities of benzothiophene and thiophene-substituted chalcone derivatives. It is hoped that this work will be helpful for new thoughts in the quest for rational designs of more active and less toxic medicinal compounds. Also, biological studies highlighting the chemical groups responsible for evoking the pharmacological activities of synthesized derivatives are studied and compared. As the world population increases and health problems expand accordingly, the need to discover new therapeutics will become even more tiring. The design of drug molecules arguably offers some of the greatest hopes for success in the present and future eras. Heterocyclic compounds are widely distributed in nature and are essential for life. There are vast numbers of pharmacologically active heterocyclic compounds, many of which are in regular clinical use. Therefore, the investigational approaches toward Structure-Activity Relationship focusing on the search of optimized candidates have become immensely important. Thus this study aim includes synthesizing different Sulphur containing chalcones in the presence of para-amino acetophenone linked to various aldehydes in the presence of aqueous alcoholic alkali. Invitro cytotoxic activity was also carried out by human breast cancer cell line MCF-7 using MTT assay. Antifungal activity was conducted by diffusion method against *Candida albicans* and *Aspergillus niger*.

1.1 Chalcones

The chemistry of chalcones has generated intensive scientific studies throughout the world. Primarily interest has been focused on the synthesis and biodynamic activities of chalcones. Kostanecki and Tambor gave the name "Chalcones." These compounds are also known as benzalacetophenone or benzylidene acetophenone. An aliphatic three-carbon chain links two aromatic rings in chalcones. Chalcone bears a very good synthon, so a variety of novel heterocycles with a good pharmaceutical profile can be designed[13]. Chalcones are -unsaturated ketone containing the reactive ketoethylenic group –CO-CH=CH-. These are coloured compounds because of the Chromophore -CO-CH=CH-, which depends on the presence of other auxochromes. Chalcones and their derivatives demonstrate wide range of biological activities such as antidiabetic, anti-neoplastic, anti-hypertensive, anti-retroviral, anti-inflammatory, anti-parasitic, antihistamine, anti-malarial, antioxidant, antifungal, anti-obesity, anti-platelet, anti-tubercular, immunosuppressant, anti-arrhythmic, hypnotic, anti-gout, anxiolytic, antispasmodic, anti-nociceptive, hypolipidemic, anti-filarial, anti-angiogenic, anti-protozoal, anti-bacterial, etc[14]. The present scheme I describe the synthesis of novel substituted methoxy derivatives of benzothiophene and their different chalcone derivatives. The compounds were prepared from malonic acids that reacted with para anisaldehyde in the presence of pyridine and piperidine to form para methoxy cinnamic acid. In the presence of a catalytic amount of pyridine, thionyl chloride gently oxidizes carboxylic acid and ketone at alpha carbon atoms to form α-chloro-α-chlorosulfenyl derivatives and their subsequent reaction products. Thus 3-phenylpropionic acid, when treated with an excess of thionyl chloride and a small amount of pyridine, can be converted to sulfenyl chloride, which undergoes further reaction to form benzothiophene and α-chloro cinnamoyl chloride, which on further refluxed with para amino acetophenone in the presence of dry acetone to form 4-(acetyl phenyl)-3-chloro-methoxy-1-benzothiophene-2-carboxamide (IV) which further on by Claisen Schmidt condensation which involves crossed aldol condensation of appropriate aldehyde in the presence of base-catalyzed reactions followed by dehydration to form different derivatives of 3-chloro-6-methoxy-N-[4-(2E)-3-phenyl prop-2-enyl]phenyl-1-benzothiophene-2-carboxamide in respectively.

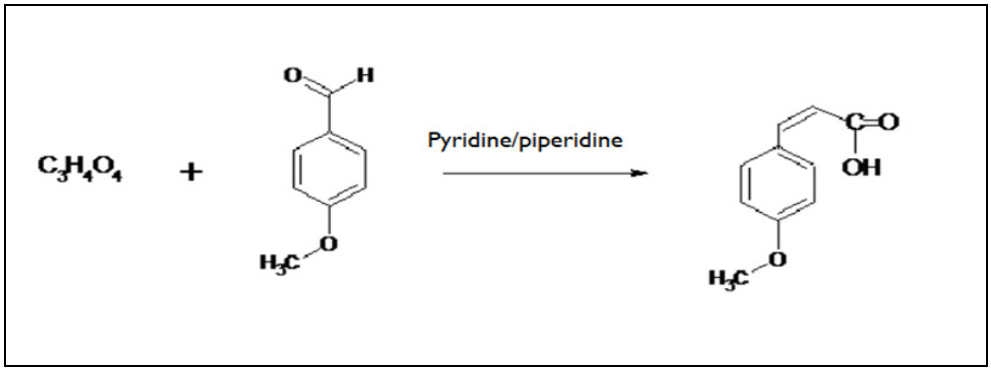
2. MATERIALS AND METHODS

General scheme (I) for the preparation of compounds-BT-IV-A, BT-IV-B, BT-IV-C, BT-IV-D, BT-IV-D, BT-IV-F, BT-IV-G, BT-IV-H, BT-IV-I, BT-IV-J.



SCHEME-I (BT-IV)

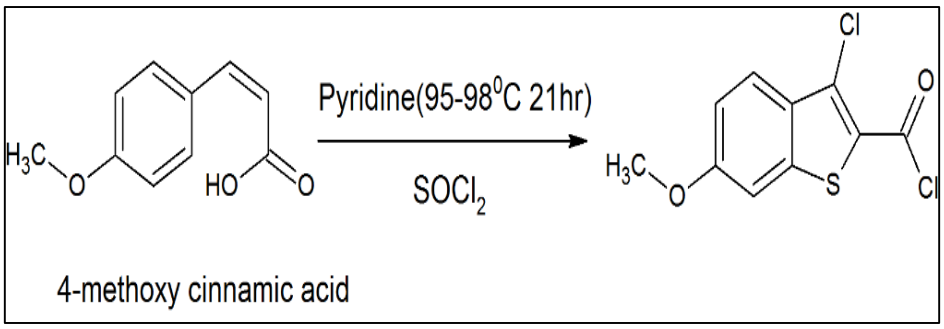
2.1 Synthesis of 3-(4-Methoxy Phenyl) Acrylic Acid (I)



Procedure

Malonic acid 60gm, para anisaldehyde 40.8gm, dry pyridine 3ml were mixed in a 500 ml 3 necked bottle equipped with a thermometer and a condenser[15]. The mixture was heated for 2hrs at 100^{0}C, then refluxed gently for 15 min at 120^{0}C. The mixture, while still hot, was poured into 180 ml of concentrated hydrochloric acid and 250 grams of chopped ice under stirring. The precipitate was filtered, washed with 25 ml of 10% hydrochloric acid, and water, and then dried. Recrystallization from ethanol afford 37.2gram (79% yields) as long white needles. The melting point was found to be 116-119^{0}C. ^{1}HNMR: δppm (DMSO.d_6): δ=3.65(s.3H.OCH3),6.01(dd.1H, J=8.HZ),6.75(dd,1H, J=4H_Z, ArH)7.43(dd,1H, J = 4H_Z, ArH),7.52(dd,1h, J=8H_Z),7.95(s,1H, OH). IR Data: (cm^{-1}):2860 (OCH3), 1650(C=O), 1428(C=C), 1080(=CCl), 1192(C=C), 1288(C-H), 1028-1596(C-C), 688(C-S-C). (M/z) 179.

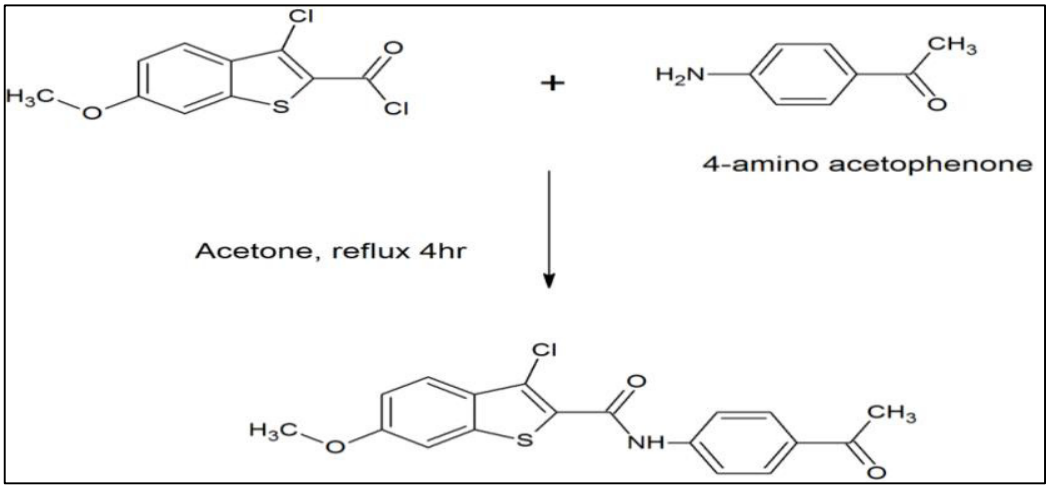
2.2. Synthesis of 3-Chloro-6-Methoxy Benzothiophene-2-Carbonyl Chloride (II)



II

To a mixture of para methoxy cinnamic acid 4.2gm, pyridine 0.5ml, and DMF 1ml was added 6ml of thionyl chloride dropwise. After stirring for 30 minutes at 140^{0}C, the reaction mixture was taken up in 100 ml of dry hexane, heated and decanted from the gummy residue, filtered the product under vacuum condition dried in an oven at 60^{0}C. TLC was checked by n-hexane and ethyl acetate in a ratio 9:1. The residue was dissolved and recrystallized in hot ethanol. The yellow decanted solution solidified to give the products 3.3gm (55%). Melting point 118^{0}C. ^{1}HNMR: δppm ($CDCl_3$): 200 MHz): 7.70 (d, 1H, J = 8.8 Hz), 7.32-7.23 (m, 2H), 3.93 (s, 3H, CH_3); IR Data: (cm^{-1}):2860(OCH3), 1650(C=O), 1562(C=C), 1080(=C-Cl), 688(C-S-C). (M/z): 260

2.3 Synthesis of 4-(Acetyl Phenyl)-3-Chloro-6-Methoxy-1-Benzothiophene-2-Carboxamide (III)



III

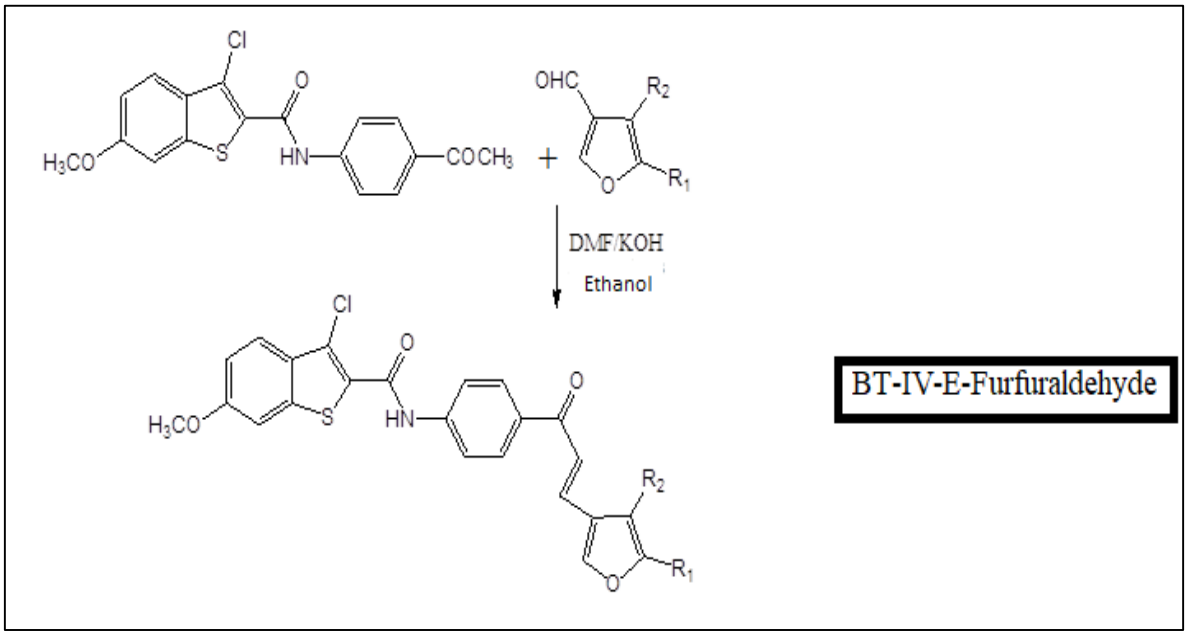
A mixture of 4-amino acetophenone 1.35gm, and 3-chloro-6-methoxy benzothiophene-2-carbonyl chloride 2.60gm dissolved in 40ml. dry acetone The reaction mixture was refluxed for 4hrs. Periodically sodium carbonate was added to neutralize HCl evolving during the reaction. Finally, the reaction mixture was cooled and poured into the crushed ice. The resulting ppt was filtered, washed with water, and recrystallized from methanol. White needles are formed. The melting point was found to be 168-170^{0}C. IR-Data: (cm^{-1}): 2860-2800(OCH3), 3220(N-H), 1650(C=O), 1562(C=C), 1080(=C-Cl), 688 (C-S-C). ^{1}H-NMRδ(ppm) (due. chloroform) 3.78and 3.85(2s,6H, OCH_3,10.89(s,1H, CONH),8.19-7.60 (m,8H, Ar-H), 2.55(s,3H, CH3): M/z: 359.0377

2.4 Synthesis of Substituted 6-Methoxy-N-{4-[(2E)-3-Phenylprop-2-Enoyl] Phenyl}-1-Benzothiophene-2-Carboxamide. (IV)

Procedure

3.6gm of 4-(acetyl phenyl)3-chloro-6-methoxy-1-benzothiophene-2-carboxamide dissolved in 20ml DMF and 1.06gm corresponding aldehydes to the reaction mixture with constant stirring at room temperature, then 40%potassium hydroxide in distilled water was added to the reaction mixture with constant stirring at room temperature. After 24 hrs the reaction mixture was poured into crushed ice and neutralized with HCl. The ppt was filtered, washed with water, dried and recrystallized in methanol to obtain the corresponding aldehyde derivatives of the compound (BT-IV). After drying in an oven at about 60^{0}C, yield 59%. The melting point was found to be 174^{0}C. Check the TLC by n-hexane and chloroform (9:1) as an eluent. BT-IV-A, BT-IV-B, BT-IV-C, BT-IV-D, BT-IV-F, BT-IV-G, BT-IV-H, BT-IV-I, BT-IV- J, T-IV-F, T-IV- G, T-IV-J were prepared by similar methods.

2.5 General Scheme (II) For The Method of Preparation Of -3-Chloro-N-[4-(3-(Furan-3-Yl) Acryloyl) Phenyl]-6-Methoxy Benzo[B]Thiophene-2-Carboxamide



SCHEME-II

Procedure

3.6gm(0.01mole) of 4-(acetyl phenyl)3-chloro-6-methoxy-1-benzothiophene-2-carboxamide dissolved in 20ml DMF, and 0.96gm furfural aldehydes were added to the reaction mixture with constant stirring at room temperature, then 40%potassium hydroxide in distilled water was added to the reaction mixture with constant stirring at room temperature. After 24 Hrs, the reaction mixture was poured into crushed ice and neutralized with HCl. The ppt was filtered, washed with water, dried, and recrystallized in methanol to obtain the corresponding aldehyde derivatives of compound BT-IV-E. After drying in an oven at about 69^{0}C, yield 69%. The melting point was found to be 171^{0}C. Check the TLC by n-hexane and chloroform (9:1) as an eluent.

2.6 Spectral Data of Synthesized Derivatives

3-chloro-N-(4-cinnamoylphenyl)-6-methoxybenzo[b]thiophene-2-carboxamide (BT-IV-A)

IR-Data: (cm^{-1}): 3342(N-H, Stretching), 1710(C=O Stretching, Ketone), 1674(C=O, Stretching, Amide), 2825(OCH_3),1587(C=C Aromatic Stretch),1060(=C-Cl, Stretch) 825(C-H Aromatic), 738(CH=CH, Stretching), 674(C-S-C, Stretch). ^{1}H-NMR-δ ppm: ($CDCl_3$, 200MHz) 9.15(s, 1H, CONH), 8.09-7.01(m.12H. Ar-H), 8.07-7.59(d, 2H, CH=CH), 3.87(s, 3H, OCH_3). ^{13}C-NMR-δ ppm:145.1,121.3-Ar-C Ethylene,55.8-CH3 Aliphatic,189.7-Ar-C(C=C) Carbonyl,135.2,128.6,128.5, 127.9-Ar-C-(C=C) Benzene, 143.7,133.5, 131.4,1 22.1-Ar-C(N-C=O) Benzene, 159.4,152.8, 143.7, 141.6,129.9,125.7-Ar-C(C=O) Cl Benzothiophene. M/z: 448.91

3-chloro-N-(4-(3-(2-chlorophenyl) acryloyl) phenyl)-6-methoxybenzo[b]thiophene2- carboxamide (BT-IV-B)

IR-Data: (cm^{-1}): 3340(N-H Stretching), 2833(OCH3 Stretching), 1710(C=O, Stretch Ketone), 1674(C=O, Stretch Amide), 1482(C=C, Aromatic stretching), 1060(=C-Cl,Stretch) 740(CH=CH, Stretching), 607(C-S-C Stretching) ^{1}H-NMR-δ ppm: ($CDCl_3$, 200MHz) 9.18- (s, 1H, CONH), 8.48-7.07(m, 14H, Ar-H), 8.30-7.48, (d, 2H, CH=CH), 3.83(s, 3H, OCH_3). ^{13}C-NMR-δ ppm: 145.1,121.3-ArC- of ethylene(C-H), 55.8- Aliphatic (CH3),189.7-(ArC) of carbonyl,161.8-ArC of N- Amide, 126.3,129.3,127.3,129.9,131.4,134.7-ArC of Benzene(C=C) Cl, 131.4,122.1,133.5, 143.7-ArC of Benzene(N-C=O),129.9,141.6,152.8,125.7-ArC of Benzothiophene (C=O) Cl,124.2 ArC of Benzothiophene (O-C), M/z 483.39

3-chloro-6-methoxy-N-(4-(3-(2-nitrophenyl) acryloyl) phenyl) benzo[b]thiophene-2- Carboxamide (BT-IV-C)

IR-Data: (cm^{-1}): 3387(N-H Stretching), 2829(OCH_3 Stretching), 1713(C=O, Stretching, Ketone), 1691(C=O, Amide), 1547(C=C, Stretch, Aromatic), 1381(NO_2 Stretch), 1063(=C-Cl, Stretching), 713(C-S-C Stretching). ^{1}H-NMR-δ ppm: ($CDCl_3$, 200MHz) 9.18- (s, 1H, CONH), 8.48-7.07(m, 14H, Ar-H), 8.30-7.48, (d, 2H, CH=CH), 3.83(s, 3H, OCH_3). ^{13}C-NMR-δ ppm: 145.1,121.3-ArC of ethylene(C-H),55.8-C Aliphatic CH3,189.7-ArC of carbonyl,161.8-ArC of N- amide, 126.3, 129.3,127.3,129.9,131.4,134.7-ArC of Benzene(C=C) Cl,131.4,122.1,133.5,143.7-ArC of Benzene (NC=O),129.9,141.6,152.8,125.7-ArC of Benzothiophene(C=O) Cl,124.2ArC-Benzothiophene (O-C). M/z 493.95

3-chloro-6-methoxy-N-(4-(3-(3-nitrophenyl) acryloyl) phenyl) benzo[b]thiophene- 2- carboxamide (BT-IV-D)

IR-Data: (cm^{-1}): 3328(N-H Stretching), 2825(OCH_3, Stretching), 1721(C=O, Stretching Ketone), 1678(C=O, stretching, Amide), 1579-C=C, Aromatic Stretching), 1339(NO_2 Stretch), 1067(=C-Cl, Stretch) 739(CH=CH, Stretching) ^{1}H-NMR-δ ppm: ($CDCl_3$, 200MHz) 9.18- (s, 1H. CONH), 8.30-7.07-(m, 11H, Ar-H), 7.90-8.87(d, 2H, CH=CH),3.83- (s,3H, OCH_3). ^{13}C-NMR-δ ppm: 129.9,141.6,

ArC- (C=O) Cl of Benzothiophene,152.8,125.7-Arc (C=O) Benzothiophene, 159.4 ArC(O-C) from benzene,147.8-ArC(-N(C=O), (C=C) Benzene, 143.7,122.1,131.4-ArC(N-C=O), (C=O) Benzene, 115.2,124.2,126.3ArC-Benzothiophene(O-C) from benzene, 137.7, 122.7, 134.6, 123.1,129.5ArC-(N(=O) =O,Benzene(C=C),Benzothiophene,161.8-ArC-(Amide),189.7-Carbonyl, 55. 8-(CH_3), 141.5,121.3-ArC(CH)Ethylene. M/z: 493.95

3-chloro-N-(4-(3-(furan-3-yl) acryloyl) phenyl)-6-methoxybenzo[b]thiophen-2-carboxamide (BT-IV-E)

IR-Data: (cm^{-1}): 3391(NH, Stretching),2817(OCH_3, Stretching),1718 (C=O, Stretching, Ketone,),1658(C=O, Amide),1482(C=C, Aromatic Stretching), 1171(C-O-C, Stretching), 1062(=C-Cl, Stretch) 756(CH=CH, Stretching), 682(C-S-C, Stretching), ^{1}HNMR: δ ppm ($CDCl_3$): 9.18 (s,1H,CONH),8.18-6.89(m,10H,ArH),7.12-7.09(d,2H,CH=CH),3.87-(s,3H,OCH_3)^{13}C-NMR-δ ppm: ($CDCl_3$,200MHz)129.9,141.6ArC(C=O) Cl, Benzothiophene, 152.8,125.7 ArC (C=O)Cl,(OC)Benzothiophene,143.0,139.0,124.4,ArC(C=C) Furan,159.4,115.8,124.2Ar(CH), Benzothiophene,143.7,133.5,122.1,131.4-ArC(CH)Benzene.161.8-ArC-(N-Amide),189.7- Carbonyl, 55.8-9CH3), 145.1, 127.3- ArC(CH)-C=O ethylene. M/z: 438.90

3-chloro-6-methoxy-N-(4-(3-p-tolylacryloyl) phenyl) benzo[b]thiophene-2- carboxamide (BT-IV-F)

IR-Data: (cm^{-1}): 3321(N-H Stretching), 2868(C-CH_3 Stretch), 2817(OCH_3, Stretch),1721(C=O, Stretch, Ketone), 1659(C=O, Stretching Amide),1590(C=C, Aromatic Stretching), 1468(CH_3 Stretching), 1060(=C-Cl, Stretch), 677(CH=CH Stretching), 636(C-S-C Stretching). ^{1}H-NMR-δ ppm: ($CDCl_3$, 200MHz) 9.18- (s,1H, CONH), 8.07-7.14 (m,11H, ArH),7.18-7.12(d,2H, CH=CH), 3.87 (s,3H, OCH_3), 2.34-2.30(s,3H, CH_3). ^{13}C-NMR-δ ppm: 121.3,145.1-ArC-(C(=O) Ethylene, 21.3, 55.8-ArC-(CH3) Aliphatic,189.7,161.8-ArC-(N-Amide), 128.9,128.5-ArC-(C=C) of Benzene, 131.4,122.1,143.7-ArC-(NC=O(C=O), 129.9, 141.6, 152.8,125.7-ArC-(C=O) Cl, Benzothiophene, 159.4,115.8,124.2(CH)-ArC-(O-C) of Benzothiophene, M/z: 461.00

3-chloro-N-(4-(3-(4-chlorophenyl) acryloyl) phenyl)-6-methoxybenzo[b]thiophene- 2- carboxamide (BT-IV-G)

IR-Data: (cm^{-1}): 3336(N-H Stretching), 2823(OCH_3 Stretching), 1715(C=O Stretch Ketone), 1659(C=O, Stretching Amide), 1596(C=C, Stretching), 1096(=C-Cl, Stretch), 976(C=C Bending), 661(C-S-C Stretching), ^{1}H-NMR-δ ppm: ($CDCl_3$, 200MHz) 9.15(s,1H, CONH), 8.09-7.60(m,11H, Ar-H),7.49-7.48(d,2H, CH=CH), 3.83.3.87(s,3H, OCH_3). ^{13}C-NMR-δ ppm: 129.9, 141.6, 152.8, 125.7-ArC-(C=O) Cl-of Benzothiophene, 133.5, 133.3-ArC-Cl(C=C) of Benzene. 128.7, 129.0-ArC-(C=C) Cl Benzene. 115.8, 124.2, 126.3(CH)-ArC-(O-C) Benzothiophene from benzene,133.5,122.1,131.4-(N-C=O)-C=O of Benzene, 189.7, 161.8-ArC-(N-Amide), 55.8-ArC-(CH3) Aliphatic, 121.3,145.1-ArC-(C(=O) Ethylene, M/z: 483.01

3-chloro-N-(4-(3-(4-hydroxyphenyl) acryloyl) phenyl)-6-Methoxybenzo[b] thiophene-2-carboxamide (BT-IV-H)

IR-Data: (cm^{-1}): 3566(O-H Stretching), 3283(N-H Stretching), 1715 (C=O Stretching Ketone),1693(C=O, Stretching Amide), 1484(C=C Stretching), 1063(=C-Cl, Stretching) 837 (C=C Stretching), 682(CH=CH Stretching) ,795(C-S-C, Stretching). ^{1}H-NMR-δ ppm: ($CDCl_3$, 200MHz) 9.18- (s,1H, CONH),8.06-6.65 (m,11H, ArH),7.54-7.53((d,2H, CH=CH), 5.35(S,1H, OH), 3.87-3.83 (s,3H, OCH3). ^{13}C NMR-δ ppm: 129.9,141.6,152.8,125.7-ArC-(C=O) Cl-Benzothiophene, 159.4ArC-,115.8 (CH) ,124.2, (CH)126.3 (CH)-ArC-(O-C) of Benzothiophene, 157.7 ArC- of Benzene(=O(C=C), 127.8 ArC, 115.8 (CH), 130.6(CH), of Benzene (=O (C=C), 143.7,133.5-ArC, 122.1,131.4, ArC-(N-C=O) of Benzene. 189.7, 161.8-ArC-(N-Amide), 55.8-ArC-(CH3) Aliphatic, 121.3,145.1-ArC-(C(=O) Ethylene M/z: 464.06

N-(4-(3-(4-bromophenyl) acryloyl) phenyl)-3-chloro-6-methoxybenzo[b] thiophene-2- carboxamide (BT-IV-I)

IR-Data: (cm^{-1}): 3338(N-H Stretching), 2828(OCH_3 Stretching), 1738(C=O Stretching Amide), 1650 (C=O Stretch Amide). 1062(=C-Cl, Stretching) 828(C=C, Stretching), 682(CH=CH Stretching),634(C-S-C, Stretching), 590(C-Br, Stretching). ^{1}H-NMR-δ ppm: ($CDCl_3$, 200MHz) 9.18 (s,1H, CONH), 8.09-6.94(m,11H, Ar-H), 7.87 7.85(d,2H, CH=CH), 3.83-3.81(m,3H, OCH_3). ^{13}C-NMR-δ ppm: 129.9,141.6,152.8.125.7ArC(C=O) Cl, 189.7,161.8 ArC-(N-Amide),145.1,121.3 ArC (C=C) Carbonyl, 122.3,134.2,131.5,128.6 (Br C=C) Benzene, 159.4 ArC. Benzothiophene(O-C) from benzene, 143.7,133.5, ArC Benzene (NC=O(C=O),122.1 (Ar-CH) 131.4 ArC (CH) (N-C=O (C=O). M/z: 527.99

3-chloro-N-(4-(3-(4-(dimethylamino) phenyl) acryloyl) phenyl)-6- methoxybenzo [b]thiophene- 2-carboxamide (BT-IV-J)

IR-Data: (cm^{-1}): 3325(N-H Stretch), 2832(OCH_3 Stretch) 2813(N-$(CH_3)_2$ Stretch), 1715(C=O, Stretch Ketone), 1659(C=O Stretch Amide), 1482(C=C Stretching Aromatic), 1060(=C-Cl, Stretch),799(C=C, Stretching), 743(CH=CH Stretching),678(C-S-C, Stretching) ^{1}H-NMR-δ ppm: ($CDCl_3$, 200MHz) 9.18- (s,1H, CONH), 8.18-7.01 (m,11H, Ar-H),7.49-7.48(d,2H, CH=CH),3.87 (m,3H, OCH_3),3.09-3.07(m,3H, CH_3). ^{13}C-NMR-δ ppm (50MHz, CDCl3): 152.8,125.7ArC-Benzothiophene (C=O) Cl-O-C from benzene,129.9,141.6ArC-(C=O) Cl of Benzothiophene, 126.3,124.2,115.8-ArC (CH) of Benzothiophene (O-C) from benzene, 129.7,111.7,129.7 ArC(CH)Benzene N(C) C(C=C), 124.7, 150.37 ArC(CH) Benzene N(C)C(C=C), 143.7,133.5,131.4,122.1ArC(N-C=O(C=O) from benzene, 41.3,55.8ArC(CH3). 189.7ArC(C=C), 161.8(N-Amide). M/z: 492.10.

N-(4-cinnamoylphenyl) thiophene-2-carboxamide(T-IV-F)

IR-Data: (cm^{-1}): 3336(N-H Stretching), 1716(C=O Stretching Ketone), 1657(C=O Stretching Amide). 1551(C=C Stretching Aromatic), 1022 (=C-Cl Stretch), 724 (C-H Bending), 692(C-S-C, Stretching), 657(CH=CH Stretching). ^{1}H-NMR-δ ppm: ($CDCl_3$, 200MHz) 9.18(s, 1H, CONH), 8.49- 7.21 (m, 13H, ArH), 7.21-6.36(d,2H, CH=CH). ^{13}C-NMR-δ ppm:139.4,130.3,131.9,129.0ArC(C=O) of Thiophene, 143.7,133.5,122.1,131.4ArC(N-C=O) Benzene, 161.8(N-Amide), 189.7-ArC(C=C), 145.1,121.3 ArC(C=O) of

Ethylene,135.2,127.9,128.5,128.6-ArC(C=C) Benzene. M/z: 334.14

N-(4-cinnamoylphenyl) thiophene-2-carboxamide (T-IV-G)

IR-Data: (cm^{-1}): 3336(N-H Stretching), 2823(OCH_3 Stretching), 1715(C=O Stretch Ketone),1659(C=O, Stretching Amide), 1596(C=C, Stretching), 1096(=C-Cl, Stretch), 976(C=C Bending), 661(C-S-C Stretching) ^{1}H-NMR-δ ppm: ($CDCl_3$, 200MHz) 9.15(s,1H, CONH),8.09-7.60(m,11H, Ar-H),7.49-7.48(d,2H, CH=CH),3.83.3.87(s,3H, OCH_3). ^{13}C-NMR-δ ppm: 138.4,130.3,131.9,126.0ArC(C=O) of Thiophene, 143.7,133.5, 122.1, 131.4 ArC (N-C=O) Benzene, 161.8(N-Amide), 189.6-ArC(C=C), 144.1,120.3ArC(C=O) of Ethylene, 133.2, 125.9,127.5,128.9-ArC(C=C) Benzene M/z: 366.87

N-(4-(3-(furan-3-yl) acryloyl) phenyl) thiophene-2-carboxamide (T-IV-J)

IR-Data: (cm^{-1}): 3310(N-H Stretching), 1713(C=O Stretching Ketone), 16769(C=O Stretching Amide), 1548(C=C Stretching Aromatic), 1170(C-O-C Stretching Aromatic), 1015(=C-Cl, Stretching), 826(CH=CH Stretching) 628(C-S-C, Stretching. ^{1}H-NMR-δ ppm: ($CDCl_3$, 200MHz) 9.15- (s, 1H, CONH), 8.31-7.12 (m, 11H, ArH), 7.59-7.13 (d,2H, CH=CH). ^{13}C-NMR-δ ppm:139.4, ArC,130.3 (CH), 131.9(CH),129.0 (CH), ArC(C=O) Thiophene, 139.0, 143.0,124.4,108.2-ArC(C=C) Furan,143.7,133.5,122.1, 131.4, 122.1,128.5-ArC (N-C=O) Benzene,161.8(N-Amide),189.7(C=C),145.1, 127.3 ArC(C=O) of Ethylene. M/z: 324.37.

Table 1: Characterization of synthesized compounds

Sl.No	Compounds	Substitutions with BT-IV	Molecular formula	Mol.wt	M.P.^{0}C	%Yield	Rf Value
1	BT-IV-A	Benzaldehyde	$C_{25}H_{18}O_3NCl$	447.94	176	69	0.65
2	BT-IV-B	Ortho-chlorobenzaldehyde	$C_{25}H_{17}O_3SNCl_2$	482.38	184	72	0.77
3	BT-IV-C	Ortho-nitrobenzaldehyde	$C_{25}H_{17}O_5SN_2Cl$	492.94	182	67	0.80
4	BT-IV-D	Meta -nitrobenzaldehyde	$C_{25}H_{17}O_5SN_2Cl$	492.94	182	73	0.81
5	BT-IV-E	Furfuraldehyde	$C_{23}H_{16}O_4SNCl$	437.93	179	71	0.59
6	BT-V-F	Para-methyl benzaldehyde	$C_{26}H_{20}O_3SNCl$	461.97	177	77	0.84
7	BT-IV-G	Para-chloro benzaldehyde	$C_{25}H_{17}O_3SNCl_2$	482.39	178	59	0.76
8	BT-IV-H	Para-hydroxy benzaldehyde	$C_{25}H_{18}O_4SNCl$	463.94	175	73	0.64
9	BT-IV-I	Para-bromo benzaldehyde	$C_{25}H_{17}O_3SNClBr$	526.84	188	72	0.73
10	BT-IV-J	Para-dimethylamino benzaldehyde	$C_{27}H_{23}O_3SN_2Cl$	491.01	180	69	0.75
11	T-IV -F	Benzaldehyde	$C_{20}H_{15}O_2NS$	333.15	147	71	0.38
12	T-IV -G	Ortho-chlorobenzaldehyde	$C_{20}H_{14}O_2SNCl$	367.87	155	68	0.49
13	T-IV-J	Furfuraldehyde	$C_{18}H_{13}O_3NS$	323.38	143	64	0.29

Table 1 illustrates that characterization and substitutions of different aldehydes with 4-(acetyl phenyl)-3-chloro-6-methoxy-1-benzothiophene-2-carboxamide and their molecular formula, molecular weight, melting points, percentage yield and Rf value.

2.7 *Docking Methodology*

Docking in Discovery Studio

In Discovery Studio there are some pre docking steps to perform docking.

Step 1: Open the files of both protein and ligand to dock in DS. For that click on, File → open → choose the file → open.

Step 2: **Protein preparation.** Prepare the protein structure before docking because in general, PDB structures contain water molecules, and all water molecules are removed except the important ones in protein preparation. Hydrogen atoms will be missing in PDB structure; many docking programs need the protein to have explicit hydrogen. Hydrogen can be added unambiguously except in the case of acid/ basic side chains through protein preparation. The PDB structure can be incorrect in some protein side chains. This is because the crystallographic structure gives electron density, not molecular structure. Click on, Macromolecule → Prepare protein → Automatic preparation → Prepare protein → Input protein (select the saved protein structure) → Run. Then save the resultant prepared structure in a new file.

Step 3: Ligand preparation. Preparation of ligand is also done because of some reasons. First, a reasonable 3D structure is needed as starting point. The protonation state and tautomeric form of a particular ligand could influence its hydrogen bonding ability. Small molecule →Prepare/ Alter-ligands →Prepare ligand →Input ligand (select the saved ligand structure) → Run. The prepared structures of ligands are saved in a new file.

Step 4: Define the binding site after the protein and ligand preparation; the next step is to define a binding site for docking. Receptor ligand interaction → Define & Edit binding site [In Define site, there are 3 options: – From Receptor Cavities, From PDB Site Records, and from Current Selection]. Selected the protein and amino acid residues (eg: ILE225, ASN226, ILE227, LEU228, SER229, GLU230, PRO231, PRO232, LYS233, ARG234, and LYS235.) Then click on → Select from Current Selection.

Step 5: Docking Click on Receptor ligand interaction → Dock Ligands → LibDock Libdock was used for docking because a target needs to dock with multiple ligands. After docking, every pose of dock results is analyzed in detail. Then the result was screened based on the presence of H-bond interaction and Libdock score and are listed out. The listed ligand poses are filtered based on the presence of H-bond interaction at GLU230 residue, and the molecular properties of these ligands are calculated in DS by ADMET descriptors and toxicity prediction. The binding energy of the ligands was also calculated.

2.8 Antifungal Activity

Antifungal activity against the following fungus

- *Candida albicans* (MTCC3018)
- *Aspergillus niger*. (MTCC2737)

The synthesized compounds were screened against two selected fungal strains, *Candida albicans,* and *Aspergillus niger*, using the diffusion method. The 48 hours old fungal culture was inoculated into nutrient broth by following aseptic techniques and incubated for 48 hours at 37±2°C in BOD incubator. This culture was mixed with well sterilized and cooled potato-dextrose agar media and poured into Petri plates by pour plate method. Four bores were made at equal distances by using a sterile steel cork borer (8 mm in diameter). Into these cups, 100µg/ml and 200µg/ml concentrations of standard drug and synthesized compounds along with control N, N'-dimethyl formamide were introduced. These plates were placed for 2 hrs. for proper diffusion. After 2 hrs. the petri plates were transferred to BOD incubator and maintained at 37 ± 2°C for 24 - 36 hours. After the incubation period, the plates were taken to measure the inhibition zone using a Hi-antibiotic zone reader. Results were evaluated by comparing the zone of inhibition shown by the synthesized compounds with standard drugs. The results were the average value of the zone of inhibition measured in millimeters of three sets. The standard drug fluconazole was dissolved in distilled water, and the synthesized compounds were dissolved in a minimum quantity of DMF and diluted to get the required concentration.

2.9 Invitro Cytotoxic Activity

MTT assay was used to determine the anticancer impact of BT-IV-H, BT-IV-E, T-IV-G, T-IV-F, and T-IV-J in vitro using MCF-7 cells (Human breast cancer cell) MCF-7 (Human breast cancer) cells were obtained from the National Centre for Cell Sciences (NCCS) in Pune, India, and were kept in Dulbecco's modified Eagles medium (DMEM) (Sigma Aldrich, USA). The cell line was grown in a 25 cm2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate, and antibiotic solution comprising Penicillin (100U/ml), Streptomycin (100g/ml), and Amphotericin B (2.5g/ml) (Merck, Germany). In a humidified 5 percent CO2 incubator, cultured cell lines were incubated at 37°C (NBS Eppendorf, Germany). The vitality of cells was determined by using an inverted phase contrast microscope to observe the cells directly, followed by the MTT assay method[16].

2.9.1 Cells Seeding in 96 Well Plate

A two-day-old confluent monolayer of cells was trypsinized and suspended in 10% growth media. A 100l cell suspension (5x103 cells/well) was seeded in a 96-well tissue culture plate and cultured at 37°C in a humidified 5% CO2 incubator. A two-day-old confluent monolayer of cells was trypsinized and suspended in 10% growth media. A 100l cell suspension (5x103 cells/well) was seeded in a 96-well tissue culture plate and cultured at 37°C in a humidified 5% CO2 incubator.

2.9.2 Preparation of Compound Stock

1mg of sample was weighed and dissolved in 1mL 0.1% DMSO using a cyclomixer. The sample solution was filtered through 0.22 µm Millipore syringe filter to ensure sterility.

2.9.3 Anticancer Evaluation

After the growth medium was removed after 24 hours, freshly prepared compounds in 5% DMEM were serially diluted five times by twofold dilution (100g, 50g, 25g, 12.5g, 6.25g in 500l of 5% DMEM) and each concentration was added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO2 incubator. Control cells that had not been treated were also kept. Methotrexate is used as a standard medication at the same concentrations as above.

2.9.4 Anticancer Assay by Direct Microscopic Observation

The entire plate was examined in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) after 24 hours of treatment, and microscopic observations were recorded as images[17]. Any visible alterations in cell shape, such as rounding or shrinkage of cells, granulation, and vacuolization in the cytoplasm, were deemed cytotoxicity indicators[18].

2.9.5 Anticancer Assay by MTT Method

15 mg MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved, then filter sterilized. After a 24-hour incubation period, the sample content in the wells was removed, and 30ml of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken, and the plate was incubated for 4 hours at 37°C in a humidified 5 percent CO2 incubator. The supernatant was removed after the incubation period and 100l of MTT was added. To solubilize the formazan crystals, Solubilization Solution (Dimethyl sulphoxide, DMSO, Sigma Aldrich, USA) was added, and the wells were gently agitated by pipetting up and down. At a wavelength of 540 nm, the absorbance values were measured using a microplate reader (Laura B. Talarico). All the experiments were repeated thrice, and data were analyzed by one-way analysis of variance followed by Duncan's multiple range tests using SPSS software. $p<0.05$ was considered statistically significant.

$$\% \text{ of viability} = \frac{(\text{Control Absorbance} - \text{Test Absorbance})}{\text{Control Absorbance}} \times 100$$

3. RESULTS AND DISCUSSION

All the selected compounds were evaluated their antifungal and invitro cytotoxic activity tabulated. The purity of tested compounds more than 95%. The phytopathogenic fungi chosen include *Aspergillus niger* and *Candida albicans.* The cytotoxic evaluation by using MCF-7 human breast cancer cell. The most significant preliminary step in the rational drug creation of new molecules was in silico molecular modification. Prior to wet lab synthesis, *Insilico* molecular modelling studies[19] was carried out in the current study to find a potential lead drug candidate for cytotoxic studies. In the lab, *insilico* experiments were carried out. molinspiration, chemsketch-12.0, and admetSAR Some of them break the Lipinski rule of five, and practically all of them are hepatotoxic but not mutagenic. Biovia Discovery Studio 2020 was used to perform docking experiments on all proposed molecules. The protein ligand mode[20] was predicted using hydrogen bond

interactions, docking score, and other factors. A compound with a docking score of more than 80 is considered for biological testing. Compounds with a high docking score EGFR was used to predict cytotoxicity activity. Figure 1, figure 2 and figure 3 representing the images of different docking interactions with 5EDP-EGFR kinase.

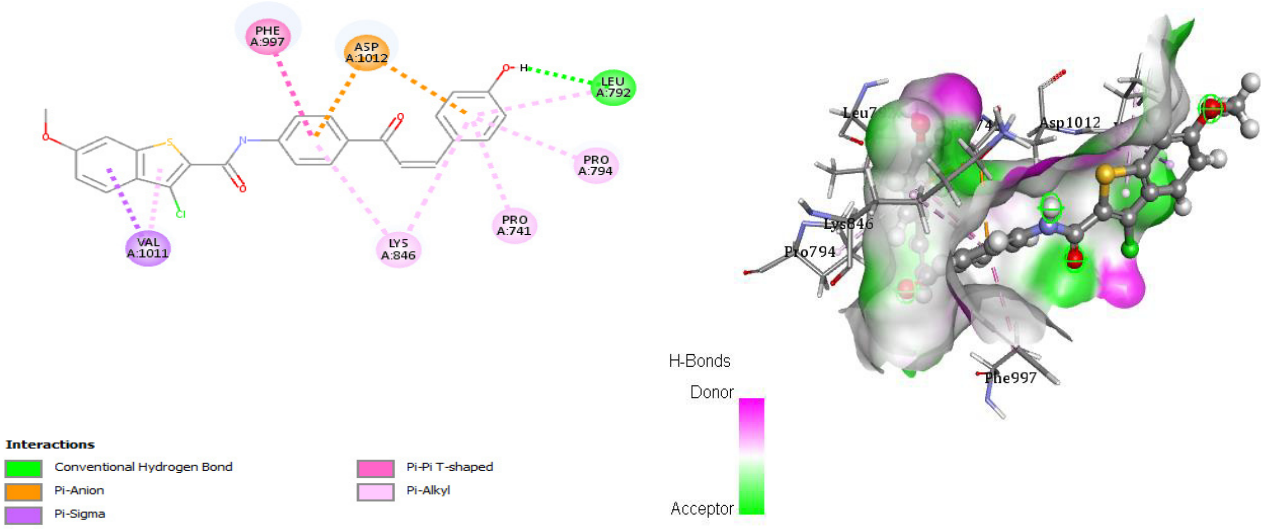


Fig1: Docking image of 5EDP-EGFR kinase with BT-IV-H

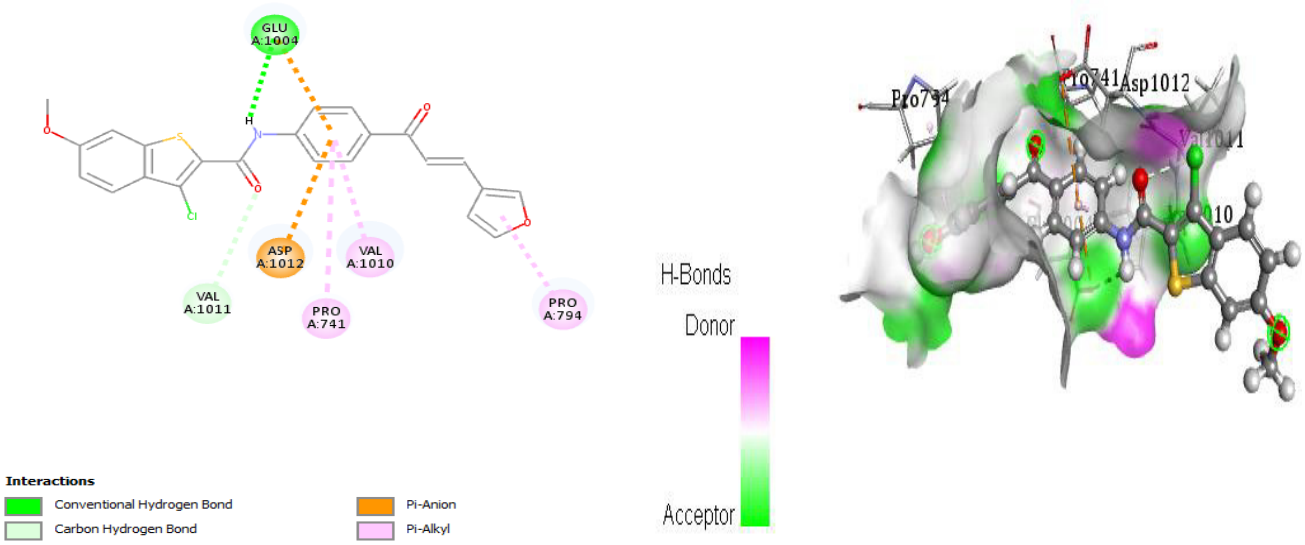


Fig 2: Docking image of 5EDP-EGFR kinase with BT-IV-E

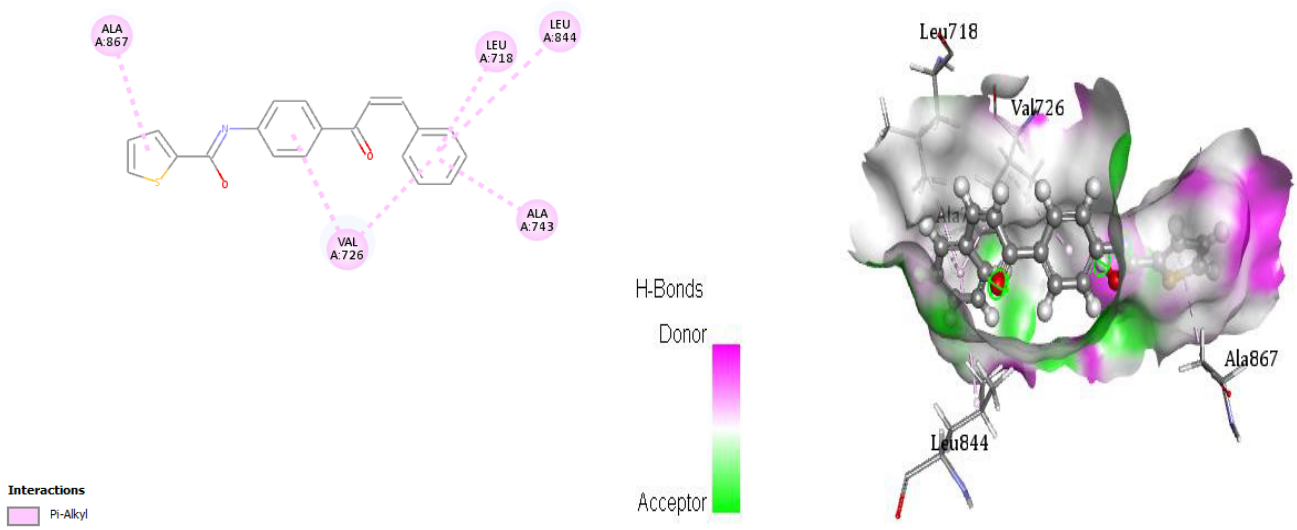


Fig 3: Docking image of 5EDP-EGFR kinase with T-IV-F

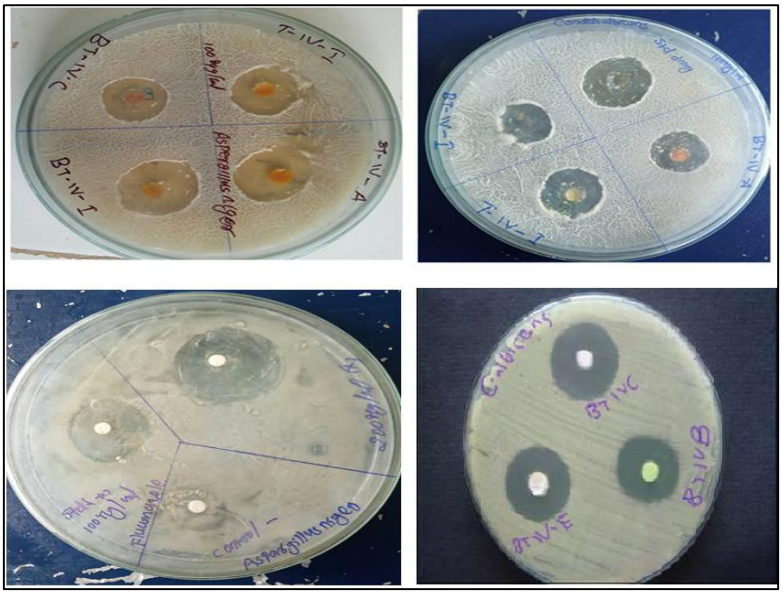


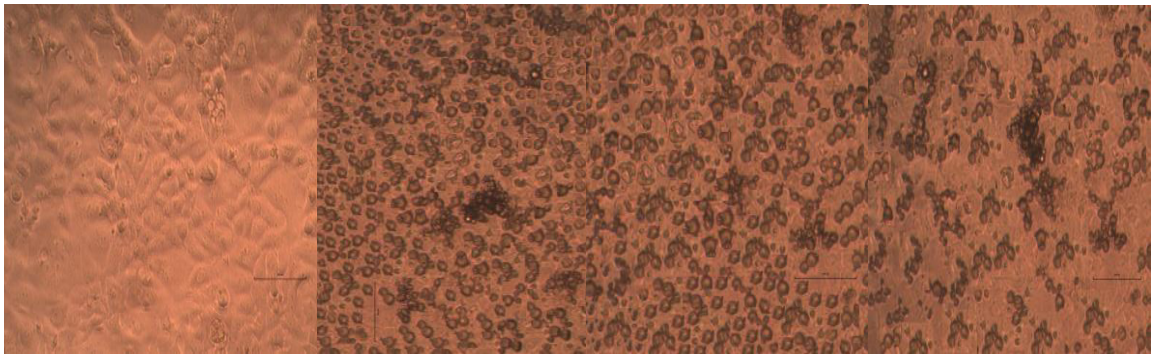
Fig 4: Antifungal activity of selected compound against *Aspergillus niger* and *Candida albicans at different concentrations* by agar diffusion method

Table 2: Antifungal activity against *Aspergillus niger* and *Candida albicans* at different concentrations

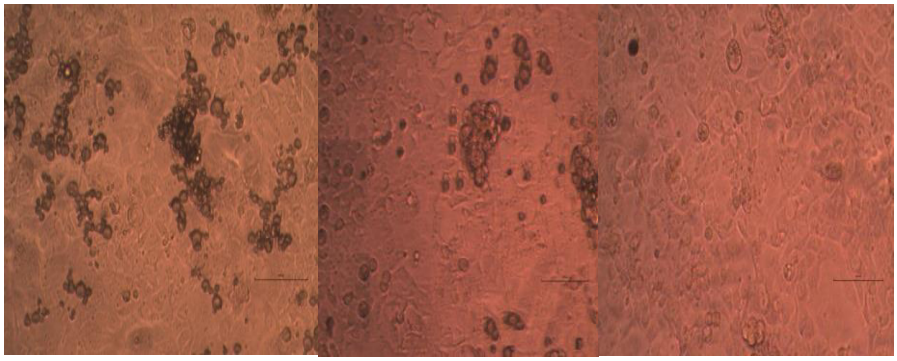
Compounds	Mean zone of inhibition in mm			
	Candida albicans		*Aspergillus niger*	
	(200µg/ml)	(100µg/ml)	(100µg/ml)	(200µg/ml)
Fluconazole	24 ± 0.1	22± 0.4	20± 0.5	22± 0.6
Control (DMF)	-	-	-	-
BT-IV-A	19± 0.7	17± 0.5	15± 0.2	17± 0.1
BT-IV-B	17± 0.3	16± 0.3	14± 0.4	15± 0.3
BT-IV-C	18± 0.2	17± 0.5	14± 0.3	16± 0.1
BT-IV-D	13± 0.4	12± 0.2	11± 0.3	12± 0.5
BT-IV-E	17± 0.5	15± 0.8	13± 0.7	14± 0.4
BT-IV-F	14± 0.3	13± 0.7	11± 0.7	13± 0.3
BT-IV-G	15± 0.7	13± 0.2	12± 0.5	13± 0.1
BT-IV-H	15± 0.3	14± 0.4	13± 0.5	13± 0.7
BT-IV-I	17± 0.1	18± 0.5	15± 0.6	15± 0.5

n=3, p<0.05

Table 2 illustrates the antifungal activity by mean zone of inhibition at concentration of 100 and 200 µ/ml. Here compounds BT-IV A, BT-IV-C, BT-IV-B, BT-IV F and BT-IV-I shows significant antifungal activity against *Candida albicans and Aspergillus niger*. Fluconazole is used as the standard drug. All the values mentioned in the table are the value of triplicate. "-" indicating no inhibition

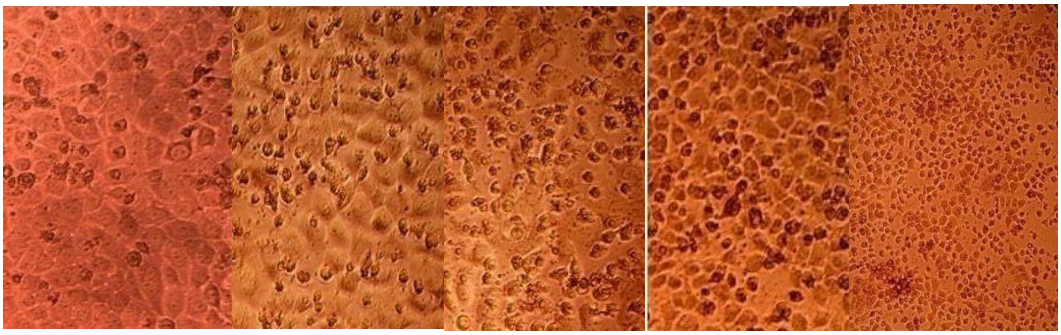


Control Methotrexate 100µ/ml 50µ/ml



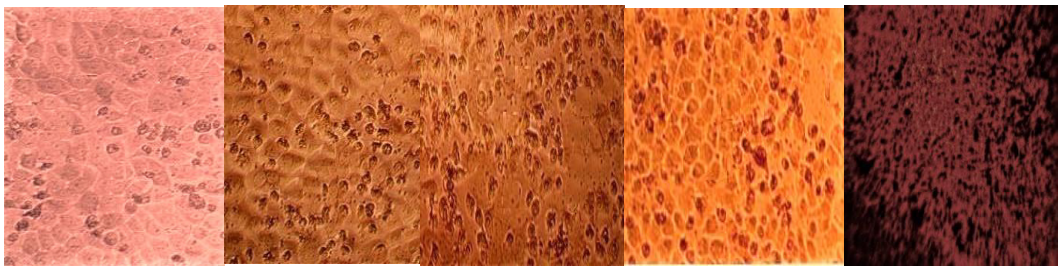
25µ/ml 12.5µ/ml 6.25µ/ml

Fig no 5: Images shows morphological features of MTT assay of BT-IV- H against MCF-7 Human breast cancer cell with control standard drug methotrexate and test compounds of different concentrations



6.25µ/ml 12.5µ/ml 25µ/ml 50µ/ml 100µ/ml

Fig no 6: Images shows morphological features of MTT assay. BT-IV-E against MCF-7 (Human breast cancer cell) at different concentrations



6.25µ/ml 12.5µ/ml 25µ/ml 50µ/ml 100µ/ml

Fig no 7: Images shows morphological features of MTT assay. T-IV-F against MCF-7 Human breast cancer cell at different concentrations

Table 3: Cytotoxicity evaluation of compounds against MCF-7Human breast cancer Cell by MTT assay

Compound	Concentration in µg/ml	Average absorbance at 540nm	% Viability	% Of inhibition	IC_{50} µg/mL
T-IV-F	6.25	0.3413	60.16 ±0.54	39.84 ±0.46	15.61
	12.5	0.2978	51.73 ±0.45	48.27 ±0.55	
	25	0.2189	39.61 ±0.37	60.39 ±0.53	
	50	0.1572	32.39 ±0.28	67.61 ±0.72	
	100	0.0821	15.65 ±0.30	84.35 ±0.70	
T-IV-J	6.25	0.5236	62.21 ±0.31	37.79 ±0.69	21.06
	12.5	0.4578	52.87 ±0.72	47.13 ±0.28	
	25	0.2743	43.81 ±0.16	56.19 ±0.84	
	50	0.1895	34.71 ±0.87	65.29 ±0.13	
	100	0.1198	18.71 ±0.44	81.29 ±0.56	
T-IV-G	6.25	0.6824	61.21 ±0.33	38.79 ±0.77	17.70
	12.5	0.5394	51.89 ±0.49	48.11 ±0.51	
	25	0.4746	40.87 ±0.56	59.13 ±0.44	
	50	0.2165	33.92 ±0.39	66.08 ±0.61	

	100	0.1337	16.76 ±0.43	83.24 ±0.57	
BT-IV-E	6.25	0.2413	59.27 ±0.55	40.73 ±0.45	12.86
	12.5	0.1978	49.57 ±0.43	50.43 ±0.57	
	25	0.1189	38.85 ±0.61	61.15 ±0.39	
	50	0.0992	31.91 ±0.44	68.09 ±0.56	
	100	0.0621	14.76 ±0.31	85.24 ±0.69	
BT-IV-H	6.25	0.1413	57.21 ±0.77	42.79 ±0.23	10.02
	12.5	0.0978	48.87 ±0.27	51.13 ±0.73	
	25	0.0789	38.31 ±0.42	61.69 ±0.58	
	50	0.0572	29.79 ±0.61	70.27 ±0.39	
	100	0.0421	12.76 ±0.11	87.24 ±0.89	
Methotraxate	6.25	0.792	55.21 ± 0.78	44.79 ±0.22	6.64
	12.5	0.723	46.87 ±0.48	53.13 ±0.52	
	25	0.509	37.91 ±0.31	62.09 ±0.69	
	50	0.429	26.83 ±0.85	73.17 ±0.15	
	100	0.312	9.26 ±0.18	90.74 ±0.82	

±SD, p<0.05

Table 3 illustrated invitro cytotoxic study report mentioned about cell variability, percentage of inhibition and IC_{50} value of selectively synthesized compounds of different concentrations against human breast cancer cell line MCF-7 using standard drug Methotrexate.

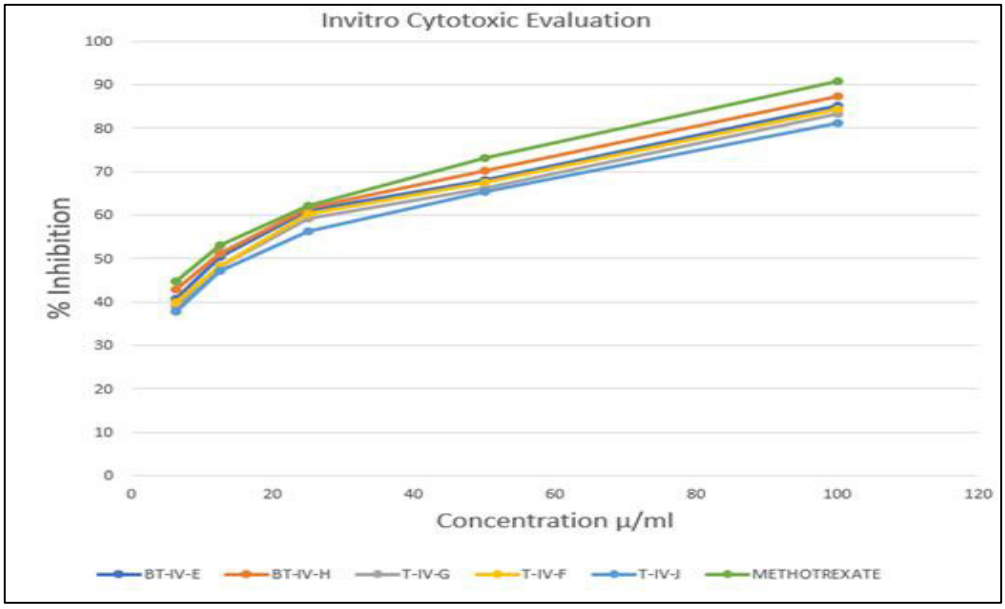


Fig 8: Invitro cytotoxic evaluation of the synthesized compounds

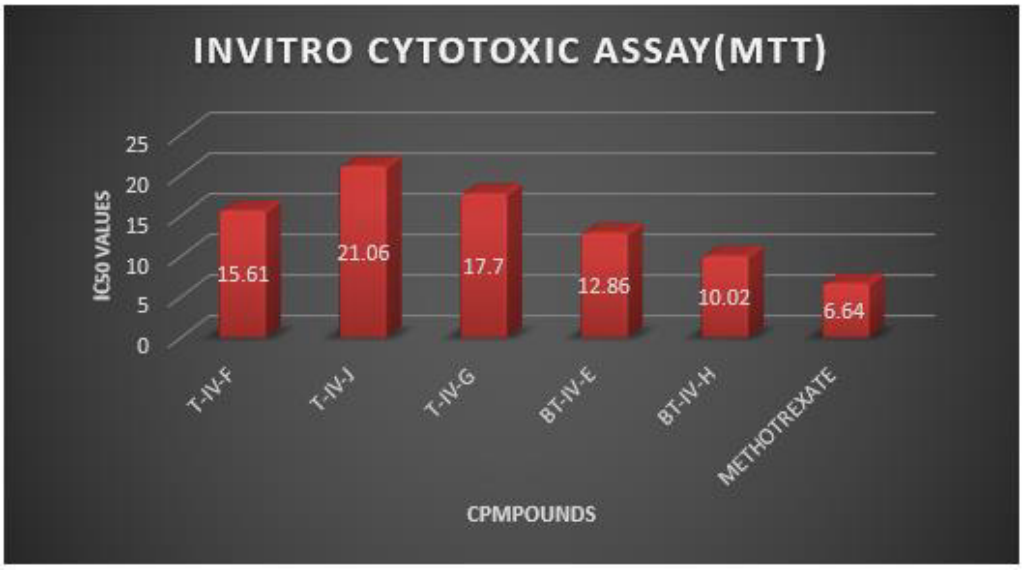


Fig 9: Graphical representation of IC_{50} values of benzothiophene and thiophene substituted chacones by MTT

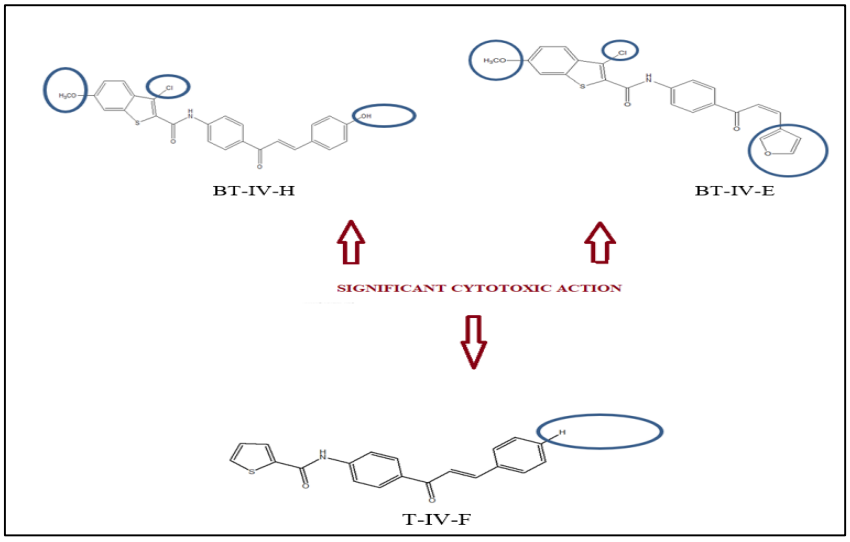


Fig 10: SAR for cytotoxic activity of selected compounds

4. CONCLUSION

A new series of benzothiophene derivatives with different chalcone moieties were synthesized successfully and evaluated for them *in vitro* antifungal and cytotoxic activities. Most newly synthesized compounds BT-IV-A, BT-IV-B, BT-IV-C, BT-IV-E, and BT-IV-I shows significant antifungal activity *Aspergillus niger* and *Candida albicans* due to the presence of different electronegative atoms. *Invitro* cytotoxic activity of synthesized compounds towards human breast cancer cell MCF-7 by MTT assay was used to test BT-IV-H (containing functional group parahydroxy benzaldehyde), BT-IV-E (containing functional group furfuraldehyde), T-IV-F (containing functional group benzaldehyde) showed great promise being much less hazardous at low concentrations. Molecular docking studies have shed light on these compounds' interaction with 5EDP-EGFR kinase. The IC_{50} values for anticancer screening were 10.02, 12.86, and 15.61, respectively. In general, our results suggest that the proposed routine is an essential orientation for the design and synthesis of new antifungal and anticancer agents from Sulphur containing heterocycles.

5. AUTHORS CONTRIBUTION STATEMENT

All authors have equally contributed to this study and publishing of this manuscript.

6. ACKNOWLEDGMENT

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7. CONFLICT OF INTEREST

Conflict of interest declared none.

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