2-(4-Aminophenyl) Benzothiazole: A Potent and Selective Pharmacophore with Novel Mechanistic Action Towards Various Tumour Cell Lines

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Abstract: 2-(4-aminophenyl) benzothiazole (CJM -126) (Table **1** (**1**) and its analogues represent a potent and highly selective class of antitumor agents. These compounds in nanomolar range elicit potent growth inhibition in human-derived breast, colon, ovarian and renal tumour cell lines. Metabolism of benzothiazole plays a central role in its mode of action. Cytocrome P450 isoform, CYP1A1, biotransforms benzothiazoles, to active, as well as inactive metabolites. *In vitro* studies had confirmed that N-oxidation and N-acetylation (only 3' halogen congener) as main active metabolic transformation (generating cytotoxic electrophilic species), while C-6 oxidation and N-acetylation (except 3' halogen congener) as inactive metabolic transformation pathway. Generation of an inactive metabolite 2-(4-aminophenyl)-6-hydoxybenzothiazole [6-OH 126, (Table **1**) (10)] is blocked by fluorinated analogue, substituted around benzothiazole nucleus, especially at 5-position. National Cancer Institute (NCI), USA, confirms this series as a unique mechanistic class distinct from clinically used chemotherapeutic agents. Benzothiazoles are potent aryl hydrocarbon receptor (AhR) agonists, binding to AhR results in induction of CYP1A1, causes generation of electrophilic reactive species which forms DNA adduct, ultimately resulting in cell death by activation of apoptotic machinery. To overcome the poor physiochemical and pharmaceutical properties (bioavailability problem) of this compounds, prodrug of benzothiazole derivatives were synthesized, which are introduced in clinical trails.

Keywords: 2-(4-Aminophenyl) benzothiazole, Antitumour agents, Aryl hydrocarbon receptor (AhR), Cytochrome P-450 isoform CYP1A1, DNA adduct.

INTRODUCTION

Over recent years, there has been steadily increasing research in the field of anticancer therapy. Many efforts have been directed towards the identification and characterization of novel, potent and selective anticancer ligand molecule. 2-(4-aminophenyl) benzothiazole [CJM 126, (Table **1** (**1**) and its analogues comprise a novel mechanistic class of antitumor agents (Fig. **1**)] [1, 2]. This nucleus comes from the related structure polyhydroxylated 2-phenylbenzothiazoles [3], flavone quercetin and the isoflavone genistein, which are tyrosine kinase inhibitors [4] having potent antitumor activity. *In vitro* unique and remarkable selectivity and characteristic pattern of benzothiazole is confirmed by National Cancer Institute (NCI) [1, 5, 6]. These agents have potent and selective activity mainly towards MCF-7 cells (including oestrogen receptor ER+ MCF-7 cells [7] and ER-cells [8]), MDA-435, MDA-MB-468 cells.

Analogues of CJM-126 substituted in 3' position [3' iodo (DF129) (Table **1**) (6), 3'methyl (DF-203) (Table **1**) (2)], 3'chloro (DF-229) (Table **1**) (11)] [1, 6, 9] and at 5 position [5 fluoro (5f-203) Table 1(13)] [10] have potent activity than parent compound. Thus all these analogues show good response across sensitive cell lines (GI_{50} value<[10]$^{-8}$M), while very less response towards insensitive cell lines (GI_{50}>10^{-4}M) [11].

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Cytochrome P-450 isoform CYP1A1 is basically required for biotransformation of benzothiazole, [12] resulting in generation of active and inactive metabolites [13]. CYPA1A1 is present only in sensitive cell lines. In benzothiazole N-oxidation leads to generation of main active metabolite [11] a reactive electrophilic species, while C-6 oxidation leads to generation of 6-OH derivative which is an inactive compound having negligible anti-tumor activity. This metabolic inactivation is successfully blocked by fluorinated analogue, substituted on benzothiazole nucleus, especially at 5-position [10].

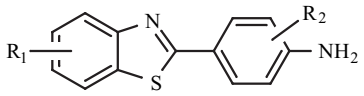

Fig. (1). Chemical structure of anti-tumor 2-(4-aminophenyl) benzothiazole.

Conjugation of exocyclic amine function of benzothiazole derivatives with amino acid results in amino acid amide prodrugs which improve the physiochemical property and bioavailability profile of benzothiazole. L- lysyl and L-alanine amino acid amide prodrugs (Fig. **7** and Fig. **8**, respectively) of benzothiazole have been synthesized by Stevens *et al*. [5, 14, 15].

CHEMISTRY

From reported literature, various substituted benzothiazole derivative which have been synthesized, are given in table (1). Prodrugs of benzothiazole, successfully synthesised by the conjugation of aryl amine with amino acids (lysine and alanine), fulfils the criteria of prodrug.

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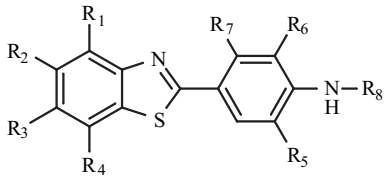
Table 1. Various Substitutions, Which Have Synthesized on 2-(4-Aminophenyl) Benzothiazole Nucleus



S.No.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	Formula
1	H	H	H	H	H	H	H	H	$C_{13}H_{10}N_2S$
2	H	H	H	H	H	Me	H	H	$C_{14}H_{12}N_2S$
3	H	H	H	H	H	Br	H	H	$C_{13}H_9BrN_2S$
4	H	H	Me	H	H	Br	H	H	$C_{14}H_{11}BrN_2S$
5	H	H	H	H	H	Br	Br	H	$C_{13}H_8Br_2N_2S$
6	H	H	H	H	H	I	H	H	$C_{13}H_9IN_2S$
7	H	H	H	H	H	Cl	Cl	H	$C_{13}H_8Cl_2N_2S$
8	H	H	H	H	H	CN	H	H	$C_{14}H_9N_3S$
9	H	H	H	H	H	OH	H	H	$C_{13}H_{10}N_2OS$
10	H	H	OH	H	H	H	H	H	$C_{13}H_{10}N_2OS$
11	H	H	H	H	H	Cl	H	H	$C_{13}H_9ClN_2S$
12	H	H	OH	H	H	Me	H	H	$C_{14}H_{12}N_2OS$
13	H	F	H	H	H	Me	H	H	$C_{14}H_{12}N_2FOS$
14	H	H	F	H	H	Me	H	H	$C_{14}H_{12}N_2FOS$
15	H	F	F	H	H	Me	H	H	$C_{14}H_{11}N_2F_2OS$

Simple and effective methods to synthesize benzothiazole derivatives include, cyclization (Jacobson cyclization) of substituted nitrothiobenzanilidine (in presence of aqueous sodium hydroxide and potassium ferricyanide), followed by reduction (in presence of stannous chloride-water and ethanol) (Fig. **2**) and secondly, by the reaction of 2-aminothiophenol with substituted benzoic acid (in presence of polyphosporic acid at 220°C) [7], (Fig. **3**).

STRUCTURE ACTIVITY RELATIONSHIP

The screening of the benzothiazole derivatives, shows cytotoxicity in tumour cell lines due to simple pharmacophore 2-(4-amino phenyl) benzothiazole (Fig. **4**), Table **1** (1).

Lead compound (benzothiazole) is 10-fold more cytotoxic against MCF-7 and MDA-468 lines than benzoxazole, but approximately 1000 fold more active than

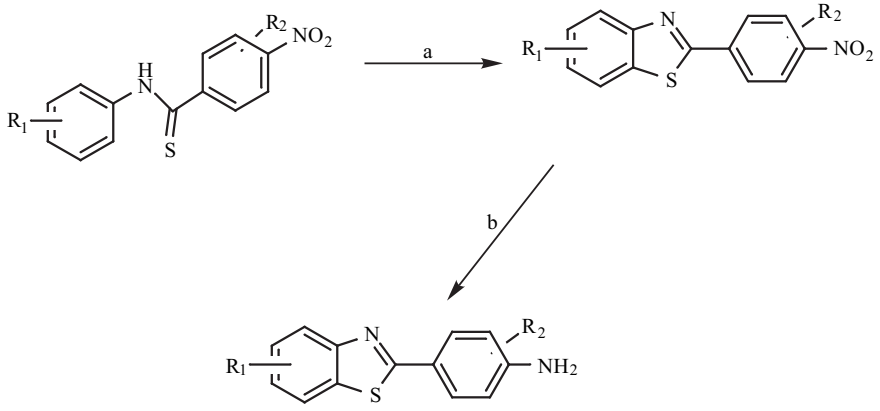


Fig. (2). Synthetic route of 2-(4-aminophenyl) benzothiazole through Jacobson cyclization.

a) $K_2Fe(CN)_6$, Aqueous NaOH, b) $SnCl_2.H_2O$ & EtOH.

the benzimidazole. As methane and ethane sulfonic acid salts, it retained the anticancer activity *in vitro*. Tertiary amine analogues acquired 50% inhibition of cancer cell growth in 0.1 to 1μg concentration range; it is more than 100 fold less active than the lead compound. Introduction of alkoxy or hydroxy groups into benzothiazole nucleus has dyschemotherapeutic effect.

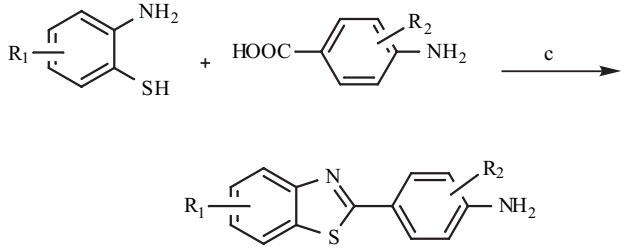


Fig. (3). Synthetic route of 2-(4-aminophenyl) benzothiazole through in presence of polyphosphoric acid.

c) Polyphosphoric acid, (PPA), 220°C.

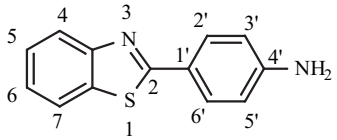


Fig. (4). Common pharmacophore of 2-(4-aminophenyl) benzothiazole, having antitumour activity.

Introduction of chlorine (Cl) at 2' position of amino phenyl group of lead (Fig. **4**), (Table **1**) (7)] gave compound with reduced activity as compared to parent amine. Substitution adjacent to the amino group i.e., at 3' position [3'-iodo (Table **1** (6), 3'-chloro (Table 1(11)), and 3'-methyl (Table 1(2))] enhances the potency in sensitive breast cell lines and in human ovarian carcinoma cells lines, while 3' cyano (Table **1** (8) and 3' hydroxy (Table **1**(9) substitution reduces activity markedly compared to parent amine. 3, 5' di-substituted compound results in dyschemotherapeutic effect.

Replacement of 2-(4 amino phenyl) group of lead compound with 2-(pyridin-4-yl) or 2-(2-aminopyridin-5-yl) residue results in a compound having cytotoxicity only at concentration >1μM. Introduction of bromo group to give 2-(2-amino-3-bromo pyridine-5-yl) benzothiazole (Table 1 (3, 4 and 5)) enhances activity >100 fold over unbrominated amino pyridine but potency is still considerably less than that of bromo aryl amine [7, 9].

Fluorinated 2-(4-amino phenyl) benzothiazole are potent cytotoxic agents which are able to block metabolic inactivation of benzothiazole to inactive metabolite [6 OH 203, (Table **1** (12)] Substitution of flouro group at 5- and 7- results in active compound towards various sensitive cells lines, while 4- and 6-fluoro analogues are inactive towards sensitive cells lines [10].

The primary aromatic amine has been derivatized as amino acid amides, by conjugating (Fig. **4**) with alanine and lysine residue as mono- (Fig. **7**) and di- (Fig. **8**) hydrochloride salts, respectively, which has increased water solubility, lipophillicity, chemical stability and bioavailability of benzothiazoles [14, 5].

MECHANISM STUDY

Selective uptake of benzothiazole into sensitive cells was followed by binding with aryl hydrocarbon receptor (AhR) [as they are potent AhR agonist] [16-18], translocation into the nucleus, induction of cytochrome P-450 isoform

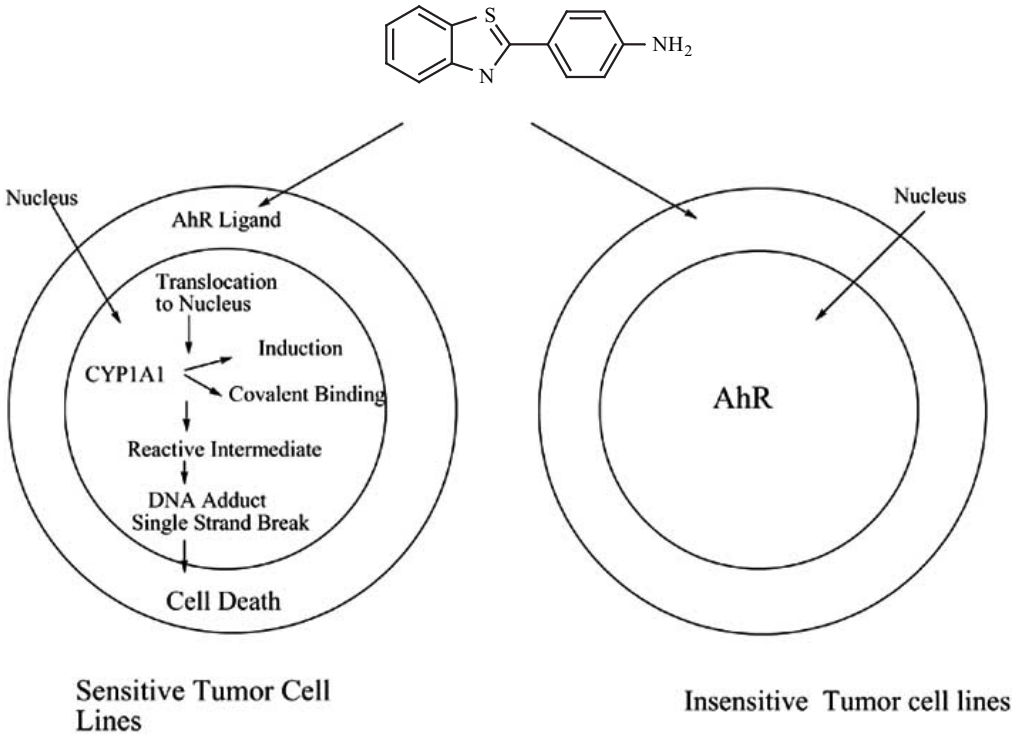


Fig. (5). Mode of action of 2-(4-amino phenyl)benzothiazole in sensitive and insensitive cell lines.

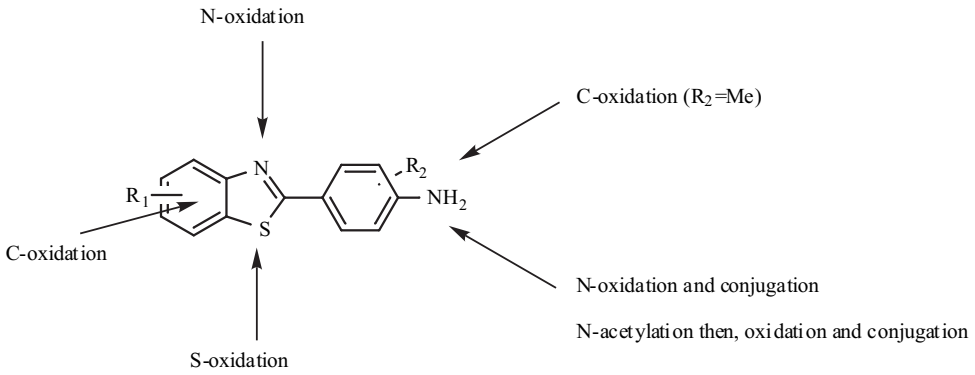


Fig. (6). Potential sites for metabolic transformation of antitumor 2-(4-amino phenyl) benzothiazoles.

(CYP1A1), increase in xenobiotic response element (XRE)-driven luciferase activity and conversion of drug into putative electrophilic species intermediate, which induces formation of benzothiazole species- DNA complexes on the XRE sequence of CYP1A1 promoter, this complex is formed due to NADPH dependent covalent binding of benzothiazole with CYP1A1 [13, 16-18].

This leads to formation of DNA adduct and causes DNA damage which activates the apoptotic machinery and ultimately leads to cell death [19], (Fig. **5**). G_1 and S phase of cell cycle arrest is induced by this molecule [20].

This mechanism occurs in sensitive cells, while insensitive cells undergo little metabolism, since they don't show drug induced CYPA1A1 activation so no DNA adduct is formed. Involvement of CYP1A1 induction is must for activity, confirmed by treatment of resveratrol, an inhibitor of CYP1A1 induction, with benzothiazole derivative, which showed decrease in sensitivity and selectivity of benzothiazole derivatives toward sensitive cell lines [21, 22].

In vitro covalent binding between recombinant CYP1A1 and benzothiazole derivative has been confirmed (bonding is reduced in presence of glutathione). Further more formation of DNA adduct were confirmed and detected before the cell death, when treated with 2-(4-amino-3-methylphenyl) benzothiazole (5f-203, Table **1** (13) at concentration >100nm [19] and c-DNA microassay is used for characterization of complete pathway [23, 24].

METABOLISM

Metabolism plays an important role in mode of action of the benzothiazole derivatives. Various biotransformation pathways are shown in (Fig. **6**).

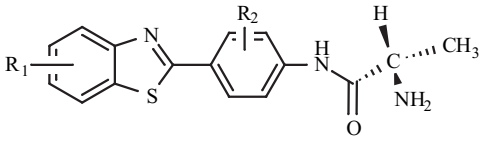


Fig. (7). Alanyl amide prodrugs of 2-(4-aminophenyl) benzothiazole.

Uptake and Development of Resistance

Uptake of benzothiazole derivatives occurs mainly in sensitive cells, negligible in insensitive cells. About 65% of benzothiazole was sequrnerd by sensitive MCF-7 cells in 24 hr and 84% after 72 hr, with negligible amount of compound remaining after 7 days in growth media. Increase in lipophilicity increases the uptake (3' chloro derivative more lipophilic). Small, lipophilic, fluorocent molecule in sensitive cells was confirmed by clonal microscopy. Reduced drug uptake and accumulation is one of the many mechanisms that may lead to acquired resistance [11].

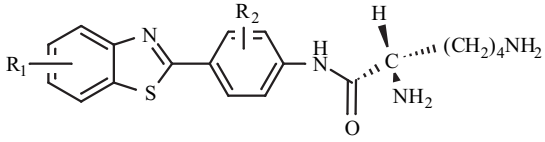


Fig. (8). Lysyl amide prodrugs of 2-(4-aminophenyl).

Biotransformation

Antitumor benzothiazoles are biotransformed by CYP1A1 to putative active or inactive metabolites. The common metabolic pathway in aryl amine metabolism includes N-acetylation, N-oxidation and C-oxidation. *In vitro* studies confirm N-acetylation and N-oxidation as main metabolic transformation pathways of 2-(4-aminophenyl) benzothiazole, with the predominant process being dictated by nature of 3' substitution. Protoamine undergoes N-acetylation while 3' substituted amine analogues undergo oxidation. N-acetylation (except for 3' halogen congener) exerts drastic dyschemotherapeutic effect. N-oxidation leads to generation of genotoxic nitrenium species responsible for the carcinogenic properties, C-6 oxidation results in formation of inactive 6-OH derivative. The 6-OH derivatives have no antitumor activity while it possesses mitogenic property at µm concentration. Moreover this metabolite antagonises cellular uptake of benzothiazole and prevails covalent binding between CYP1A1 and benzothiazole. *Ab intio* frontier molecular orbital calculations have been used to predict the presence or absence of hydroxy metabolite. Metabolic inactivation of molecule has been thwarted by isosteric replacement of hydrogen by flourine atoms at position around benzothiazole nucleus [1, 11].

PHYSICOCHEMICAL STUDY

Exocyclic primary amine function of 2-(4-amino phenyl) benzothiazole has been conjugated successfully to alanine and lysine residue as mono- and di- hydrochloride salts, respectively, fulfilling the criteria for suitable prodrug i.e.,

increasing water solubility, lipophilicity, chemical stability, parentral applicability, ultimately improving bioavailability. Metabolic conversion of prodrug lysamide (NSC D710305) into 2-(4-amino3-methylphenyl)-5 fluorobenzothiazole occurs in cell and this active moiety binds with cytosolic AhR and follows the same mechanism as the normal derivatives followed [5, 14, 15, 17].

CONCLUSION

NCI has confirmed that 2-(4-aminophenyl) benzothiazoles and their series of compounds have unique *in vitro* selective fingerprint i.e., they represent a mechanistic class, distinct from clinically used chemotherapeutic agents. Activation by CYP1A1 is an integral part of the antitumor activity of this novel compound. N-acetylation (except halogen congener) and C-6 oxidation results in deactivation, while N-acetylation (only for halogen congener) and N-oxidation results in activation of compound. The prodrug approach for benzothiazole conjugate with amino acids produce desired physiochemical and pharmaceutical properties. Due to high selectivity of the molecule and improved physiochemical properties, the lysyl amide prodrug (NSC D71305) has been selected for phase-I clinical trials.

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