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SYNTHESIS CHARACTERIZATION AND ANTIANGIOGENIC ACTIVITY OF 2-(6-SUBSTITUTED 1-3- BENZOTHIAZOL-2-YL)-N-(4-HALOPHENYL) HYDRAZINECARBOTHIOAMIDE DERIVATIVES.

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ABSTRACT

Compounds having benzothiazole moiety possess diverse pharmacological activities that is antibacterial[1-2], anticonvulsant[3], antitumour[4], anti-inflammatory[5], antitubercular[6], etc. In this study a series of 2-(6-substituted 1-3- benzothiazol-2-yl)-N-(4-halophenyl) hydrazinecarbothioamide (5a-o) have been synthesized by refluxing equimolar quantity of 2-Hydrazino-6-substituted-1,3- benzothiazole (3a-e) and 4-substituted phenylisothiocyanate (4a-c). 2-Hydrazino-6-substituted-1,3- benzothiazole (3a-e) have been synthesized by refluxing of 6-substituted-1,3- benzothiazol-2-amine (2a-e)with hydrazine hydrate in the presence of con. Hydrochloric acid and ethylene glycol. 4-substituted phenylisothiocyanate (4a-c) werebprepared by reaction of 4-substituted aromatic amines with carbon disulfide in the presence of ammonia.[7] 2-(6-substituted 1-3- benzothiazol-2-yl)-N-(4-halophenyl) hydrazinecarbothioamide (5a-o) yielded according to the scheme. A number of new 2-(6-substituted 1-3- benzothiazol-2-yl)-N-(4-halophenyl) hydrazinecarbothioamide (5a-o) have been synthesized and screened for antiangiogenic activity.

KEYWORDS: Benzothiazole, Synthesis, Antiangiogenic Activity.

INTRODUCTION

Angiogenesis or neovascularization is a complex process involving the activation, adhesion, proliferation and transmigration of endothelial cells from pre-existing blood vessels. It plays a critical role in normal physiological processes such as wound healing, but also in a number of pathological processes, for instance diabetes retinopathy, arthritis and the growth of solid tumors. Therefore angiogenesis is considered as a potential target for antitumor (anticancer)

activity. 2-aminobenzothiazole is an important pharmacophore. Literature survey reveals that 2-aminobenzothiazoles possess anticancer activity along with other pharmacological activities like diuretic, anti-ulcer, anti-histaminic, anticonvulsant, antileishmanial, antidiabetic and antitubercular activity.[1-8] In view of anticancer activity shown by 2-aminobenzothiazoles, we have synthesized some novel derivatives of 2-aminobenzothiazoles, and tested them for antiangiogenic activity by CAM (Chorioallantoic Membrane) assay method.

MATERIAL AND METHOD

All melting points were determined in open capillaries in liquied paraffin and are uncorrected IR spectra (KBr) were recorded on Shimadzu IR 200 spectophotometer. H^1 NMR spectra recorded Bruker Avance II 400 spectometer. Mass spectra recorded on a Jeol mass spectrophotometer at 70 eV.

6-Chloro-1,3-benzothiazol-2-amine(2a)

To glacial acetic acid (150 ml) precooled to 5^0C were added to potassium thiocyanate (0.06 mol) and 4-sunstituted aniline (0.06 mol) (1a). The mixture was placed in freezing mixture of ice and salt and mechanically stirred while bromine (0.02 mol) in 10 ml glacial acetic acid was added from a dropping funnel at such rate that temp. does not rise beyond 0-5^0C. After all the bromine has been added (105 min), the solution was stirred for an addition 2 hr at 0-10^0C. The residue was filtered and dissolved in hot water (150 ml). The solution was filtered and filtrate was neutralized with conc ammonia solution to pH 6. The precipitates was collected and crystallized with ethanol, yield: 78%; m.p 170^{o}c; Vmax 3425(1^o NH), 1530 (aromatic C=C), 1442 (thiazole), 1320 (C-N), 716 (C-Cl); DMSO 6.8 (2H, s, NH2), 7.1-7.5 (3H, m, Ar-H). The other compounds of the series were prepared by similar procedure (Yield: 67-75 %).

2-Hydrazino-6-chloro-1, 3-benzothiazole (3a)

Conc. Hydrochloric acid (10 ml) was added dropwise with stirring to hydrazine hydrate (10.0g, 0.2 mol) at 5-10^0C followed by ethylene glycol (40 ml). Thereafter, 6-substituted-1,3-benzothiazol-2-amine (0.01 mol) (2a-e) was added in portion and the resultant mixture refluxed for 2hr and cooled. The fine crystalline solid separated was filtered, washed with water and recrystallization from ethanol. yield: 72%; m.p 198^{o}c; Vmax 3425(1^o NH), 3200 (2^o NH), 1632 (C=N), 1530 (aromatic C=C), 1442 (thiazole), 1320

(C-N), 716 (C-Cl); DMSO 4.8 (2H, s, NH2), 7.1-7.5 (3H, m, Ar-H), 8.9 (1H, s,NH) The other compounds of the series were prepared by similar procedure (Yield : 60-75%).

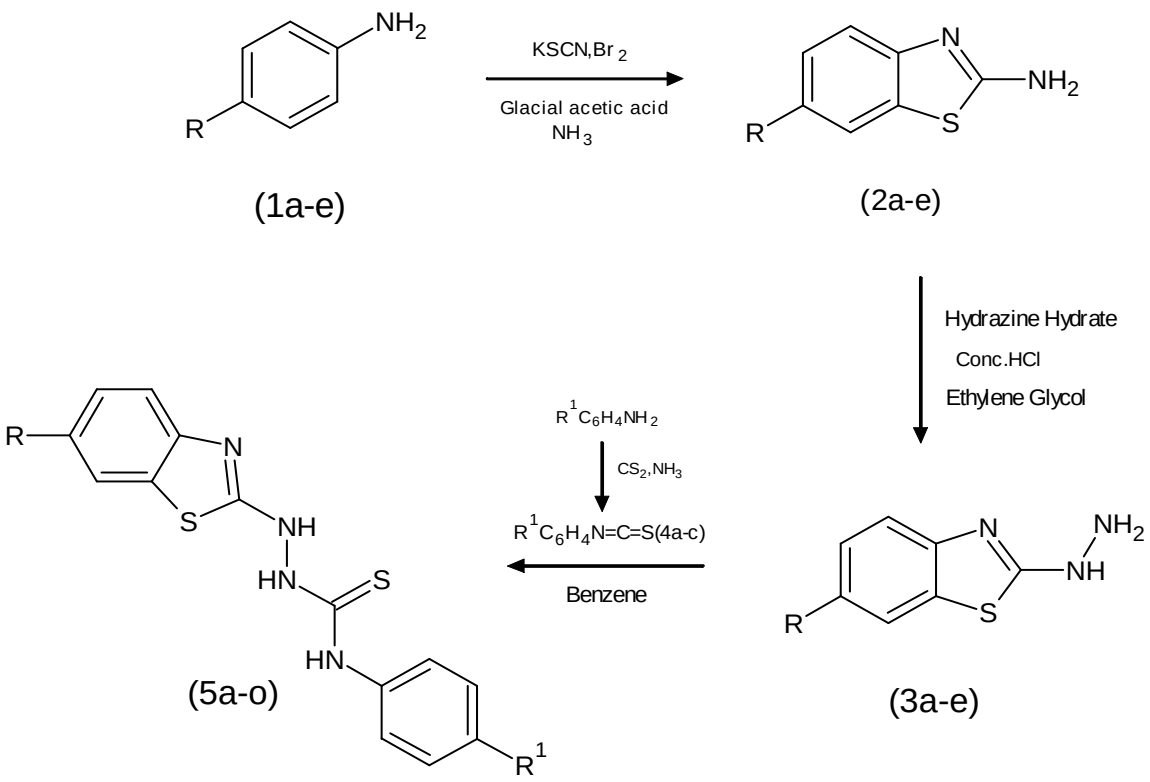
P-Substitutedphenyl isothiocyanate(4a)

Add 41ml of conc. ammonia solution (d 0.88) slowly with stirring to a solution of 0.26 mol of p-substituted aniline, 0.396 mol of carbon disulphide and 40ml of rectified spirit at 10-15^{0}C. Considerable heat is evolved; cool the flask in a freezing mixture from time to time so that temp. does not rise above 30^{0}C. The original milky suspension becomes clear and the intermediate dithiocarbamate soon crystallises out. Allow to stand overnight, filter the crystals, wash with little ether, dissolve in 1500ml water and stir mechanically while a solution of 0.262mol of lead nitrate in 175ml of water is slowly added. Continue the stirring for 20 minutes, and isolate the p-substitutedphenyl isothiocyanate by steam distillation into a receiver containing 5ml of 0.5M sulphuric acid; if the substances solidifies in the condenser, stop the cooling water until the solid has melted and run into receiver. Filter the cold solid product, wash with little water and dry in air upon filter paper.

2-(6-substituted-1,3-benzothiazol-2-yl)-N-(4-halophenyl) hydrazinecarbothioamide (5a-O)

A equimolar mixture of 2-hydrazino-6-substituted-1, 3-benzothiazole (3a) and p-substitutedphenyl isothiocyanate (4a) in benzene (50 ml) and refluxed for 3-6hr. A white product gradually separated out. It was filtered hot, dried and crystallization in methanol. yield: 96%; m.p 126^{o}c; Vmax,3200 (2^o NH), 1632 (C=N), 1530 (aromatic C=C), 1442 (thiazole), 1320 (C-N), 716 (C-Cl), 1220 (C=S), 1100 (aromatic F); DMSO, 7.1-7.7 (7H, m, Ar-H), 9.3 (1H, s, NH), 9.8 (1H, s, NH), 10.1 (1H, s, NH); m/z 352 (M^+) (Found: C, 51.71; H, 5.03 ; N, 18.05, Calcd. C, 51.80; h, 5.15; n, 18.27 %). The other compounds of the series were prepared by similar procedure(Yield : 90-96 %).

Scheme -1



Where,

R^1 = F R = Cl, Br, F, CH_3, OCH_3 **(5a-e)**

R^1 = Br R = Cl, Br, F, CH_3, OCH_3 **(5f-j)**

R^1 = Cl R = Cl, Br, F, CH_3, OCH_3 **(5k-o)**

Table 1: Physical Data of compounds(5a-o)

Compd	R	R^1	M.pt (^{0}C)	Yield (%)	*R_f	Calculated LogP# value	Mol.Formula (Mol.weight)
5a	Cl	F	126	96	0.32	4.88 ± 0.66	$C_{14}H_{10}ClF\ N_4S_2$ (352.84)
5b	Br	F	140	94	0.36	5.06 ± 0.68	$C_{14}H_{10}BrFN_4S_2$ (397.29)
5c	F	F	142	92	0.32	4.34 ± 0.68	$C_{14}H_{10}F_2N_4S_2$ (336.38)
5d	CH_3	F	133	95	0.38	4.75 ± 0.65	$C_{15}H_{13}FN_4S_2$ (332.42)
5e	OCH_3	F	141	90	0.33	4.20 ± 0.92	$C_{15}H_{13}FN_4OS_2$ (348.42)
5f	Cl	Br	135	95	0.30	5.60 ± 0.66	$C_{14}H_{10}BrClN_4S_2$ (413.74)
5g	Br	Br	138	94	0.34	5.78 ± 0.68	$C_{14}H_{10}Br_2N_4S_2$ (458.19)

5h	F	Br	135	92	0.34	5.06 ± 0.68	$C_{14}H_{10}BrFN_4S_2$ (397.29)
5i	CH_3	Br	130	96	0.36	5.47 ± 0.65	$C_{15}H_{13}BrN_4S_2$ (393.32)
5j	OCH_3	Br	137	94	0.35	4.92 ± 0.92	$C_{15}H_{13}BrN_4OS_2$ (409.32)
5k	Cl	Cl	132	96	0.30	5.43 ± 0.64	$C_{14}H_{10}Cl_2N_4S_2$ (369.29)
5l	Br	Cl	139	94	0.35	5.60 ± 0.66	$C_{14}H_{10}BrClN_4S_2$ (413.74)
5m	F	Cl	140	93	0.33	4.88 ± 0.66	$C_{14}H_{10}ClF\ N_4S_2$ (352.84)
5n	CH_3	Cl	128	95	0.39	5.29 ± 0.63	$C_{15}H_{13}ClN_4S_2$ (348.87)
5o	OCH_3	Cl	139	93	0.34	4.75 ± 0.89	$C_{15}H_{13}ClN_4OS_2$ (364.87)

*Benzene(9): Ethanol (1), # Caluated by ACD/Chem Sketch.

Antiangiogenesis study by chorioallantoic membrane (CAM) assay

Twelve eggs were used per experiment to test one compound as a given dose. The eggs were fertilized at 37^0C and 80% relative humidity in ideal conditions. The shells of eggs were cleaned with 70% EtOH to avoid infections. After 72 hrs 8-10 ml of albumin was removed with a syringe at the lower side of the egg and the hole was sealed with tape. Subsequently the upper part of the shell was removed and the eggs were covered with a plastic film and incubated for another 72 hrs. At this point of time, when the diameter of CAM is between 1.8 and 2.6 cm, the pellets containing the test substances were placed on the CAM. Test substances were dissolved or suspended in a 2.5% agarose solution. After gel formation, the volume of agarose gel corresponding to the dose of the test compound to be applied to the CAM was taken by means of a micropipette for viscous solutions. Therefore the agarose pellets do not have a uniform size. The half-cone-shaped agarose pellets are fixed becausev they slightly sink into the CAM. After 24 hrs the antiangiogenic effect was measured after addition of cream as a contrast fluid, by means of a stereomicroscope, by observing the avascular zone surrounding the pellet. Antiangiogenic activity is expressed as a score where 0 = no or weak effect, 1 = medium effect and 2 = strong effect (capillary free zone is at least twice as large as the pellet). Also membrane irritation and embryotoxicity can be evaluated. B-1,4-galactan sulfate (LuPS S5) with an average molecular weight of 20000 was used as positive control[46] and an agarose pellet as a blank.

Table 2: Antiangiogenic activity of compounds in the CAM assay

Test Compounds	Concentration (µg/pellet)	Antiangiogenic score[b] ± sd (n = no. of experiment)
5a	10	0.8± 0.1 (n =2)
5b	10	0.9± 0.1 (n =2)
5c	10	0.8± 0.1 (n =2)
5d	10	1.0± 0.1 (n =2)
5e	10	1.2± 0.1 (n =2)
5f	10	1.0± 0.1 (n =2)
5g	10	1.3± 0.2 (n =2)
5h	10	1.0± 0.1 (n =2)
5i	10	0.8± 0.1 (n =2)
5j	10	1.1± 0.1 (n =2)
5k	10	0.9± 0.1 (n =2)
5l	10	1.2± 0.1 (n =2)
5m	10	0.9± 0.2 (n =2)
5n	10	1.3± 0.1 (n =3)
5o	10	0.9± 0.1 (n =2)
Agarose pellet	10	0.1 ± 0.1 (n =3)
Standard {β-1,4-galactan sulphate (LuPS S5)}	50	1.4 ± 0.1 (n =3)

RESULT AND DISCUSSION

A Series of 2-(6-substituted 1-3- benzothiazol-2-yl)-N-(4-halophenyl) hydrazinecar-bothioamide (5a-o) was synthesized and their structure was elucidated by elemental analysis, IR, H-NMR and mass spectra. Yields, melting points. Rf values, calculated log P value and molecular formulas and Compounds 5e, 5g, 5j, 5l and 5n showed an antiangiogenic score of more than 1. Compounds 5g and 5n were found to be most potent with a score of 1.4 ± 0.1. The result shows that synthesized compounds have significant antiangiogenic activity.

CONCLUSION

All the synthesized compounds were screened for antiangiogenic activity by chorioallantoin membrane (CAM) assay method. β-1,4-galactan sulphate (LuPS S5) as taken as a standard drug. All the compounds were tested at a dose of 10 µg/pellet because at higher dose most of compounds showed a toxic effect Result showed that none of the synthesized compounds was more active than the standard compound. Compounds 5g and 5n should remarkable activity which was comparable to standard compounds.

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