Chapter

2

Literature Review

2. LITERATURE REVIEW

2.1. BENZOTHIAZOLES: A PROMISING LEAD FOR DRUG DEVELOPMENT

Benzothiazoles have been identified as large class of compounds endowed with rich and interesting chemistry. Benzothiazoles are the model structures, with their inherent affinity for diverse biological receptors and represent an ideal source of core scaffolds and capping fragments for the design and synthesis of targeted molecules on a reasonable time scale. These molecules represent a class of molecules capable of binding to multiple receptors with high affinity. Benzothiazoles play pivotal role in biological functions including oxidative phosphorylation. They are generally catalyzed by microsomal enzyme cytochrome P450 CYP1A1 and get metabolized resulting in the formation of reactive chemical intermediate nitrenium ion species. These species are mainly responsible for their anticancer property by interfering with DNA and RNA replication as well as responsible for the generation of DNA adducts. Structures of the benzothiazole derivatives along with their diverse biological activities are summarized below:

2.1.1. Benzothiazoles as anti-microbial agents

The discovery and development of new antimicrobial drugs to deal with resistant microbes have become one of the most important areas of antimicrobial research today. In recent decades, problems with multi-drug resistant microorganisms have reached an alarming level in many countries around the world. Thus, the discovery of novel and potent antimicrobial agents is more demanding and challenging for the scientific community nowadays. Despite numerous attempts to develop new structural models in the search for more effective antimicrobials, Benzothiazole derivatives still remain as one of the most versatile class of compounds against microbes [Bujdakova *et al.,* 1994]. These are useful substructures for further molecular exploration. Many studies have revealed the significant potential of benzothiazole derivatives as antimicrobial agents.

Sahu *et al.,* synthesized a series of 4H-pyrimido[2,1-b][1,3]benzothiazole derivatives (32) and evaluated their antibacterial activities against several gram-positive and gram-negative bacteria like *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli, Bacillus cereus,* and *Providencia rettegeri* [Sahu *et al.,* 2012].

Amir *et al.,* reported a new class of 4-arylhydrazono-1-benzothiazolyl-3-methylpyrazolin-5-ones and 4-arylazo-1-benzothiazolyl-3,5-dimethylpyrazoles (33) which were designed as pharmacophore hybrids between pyrazolinone/pyrazole and benzothiazole moiety and screened for antimicrobial activity [Amir *et al.,* 2012].

Soni *et al.*, reported Schiff bases of Benzothiazole-triazole conjugates (34) and evaluated for their antimicrobial activities against a panel of bacterial and fungal stains. The compounds with a 4-hydroxy, 4-dimethylamino and 3,4-dimethoxy group on the aromatic ring showed good antibacterial activity. The substitution at position 2 on the aromatic ring by a chloro (-Cl) group and 3,4-dimethoxy (3,4-OCH_3) increases antifungal activity and substitution at position 2-nitro or 3-nitro group decreases the activity [Soni *et al.*, 2010].

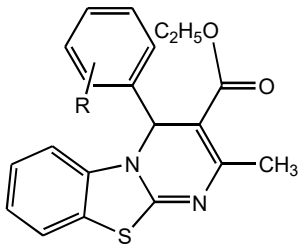
Bolelli *et al.,* synthesized a new series of 2-[4-(4-substitutedbenzamido/ phenylacetamido)phenyl]benzothiazole derivatives (35) and evaluated for antibacterial and antifungal activities. The compounds possessed a broad spectrum of activity against the tested microorganisms at MIC values ranging 100-6.25 μg/mL[Bolelli *et al.*, 2012].

Sharma *et al.,* have synthesized 4-Phenyl-2H-pyrimido[2,1-b]benzothiazol-2-ones (36) in quantitative yields by the reaction of 2-aminobenzothiazoles with alkynoic acid. Fusion of two biodynamic heterosystems, Benzothiazole and pyrimidine, resulted in formation of a new heterocyclic scaffold with significant activity. The antimicrobial activity of the synthesized compounds was tested against bacterial species, *Bacillus coagulans, Bacillus subtilis, Staphylococcus aureus*, and *Pseudomonas aeruginosa* [Sharma *et al.,* 2010].

Bondock *et al.,* reported synthesis of benzothiazole derivatives incorporating pyrazole, isoxazole and pyrimidine moiety, and evaluated *in-vitro* antimicrobial activity. Benzothiazole-pyrazole conjugates (37) exhibited better antibacterial potentials than the transamination adduct. The pyrimidine conjugated Benzothiazole (38) exhibited broad spectrum antibacterial profile against Gram positive bacteria and were more efficacious than their pyrazole counterparts. Compound (38) was equipotent to streptomycin in inhibiting the growth of *B. subtilis* (MIC 3.125 μg/mL) [Bondock *et al.,* 2009].

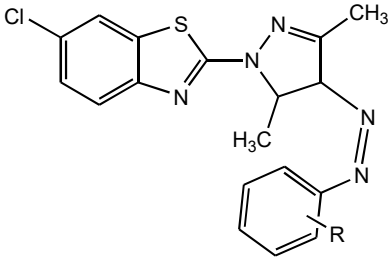
Singh *et al.,* reported the synthesis of benzothiazole analogs using click chemistry. Compound (39a) evidenced maximum potency against all Gram positive and Gram negative bacterial strains with a MIC value of 3.12 μg/mL, in comparision to standard drug Ciprofloxacin (MIC 6.25 μg/mL). Compound (39b) was found most active against all fungal strains with MIC value in the range of 1.56-12.5 μg/mL, while the remaining compounds demonstrated moderate to weak antifungal activity [Singh *et al.,* 2013].

Sahu *et al.,* synthesized novel 4H-pyrimido[2,1-b]benzothiazole derivatives (40) of curcumin under solvent and solvent free conditions in microwave with good yields. All the synthesized compounds were screened for their antibacterial activity against various gram-positive and gram-negative bacteria [Sahu *et al.,* 2012].



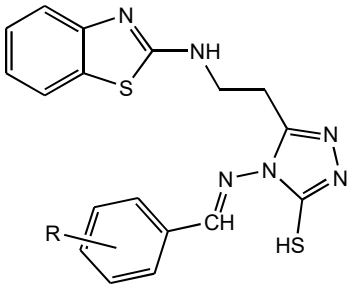
R= phenyl, 4-hydroxyl phenyl, 4-methoxyphenyl

(32)



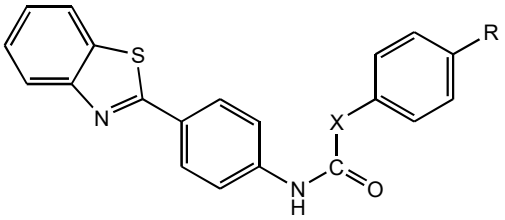
R=o-OH, p-OCH_3,o-COOH, p-COOH

(33)



R=hydroxy, 2-chloro, 3,4-dimethoxy

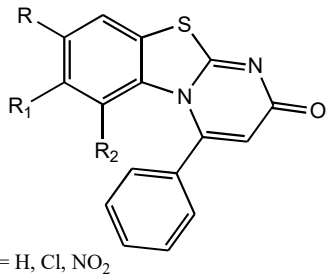
(34)



X= -CH_2

R= F, Cl, Br, CH_3, C_2H_5

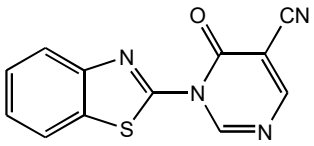
(35)



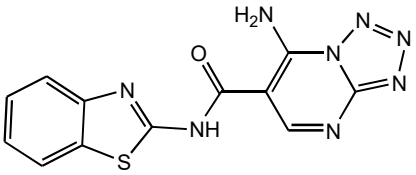
R= H, Cl, NO_2

R_1= H, Cl

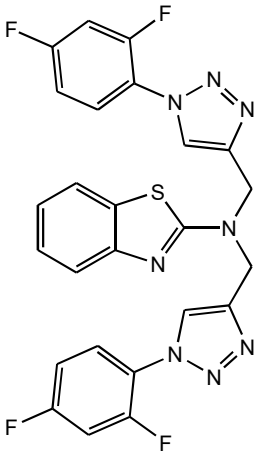
(36)



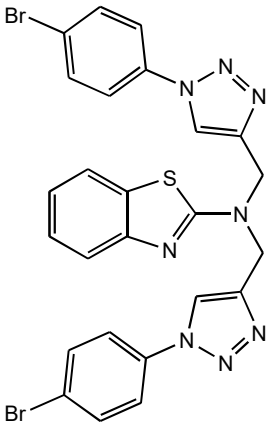
(37)



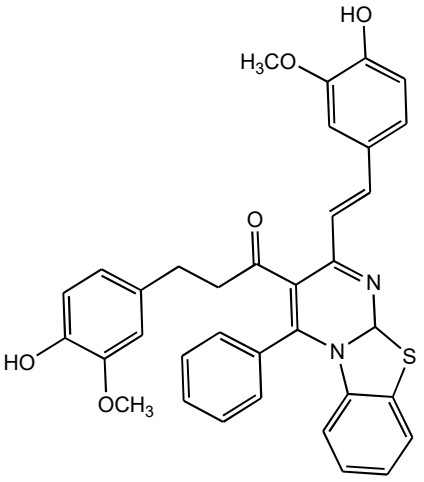
(38)



(39a)



(39b)



(40)

Benzothiazoles having antimicrobial action

Tomi *et al.,* have reported novel derivatives containing five-membered heterocyclic benzothiazole (41) and oxazole rings. This study was designed to illustrate the bioactivity comparision for two different types of synthesized analogs, and it has been observed that benzothiazoles are more active than the oxazole derivatives in antimicrobial assay against *Escherichia coli, Staphylococcus aureus*, *Pseudomonas aeruginosa, Aspergillus niger, Candida albicans* [Tomi *et al.,* 2012].

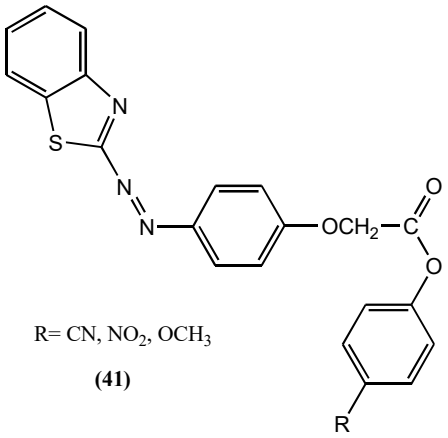
Padalkar *et al.,* investigated antibacterial and antifungal activity of new analogues of a series of 2-(1,3-benzothiazol-2-yl)-5-(diethylamino)phenol (42). The synthesized compounds were evaluated for *in-vitro* antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* strains and *in-vitro* antifungal activity against *Candida albicans* and *Aspergillus niger* strains by using serial dilution method [Padalkar *et al.,* 2012].

Gilani *et al.,* investigated the antimicrobial property of a series of novel thiazolidin-4-ones and azetidin-2-ones (43) benzothiazole compounds. These derivatives were synthesized from N-(6-chlorobenzo[d]thiazol-2-yl)hydrazine carboxamide derivatives of the benzothiazole class [Gilani *et al.,* 2012].

Sheng *et al.,* demonstrated and applied molecular docking and 3D-QSAR methods, CoMFA and CoMSIA to a set of novel benzothiazole (44) *Candida albicans* Nmyristoyltransferase (CaNmt) inhibitors. The binding mode of the compounds at the active site of CaNmt was explored using flexible docking method, and various hydrophobic and hydrogen bonding interactions were observed between the benzothiazole inhibitors and the target enzyme [Sheng *et al.,* 2007].

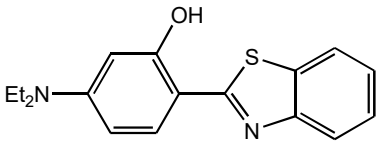
Bhusari *et al.,* reported benzothiazole-benzenesulphonamide derivatives as antimicrobial and anti-TB agents. The compounds with chloro and carboxyl substitutions evidenced considerable antibacterial activity against *B. subtilis* and *E. coli*. The chloro and methoxy substituted compounds showed pronounced antifungal activity against *C. albicans*. The compounds having electron withdrawing substitutions (-Cl and -Br) were found more active than nitro ($-NO_2$) substituted derivatives in the case of anti-TB activity. The compound (45) displayed prominent antibacterial, antifungal and antimycobacterial activities [Bhusari *et al.,* 2008].

Patel *et al.,* identified and studied Quinoline based benzothiazolyl-1,2,4-triazoles as antimicrobial analogues. Compound (46a) containing electron withdrawing fluorine substituents, showed potent action against *S. aureus* at 25 µg/mLof MIC. Another analog (46b) with bromine substituent indicated 25 µg/mLof MIC against Gram-positive bacteria *B.cereus*. These two derivatives exhibited half fold potency against both Gram positive strains compared with the control drug, ampicillin (MIC 12.5 µg/mL) [Patel *et al.,* 2014].

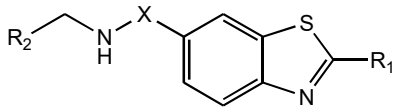


R= CN, NO_2, OCH_3

(41)

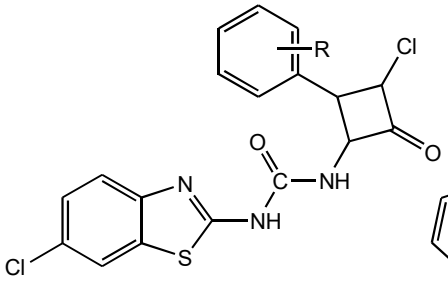


(42)

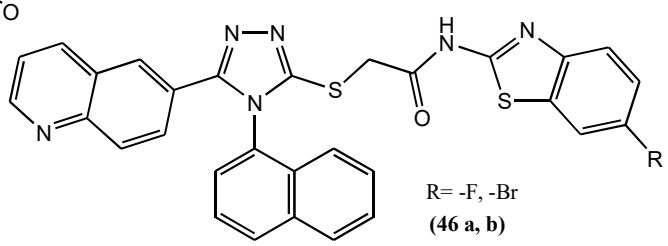


X= $(CH_2)_2NHCO$-, $(CH_2)_3NHCO$-, CH_2CONH
R_1= $NHCOC_6H_{11}$, R2= $C_{10}H_7$

(44)

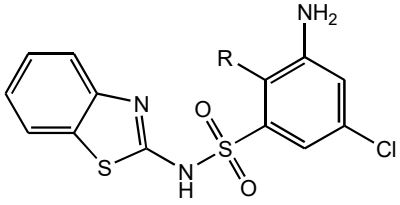


(43)



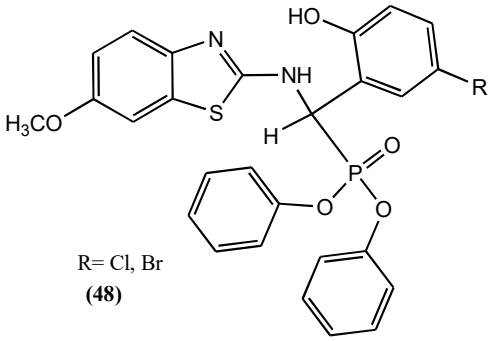
R= -F, -Br

(46 a, b)



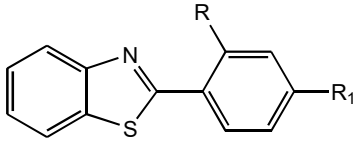
R= -COOH, $-OCH_3$, $-CH_3$

(45)



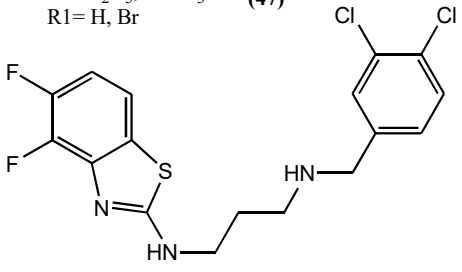
R= Cl, Br

(48)

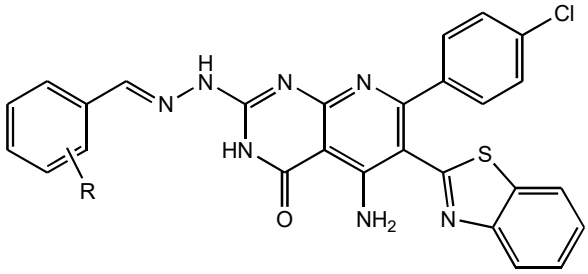


R= $-C_2H_5$, $-OCH_3$
R1= H, Br

(47)



(49)



R= 4-Cl, 4-OCH_3, 4-F, 4-NO_2, 2,4-$(CH_3)_2$, 4-C_2H_5

(50)

Benzothiazoles having antimicrobial action

Bandyopadhyay *et al.,* reported synthesis of 2-substituted benzothiazole using Al_2O_3-Fe_2O_3 nanocrystals as heterogeneous catalyst and screened for antibacterial activity. Compounds (47) demonstrated moderate to good antibacterial activity compared with standard antibacterial drug, ciprofloxacin, against *V. cholerae, Bacillus cereus,* and *S. dysenteriae*, respectively [Bandyopadhyay *et al.,* 2011].

Rao *et al.,* synthesized BTA-α-aminophosphonates derivatives by using the Kabachnike Fields reaction, screened for antibacterial and antioxidant activities. The compound (48) was found most potent for antibacterial and antioxidant activities and was more effective than the standard Penicillin [Rao *et al.,* 2010].

Ouyang *et al.,* synthesized novel Benzothiazole-2-amine derivatives and tested *in-vitro* antibacterial activity against a panel of bacterial strains. 5,6-difluorosubstituted Benzothiazole analogue (49) was found to be potent inhibitor of Gram positive pathogens and also some of them exhibited exquisite potential against drug-resistant bacteria, without cytotoxicity in therapeutic concentrations. The activity was significantly affected by the length of the linker and halogen atom in the heterocyclic ring and also played a crucial role [Ouyang *et al.*, 2012].

Maddila *et al.,* reported a series of 6-(benzothiazol-2-yl)pyrido[2,3-*d*]-pyrimidine (50**),** and evaluated for their antimicrobial activity. The synthesized compounds exhibited significant activity against various strains as compared to the standard drugs ciprofloxacin and clotrimazole [Maddila *et al.*, 2013].

In recent years, a good number of patents of benzothiazole derivatives exhibiting interesting antibacterial and antifungal activities have been filed. Some of them are:

Haydon *et al.,* have reported a patent of pyridine substituted benzothiazoles as antibacterial agent. The compounds were synthesized in a multistep synthetic route and were evaluated for antibacterial activity against several bacterial strains. From the series, compound (51) showed excellent antibacterial activity with MIC<0.25 µg/mLagainst several bacterial strains such as *Chlamydophila pneumonia, Enterococcus faecalis, Staphylococcus aureus* and so on [Haydon *et al.*, 2012].

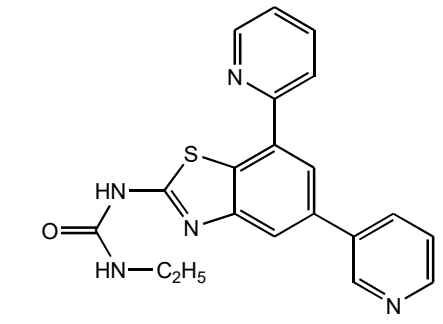
Luniss *et al.,* developed pyrimidine substituted benzothiazoles and screened for their antibacterial activity and compound (52) exhibited MIC ≤1 µg/mL [Luniss *et al.*, 2013].

Lavoie *et al.,* have discovered various benzamide-linked benzothiazole conjugates connected through an ether linkage. All the compounds claimed in the invention were screened for their antimicrobial activity, compound (53) was found to be most effective with IC_{50} < 0.125 µM [Lavoie *et al.*, 2013].

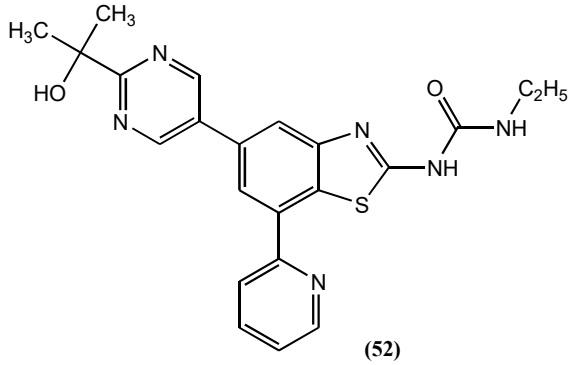
Ding *et al.,* claimed pyridothiazole and benzothiazole aryl ether derivatives as antibacterial agents. Compound (54) showed excellent antibacterial activity exhibiting MIC value 0.39 µg/mL [Ding *et al.*, 2014].

Sheng *et al.,* claimed cycloalkylamido benzothiazole derivatives as potential antifungal agents. One of the compounds from the series (55) with quinoline substituent exhibited MIC value < 0.125 µg/mL against *Candida albicans* and 4 µg/mL against most of the tested fungi species. Many plants including crops of human use are prone to fungal attack leading to their spoilage [Sheng *et al.*, 2013].

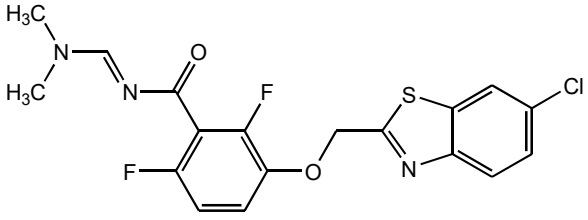
Bayer CropScience AG developed substituted benzoxazoles and benzothiazole derivatives of general formula (56) and filed two patents claiming them as fungicides. All the invention compounds were evaluated for their fungicidal activity and were found to impart significant protection from various types of fungi [Bayer CropScience AG, 2011].



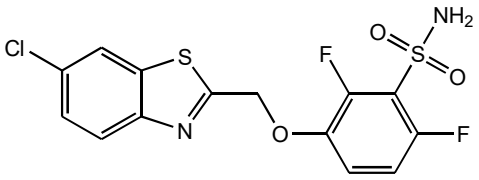
(51)



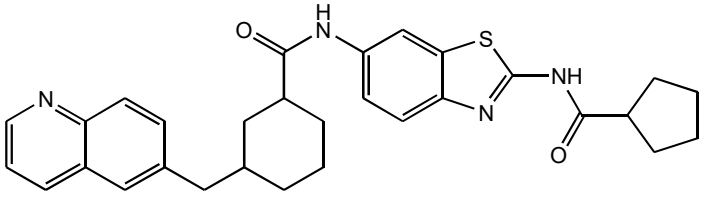
(52)



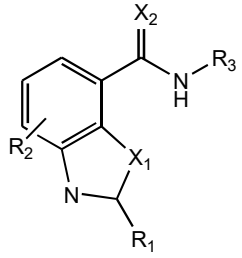
(53)



(54)



(55)



X_1, X_2= O, S

(56)

Patented Benzothiazoles having antimicrobial action

2.1.2. Benzothiazoles as anticancer agents

Cancer is a disease of striking significance in the world today. It is one of the most serious worldwide health threats, killing almost seven million people a year, and poses great challenges to medical science. The extensive global research efforts in this field are focused both on the development of effective anticancer therapeutics and on the discovery of novel biological targets. In the efforts to develop suitable drugs with such capabilities, scientists have focused upon different aspects of cancer biology. Benzothiazole derivatives attracted considerable attention towards anticancer research, and several attempts were made to modify the benzothiazole nucleus to improve their antitumor activity. Continuous effort has been directed towards development of new benzothiazole-based anticancer agents targeting various enzymes or receptors such as topoisomerases, microtubule cytochrome P450 enzyme, rapidly accelerated fibrosarcoma (RAF) kinases, transforming growth factor-β (TGF- β), farnesyltransferase, and DNA. They have great potential to overcome the diverse drawbacks of currently available clinical drugs and to be developed as anticancer drugs. A large number of benzothiazole derivatives have shown potent anticancer activity. Some of the recent literature reports are summarized as under.

Gill *et al.,* reported novel derivatives of N-alkylbromo-benzothiazoles (57) and evaluated their anticancer potency. Most of the compounds in this series have shown significant cytotoxic activity. However, compound (57), (3-bromo-propyl)-(6-methoxy-benzothiazol-2-yl) amine has been found to be the most promising anticancer agent against the PC-3 (IC_{50} 0.6μM), THP-1 (IC_{50} 3μM) and Caco-2 cell lines (IC_{50} 9.9μM), respectively [Gill *et al.,* 2013].

Wang *et al.,* reported novel benzothiazole-2-thiol derivatives (58), and investigated their antiproliferative activities on HepG2 and MCF-7 cells. Most of the compounds had inhibitory effects on cell growth, and some of them were more effective than cisplatin **[**Wang *et al.,* 2011].

Kumbhare *et al.,* synthesised benzothiazolyl thiocarbamides (59) using a catalytic amount of 4-dimethylaminopyridine (DMAP) followed by its chemoselective oxidative cyclization with 1,3-di-*n*-butylimidazolium tribromide [bbim][Br3] to afford the N-bis-benzothiazole derivatives. All the synthesized compounds were evaluated for cytotoxic activity against two human monocytic cell lines (U 937, THP-1) and a mouse melanoma cell line (B16-F10) [Kumbhare *et al.,* 2012].

Jin *et al.,* synthesized γ-aminophosphonates (60) containing benzothiazole and fluorine moiety by Mannich-type addition in ionic liquid media with high yield and short reaction time. The newly synthesized compounds were evaluated for their anticancer activity

against PC3, A431, A375, and Bcap37 cells *in-vitro* by the MTT assay method [Jin *et al.,* 2006].

Racané *et al.,* reported new amidino derivatives of phenylene-bisbenzothiazoles and evaluated their antiproliferative activity against several human cancer cell lines, as well as DNA binding properties. All the compounds exhibited significant activity on tumor cells in concentration dependant manner. The most cytotoxic compound was diimidazolinyl substituted phenylene-bisbenzothiazole (61) with IC_{50} 5.3, 0.87, 6.19, 1.49, 6.63, 7.38 μM against MCF-7, SK-BR-3, SW620, MiaPaCa-2, WI38 and HeLa cancer cell lines, respectively. [Racané *et al.,* 2013].

Saeed *et al.,* synthesized five series of thiourea derivatives (62) bearing benzothiazole moiety and evaluated for antimicrobial and anticancer activities [Saeed *et al.*, 2010].

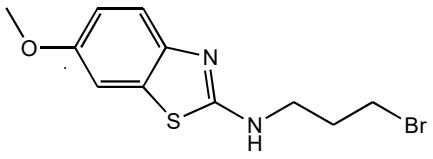
Havrylyuk *et al.,* have performed antitumor screening of several novel 4-thiazolidinones with benzothiazole moiety (63) on leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancers cell lines [Havrylyuk *et al.,* 2010].

Solomon *et al.,* described hybrid pharmacophore approach which was used to design and synthesize isatin–benzothiazole analogs (64) and to examine their anti-breast cancer activity. The cytotoxicity of these compounds was determined using three different human breast tumor cell lines, MDAMB231, MDA-MB468, and MCF7, and two non cancer breast epithelial cell lines, 184B5 and MCF10A [Solomon *et al.,* 2009].

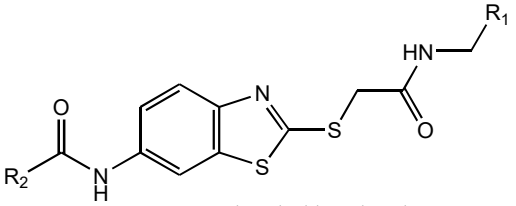
Aziz *et al.,* reported a novel series of N-(pyridine-2-yl-methylene)benzo[d]thiazol-2-amine and its Cu(II), Fe(III), Co(II), Ni(II) and Zn(II) complexes and evaluated their anticancer potential. Zn complex (65) has been found to be most active in human breast carcinoma (MCF-7), liver carcinoma (HEPG2), colon carcinoma (HCT116) and larynx carcinoma (HEP2) cell lines [Aziz *et al.,* 2013].

Kamal *et al.,* synthesized a series of novel benzothiazole linked pyrrolobenzodiazepine conjugates (66) attached through different alkane or alkylamine spacers and investigated their anticancer activity, DNA thermal denaturation studies, restriction endonuclease digestion assay, and flow cytometric analysis in human melanoma cell line (A375) [Kamal *et al.,* 2010].

Kamal *et al.,* reported substitution of pyrazolo[1,5-a]pyrimidine carboxylic acid at position-2 of amino benzothiazole scaffold through an amide functionality. Compounds (67a,b) were found to possess appreciable anticancer activity. Both of these compounds also have potential to arrest G2/M cell cycle in A549 cancer cell line and cause reduction in Cdk1 expression level [Kamal *et al.,* 2013b].

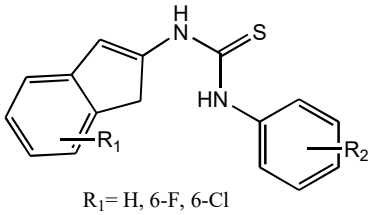


(57)



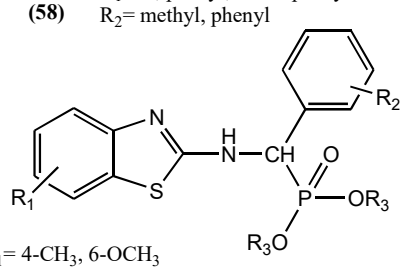
R_1= H, phenyl, chlorophenyl
R_2= methyl, phenyl

(58)



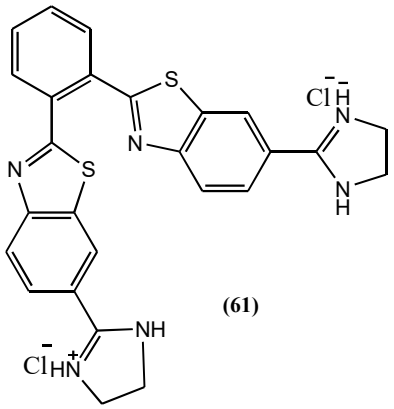
R_1= H, 6-F, 6-Cl
R_2= H, F, Cl

(59)

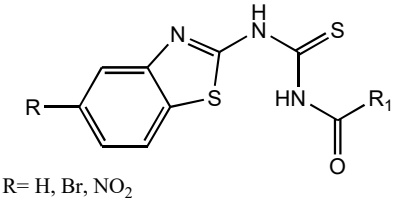


R_1= 4-CH_3, 6-OCH_3
R_2= 2-F, 4-F, 4-CH_3
R_3= CH_3, C_2H_5, nC_3H_7

(60)

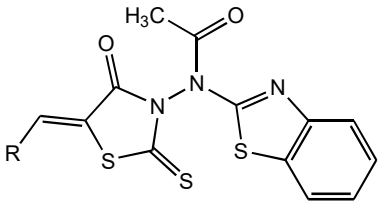


(61)

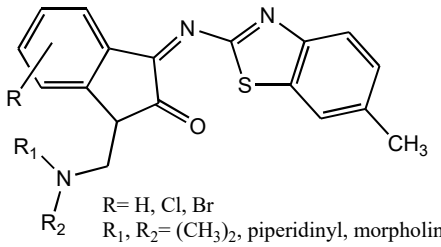


R= H, Br, NO_2
R_1= phenyl, n-butyl, 4-nitrophenyl

(62)

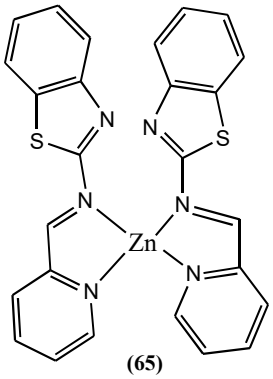


(63)

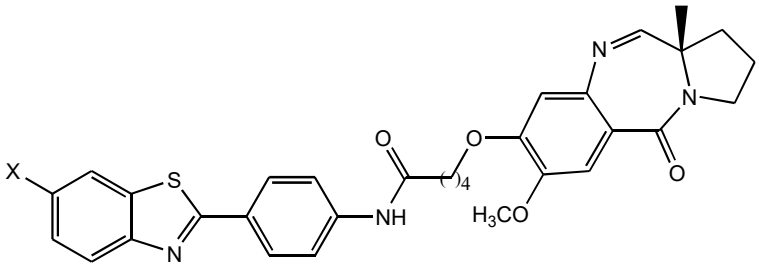


R= H, Cl, Br
R_1, R_2= $(CH_3)_2$, piperidinyl, morpholinyl

(64)



(65)



X= H, F

(66)

Benzothiazoles having anticancer potential

Prabhu *et al.,* synthesized a novel series of 2-(3-(4-oxo-2-substituted phenylthiazolidin-3-yl)phenyl)benzo[d]thiazole-6-carboxylic acid derivatives PP1–PP8 (68) by various benzothiazole Schiff 's bases by reaction with thioglycolic acid and evaluated *in-vitro* anticancer activity on human cervical cancer cell line (HeLa) [Prabhu *et al.,* 2012].

Caputo *et al.,* synthesised two sets of benzothiazole derivatives bearing an arylamide or an aryl urea moiety at C-2 (69) and performed *in-vitro* primary anticancer assay against a panel of 60 human tumor cell lines [Caputo *et al.,* 2012].

Chen *et al.,* have performed a quantitative structure activity relationship (QSAR) of a series of benzothiazole derivatives (70) showing a potent and selective cytotoxicity against a tumorigenic cell line by using the density functional theory (DFT), molecular mechanics (MM+), and statistical methods, and the QSAR equation was established *via* a correlation analysis and a stepwise regression analysis [Chen *et al.,* 2008].

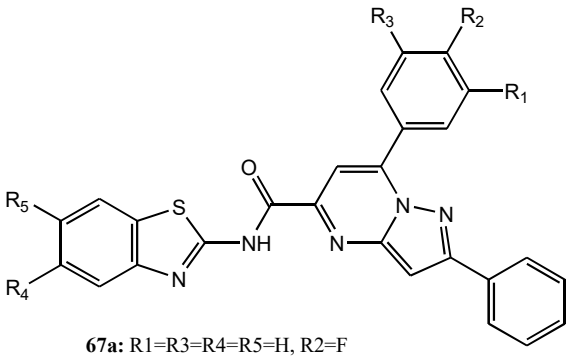
Kamal *et al.,* reported a series of new benzothiazole-pyrrole based conjugates 71a,b and evaluated for cytotoxic activity against MCF-7 cell line. Compounds 71a and 71b were found effective in inducing apoptosis in MCF-7 cells. Compound 71a has also shown down-regulation of oncogenic expression of Ras and its downstream effector molecules such as MEK1, ERK1/2, p38MAPK and VEGF [Kamal *et al.,* 2013b].

Mortimer *et al.,* synthesized a series of new 2-phenylbenzothiazoles (72) on the basis of the discovery of the potent and selective *in-vitro* antitumor properties of 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole [Mortimer *et al.,* 2006].

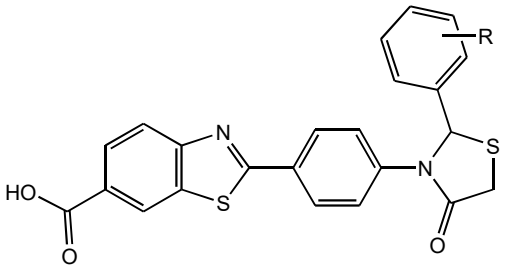
Oanh *et al.,* prepared two series of benzothiazole containing analogues of SAHA (73). It was found that several compounds with 6C-bridge linking benzothiazole moiety and hydroxamic functional groups showed good inhibition against HDAC3 and HDAC4 and exhibited potent cytotoxicity against five cancer cell lines with average IC_{50} values of as low as 0.81 μg/mL, almost equipotent to SAHA [Oanh *et al.,* 2011].

Kok *et al.,* described the "one pot" condensation reaction for the synthesis of phthalic imide derivative (benzothiazole containing phthalimide) (74), exhibiting *in-vitro* cytotoxic potential on human cancer cell lines. They further demonstrated that both caspase-dependent and independent pathways are involved in novel benzothiazole containing phthalimide induced apoptosis on cancer cells. [Kok *et al.,* 2008]

Karmakar *et al.,* synthesized cadmium and indium complexes of benzothiazoles and tested for their anticancer activity by cell-based cytotoxicity assays. It has been found that ligands (2-(pyridin-2-yl)benzo[d]thiazole and 2-(pyridin-4-yl)- benzo[d]thiazole) are biologically inactive, while the cadmium complex (75) exhibited significant cytotoxic activity in pancreatic cancer cell lines with IC_{50} values <16.0 μM [Karmakar *et al.,* 2013].

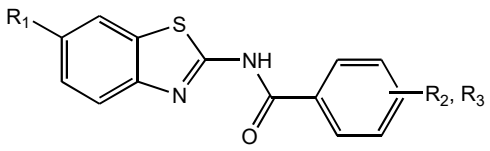


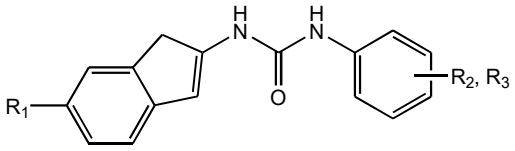
67a: R1=R3=R4=R5=H, R2=F
67b: R1=R2=R3=OCH3, R4=R5=H



R= H, p-Cl, m-F, p-NO_2, p-OCH_3

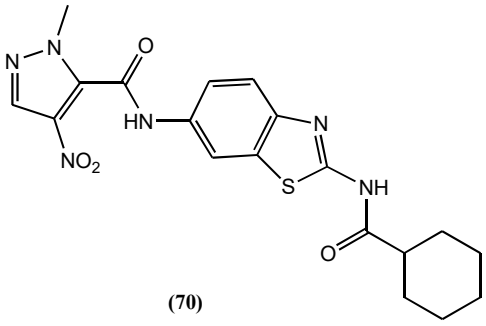
(68)



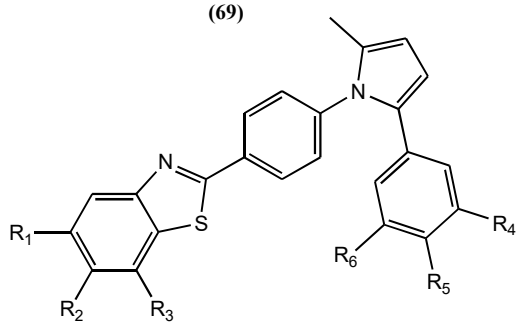


R_1= OCH_3, OCF_3
R_2= 4-F, 2-F, 4-OCH_3

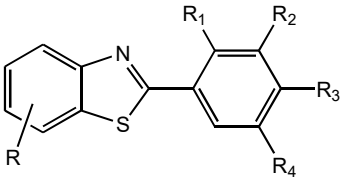
(69)



(70)

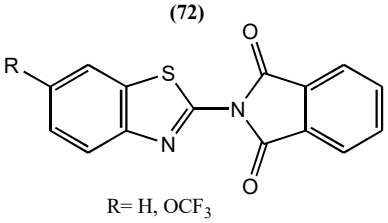


71a: R_1=R_3=H, R_2=OCH_3, R_4=R_5=R_6= 3,4,5-OCH_3
71b: R_1=R_2=R_3= 3,4,5-OCH_3, R_4=H, R_5=OCH_3



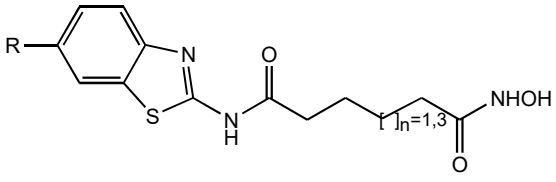
R= H, 5-Cl, 5-Br
R_1= H, R_2= OMe, OEt
R_3= OMe, R_4= H, OH

(72)



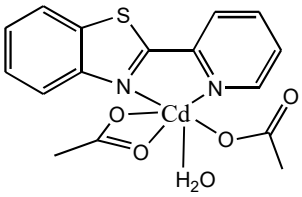
R= H, OCF_3

(74)



R= H, CH_3, OMe, OEt

(73)



(75)

Benzothiazoles having anticancer potential

Swinnen *et al.,* patented fused bicyclic derivatives as inhibitors of PI3K. The 2-acetamido benzothiazole (76) with sulfonamido aryl substitution at 6th position was found to significantly inhibit PI3K activity with IC_{50} value ranging between < 1 and < 5 μM [Swinnen *et al.,* 2010].

Damour *et al.,* patented triazolo pyridazinyl benzothiazole derivatives as c-Met inhibitors. On biological evaluation, compound (77) exhibited good inhibition of c-Met with an IC_{50} value lower than 100 nM [Damour *et al.,* 2011].

Li *et al.,* claimed compounds having sulfonamido benzamide substitution at 6th position of benzothiazole as potent antitumor agents. One of the compounds (78) from this series effectively inhibited tumor cell proliferation with IC_{50} value as low as 0.5 μM against the breast cancer cell line (MCF-7). In addition, it exhibited excellent *in-vivo* profile compared to previously reported counterparts [Li *et al.,* 2014].

Li *et al.,* patented acenaphtho heterocyclic-benzothiazole congeners linked through a sulfur atom at the C-2 position of benzothiazole ring. One of the compounds from the series (79) exhibited significant cytotoxicity with an IC_{50} of 5.05 μM against the breast cancer cell line MCF-7 [Li *et al.,* 2013].

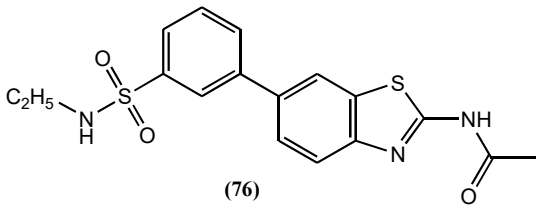
Hong *et al.,* synthesized series of sulfur-bridged benzothiazole cyclohexylamide-isoxazole congeners as cytotoxic agents. Compound (80) showed good cytotoxicity against the cell lines specifically against the lung cancer cell line A549 [Hong *et al.,* 2013].

Zou *et al.,* patented pyrazolyl benzothiazoles as protein kinase inhibitors. Compound (81) was found to be most active compound from the series exhibiting an IC_{50} value of 1.1 μM [Zou *et al.,* 2013].

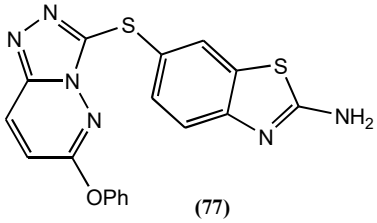
Panicker *et al.,* reported a series of imidazolyl benzothiazoles as potent inhibitors of CYP thereby inducing antiproliferative effects. Most of the compounds from this series demonstrated an IC_{50} value of < 1 μM in a CYP 26 assay, and compound (82) was found to be one of the active compounds [Panicker *et al.,* 2011].

Li *et al.,* synthesized naphthalimide aryl benzothiazole conjugates by a multistep procedure and tested for their anticancer activity. Among the series, compound (83) demonstrated potent cytotoxicity exhibiting IC_{50} value of 3.81 μM against the cell line SMMC-7721 [Li *et al.,* 2013].

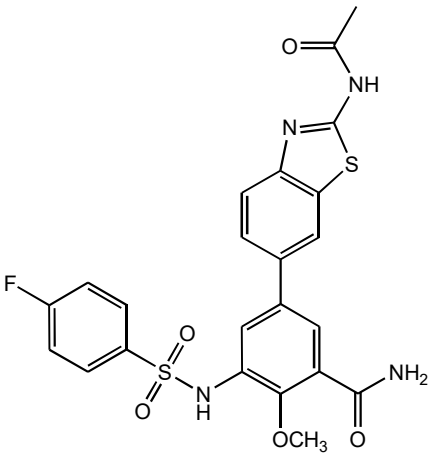
Wang *et al.,* claimed pyridinyl-tetrahydroisoquinoline benzothiazole amides as apoptosis-inducing agents used for the treatment of cancer, immune and autoimmune diseases. These agents induced apoptosis by modulating Bcl-xL levels, which is evident from excellent inhibitory effects of compound (84) which exhibited a Ki value of 12 nM and EC_{50} of 425 nM [Wang *et al.,* 2013].



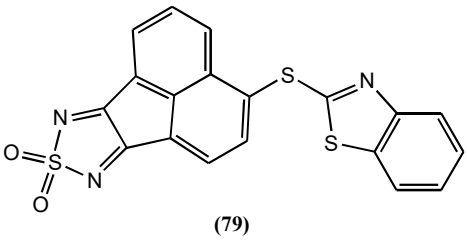
(76)



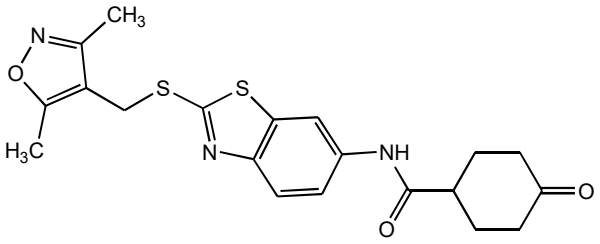
(77)



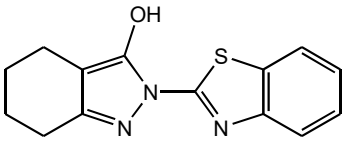
(78)



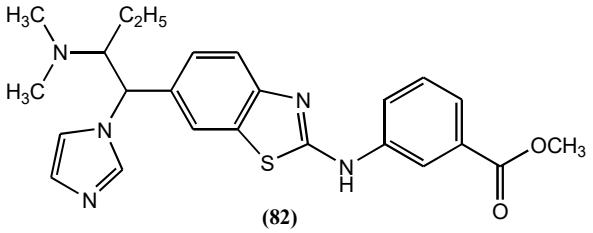
(79)



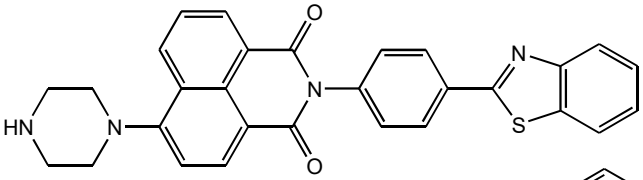
(80)



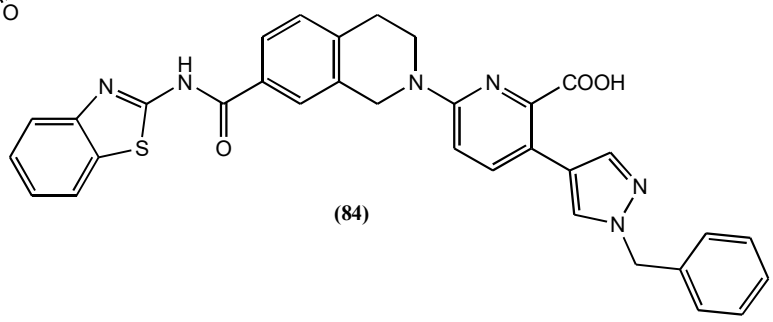
(81)



(82)



(83)



(84)

Patented Benzothiazoles having anticancer potential

A work by Dr. Malcolm Stevens of the Cancer Research UK Group at Nottingham University showed the potential of benzothiazole (NSC 674495) and related compounds as anticancer agents. Phortress (26) (NSC 710305) is the lead compound from this work. This agent has demonstrated activity against breast tumors, regardless of estrogen receptor status, and against ovarian, renal, lung, and colon cancer cells. The mechanism of action of phortress can be described as follows:

In the absence of cells, no spontaneous hydrolysis of the amide bond occurs, but L-lysyl prodrug rapidly and quantitatively hydrolysed by liver P450 enzymes and reverts to their parent amine in the presence of sensitive and insensitive cells *in-vitro*. However, in presence of sensitive tumor cells only, prodrug rapidly liberates their parent amine which are then sequestered and metabolised. The parent amine (5F203) undergoes selective uptake into sensitive cells followed by Aryl hydrocarbon Receptor (AhR) binding and translocation into the nucleus, there upon induction of the cytochrome P450 isoform CYP1A1 occurs and then conversion of the drug into an electrophilic reactive intermediate, which covalently bind to DNA, exacting lethal damage to sensitive tumor cells that leads to formation of DNA Single Strand Break (SSB) and Double Strand Break (DSB), *in-vitro* and *in-vivo.* These DNA lesions, in turn, evoke manifold biological/cellular sequel, including DNA damage recognition, cell cycle arrest, and apoptosis. The treatment-induced DNA damage reveals clinical relevance is shown in Fig. 2.1 [Bradshaw and Westwell, 2004].

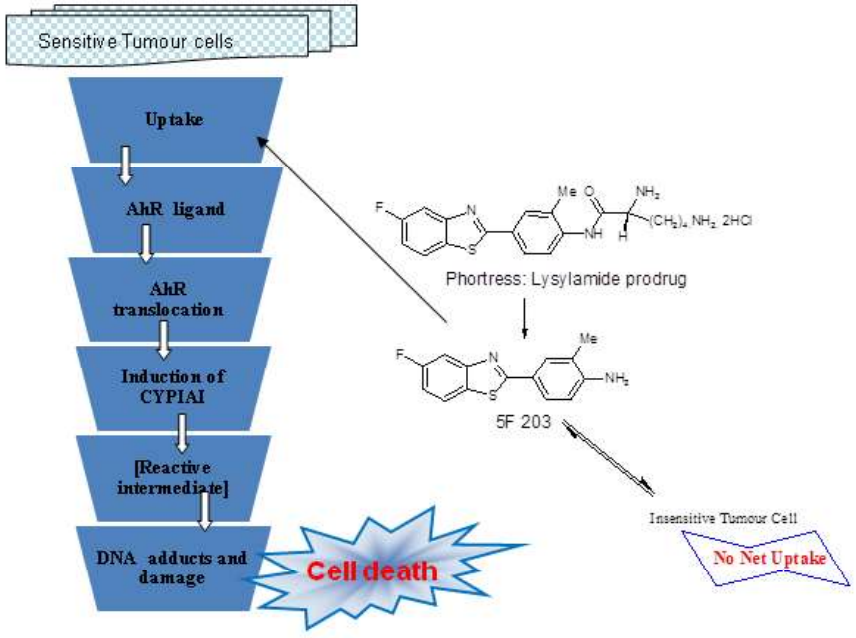


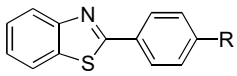
Fig. 2.1 Mode of action of Phortress.

Structure-activity relationship (SAR) analyses with respect to anticancer activity

Analysis of structure activity relationships identified the benzothiazole nucleus as being essential for potent activity. Also, the studies interestingly revealed that change in the nature of substituent group at C-2 position commonly results in the change of its bioactivity. Among those, halogen substituted molecules have already received considerable attention due to their potential bioactivities.

Some of the salient features of the SAR study are as under:

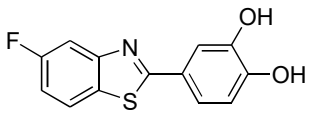
1. Anticancer activity was found to be influenced by the substitution at position 3' in the phenyl ring with a halogen atom or an alkyl group with enhanced potency in the breast carcinoma panel and thus extended the *in-vitro* spectrum of activity to include certain human ovarian, lung, renal and colon carcinoma cell lines [Kumbhare *et al.*, 2012].
2. Modification of the heterocyclic nucleus to generate benzoxazole or benzimidazole congeners had a dyschemotherapeutic effect [Kumbhare *et al.*, 2012].
3. Presence of lipophillic moieties for e.g., amino, hydroxyl and chloro group in molecule is crucial for cytotoxic activity of benzothiazole derivitives against cancer cell lines (85) [Bradshaw *et al.,* 2005; Mukherjee *et al.,* 2005].



R=-NH_3, -OH, -Cl

(85)

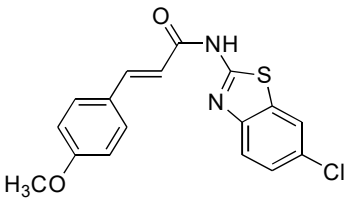
4. Removal of fluro group or its replacement with other halogens, minor modification of dihydroxyphenyl group, had a profoundly dyschemotherapeuitic effect with respect to *in-vitro* cancer cell growth inhibitory activity (86) [Henriksen *et al.,* 2007].



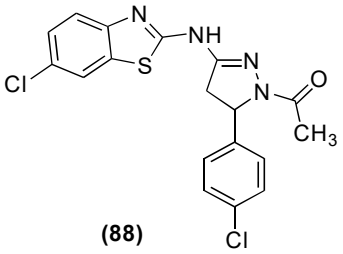
(86)

5. Compound (87) with prop-2-enamido derivative at position 2nd of aminobenzothiazole and p-methoxyphenyl substitution demonstrate most marked effect and possess significant anticancer activity (Fig. 2.2) [Chua *et al.,* 1999; Kashiyama *et al.,* 1999].
6. Compounds with pyrazoline and thiazole substitution at position 2nd of aminobenzothiazole (88-91) were tending to have moderate anticancer activity (Fig. 2.2) [Chua *et al.,* 1999; Kashiyama *et al.,* 1999].

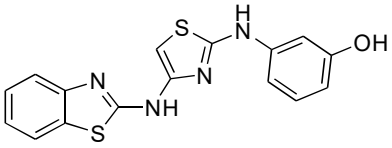
7. Chloro substituted amino benzothiazoles were found to have significant sensitivity to cancer cell lines compared to fluro substituted benzothiazoles [Chua *et al.,* 1999; Kashiyama *et al.,* 1999].



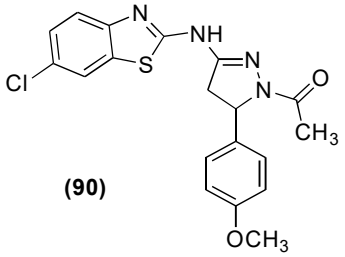
(87)



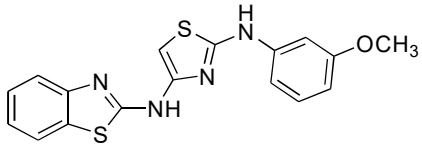
(88)



(89)



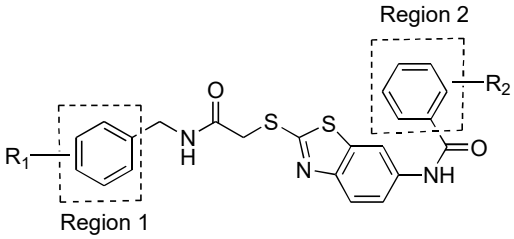
(90)



(91)

Set of Benzothiazole derivatives for SAR analysis

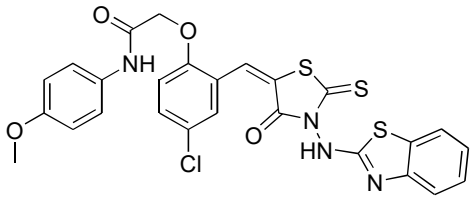
8. Anticancer inhibition was found to depend on substitution pattern of the side chains, especially in region 2, and chloromethyl played a crucial role in this series of benzothiazole-2-thiol derivatives (92) [Bradshaw *et al.,* 2002].



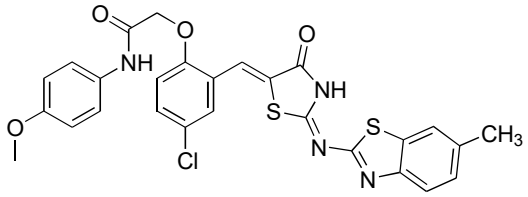
(92)

9. Coupling of 4-thiazolidinone and benzothiazole moieties in a single molecule is a promising approach for exploration of anticancer agents and its potency is sensitive to the nature of substituent in position 5 of 4-thiazolidinone cycle [Patterson and Murray, 2002].

10. Introduction of 4-chlor-ophenoxy-N-(4-methoxyphenyl)-acetamide group (compounds 93, 94) in 5-position of 4-thiazolidinone core enhanced potency [Patterson and Murray, 2002].



(93)



(94)

Four conclusions emerged from the SAR analyses of the above-mentioned study:

1. The presence of free nitrogen is important for the antineoplastic activity.
2. The planarity of molecules is important, may be because of the DNA-interaction possibility.
3. The free nitrogen should not be sterically hindered.
4. The presence of halogen, alkyl functions at proper positions is important for the activity, may be because of metabolism-related issues.

SAR evaluation of the structural features necessary to impart good biological activity was performed to identify improved physicochemical properties of pharmacophore. Diagrammatic representation of SAR analysis, planned to install a variety of substituents at the C2, C5 and C6 positions is shown in Fig. 2.2. In this, modifications at different positions are clearly demonstrated.

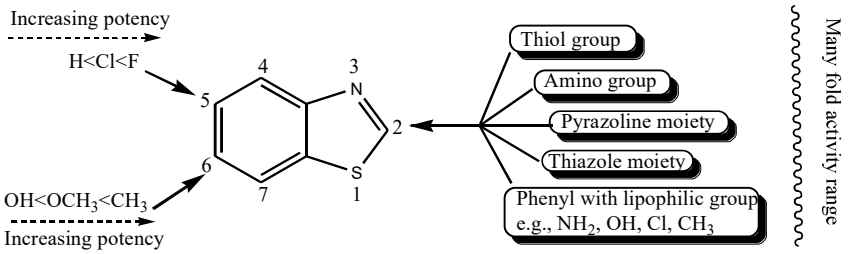


Fig. 2.2 Structure-activity relationship of benzothiazole scaffold.

2.2. CHEMISTRY OF BENZOTHIAZOLES

Benzothiazole is a privileged bicyclic ring system. The ring system in which benzene ring is fused to the 4, 5 positions of Thiazole ring are designated as Benzothiazole and are completely planar. The various positions on the Benzothiazole ring are numbered in the manner indicated as shown in Fig. 2.3.

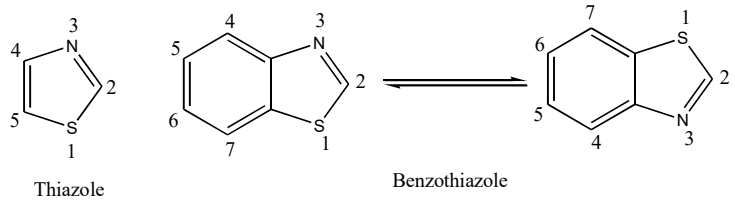


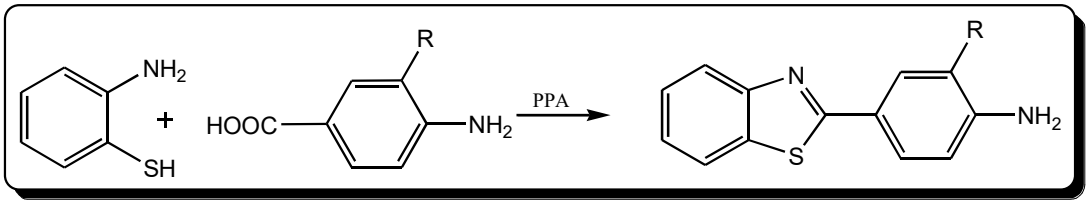
Fig. 2.3 Numbering tautomerism in benzothiazole

Benzothiazole is a colorless, slightly viscous liquid with a melting point of 2ºC and a boiling point of 227-228ºC. The density of benzothiazole is 1.24 g/mL, and its molecular mass is 135.19 $gmol^{-1}$. Benzothiazole has no household use. It is used in industry and research. Being a heterocyclic compound, benzothiazole finds use in research as a starting material for the synthesis of larger, usually bioactive structures. Its aromaticity makes it relatively stable; although, as a heterocycle, it has reactive sites, which allow for functionalization.

In 1887, 2-substituted Benzothiazole was first synthesized by A. W. Hofmann because of diversified activity as well as simple cyclization mechanism; numbers of synthetic routes have been adopted and reported [Fan *et al.,* 2011]. Traditional methods for preparation of the Benzothiazole framework include condensation reactions of 2-aminothiophenols with substituted nitriles, aldehydes, carboxylic acids, acyl chlorides, or esters [Ben-Alloum *et al.*, 1997; Seijas 2007]. Some of the important syntheses and reactions of 2-substituted benzothiazoles are discussed below:

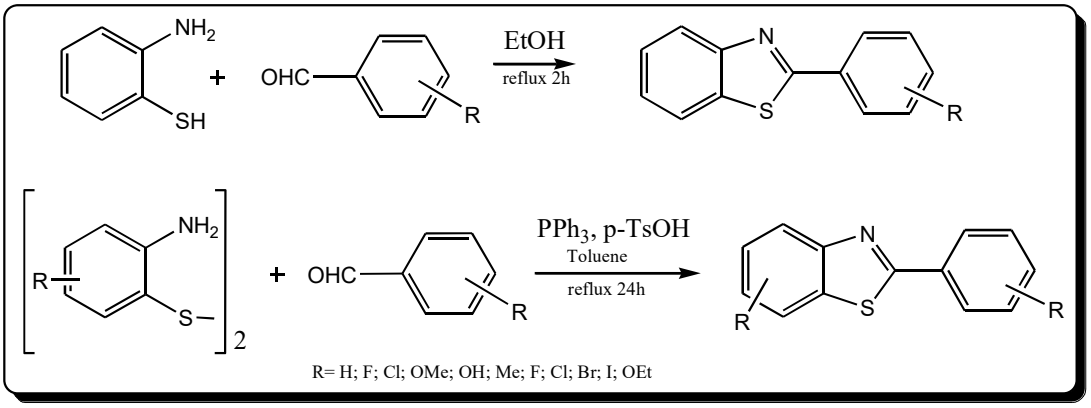
2.2.1 Acid catalyzed condensation.

Ian, H. *et al.,* have been reported the reaction of 2-thiolaniline with aromatic acid in the presence of polyphosphoric acid at 110ºC for the synthesis of 2-(4-aminophenyl)benzothiazole derivatives (Scheme 2.1) [Ian *et al.,* 2001].



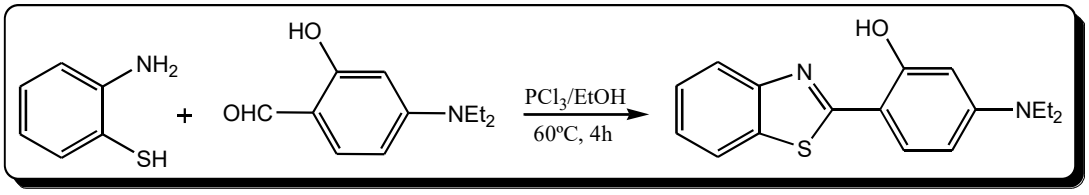
Scheme 2.1

Mortimer *et al.,* synthesized a series of novel 2-phenylbenzothiazoles by the reaction of ortho-aminothiophenol disulfides and substituted benzaldehydes under reducing conditions in ethanol (Scheme 2.2) [Mortimer *et al.,* 2006].



Scheme 2.2

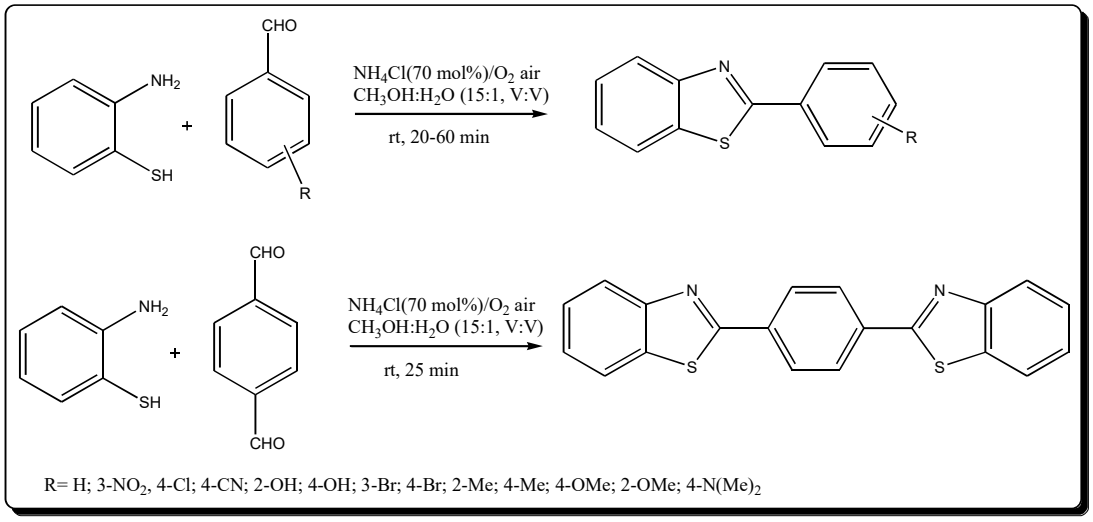
Padalkar *et al.,* reported the condensation of ortho-aminothiophenol and 4-(diethylamino)-2-hydroxybenzaldehyde using PCl3 as a catalyst in ethanol (EtOH) (Scheme 2.3) [Padalkar *et al.,* 2011].



Scheme 2.3

2.2.2 Base catalyzed condensation.

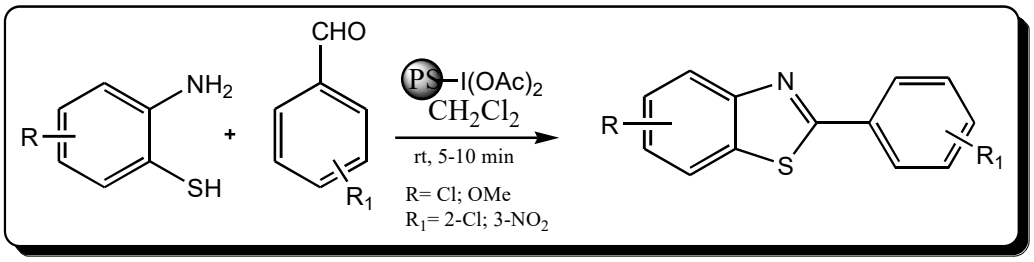
Maleki and his co-worker have developed a mild and efficient protocol for the synthesis of 2-arylbenzothiazole derivatives from condensation of ortho-aminothiophenol with aromatic aldehydes employing ammonium chloride as a catalyst in methanol–water (15:1 v/v) as a dual solvent system at room temperature, which afforded high yields of the products (Scheme 2.4). The use of NH_4Cl as a very inexpensive, metal-free and readily available reagent is the superior feature of this protocol [Maleki and Salehabadi, 2010].



Scheme 2.4

2.2.3 Solid supported condensation.

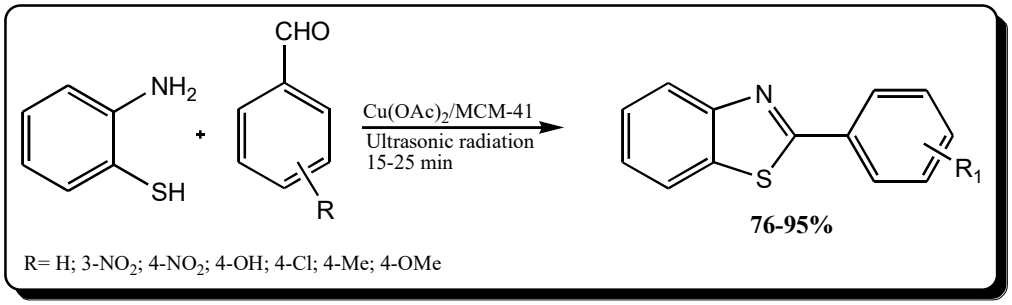
Kumar *et al.,* have attempted a synthesis of a library of 2-substituted benzothiazoles via the condensation of various substituted amines with different aldehydes in the presence of poly[4-diacetoxyiodo] styrene (PDAIS) as a solid supported hypervalent iodine reagent in dichloromethane (DCM), which afforded an excellent yield of the products (Scheme 2.5). In this, PDAIS is converted to polymer supported iodobenzene which is recovered by filtration is the major benefit of this protocol [Kumar *et al.,* 2009a].



Scheme 2.5

2.2.4 Resin supported condensation.

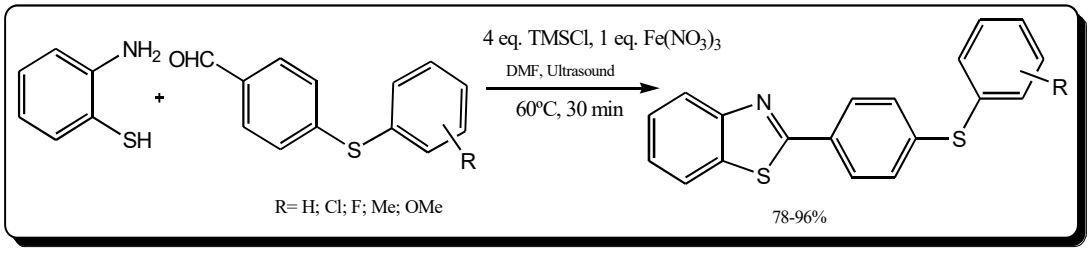
Sadjadi and his co-worker have found a new solvent free approach for the synthesis of 2-arylbenzothiazole in the existence of MCM-41 supported $Cu(OAc)_2$, as a catalyst under ultrasonic irradiation to afforded excellent yield of the products (Scheme 2.6) [Sadjadi and Sepehrian, 2011].



Scheme 2.6

2.2.5 Organo-silicon supported condensation.

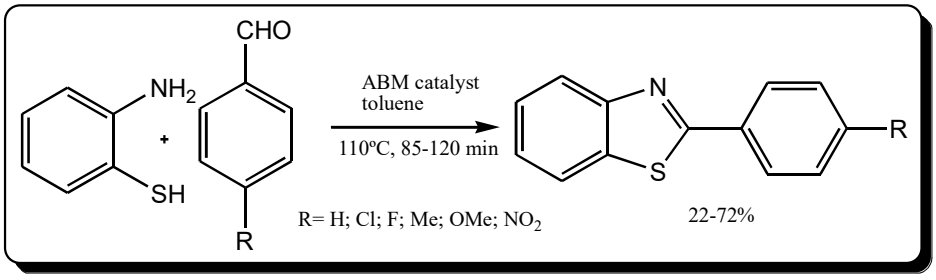
Yuan and his co-worker reported a mild and efficient reagent chlorotrimethylsilane (TMSCl) in dimethylformamide (DMF) as a promoter and water scavenger and $Fe(NO_3)_3$ as catalyst for the one-pot preparation of benzothiazoles from aromatic ortho-aminothiophenols and aldehydes under ultrasonic irradiation with good yield (Scheme 2.7). The authors performed a set of experiments on 4-phenylsulfanyl-benzaldehyde and ortho-aminothiophenol by applying various amounts of TMSCl and $Fe(NO_3)_3$ at different reaction temperatures and deduced that the best result was achieved by carrying out the reaction with TMSCl and $Fe(NO_3)_3$ (4 : 1) at 60ºC under ultrasonic irradiation in DMF or without solvent [Yuan and Guo, 2011].



Scheme 2.7

2.2.6 Using bio-reagent.

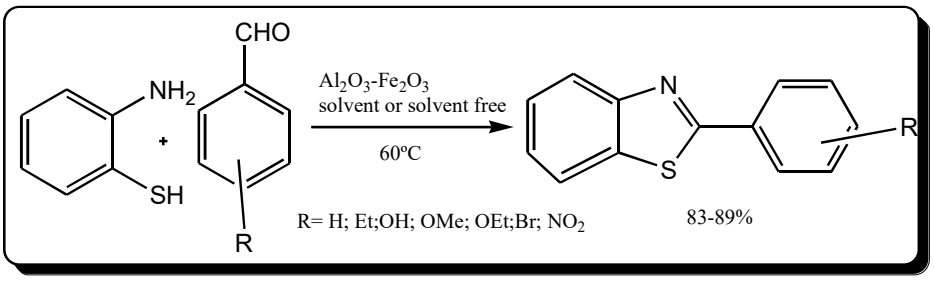
Riadi *et al.,* have efficiently synthesized a series of benzothiazoles by the condensation of ortho-aminothiophenol with aromatic aldehydes in the presence of catalytic amounts of Animal Bone Meal (ABM) and Lewis acids doped ABMs under reflux conditions in air (Scheme 2.8). The remarkable features of this new protocol are high conversion, short reaction times, cleaner reaction profiles, straight forward procedure and reduction in catalyst toxicity [Riadi *et al.,* 2011].



Scheme 2.8

2.2.7 Metal nanoparticle catalysed condensation.

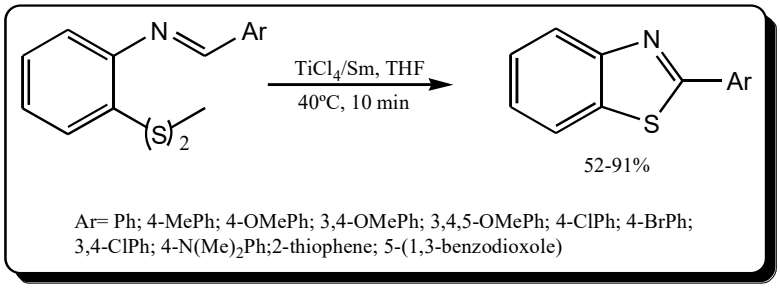
Bandyopadhyay *et al.,* reported an efficient procedure for the synthesis of 2-substituted benzothiazoles using ortho-aminothiophenol and substituted aldehydes in the presence of Al_2O_3–Fe_2O_3 nanocrystals (5% w/w of ortho-aminothiophenol), as a heterogeneous catalyst at 60ºC (Scheme 2.9) [Bandyopadhyay *et al.,* 2011].



Scheme 2.9

2.2.8 Cyclization reaction

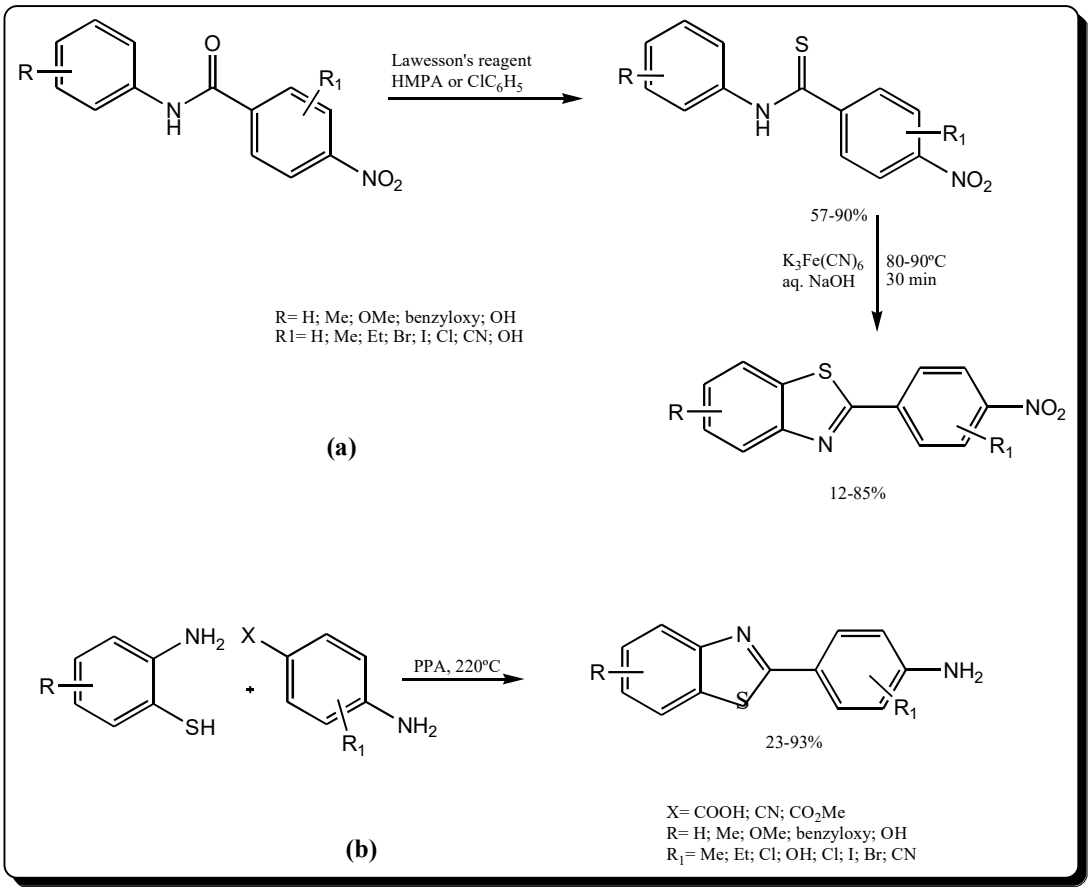
Shi *et al.,* have determined a novel reductive cyclization of bis-(2-benzalaminophenyl)disulfide promoted by a titanium tetrachloride (TiCl4)/samarium (Sm) in 1:2 ratio of $TiCl_4$ and Sm at 40ºC system using tetrahydrofuran (THF) as a solvent for the synthesis of 2-arylbenzothiazole derivatives in good yields (Scheme 2.10). Low-valent titanium was prepared from titanium tetrachloride and samarium powder. The advantages of this new method are easily accessible starting materials, short reaction time and moderate to good yields [Shi *et al.,* 2010].



Scheme 2.10

2.2.9 Using Lawesson's reagent and $K_3Fe(CN)_6$

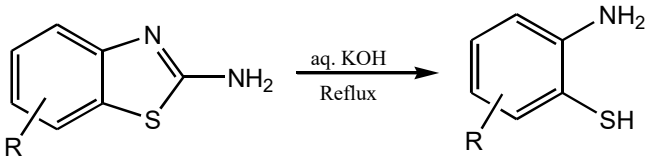
Shi *et al.,* synthesized a new library of 2-(4-aminophenyl)benzothiazoles using Lawesson's reagent in hexamethylphosphoramide (HMPA) or chlorobenzene (ClC_6H_5) followed by the treatment of potassium ferricyanide ($K_3[Fe(CN)_6]$) in aq. sodium hydroxide at 80-90ºC, which afforded excellent yield of the products (Scheme 2.11) [Shi *et al.*, 1999].



Scheme 2.11

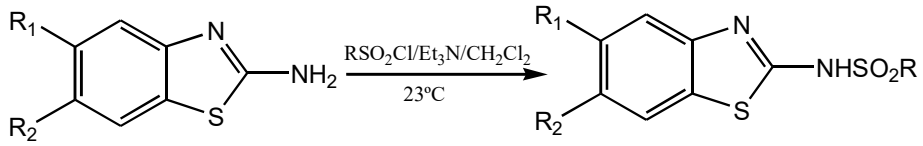
2.2.10 Reactions of 2-amino benzothiazole:

Catriona *et al.,* reported the hydrolysis of 2-aminobenzothiazole using strong base aqueous potassium hydroxide on reflux condition for 6-8 hrs. The reaction scheme is given in Scheme 2.12 [Catriona *et al.*, 2006].



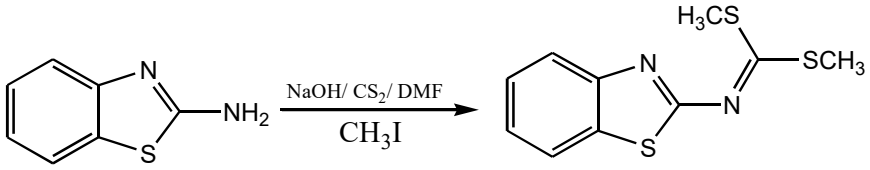
Scheme 2.12

Al-Soud *et al.,* disclosed the sulphonation reaction of 2-aminobenzthiazole with alkylthionyl chloride in presence of organic base and dichloromethane (DCM) as a solvent of the reaction at room temperature The reaction scheme is given in Scheme 2.13 [Al-Soud *et al.,* 2008].



Scheme 2.13

Alejandro *et al.,* reported the reaction of amino group of 2-amino benzothiazole with carbondisulphide and methyl iodide in the presence of basic medium. Dimethylformamide (DMF) was used as a solvent in the reaction. The reaction scheme is given in Scheme 2.14 [Alejandro *et al.*, 2008].

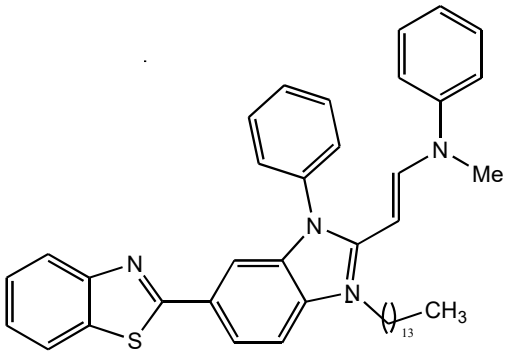


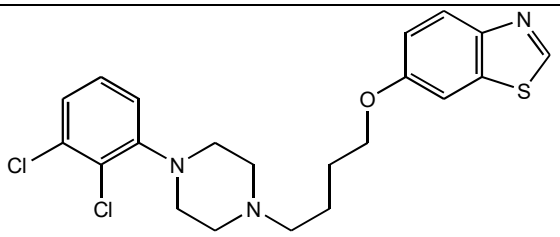
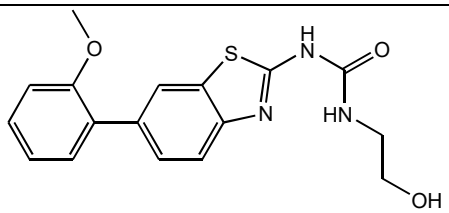
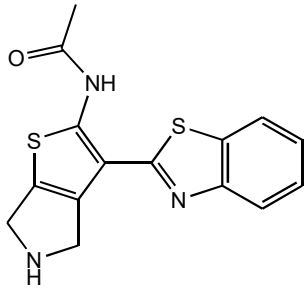
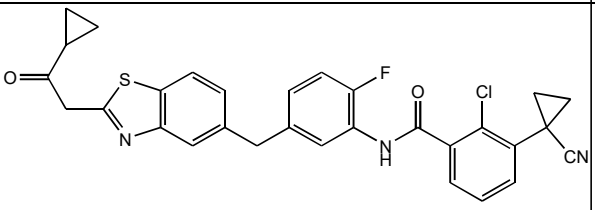
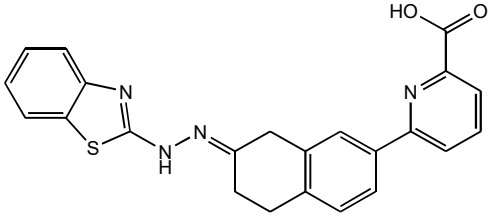
Scheme 2.14

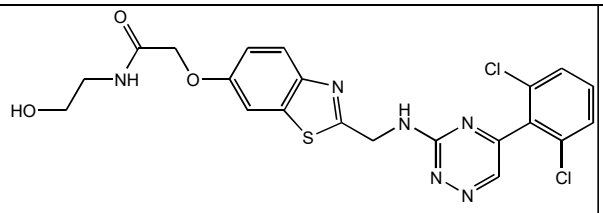
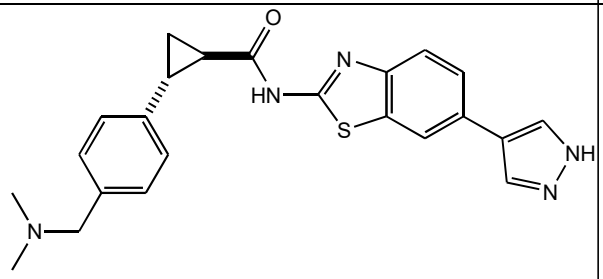
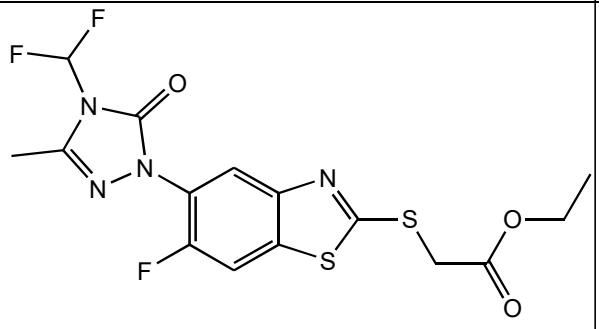
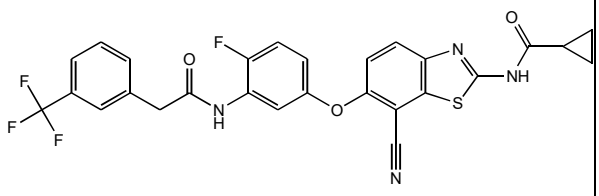
2.3. BENZOTHIAZOLES AS ENZYME AND RECEPTOR AGONISTS/ ANTAGONISTS

Some examples of BTA derivatives acting as agonists or antagonists of various receptors and enzymes are as follows:

Table 2.1 Benzothiazole derivatives that act on enzymes/receptors.

Sr. No	Structures	Enzyme/ Receptors	Reference
1	 **(95)**	AKT (Protein Kinase B) inhibitor-IV	Sun *et al.,* 2011

2	 EC_{50}(β-Arrestin)= 63μM **(96)**	β-Arrestin-biased dopamine D2 receptor	Chen *et al.,* 2012
3	 IC_{50} (AB1T3151 kinase)= 0.064nm **(97)**	Inhibitors of breakpoint cluster region-abelson kinase	Hong *et al.,* 2013
4	 IC_{50} (APE1 inhibitor)= 3.3μM **(98)**	Apurinic/ apyrimidinic endonuclease 1 inhibitors	Rai *et al.,* 2012
5	 IC_{50} (Kinase BRAF)= 7nm IC_{50} (Kinase VEGFR2)= 2.2nm **(99)**	Vascular endothelial growth factor receptor 2 (VEGFR2) inhibitors	Okaniwa *et al.*, 2012
6	 IC_{50} ($BclX_L$)= 0.023μM **(100)**	Inhibitors of $BclX_L$	Sleebs *et al.,* 2013

7	 IC_{50} (HldE-K)= 55µM **(101)**	HldE-K inhibitors	Atamanyuk *et al.,* 2013
8	 ITK Ki= 0.7nm LCK Ki= 0.055 µM IC_{50} (pPLC-γ1)= 0.091 µM **(102)**	Interleukin-2 inducible T cell kinase (ITK)	MacKinnon *et al.,* 2013
9	 Ki(mtPPO)= 0.06 µM **(103)**	Protoporphy-rinogen oxidase (PPO) inhibitors	Yang *et al.,* 2013
10	 IC_{50} (Kinase BRAF)= 2.4nmol/L IC_{50} (Kinase VEGFR2)= 160nmol/L **(104)**	Selective kinase inhibitor (TAK-632)	Okaniwa *et al.*, 2013

2.4. TARGETS FOR ANTIMICROBIAL AND ANTICANCER DRUGS

2.4.1. Glucosamine-6-phosphate synthase (Glc-6-PS): a novel target for antimicrobial agents

Wojciechowski *et al*., has reported L-Glutamine: D-fructose-6-phosphate amidotransferase, known under trivial name of glucosamine-6-phosphate synthase, as the only member of the amidotransferase subfamily of enzymes which does not display any ammonia-dependent activity (Fig. 2.4). Glucosamine-6-phosphate synthase (GlmS) catalyzes the formation of D-glucosamine 6-phosphate from D-fructose 6-phosphate using L-glutamine as the ammonia source. This protein is a complex enzyme, catalysing the first committed step in a pathway leading to the eventual formation of uridine 5'-diphospho-N-acetyl-D-glucosamine (UDP-GlcNAc) an important point of metabolic control in biosynthesis of amino sugar-containing macromolecules. Substantial alterations to the enzyme structure and properties have been detected in different neoplastic tissues. Obviously, glucosamine-6-phosphate, the product of this enzyme, is indispensable for fungi as well as for human cells, yet the consequences of its deficiency in both species are very different. It has been shown that even a short-time inactivation of GlcN-6-P synthase in fungal cells is lethal for the pathogen (it induces morphological changes, agglutination and lysis), while in mammals depletion of the amino sugar pool for a short time is not lethal, because of the much longer lifespan of mammalian cells. Long half lifetime of GlcN-6-P synthase, and rapid expression of the mammalian gene encoding the enzyme. N-acetylglucosamine is an essential building block of both bacterial cell walls and fungal cell wall chitin and thus being a potential target for antibacterial and antifungal agents [Milewski *et al.,* 1988]. The most potent carbohydrate-based inhibitor of GlcN-6-PS reported till date is 2-amino-2-deoxy-D-glucitol 6-phosphate, an analogue of the putative cis-enolamine intermediate formed during catalysis [Wojciechowski *et al*., 2005]

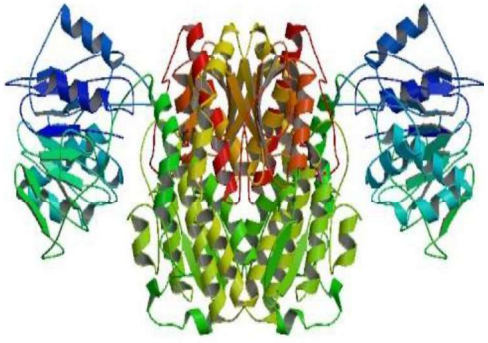


Fig 2.4. Biological Assembly Image for Glucosamine 6-phosphate synthase with Glucose 6-phosphate (1JXA)

2.4.2. Epidermal Growth factor Receptor-Tyrosine Kinase (EGFR-TK): a novel target for anticancer agents

Receptor tyrosine kinases (RTKs) are high affinity cell surface receptors that bind polypeptide growth factors, cytokines and hormones. They have been shown to be key regulators of normal cellular processes and additionally play a critical role in the development and progression of many types of cancer [Zwick *et al.,* 2001]. Protein tyrosine kinases occupy a central position in the control of cellular proliferation. Over expression of certain RTKs show association with promotion and maintenance of malignancies. For example, the epidermal growth factor (EGF) receptor tyrosine kinases of the erbB family (which includes erbB1-erbB4) is frequently expressed at high levels in certain carcinomas and show an inverse correlation with survival (particularly breast, colon and bladder cancers) [Gullick *et al.,* 1991]. Thus, inactivation of the specific tyrosine kinases responsible for the malignant phenotype of certain cancers represents a potential approach to design the antiproliferative drugs [Yardern and Ullrich, 1988].

The epidermal growth factor receptor (EGFR) is a member of the ErbB family of receptor tyrosine kinases together with HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4) (Fig. 2.5). The ErbB receptors are normally expressed in various tissues of epithelial, mesenchymal and neural origin. The pivotal role of ErbBs in normal individual development has been largely investigated by multiple research groups through their involvement in the regulation of important tumorigenic processes, such as proliferation, apoptosis, angiogenesis, and invasion. However, it is easy to imagine that dysregulation of such genes or proteins can lead to cancer development. The ErbB receptors are plasma membrane glycoproteins composed of an extracellular ligand-binding domain, a single transmembrane region and an intracellular TK-domain [Yoshida *et al.,* 2010]. These molecules exists as inactive monomers and, on binding to ligands, they undergo conformational changes that allow homo- or heterodimerization with the other members of the family of receptors [Schlessinger, 2004; Yarden and Sliwkowski, 2001]. Dimerization, an essential requirement for the transactivation of the intrinsic kinase domain, leads to the phosphorylation on specific tyrosine residues within the cytoplasmic region of the receptor [Schlessinger, 2002]. Activated ErbB dimers stimulate many intracellular signalling pathways and, despite the extensive overlap in the recruited proteins, different ErbBs preferentially modulate specific signalling pathways (Fig. 2.5). Among the ErbBs downstream pathways, the phosphatidylinositol 3-kinase (PI3K)-Akt-mammalian target of apamycin (mTOR) and the mitogen-activated protein kinase (MAPK) pathways have been

demonstrated to play a key role in the control of numerous fundamental cellular processes [Bjornsti and Houghton, 2004; Normanno *et al.,* 2006; Scaltriti and Baselga, 2006].

Two classes of EGFR inhibitors are available for clinical use in many countries. They are small-molecule TK inhibitors (TKIs) which compete with ATP for the interaction with the intracellular domain, and monoclonal antibodies (mAbs) which contend with ligands at the extracellular domain. Both the above classes exert antitumor activity with various potential implications for clinical efficacy correlated to the different administration way and mechanism of action. In addition to the receptor, kinase inhibitors against other proteins within the EGFR pathway, such as Raf, MEK, PI3K, Akt and mTOR, are also in clinical development. Gefitinib (Iressa®, AstraZeneca, and Wilmington, DE, USA) and erlotinib (Tarceva®, Hoffman-La Roche, Basel, Switzerland) are the two EGFR-TKIs currently approved for the treatment of advanced NSCLC in many countries. Both first-generation quinazoline- derived drugs were designed as ATP-mimetics to reversibly block wild-type receptor phosphorylation. Their clinical development in NSCLC treatment preceded the discovery of EGFR activating mutations as key markers of sensitivity.

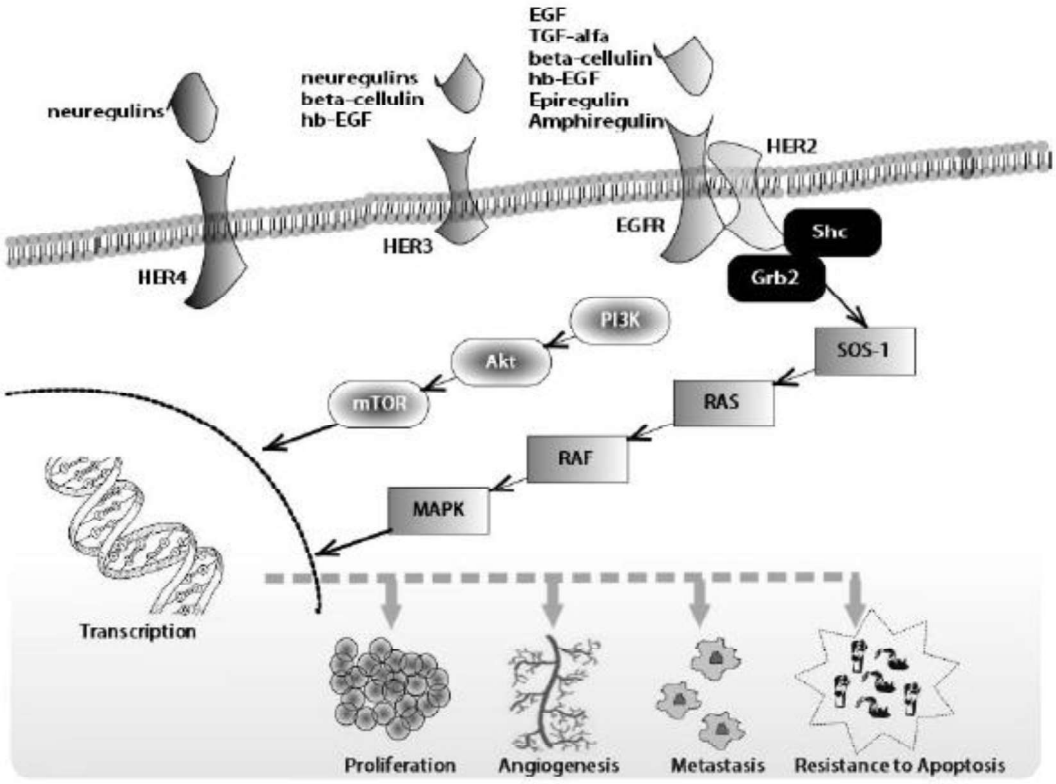


Fig. 2.5 EGFR signaling pathways: Signaling pathways and epidermal growth factor tyrosine kinase receptors involved in the tumorigenesis of NSCLC.