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ULTRASOUND-ASSISTED EFFICIENT SYNTHESIS OF NOVEL THIAZOLIDINONE DERIVATIVES AS ANTICANCER AGENTS

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ABSTRACT

A series of N-(2-substituted phenyl)- 4-oxothiazolidine-3-carbonothioyl benzamide derivatives (PG1-PG10) were designed and synthesized using Ultrasonic processor as an eco-friendly synthetic route, keeping in view the structural requirement of pharmacophore and evaluated for anticancer activity. N-carbamothioylbenzamide was prepared and thiazolidinone moiety was developed on it by cyclization of intermediate N-(benzylidenecarbamotioyl)-benzamide with thioglycolic acid in N,N- dimethyl formamide as solvent in presence of catalytic amount of zinc chloride, so as to get the novel final derivatives. Out of the 10 derivatives, compound number PG1,PG2 and PG5 were found to have potential activity against MCF-7 cell line whereas compound PG7 exhibited potential activity against HeLa cell line. As the synthesized derivatives showed good anti breast cancer and anti cervical cancer activity, these compounds can be further studied and optimized to help in development of anticancer drugs.

KEYWORDS: A series N-carbamothioylbenzamide HeLa anti cervical drugs.

1. INTRODUCTION

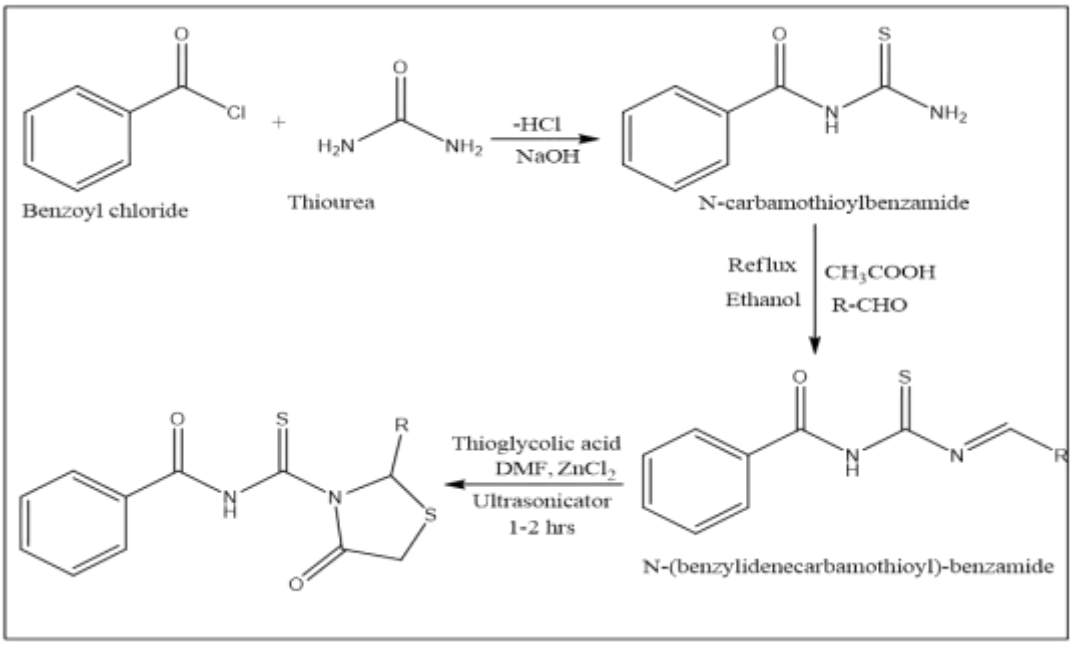
Around the world, tremendous resources are being invested in prevention, diagnosis and treatment of cancer. Development of anticancer drugs with fewer or no side effects is important for the treatment of cancer. The search for such potential anticancer drugs have led

to the discovery of synthetic small molecules with anti-carcinogenic activity and limited harmful side effects particularly with respect to the immune system.[1] According to the data provided by the WHO, cancer figures among the leading cause of morbidity and mortality worldwide with approximately 14 million new cases and 8.2 million cases related deaths in 2012. The number of new cases is expected to rise by about 70% over the next two decades[2]; among the women death due to breast cancer and cervical cancer is very common in developing countries and under developed countries.[3] Thiazolidinone, a saturated form of thiazoles with carbonyl group on fourth carbon, has been considered as a moiety of choice as it possesses a broad spectrum of pharmacological activities against several targets. This array of biological response profile has attracted the attention of scientists the world over to further investigate the potential of this organic motif. 4-Thiazolidinone ring system is a core structure in various synthetic compounds displaying broad spectrum of biological activities[3], including an anticancer effect.[4-8] The combination of two pharmacophores into a single molecule is an effective and commonly used direction in modern medicinal chemistry for the exploration of novel and highly active compounds.[9] The use of ultrasound to promote chemical reactions is called sonochemistry. Ultrasonic-assisted organic synthesis is a green synthetic approach and it is a powerful technique towards the increase in reaction rate.[10-12] It can also be considered as an important tool for conservation of energy and minimization of waste as compared to the conventional techniques.[13-14] Herein, in continuation of our earlier efforts[15,16,17] to synthesize novel thiazolidinone derivatives, we report ultrasound- promoted heterocyclization reactions of suitably functionalized substrates, which can allow the regioselective synthesis of thiazolinedinone derivative from the readily available starting materials under mild and selective conditions and their evaluation as potent anti-breast cancer and anti-cervical cancer.

2. Chemistry

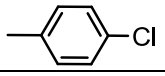
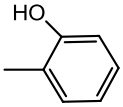
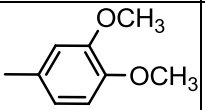
The synthetic protocol employed for the synthesis of N-(2-substituted phenyl)- 4-oxothiazolidine-3-carbonothioyl benzamide derivatives (PG1-PG10) are presented in scheme1. In the first step we obtained N-carbamothioylbenzamide via the reaction of benzyl chloride and thiourea in presence of base. This is simple nucleophilic substitution and results in the formation of intermediate Schiff's base i.e. N-(benzylidenecarbamotioyl)-benzamide(SB-1 to SB-10) by condensation of substituted aromatic aldehydes with N-carbamothioyl benzamide in presence of catalytic amount of glacial acetic. The final step is critical and give good yield in 1-2 hrs of ultrasound irradiation at room temperature, as

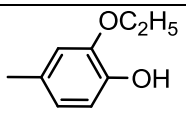
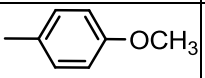
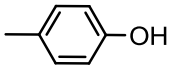
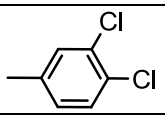
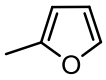
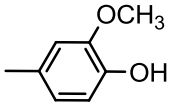
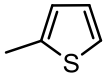
against that of the conventional method, which requires 8-12 hrs. In solvent benzene, which is proven carcinogen. Intermediate benzamides (SB-1 to SB-10) undergoes intramolecular cyclization with thioglycolic acid in presence of zinc Chloride as a catalyst in solvent DMF to obtain N-(2-substituted phenyl)- 4-oxothiazolidine-3-carbonothioyl benzamide derivatives. The purity of the synthesized compounds was checked by TLC and melting points were determined in open capillary and are uncorrected. The assignments of the structures were based on elemental and spectral data. The physical data of the synthesized compounds are presented in Table 1. The data obtained from IR, 1H NMR, Mass and elemental analysis data confirmed the proposed structures.



Scheme I. Synthetic protocol for titled compounds.

Table No. 1 Physical data of N-(2-substituted phenyl)- 4-oxothiazolidine-3-carbonothioyl benzamide derivatives (PG1-PG10)

Code	Ar	Mol. Formula	Mol. Wt	%Yield	M. P. (°C)	Rf Value
PG-1		$C_{17}H_{13}ClN_2O_2S_2$	376.88	90	68-70	0.36
PG-2		$C_{17}H_{14}N_2O_3S_2$	358.43	84	156-158	0.72
PG-3		$C_{19}H_{18}N_2O_4S_2$	402.49	81	152-154	0.71

PG-4		$C_{19}H_{18}N_2O_4S_2$	402.49	79	178-180	0.83
PG-5		$C_{18}H_{16}N_2O_3S_2$	372.46	74	176-178	0.54
PG-6		$C_{17}H_{14}N_2O_3S_2$	358.43	69	130-132	0.30
PG-7		$C_{17}H_{12}Cl_2N_2O_2S_2$	411.33	70	100-102	0.67
PG-8		$C_{15}H_{12}N_2O_3S_2$	332.40	88	155-157	0.44
PG-9		$C_{18}H_{16}N_2O_4S_2$	388.46	84	124-126	0.89
PG-10		$C_{15}H_{12}N_2O_2S_3$	348.46	82	148-150	0.51

Solvent of re crystallization was ethanol; Eluants used in TLC were petroleum benzene: methanol (8:2) for all compounds.

3. Pharmacology

The new derivatives obtained by the above mentioned procedure were undertaken for the anticancer studies. The profile of anticancer activity was established by MTT Assay. Each synthesised compounds from PG-1 to PG-10 were tested at 4 dose levels (10, 20, 40, 80µg/ml). These data are presented in Table 2.

Table no. 2:-Anticancer screening

Comp ID	Cell Line MCF7			Cell Line HeLa		
	LC50	TGI	GI50	LC50	TGI	GI50
PG1	>80	>80	**40.7**	>80	>80	>80
PG2	>80	>80	**50.6**	>80	>80	>80
PG3	>80	>80	>80	>80	>80	>80
PG4	>80	>80	>80	>80	>80	>80
PG5	>80	>80	**67.6**	>80	>80	>80
PG6	>80	>80	>80	>80	>80	>80
PG7	>80	>80	>80	>80	41.6	**21.0**
PG8	>80	>80	>80	>80	>80	>80
PG9	>80	>80	>80	>80	>80	>80
PG10	>80	>80	>80	>80	>80	>80
ADR	63.6	26.1	**<10**	47.9	12.4	**<10**

Drug concentrations (µg/ml) calculated from graph.

4. RESULTS AND DISCUSSIONS

A series of of N-(2-substituted phenyl)- 4-oxothiazolidine-3-carbonothioyl benzamide derivatives (PG1-PG10) were obtained by using ultrasonic processor ator which give good yield and require shorter reaction times, the solvent used in conventional synthesis of thiazolidinones is benzene, which is carcinogenic and hence avoided in present investigation. All the synthesized compounds were evaluated for anticancer activity and some of them have shown promising anticancer activities.

4.1 Anticancer screening

The compound PG1, PG2 & PG5 showed moderate inhibitory activity on MCF-7 cell line compared to the standard drug Adriamycin. The compound PG7 possess moderate inhibition on HeLa cell line. Positive controls are run in each experiment and each experiment is repeated thrice. A plate reader was used to read the optical densities and a microcomputer processed the optical densities into the special concentration in terms of GI50, TGI and LC50 values.

5. Experimental protocols

5.1. All reagents and solvents were used as obtained from the supplier or recrystallized/redistilled unless otherwise noted. Ultrasonic processor Vibra cell P/No. VCX 500-220 with solid probe was used for synthesis of final compounds. Infrared (IR) and proton nuclear magnetic resonance (1H NMR) spectra were recorded for the compounds on JASCO FTIR (PS 4000) using KBr pallet and Brucker Advance II (400 MHz) instruments, respectively. Chemical shifts are reported in parts per million (ppm), using TMS as an internal standard. The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) on silica gel-G (Merck) coated aluminumplates, visualized by iodine vapor.

5.1.1. General procedure for Synthesis of N-(benzylidenecarbamothioyl) benzamide (Schiff's base)(SB-1 to SB-10)

Equimolar concentration of N-carbamothioylbenzamide the product of the first step and substituted aromatic aldehydes (0.01 mol) was taken and to this 0.01 mol of glacial acetic acid was added Then 0.01 mol of thioglycolic acid was added and this was attached to a reflux for about 7-8 hours. The progress of the reaction was checked with the help of TLC. It was removed from reflux after getting a single spot on the TLC. The product was taken in a petri plate and was allowed to dry overnight. A solution of sodium metabisulphite was

prepared fresh and the dried product was poured into it. It was allowed to stir on a mechanical stirrer for 30 minutes. The product was filtered and allowed to dry.

5.1.2. General procedure for Synthesis of N-(2-substituted phenyl-4-oxathiazolidine-3-carbonothioyl) benzamide (PG1-PG10)

Equimolar concentration of respective Schiff's base (SB_1 to SB-10) and thioglycolic acid (0.01mol) and anhydrous Zinc Chloride (0.04 mol) were taken in DMF (20 ml). The reaction mixture was kept inside an Ultrasonicator for about 1-2 hours during the reaction. After completion of the reaction (monitored by TLC), the mixture was poured into ice cold water. Then this mixture was washed with sodium bicarbonate wash away unreacted thioglycolic acid and allowed to settle for 10min. Now ethyl acetate is poured in the mixture and it is separated using a separator. It is allowed to stand after vigorous shaking. The ethyl acetate forms the upper portion of the mixture. This layer contains the product dissolved in it. The water layer isdecanted. It contained the unreacted zinc chloride in the form of zinc hydroxide. Ethylacetate layer in poured in a beaker and sodium nitrite is added to it. The sodium nitrite absorbs the water. Now the ethyl acetate containing the product is taken in petriplate n allowed to evaporate. The product obtained after the evaporation of the ethyl acetate is the pure crystallized thiazolidinone.

5.1.2.1.N-(2-(3,4-dimethoxyphenyl)-4-oxothiazolidine-3carbonothioyl)benzamide (PG3):% Yield:81; M. P.:152-154;IR (KBr) cm^{-1}: (N-H str.) 3445; (C-H str. aromatic) 3130;(C-H str. aliphatic) 3030, 2900;(C-H str. imine) 2880; (C=N str.) 1624; (C=C str. Arom 1600,1475; ^{1}H NMR (DMSO, 400MHz) δ: 3.83 (s, 6H, $-OCH_3$), 6.6-8.0 (m, 8H, Aromatic ring), 8.1 (s, 1H, -NH), 4.0 (s, 2H, $-CH_2$ of thiazolidinone).

5.1.2.2. N-(2-(3-ethoxy-4-hydroxyphenyl)-4-oxothiazolidine-3-carbonothioyl) benzamide (PG4):% Yield:79; M. P.: 178-180; IR (KBr) cm^{-1}:**(**N-H str.) 3444; (C-H str.Aromatic) 3331; (C-H str.Aliphatic) 3035;(C-H str.Imine) 2900; (C=N str.) 1630; (C=C str.Aromatic) 1633,1470; ^{1}H NMR (DMSO, 400MHz) δ: 1.3 (s, 3H, $-CH_3$), 4.0(s, 2H, $-OCH_2$), 7.2-7.9 (m, 8H, Aromatic ring), 8.0 (s, 1H, -NH), 3.9 (s, 2H, $-CH_2$ of thiazolidinone).

5.1.2.3. N-(2-(4-methoxyphenyl)-4-oxothiazolidine-3-carbonothioyl) benzamide (PG5):% Yield: 74; M. P.: 176-178; IR (KBr) cm^{-1}: **(**N-H str.) 3450;(C-H str.Aromatic) 3200; (C-H str.Aliphatic) 3038;(C-H str.Imine) 2890;(C=N str.) 1622; ^{1}H NMR (DMSO, 400MHz) δ: 3.8

(s, 3H, -OCH_3), 7.1-7.9 (m, 9H, Aromatic ring), 8.0 (s, 1H, -NH), 3.9 (s, 2H, -CH_2 of thiazolidinone).

5.2. Pharmacology

MTT assay

HeLa cervical cancer cells were cultured in a 96-well plate at a density of 2 × 10^4 cells per well, incubated for 24 h. The cells were then treated with varying concentrations of calanone and 5-FU. After 24 h, the cells were washed and treated with 0.01 mL MTT per well. Plates were incubated at 37 °C in a 5% CO2 atmosphere for 4 h, and 0.1 mL of the extraction buffer (10% sodium dodecyl sulfate in 0.01% HCl) was added. After an overnight incubation at 37°C, the absorbance was measured at 595 nm using an ELISA reader (Bio-Rad) and was compared with the control cultures without compound. To determine cell viability, percent viability was calculated as [(absorbance of drug-treated) sample/(control absorbance)] × 100. The synthesized compounds were subjected to preliminary testing for anticancer screening by using MCF-7 and HeLa cell lines. Cell suspensions that were diluted according to particular cell type and the expected target cell density (5000-40,000 cells pre well based on cell growth characteristics) were added by pipette (100 µL) into 96- well microtiter plates assayed by using the sulforhodamine B assay. Inoculates were allowed a pre incubation period of 24 h at 37^0C for stabilization.

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