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RESEARCH ARTICLE

IN-SILICO DOCKING INVESTIGATION, SYNTHESIS AND *INVITRO* ANTICANCER STUDY OF BENZOXAZOLE DERIVATIVES

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ABSTRACT:

Benzoxazole derivatives display broad spectrum of biological and pharmacological activities. The main objective of the present study was to explore newer molecules with potent biological activity like anticancer activity. *Insilico* screening was done and compound with no violation in Lipinski rule of five and good docking score are selected for wet lab synthesize. Benzoxazole derivatives were synthesized and are evaluated for *invitro* anticancer studies. Derivatives were synthesized using different benzaldehyde and anhydride derivative. The synthesized compounds were then established on the basis of IR, MASS and ^{1}H NMR spectral data and screened for anticancer activity on Human breast cancer cell line (MCF-7). The derivative showed significant activity on cell line.

Keywords: Benzoxazole, dihydrophthalazine, anticancer, MTT assay.

INTRODUCTION:

The main objective of medicinal chemistry is to synthesize the compounds that show promising activity as therapeutic agents with lower toxicity. Benzoxazoles have been reported to show abroad spectrum of biological activity.[1,2] The substituted benzoxazole have been shown to exhibit antitumor,[3] antihistaminic, anti-inflammatory,[4] herbicidal, antiallergic, antihelmintic, COX-2inhibitory,[5] antifungal,[6] antibacterial,[7] antitubercular,[8] anticonvulsant,[9] diarrhea redominant irritable bowel syndrome, hypoglycemic, HIV-1 reverse transcriptase inhibitor & insecticidal activities. *Insilico* modeling of different derivatives will be carried out by using software such as Chemsketch and Molinspiration. Compounds having drug likeness and molecular descriptors, resembling those of standard molecules, and which obeys the Lipinski Rule of Five will be selected for wet lab synthesis. Synthesized compounds were screened for anticancer activity.

MATERIALS AND METHODS:

All the chemicals and reagents used in the research work were of analytical grade or synthetic grade. Melting point were determined by melting point apparatus (KHERA) and TLC plate were prepared by using silica gel G. Spots were visualized by exposure to iodine vapour or UV light. IR spectra of the synthesized compounds were recorded using FTIR in the range of 4000-500 cm^{-1} on FTIR-α-zn Se ATR -BRUKER Spectrophotometer.^{1}HNMR spectra were measured with a broker spectrophotomerter (500 MHz) in $CDCl_3$ using TMS as an internal standard .Mass spectra were obtained with LC-MSD Trap-SL 2010 A-Shimadzu.

Synthetic procedure

(i) Synthesis of 4-Hydroxy-3-nitro-benzoic acid methyl ester (p_1)

Mixture of 12.4ml of concentrated suphuric acid and concentrated nitric acid (1:1) was added to p- hydroxy methyl benzoate (10 g, 0.74 mol) in a temperature 0-10˚c with continuous stirring. Temperature of the reaction maintained between 5 to 15˚c for 1 hour and then poured it into crushed ice (70g). From that crude m-nitro and p-hydroxy methyl benzoate were filtered off. Washed product was then added to ice cold methanol and stirred and filtered to remove the trace of ortho-isomer and other impurities. The purity of the compound was established by single spot on TLC plate.

(ii) Synthesis of 3-Amino-4-hydroxy-benzoic acid methyl ester (p_2)

In a 500 ml three necked flat bottom flask equipped with reflux condenser, Compound p_1 (10 g) was dissolved in boiling alcohol (50%, 100 ml) and sodium

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dithionate was added to this boiling alcohol solution until it becomes almost colorless. Then the alcohol was reduced to one third of its volume by distillation and the residual liquid was triturated with ice cold water. The resulting colorless, shiny product was filtered, washed with cold water, dried and recrystallized using methanol as solvent

(iii)Synthesis of 2-subtituted benzoxazole-5-carboxylic acid methyl ester (p_3)

Compound p_2 (0.01mol) was heated with an appropriate aliphatic acid (formic acid and acetic acid) in excess under reflux for 2h. The reaction mixture was cooled and poured in crushed ice (100 gm) with stirring. The product thus separated was filtered under suction and washed with cold water. The products were recrystallized by using methanol as a solvent.

(iv)Synthesis of 2-substituted benzoxazole-5-carboxylic acid hydrazide (p_4)

A mixture of an appropriate 2-subtituted benzoxazole-5-carboxylic acid methyl ester p_3 (0.001 mol) in alcohol (25 ml) and hydrazine hydrate (99%, 0.015 mol) was heated under reflux on water bath for 4 hours. The alcohol was reduced to half of its volume and cooled. The product separated was filtered and washed with small portions of cold alcohol and then with cold water, repeatedly and dried. The resultant product was recrystallized using methanol as solvent. Using above mentioned procedure following two compounds was synthesized.

(v_a)Synthesis of 2-[(2-substituted-1, 3-benzoxazol-5-yl) carbonyl]-2, 3-dihydrophthalazine-1,4-dione(BCH-b2)

11.29 mmol of 1, 3-benzoxazole-5-carbohydrazide dissolved in 20 mL of acetic acid and 11.29 mmol of phthalic anhydride added to the above and content refluxed for 8 hrs and the reaction monitored by TLC (Methanol: EtOAc/6: 4), quenched in to the ice under stirring to get the solid, filtered the solid, washed the solid with chilled water and recrystallized from minimum amount of methanol to get the white crystalline powder.

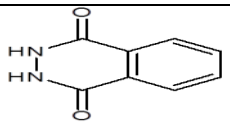
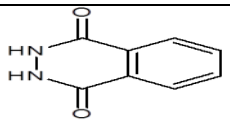
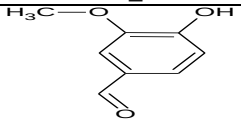
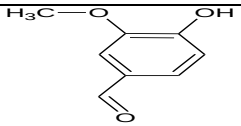
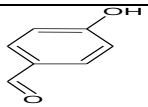
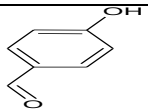
(v_b)Synthesis of N'-[(E)-(4-hydroxy-3-methoxyphenyl)methylidene] -2-substituted-1, 3-benzoxazole5-carbohydrazide .(BCH-a1&a2)

A mixture of compound-p_4 (0.01mol) in 50ml ethanol, 4-Hydroxy-3-methoxy-benzaldehyde (0.01mol) and a few drops of acetic acid were refluxed for 2.5 hours at 60˚ C. The resulting mixture was poured into ice cold water and then it was filtered. Pure compound was obtained from DMF and followed a column chromatography. The purity was checked by single spot on TLC plate, consistency in melting point and $R_f$value. Solvent system used: methanol and ethyl acetate-1:1

(v_c)Synthesis of N'-[(E)-(4-hydroxyphenyl)methylidene] -2-substituted-1, 3-benzox azole5-carbohydrazide. (BCH-a3&a4)

A mixture of compound-p_4 (0.01mol) in 50ml ethanol, 4-Hydroxybenzaldehyde (0.01mol) and a few drops of acetic acid were refluxed for 2.5 hours at 60˚ C. The resulting mixture was poured into ice cold water and then it was filtered. The purity was checked by single spot on TLC plate, consistency in melting point and R_f value.[8]

Table 1: List of synthesized compounds

Compound code	R	Ar	R^1
BCH-b1	H	–	
BCH-b2	CH_3	–	
BCH-a1	H		–
BCH-a2	CH_3		–
BCH-a3	H		–
BCH-a4	CH_3		–

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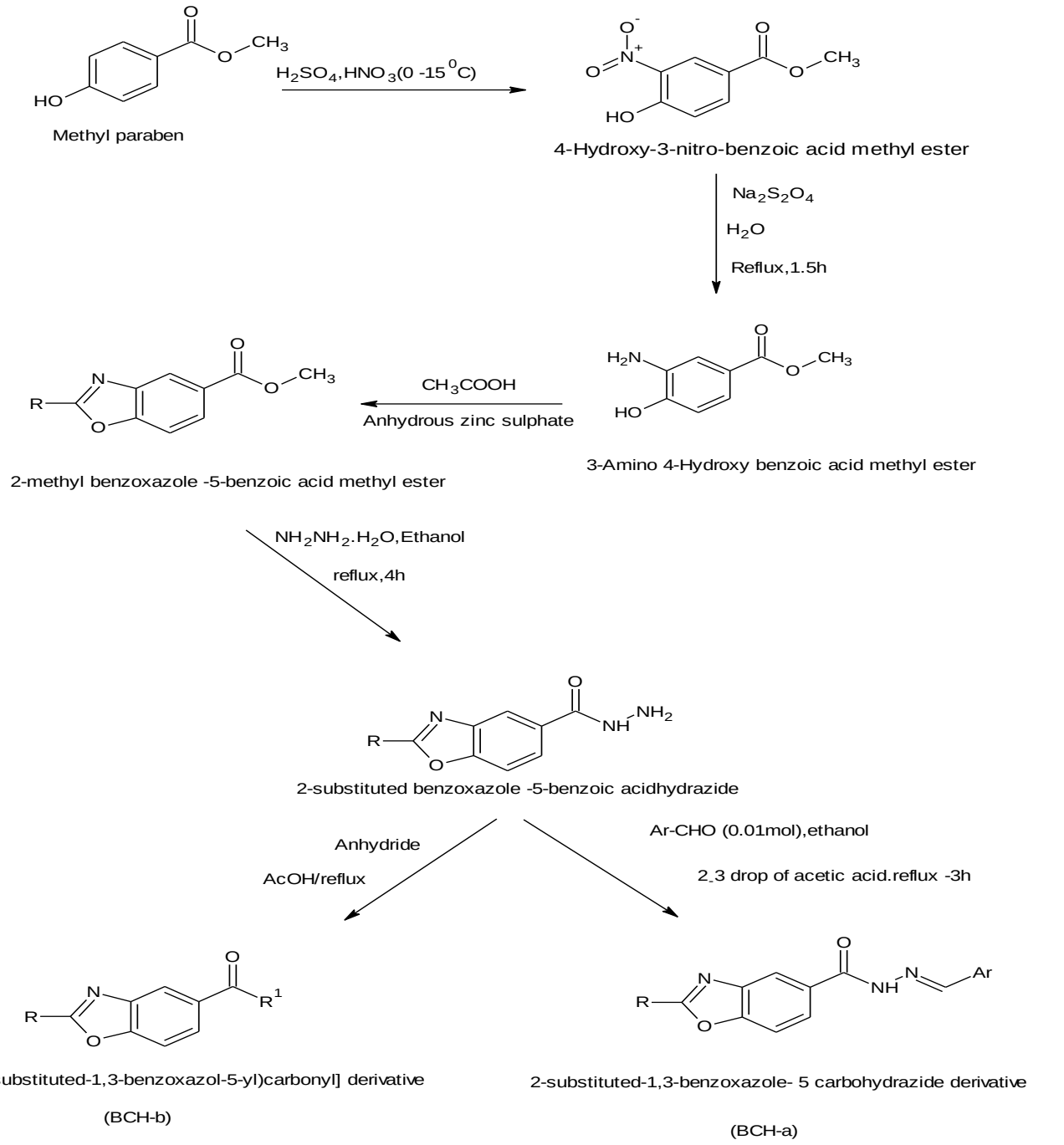
Scheme of synthesis



Pharmacological screening

The human breast cancer cell line (MCF7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37^0 C, 5% CO_2, 95% air and 100% relative humidity.

MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

After 48h of incubation of cell lines treated with standard as well as the synthesized compounds, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The percentage cell inhibition was determined using the

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following formula,

$$\%\ cell\ inhibition = \frac{100 - \text{Abs sample}}{\text{Abs control}} X\ 100$$

Nonlinear regression graph was plotted between % Cell inhibition and Log_{10} concentration and IC_{50} was determined using Graph Pad Prism software.[10]

RESULTS AND DISCUSSION:

Table 2: Lipinski rule analysis of some derivatives by Molinspiration

Compound code	milogP	MW	nON	nOHNH	nrotb	nviolation
BCH-b1	1.937	307.265	7	1	1	0
BCH-b2	2.158	321.292	7	1	1	0
BCH-a1	2.205	311.297	7	2	4	0
BCH-a2	2.427	325.324	7	2	4	0

Table 3: Docking Score for some derivatives against Tyrosine kinase

Compound Code	Tyrosine kinase	
	Energy Score (kcal/mol)	Best Pose
BCH-b1	-9.0856	98
BCH-b2	-9.5872	99
BCH-a1	-10.0721	95
BCH-a2	-10.6572	69

Table 4: Preliminary characterizations of newly synthesized compounds

Compound Code	Molecular formula	Molecular weight	Melting point (˚c)	Percentage Yield (%)	Rf value
BCH-b1	$C_{16}H_9N_3O_4$	307.265	220	60	0.78
BCH-b2	$C_{17}H_{11}N_3O_4$	321.292	221	58	0.82
BCH-a1	$C_{16}H_{13}N_3O_4$	311.297	220	61	0.70
BCH-a2	$C_{17}H_{15}N_3O_4$	325.324	228	60	0.73

Table 5: spectral value of synthesized compounds

compounds	Mass value	IR Spectra	^{1}HNMR
BCH-a1	311	3215(O-H str of phenolic OH), 1708(C=O str of amide), 1630(C=N str of benzoxazole), 1257(C-O-C).	1.83(s, 3H of OCH_3), 6.45-6.49(m, 3H ArH of hydroxyl anisole),7.02(s, 1H of CH), 6.56(s, 1H, Phenolic OH, D_2O Exchangeable), 7.04-7.25(m, 4H, ArH of benzoxazole), 9.05(s, 1H, NH, D2O Exchangeable).
BCH-a2	326	3470(N-H str of amide), 3275(O-H str of phenolic OH), 1708(C=O str of amide), 1630(C=N str of benzoxazole), 1225(C-O-C).	2.30(s, 3H of CH_3), 3.8(3H of OCH_3)7.02-7.04(m, 3H ArH of hydroxyl anisole), 7.05(s, 1H of CH),7.26(s, 1H, Phenolic OH, D_2O Exchangeable), 7.79-7.82(m, 3H, ArH of benzoxazole), 9.77(s, 1H, NH, D2O Exchangeable).
BCH-b2	322	3271(N-H str of Phthalazinone), 1713(C=O str of amide), 1226(C-O-C str of benzoxazole).	2.29(s, 3H CH_3 of benzoxazole), 6.5-7.76(m, 4H ArH of phthalazine), 7.81-8.04(m, 3H, ArH of benzoxazole), 9.79(s, 1H, NH, Phthalazinone, D_2O Exchangeable).

Pharmacological screening

All tested compound show significant cytotoxicity towards human breast cell line. Among them BCH-a2 exhibit comparatively good activity.

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Table 6: IC_{50} values of tested compounds on MCF-7

Compound Code	% Cell Inhibition					IC_{50} Value
	0.1µM	1µM	10µM	50µM	100µM	
BCH-b2	1.4845	3.7425	12.4514	39.7542	54.9826	80
BCH-a1	0.9523	1.4285	8.9523	46.4255	63.7142	52
BCH-a2	1.0481	7.1694	18.2081	53.2712	69.3126	47

Anticancer activity of BCH-a2 on MCF-7 cell line

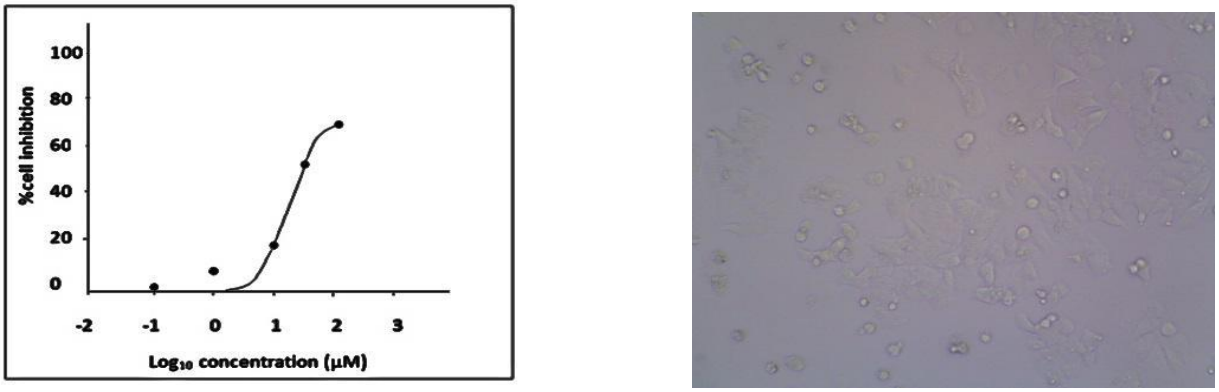

Figure 1: Showing Anticancer activity of BCH-a2 on MCF-7 cell line

CONCLUSION:

The preliminary *insilico* screening of various analogues was performed to assess the drug like properties using Molinspiration software. Drug likeness properties and bio activity of the proposed analogues were studied and all the compounds obeyed Lipinski rule of five were selected for further studies. Docking score of derivatives find out using Argus lab software and depending on good docking scores compound were selected for wet lab synthesis and further pharmacological screening. The prepared compounds were characterized using FTIR, ^{1}HNMR and MASS spectral analysis.

Cytotoxicity studies were carried out by MTT assay. The values obtained were plotted against percentage cell inhibition and concentration gives a nonlinear graph which result in the IC_{50} values of each compound. All tested compound show significant cytotoxicity towards human breast cell line. Among them BCH-a2 exibit comparatively good activity.

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