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Journal of Chemical and Pharmaceutical Research, 2013, 5(6):64-70

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis, characterization and *invitro* anticancer screening of novel thiazole-1,3,4-oxadiazole hybrid analogues

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ABSTRACT

The aim and objective of the work was to develop novel thiazole-1, 3, 4-oxadizole hybrid analogues and to evaluate their invitro cytotoxic activity. In this study, 9 novel thiazole-1,3,4-oxadiazole hybrid analogues were synthesized by cyclodehydrogenation reaction of 2-[(4-phenyl-1,3-thiazol-2-yl)amino]acetohydrazide with substituted aliphatic or aromatic acids using phosphorusoxychloride as dehydrating agent to yield N-[(5-substituted-1,3,4-oxadiazol-2-yl)methyl]-4-phenyl-1,3-thiazol-2-amine. The synthesized compounds were then established on the basis of IR,[1]HNMR spectral datas and screened for invitro anticancer activity on human breast cancer cell line MCF-7 and lymphoma cancer cell line DLA . The derivatives showed moderate activity on both cell lines.

Keywords: Thiazole-1, 3, 4-oxadizole hybrid analogues, anticancer activity, cyclodehydrogenation, MCF-7, DLA.

INTRODUCTION

Heterocycles are common structural units in marketed drugs and in medicinal chemistry targets in the drug discovery process [1]. Heterocycles with a variety of shapes and electronic and physicochemical properties provide fertile grounds for optimization of drug candidates. Our approach is to take highly halogenated heterocyclic systems and use them as scaffolds for the synthesis of novel compounds by the sequential replacement of halogen atoms with other functionalities. This approach has led to the generation of a number of novel highly substituted heterocyclic species [2]. According to literature survey, thiazoles containing N=C-S moiety were reported to possess anti-microbial [3], analgesic [4], anti-inflammatory [5], anti-cancer [6], anti-tubercular [7], anthelmintic [8] and diuretic activities [9]. In addition 1, 3, 4-oxadiazoles have been reported to have broad biological activities like analgesic [10], anti-inflammatory [11], anti-cancer [12], anti-HIV [13], anti-parkinson [14], anti-bacterial [15], anti-fungal [16] and anti-tubercular agents [17]. The synergism of both the heterocyclic moieties in a single entity may result in the formation of some worthwhile molecules with promising biological activities. Promoted by the above observations, it was aimed to synthesize novel thiazole-1, 3, 4-oxadiazole hybrid analogues. The proposed lead molecule of novel thiazole-1, 3, 4-oxadiazole hybrid analogue was *N*-[(5-substituted-1, 3, 4-oxadiazol-2-yl) methyl]-4-phenyl-1, 3-thiazol-2-amine that envisages a meaningful exploration for newer anticancer activities with minimum toxicity and high potency.

EXPERIMENTAL SECTION

Synthesis and characterization

All the chemicals and reagents used in this research work were of analytical or synthetic grade. Melting point of the synthesized compounds was determined by open capillary method and is uncorrected. The IR spectra of the synthesized compounds were recorded using Perkin Elmer FT-IR Spectrophotometer in the range of 3500 – 500 cm^{-1}. ^1H NMR of the synthesized compounds was recorded in DMSO on Bruker Ultra Shield DPX 500. Chemical shifts were reported in δ (ppm) relative to Tetra Methyl Silane (TMS) as internal standard. The reactions were monitored by thin layer chromatography over precoated preactivated glassplates with solvent system Chloroform: methanol (9:1).

Synthetic procedure:

Step 1: Preparation of 4-phenyl-1, 3-thiazol-2-amine (1)

A mixture of 0.1 mole of acetophenone, 0.1 mole of iodine and 0.2 mole of thiourea was taken in a 250 ml round bottom flask and heated at 110°C for 4 hours. The reaction mixture was cooled to room temperature and diluted with 100 ml of water and extracted with ether to remove unreacted iodine and acetophenone. Excess of ether was distilled off. This residue then dissolved in boiling water and filtered to remove sulphur. It was allowed to stand for 30 minutes. Make the reaction mixture alkaline (up to pH 8-9) using ammonium hydroxide solution. The solid obtained was filtered and washed successively with water. The separated solid was recrystallized using ethanol. m.p 148°C. Percentage yield was found to be 84% [18].

Step 2: Preparation of ethyl [(4-phenyl-1, 3-thiazol-2-yl) amino] acetate (2)

A mixture of compound 1 (0.01 mole) and ethylchloroacetate (0.01 mole) with potassium carbonate (0.015mole) in methanol (25ml) was refluxed on a waterbath for about 12 hours. The reaction mixture was then cooled, filtered and the solvent was then distilled off under reduced pressure. The solid thus obtained recrystallized using methanol. m.p 159^0c. Percentage yield was found to be 74% [19].

Step 3: Preparation of 2-[(4-phenyl-1, 3-thiazol-2-yl) amino]acetohydrazide (3)

A mixture of compound 2 (0.01 mole) and hydrazine hydrate (0.1 mole) was taken in 250ml round bottom flask attached to reflux condenser and refluxed with 50ml of 95% ethanol for 15 hrs. After 15hrs the reaction mixture was poured on to the crushed ice to separate the product. The solid thus obtained recrystallized using ethanol. m.p 133^0c. Percentage yield was found to be 61% [20].

Step 3: N-[(5-substituted-1,3,4-oxadiazolyl-2yl)methyl-4-phenyl-1,3-thiazol-2-amine (4)

To a mixture of compound 3 (0.01mole), substituted acid (0.1mole), phosphorous oxychloride (6ml) was added dropwise. The mixture was then refluxed for 4-5hrs at 120^0c. The reaction mixture was cooled, poured into crushed ice and made alkaline by 20% sodium hydroxide solution. The solid separated was filtered and recrystallized from ethanol [21].

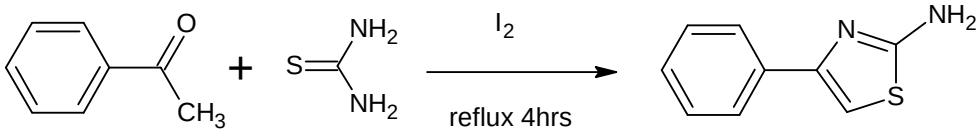
Pharmacological screening

The human breast cancer cell line (MCF-7) and the lymphoma cancer cell line (DLA) were obtained from National Centre for Cell Science (NCCS), Pune. All the cell lines were grown in Dulbecco's Modified Eagles Medium containing 10% fetal bovine serum (FBS) and maintained at 37^0C, 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, 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 % cell inhibition was determined using the following formula.

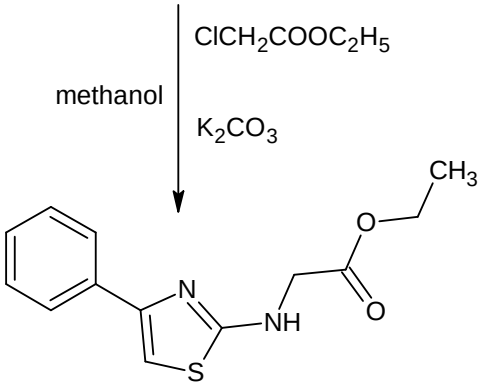
% cell Inhibition = 100- Abs (sample)/Abs (control) x100

Nonlinear regression graph was plotted between % cell inhibition and $\log_{10}$ concentration and LD50 was determined.

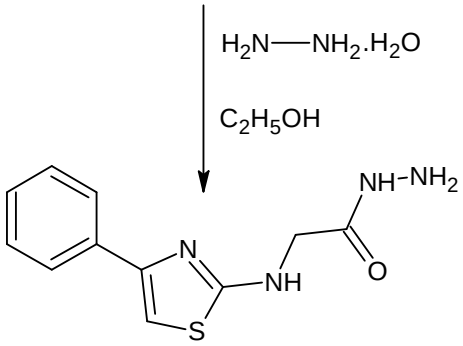
Synthetic scheme



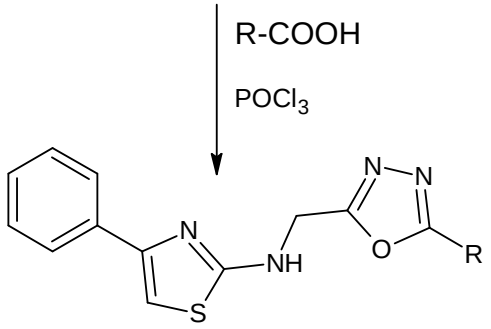
Acetophenone Thiourea 4-phenyl-1,3-thiazol-2-amine **(1)**



ethyl [(4-phenyl-1,3-thiazol-2-yl)amino]acetate **(2)**

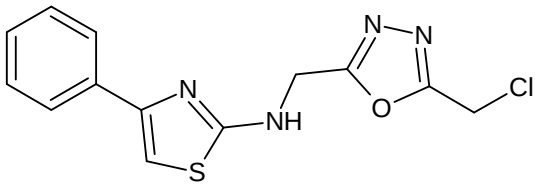
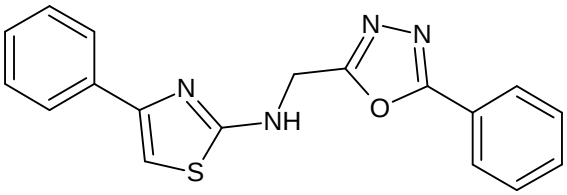
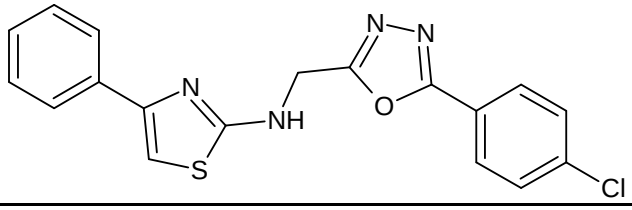
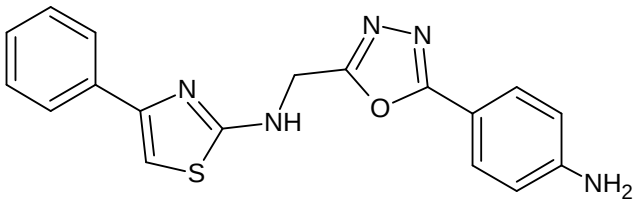
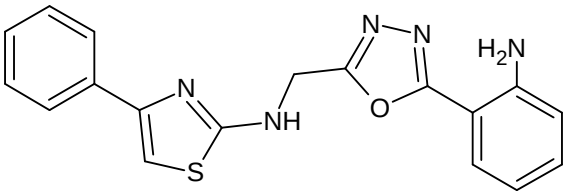
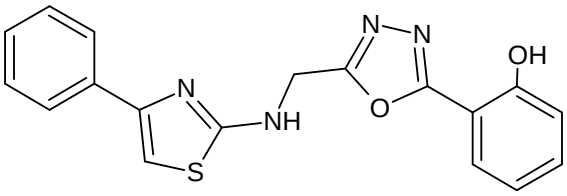


2-[(4-phenyl-1,3-thiazol-2-yl)amino]acetohydrazide **(3)**



N-[(5-substituted-1,3,4-oxadiazol-2yl)methyl]-4-phenyl-1,3-thiazol-2-amine **(4)**

Table 01: List of synthesized compounds

Compound Code	Name of the Compound	Structure of the Compound
APTOM-4a	*N*-{[5-(chloromethyl)-1,3,4-oxadiazol-2-yl] methyl}-4-phenyl-1,3-thiazol-2-amine	
APTOM-4b	4-phenyl-*N*-[(5-phenyl-1,3,4-oxadiazol-2-yl) methyl]-1,3-thiazol-2-amine	
APTOM-4c	*N*-{[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl] methyl}-4-phenyl-1,3-thiazol-2-amine	
APTOM-4d	*N*-{[5-(4-aminophenyl)-1,3,4-oxadiazol-2-yl] methyl}-4-phenyl-1,3-thiazol-2-amine	
APTOM-4e	*N*-{[5-(2-aminophenyl)-1,3,4-oxadiazol-2-yl] methyl}-4-phenyl-1,3-thiazol-2-amine	
APTOM-4f	2-(5-{[(4-phenyl-1,3-thiazol-2-yl)amino]methyl}-1,3,4-oxadiazol-2-yl)phenol	

RESULTS AND DISCUSSION

Table 02: Preliminary characterization of newly synthesized compounds

Compound code	Molecular formula	Molecular weight	Melting point (^{0}c)	Percentage yield (%)	R_f value
APTOM-4a	$C_{13}H_{11}ClN_4OS$	306.77064	90	64	0.57
APTOM-4b	$C_{18}H_{14}N_4OS$	334.9496	130	52	0.6
APTOM-4c	$C_{18}H_{13}ClN_4OS$	368.84002	134	47	0.62
APTOM-4d	$C_{18}H_{15}N_5OS$	349.4096	196	92	0.73
APTOM-4e	$C_{18}H_{15}N_5OS$	349.4096	154	71	0.71
APTOM-4f	$C_{18}H_{14}N_4O_2S$	350.39436	168	57	0.615

Spectral data of synthesized compounds

The synthesized compounds were confirmed with IR and ^{1}H NMR spectra.

Table 03: IR spectral analysis

Compound code	IR peaks (cm^{-1})
APTOM-4d	Ar–CH Str (3222), C=C Str (1594), C-N Str (1278), C=N Str (1594), C-S Str (702), NH Str (3337), CH_2 Str (2975), C-O-C (1022), NH_2 Str (3422)
APTOM-4f	Ar–CH Str (3111), C=C Str (1594), C-N Str (1331), C=N Str (1594), C-S Str (708), NH Str (3250), CH_2 Str (2919), C-O-C (1027), Phenolic OH(3430)

Table 04: ^{1}H NMR spectral analysis

Compound code	^{1}H NMR (DMSO) δ(ppm)
APTOM-4d	δ 1.26-1.28 (d, 2H, CH_2 of $NHCH_2$), δ 3.37 (s, 2H, NH_2, D_2O exchangeable), δ 4.17 (s, 1H, NH, D_2O exchangeable), δ 5.96 (s, 1H, CH of thiazole), δ 6.55-7.06 (m, 4H, ArH of aniline), δ 7.25-7.80 (m, 5H, ArH).
APTOM-4f	δ 1.26-1.28 (d, 2H, CH_2 of $NHCH_2$), δ 3.35 (s, 1H, NH, D_2O exchangeable), δ 5.96 (s, 1H, CH of thiazole), δ 7.00 (s, 1H, phenolic OH), δ 7.06-7.27(m, 4H, ArH of phenol), δ 7.34-7.80 (m, 5H, ArH).

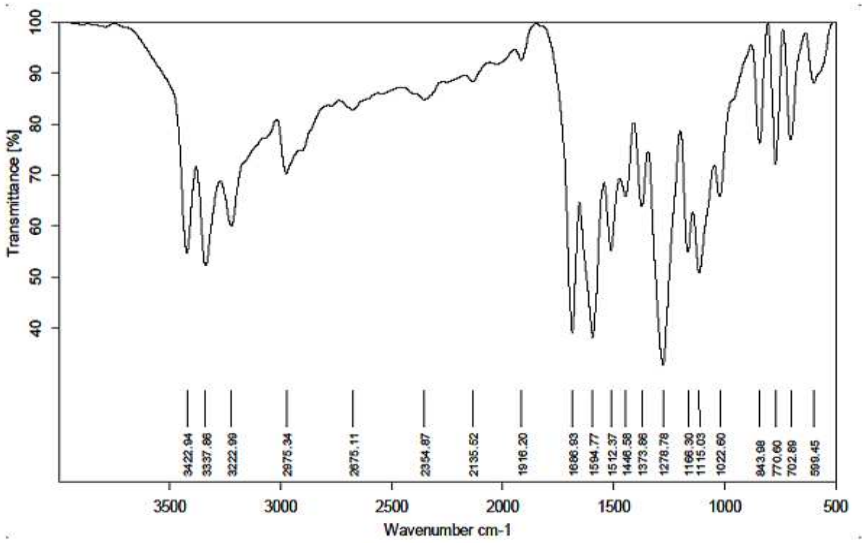


Fig 01: IR spectrum of *N*-{[5-(4-aminophenyl)-1, 3, 4-oxadiazol-2-yl] methyl}-4-phenyl-1, 3-thiazol-2-amine [APTOM-4d]

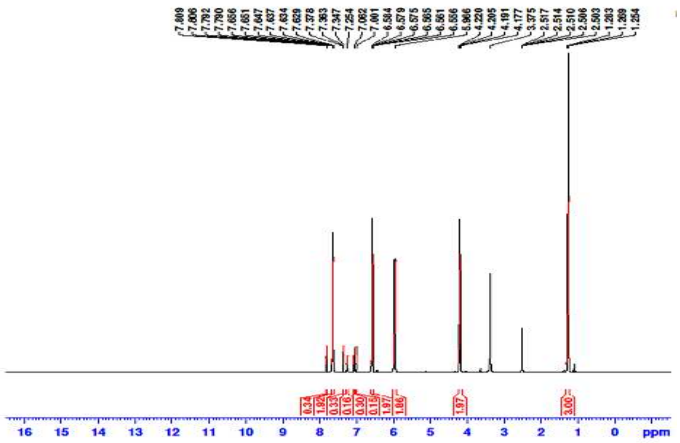


Fig 02: ^{1}H NMR spectrum of *N*-{[5-(4-aminophenyl)-1, 3, 4-oxadiazol-2-yl] methyl}-4-phenyl-1, 3-thiazol-2-amine [APTOM-4d]

Pharmacological screening

All the synthesized compounds showed moderate cytotoxic activity towards both the cell lines. Among them APTOM-4c exhibited significant activity against MCF-7 and DLA cell lines

Table 05: LD50 values of tested compounds on MCF-7

Compound code	% Cell Inhibition					LD50 value (µg/ml)
	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml	
APTOM-4b	15.75	37.75	45.75	59.88	64.75	186
APTOM-4c	24.76	39.75	54.76	59.74	67.98	132
APTOM-4e	11.45	39.75	54.74	59.77	61.75	140
APTOM-4g	11.75	24.76	34.76	49.74	54.75	200
APTOM-4h	10.75	22.78	33.75	47.24	53.75	220

Table 06: LD50 values of tested compounds on DLA

Compound Code	% Cell Inhibition					LD50 value (µg/ml)
	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml	
APTOM-4b	16.75	31.78	39.75	49.77	58.77	200
APTOM-4c	13.75	34.34	53.75	57.44	63.75	136
APTOM-4e	16.75	39.75	44.74	55.75	59.88	176
APTOM-4g	13.75	19.76	25.74	45.76	52.75	236
APTOM-4h	12.75	17.76	24.75	44.78	52.78	240

CONCLUSION

The present work describes the synthesis of novel thiazole-1, 3, 4-oxadizole hybrid analogues and their *invitro* cytotoxic activity. The purity of the compounds and the completion of reaction thus synthesized were ascertained by consistency in melting point and by TLC and the structures of the synthesized compounds were assigned on the basis of the spectral data.

Acknowledgement

The author is thankful to the management of Devaki Amma Memorial College of Pharmacy, Malappuram for providing the facilities to carry out this research work.

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