RESEARCH PAPER

Design and Evaluation of Novel Benzothiazole–Benzimidazole Hybrids as Potential Anticancer Agents

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ABSTRACT

Aromatase inhibitors, especially in low-supply postmenopausal women with hormone-dependent breast cancer, are highly susceptible to resistance development due to prolonged usage. Among the novel aromatase inhibitors, benzimidazole and benzothiazole ring systems are found to be of great interest in this study. The study was performed to synthesize benzothiazole derivatives fused with the benzimidazole ring system and to evaluate their anticancer activity against breast cancer. A total of three derivatives containing benzothiazole substituted with benzimidazole were synthesized. The drug-likeness and toxicity study was performed using SWISS ADME and PROTOX III server. The derivatives were characterized using H1-NMR and Mass spectrophotometry. The antitumor property of all novel developed derivatives was evaluated by MTT assay using MCF-7 cell lines. From the MTT assay results, it was found that the novel synthesized compounds are potent anticancer molecules against breast cancer.

Keywords: Aromatase inhibitors, Anticancer drugs, Benzothiazole, Benzimidazole, Molecular Docking, MTT assay

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INTRODUCTION

Benzothiazole is a versatile moiety.[1] With some modifications, the nucleus of benzothiazole showed a broad range of pharmacological activities, from antibacterial[2], anti-HIV[3], anthelmintic[4], antidiabetic[5], antiviral[6], anticonvulsant[7], larvicidal[8], and antimalarial[9], to anticancer[10] activity. Due to the wide spectrum of pharmacological activities, benzothiazole is a molecule of interest to medicinal chemists. Benzothiazole as a parent moiety contains antitumor activity and the substituent heterocycle benzimidazole also possesses antitumor activity. Hence, this research prompted the decision to integrate the two ring systems to create a more potent anticancer agent through synergy.

Aromatase is an enzyme crucial for the biosynthesis of estrogen. The conversion of androgens into estrogen is catalysed by aromatase activity.[11] Estrogen promotes the growth of tumor cells in hormone receptor-positive types of breast cancer. Hence, aromatase inhibition is the cornerstone of therapeutic interventions in the management of breast cancers.[12]

MATERIAL AND METHODS

All the chemicals utilized were imported from Merck. All the chemicals were of AR grade. The structures of all the intermediates and products were verified by LC-MS, Nuclear Magnetic Resonance spectrophotometry, and Mass spectrophotometry. Melting points were determined using the Equiptronics type EQ-730 device. For TLC, Merck silica gel 60 F254 plates were utilized. The plates were viewed using a UV lamp. 1H NMR spectra were obtained using a Bruker 400MHz NMR spectrometer in DMSO-d6 solution and TMS as an internal standard. The infrared spectra were recorded using the Bruker alpha FTIR spectrometer. Mass spectral data was recorded using a Shimadzu LC2010 mass analyzer, and C, H, and N analyses were performed using a Perkin Elmer PE 2400.

General procedure:

Benzothiazole is a heterocyclic compound

consisting of a benzene ring fused to a thiazole ring, while Benzimidazole is a heterocyclic compound consisting of a benzene ring fused to an imidazole ring as shown in Figure 1. Both these heterocycles contain anticancer activity. Therefore, the goal is to synergize their antitumor activity by fusing them.[13]

Synthesis of Compound 1 [7-chloro-6-fluorobenzo[d]thiazol-2-amine] from 3-fluoro,4-chloro aniline

To a solution of 3-fluoro, 4-chloro aniline, (30 g, 206.89 mmol, 1.0 eq.) in AcOH (400mL) which was maintained at 0˚C, potassium thiocyanate (40.0 g, 413.79 mmol, 2.0 eq.) was added. Bromine (49.34 g, 310.33 mol, 1.5 eq.) was dissolved in AcOH (400 mL) and added dropwise at 0˚C in the previous solution. The reaction mixture was agitated and left to warm at room temperature for four hours. TLC tracked the reaction's development. The reaction mixture was filtered using a Buchner funnel once the reaction was finished. The precipitate was filtered through a Buchner funnel and dried under reduced pressure to produce a crude compound, which was then purified by methanol trituration to produce pure compound 1 (10 g). The precipitates were obtained by pouring solid precipitate into ice-cold water and basifying it with aqueous ammonia solution.

Synthesis of Compound 2 [N-[7-chloro-6-fluorobenzo[d]thiazol-2-yl]hydrazinecarbothioamide]

Compound 1(10 g, 49.50 mmol, 1.0 eq) was dissolved in Tetrahydrofuran (100 ml) at 0˚C and 60% sodium hydride in mineral oil (2.97 g, 74.25 mmol, 1.5 eq) was added portion-wise in it. The mixture was stirred by maintaining 0˚C for 45 min. Drop by drop, at 0°C, carbon disulfide (5.64 g, 74.25 mmol, 1.5 eq) was added, and the solution was agitated for 45 minutes at that temperature. After a dropwise addition of methyl iodide (10.4 g, 74.25 mmol, 1.5 eq) at the same temperature, the solution was allowed to be heated at room temperature while being shaken for three hours. The progress of the reaction was monitored by TLC. The mixture was put in water after it was complete, and ethyl acetate was utilized to extract the product. Crude was yielded by drying the organic layer with sodium sulfate, washing it with brine, and concentrating it. To acquire pure compound 2, this was cleaned up with silica gel

column chromatography, and the product was separated using 8% ethyl acetate in hexane.

Synthesis of Benzothiazole derivatives substituted with benzimidazole (Compound 3a, 3b, 3c)

To a mixture of compound 3 (0.3 g, 1.0 eq) in N, N-dimethylformamide (10 mL) was cooled added substituted phenylene diamine (1.5 eq). The reaction mixture was stirred at 140°C for 16 hours. The course of the reaction was monitored by TLC. After the reaction, the reaction mixture was immersed in water and the product was extracted with ethyl acetate. For the crude, the organic layer was washed with brine, dried over sodium sulfate, and concentrated. In order to get a pure final product, this was purified by silica gel column chromatography and the product was eluted in 2% methanol in dichloromethane. (scheme 1, Figure 2)

Detection Method:

All the compounds were characterizied by proton NMR (Nuclear Magnetic Resonance),

mass spectrophotometry and LC-MS (liquid chromatography – mass spectrophotometry)

Characterization data for compound 3a, 3b, 3c

N-(1H-benzo[d]imidazol-2-yl)-7-chloro-6-fluorobenzo[d]thiazol-2-amine(3a)- Qty: 90mg, LCMS: 96.49%, IR 3320 cm^{-1} (N–H stretching), 3080 cm^{-1} (aromatic C–H), 1630 cm^{-1} (C=N stretching), 1590–1515 cm^{-1} (aromatic C=C), 1340 cm^{-1} (C–N), 1275 cm^{-1} (C–F), 1150 cm^{-1} (C–S), and 1015 cm^{-1} (C–Cl) vibrational modes. 1 H NMR (400 MHz, DMSO-d 6) δ (ppm): 12.13 (s, 2H), 7.946-7.923(d, J=9.2Hz, 1H), 7.729-7.717 (d, J=4.8Hz, 1H), 7.425-7.403 (m, 2H), 7.192-7.170 (m, 2H).ESI-MSm/z: 319.1 [M +H] +.

7-chloro-6-fluoro-N-(6-methyl-1H-benzo[d]imidazol-2-yl)benzo[d]thiazol-2-amine (3b)-

Qty: 68mg, LCMS: 94.91%, IR 3320 cm^{-1} (N–H stretching), 3080 cm^{-1} (aromatic C–H), 1630 cm^{-1} (C=N stretching), 1590–1515 cm^{-1} (aromatic C=C), 1340 cm^{-1} (C–N), 1275 cm^{-1} (C–F), 1150 cm^{-1} (C–S), and 1015 cm^{-1} (C–Cl) vibrational modes.1 H NMR (400 MHz, DMSO-d 6) δ (ppm): 12.03 (s, 2H), 7.920-7.897 (d, J=9.2Hz, 1H), 7.703-7.687 (d, J=6.4Hz, 1H), 7.299-7.279 (d, J=8Hz, 1H), 7.195 (s, 1H), 7.001-6.981 (d, J=8.0Hz, 1H), 2.388 (s, 3H). ESI-MSm/z: 333.3 [M +H] +.

7-chloro-N-(5,6-difluoro-1H-benzo[d]imidazol-2-yl)-6-fluorobenzo[d]thiazol-2-amine (3c):

Qty: 58mg, LCMS: 93.76%, IR 3315 cm^{-1} (N–H stretching), 3075 cm^{-1} (aromatic C–H), 1628 cm^{-1} (C=N stretching), 1590–1515 cm^{-1} (aromatic C=C), 1338–1340 cm^{-1} (C–N), 1260–1275 cm^{-1} (C–F), 1148–1150 cm^{-1} (C–S), and 1012–1015 cm^{-1} (C–Cl) vibrational modes.1 H NMR (400 MHz, DMSO-d 6) δ (ppm): 12.21 (s, 2H), 8.003-7.980 (d, J=9.2Hz, 1H), 7.675 (s, 1H), 7.456-7.412 (m, 2H). ESI-MSm/z: 355.0 [M +H] +.

RESULTS

Chemistry

The synthesis of benzothiazole derivatives (3a-3c) were prepared by condensation of N-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)hydrazinecarbothioamide with substituted o-phynelene diamine. All the derivatives were confirmed by ^{1}H-NMR and Mass analyses. Reaction scheme is given in figure.

Anticancer study

MTT assay

A cell line study as an MTT assay was performed to screen the anticancer study of the novel synthesized benzothiazole derivatives. Because the anticancer study was aimed to perform against breast cancer, the cell line here used was the MCF-7 cell line.

Cell lines and cell culture

The MCF-7 cell line[14] of breast cancer was utilized to evaluate the anticancer potential of newly designed drugs in vitro. The cell line MCF-7 (for breast cancer) was procured from NCCS, Pune, India. The cell line was upheld as one-layer culture at 37 °C under moistened conditions with 5% CO2/95% air in 10% fetal bovine serum containing DMEM/F12 and penicillin (50 U/mL) and streptomycin (50 µg/mL).

Cytotoxicity measurement by MTT assay

The cytotoxicity experiment was conducted in response to the treatment of produced chemicals. In short, 96-well plates of culture were filled with 10,000 cells per well, and the plates were incubated for a full day. [15] Cells were subjected to various dosages of compounds in order to identify the optimum IC50 value of each novel synthesized compound. IC50 value can be defined as 50% of the maximum inhibitory concentration, which is a compound's concentration, such as a drug, inhibitor, or toxin required to inhibit the biological or biochemical function by 50%.[16] The next day, 10 µL of MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-

Diphenyltetrazolium Bromide) (5 mg/mL) was added to every well. After that, the wells were incubated for between three and four hours at 37 °C in a CO2 incubator. The wells contained 100 µL of culture. Following the incubation, the developed purple crystals were solubilized in 100 µL of molecular grade DMSO, and the culture media was discarded. A microplate reader was employed to quantify absorbance at 570 nm. The formula given below was employed to calculate the cell viability percentage. With n = 3, the trial was replicated twice.[17]

$$\%cell\ viability = \frac{mean\ absorbance\ of}{mean\ absorbance\ of\ }$$

The results of MTT assay are given in table 1.

Drug Likeness and Drug Toxicity Studies

The drug-likeness and drug-toxicity studies parameters are shown in Tables 2 and table 3.

In drug-likeness, the parameters like no of rotatable bonds, hydrogen bond acceptors, hydrogen bond donors, log P, and log S value as well as GI permeability, are performed using SWISS ADME.[18] No of rotatable bonds determines the flexibility of the compound to bind with its target. It should be less than 10 as per Veber's rule[19] for good oral bioavailability. Hydrogen bonding affects the molecule's solubility, permeability, and binding capacity. As per Lipinski's rule[20], hydrogen bond acceptors should be less than 10 and hydrogen bond donors should be less than 5. Log P stands for the octanol-water partition coefficient. It should be less than 5 as per Lipinski's rule of 5. A log P value of more than 5 shows poor solubility, increased metabolism, and potential toxicity because of high lipophilicity. Log P value less than zero denotes poor absorption and permeability because of high hydrophilicity. Log S stands for aqueous solubility. Log S value greater than -4 indicates good solubility.

As per the data in Table 2, all the compounds are within the range of standard data and as they have high GI permeability, they can be formulated as an oral dosage form as they would have good absorption in systemic circulation through GI wall. Toxicity study is a very important parameter while developing a new drug moiety. A toxicity study was performed by the PROTOX III server. It

indicates the possible toxicities of the molecule. As per the data denoted in table 4, there is very low hepatic toxicity[21] for all the novel derivatives, which indicates that all the novel derivatives are under the category of class IV[22] which is non-irritating and non-toxic compounds. These results indicate that all the novel benzothiazole derivatives are safe to administer.

DISCUSSION

The following discussion integrates literature comparisons to contextualize findings and highlight the novelty and performance of the synthesized benzothiazole–benzimidazole hybrids relative to existing anticancer scaffolds.

The synthesized benzothiazole–benzimidazole hybrids (3a–3c) demonstrated clear structure–activity trends supported by both in vitro and in-silico results. Compound 3a showed the lowest IC_{50} value among the series (70.05 ± 0.55 μM), closely matching the standard drug exemestane (69.12 ± 0.35 μM), indicating strong anticancer potential. The superior performance of 3a may arise from minimal steric hindrance around the benzimidazole moiety, which favors target engagement. Docking results corroborated the biological profile, as 3a exhibited strong binding affinity (–8.1 kcal/mol) and replicated essential aromatase interactions seen with exemestane. Comparative analysis with studies on benzothiazole and benzimidazole derivatives indicates that the hybridization strategy adopted here provides a distinct advantage by directly targeting the aromatase active site, a mechanism scarcely explored in current literature. ADME and toxicity predictions further support the drug-likeness of the compounds, with all derivatives meeting Lipinski's and Veber's rules, showing high GI permeability and falling into toxicity Class IV (non-toxic, non-irritant). Collectively, these data establish compound 3a as a strong lead candidate and demonstrate the novelty of targeting aromatase using a benzothiazole–benzimidazole fused scaffold.

CONCLUSION

Novel benzothiazole derivatives were synthesized by merging benzothiazole with benzimidazole moiety by synthetic pathway. From the results of MTT assay, IC50 value of compound 3a is least in all three compounds and is nearly same as the standard drug. Therefore, compound 3a is the most

potent anticancer compound from all the three compounds. As per the results of drug likeness, all the derivatives are highly permeable through GI membrane; therefore, oral dosage form can be developed and from the results of toxicity studies, all the drugs are under class IV i.e. non-toxic and non-irritating compounds.

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Table 1 IC50 value of Novel compounds and Standard drug

Sr No.	Compounds Name	IC50 Value
1	3a	70.05 ± 0.55
2	3b	127.30 ± 0.75
3	3c	95.18 ± 0.38
7	Standard drug: Exemestane	69.12 ± 0.35

Table 2. Results of drug-likeness

Sr no	Code	MW [g/mol]	No of Rotatable bonds	H-bond Acceptors	H-bond Donors	Log P	Log S	GI Permeability
1	3a	318.76	2	3	2	3.96	5.39	High
2	3b	332.78	2	3	2	4.33	5.68	High
3	3c	354.74	2	5	2	4.86	5.69	High

Table 3. Results of toxicity studies

Sr no	Code	Hepatotoxicity	Nephrotoxicity	Cardiotoxicity	Carcinogenicity	Mutagenicity
1	3a	Low toxicity	No Toxicity	No Toxicity	No Toxicity	No Toxicity
2	3b	Low toxicity	No Toxicity	No Toxicity	No Toxicity	No Toxicity
3	3c	Low toxicity	No Toxicity	No Toxicity	No Toxicity	No Toxicity

Figure Captions

Fig. 1 Structures of Benzothiazole and Benzimidazole

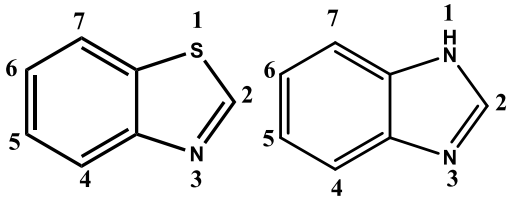
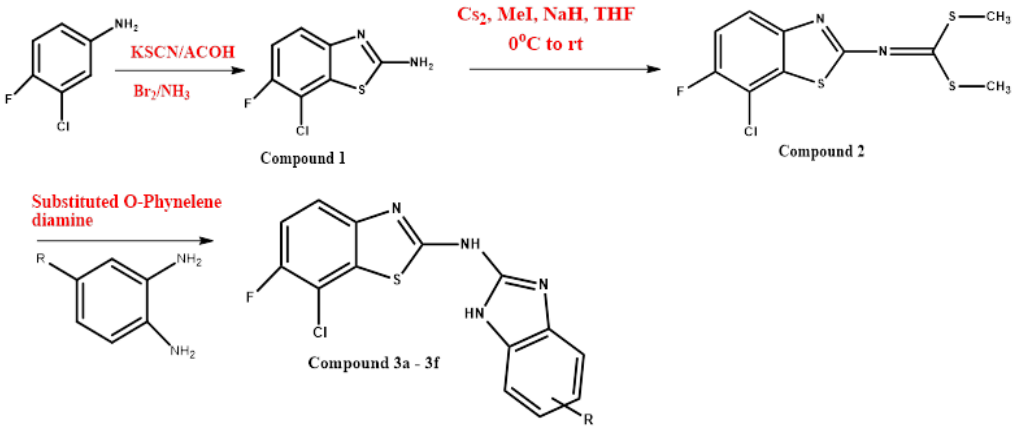


Fig. 2 Synthetic Pathway of Benzothiazole derivatives fused with Benzimidazole


KSCN/ACOH
Br2/NH3
Cs2, MeI, NaH, THF
0°C to rt
Compound 1
Compound 2
Substituted O-Phynelene diamine
Compound 3a - 3f