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Research Article

SYNTHESIS OF SOME NOVEL 2, 4-THIAZOLIDINEDIONE INCORPORATED PYRAZOLE DERIVATIVES AS ANTI CANCER AGENTS

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ABSTRACT

Objective: To synthesize pyrazolyl thiazolidinediones i.e., 2, 4-thiazolidinedione incorporated pyrazole derivatives anticipating effective anti-cancerdrugs.

Methods: Different substituted pyrazole carbaldehydes which were synthesized by Vilsmeier-Haack reaction of substituted phenyl hydrazones with $POCl_3$ and dimethyl formamide, hybridized with 2, 4-thiazolidinedione in the presence of acetic acid and catalytic amounts piperidine. The synthesized hybrids were evaluated against three different cancer cell lines (human lung cancer cell line A549, human breast cancer cell line MCF-7 and human prostate cancer cell line DU145) by employing MTT assay method.

Results: The chemical structures of the synthesized compounds were confirmed using FTIR, Mass and ^{1}H NMRspectral data. The MTT assay of synthesized hybrids showed promising and effective anti-cancer activity against the cell lines comparable to standard drug Doxil.

Conclusion: Among the hybrids, p-chloro substituted derivative (3b)showed highest activity against three cell lines namely, human lung cancer cell line A549, human breast cancer cell line MCF-7 and human prostate cancer cell line DU145 with IC_{50} values 4.63, 1.32 and 5.25µg respectively. Compounds 3c and 3h were found to be effective against both lung and breast cancer cell lines with IC_{50} values ranging from 4.44 to 9.16µg.

Keywords: Pyrazole carbaldehydes, 2, 4-thiazolidinedione, Anti-cancer activity, MTT assay method.

INTRODUCTION

The PPAR-gamma (PPAR-γ) activating thiazolidinediones (TZDs) such as rosiglitazone, pioglita zone, troglita zone, ciglita zone etc. are a new class of anti-diabeticdrugs used to improve lipid and glucose metabolism in type-2 diabetes. Recent reports indicate that besides insulin sensitization action, these drugs have tumor suppressor action which was proved in several invitro/*in vivo* models [1-5]. Sato et al. [6] and Takashima et al. [7]have demonstrated in animal experiments that PPAR-γ activation will have anti-cancer properties. Further, the anti-tumor activity and mechanism of TZDs against lung, breast and colon carcinomas was reported by Carmello Blanquicett et al. [8]. On the other hand, pyrazoles and pyrazolines area n interesting class of heterocyclic compounds which attract continuing attention over the years because of their broad spectrum biological activities such as anti-inflammatory[9], anti-convulsant and anti-depressant[10], ACE-inhibitor[11],anti-microbial[12], anti-viral[13] and anti-cancer[14-17] properties. In recent years, they are identified as potential anti-cancer agents with B-Raf kinase inhibitor activity[18-21], and also as targets for EGFR Tyrosine kinases[22].

Hybridization is one of the approaches to design new drug molecules. Inspired by the diverse biological properties of thiazolidinedione moiety and pyrazoles, in the present study, an attempt has been made to synthesize title compounds by employing hybridization approach with the hope that the resulting new molecules will have anti-cancer activity.

MATERIALS AND METHODS

All the reagents used in the present work were of analytical grade and were used without any further purification. The reactions were carried out under controlled anhydrous conditions. Melting points were recorded on Analab melting point apparatus by open capillary method and are uncorrected. The IR spectra were recorded on Schimadzu FTIR spectrophotometer using 1% potassium bromide discs. ^{1}H NMR spectra were recorded on Varian 400MHz spectrophotometer using DMSO-d_6 as solvent and TMS as an internal standard. Mass spectra were taken on Agilent 6430 triple quadruple EI-MS system. Thin layer chromatography was performed using E. Merck 0.25mm silica gel plates, and the spots were visualized under UV light at 256nm.

General procedure for synthesis of 3-(Substituted aryl)-1-phenyl-1*H*-pyrazole-4-carbaldehydes (2a-h)

To an ice cold solution of DMF(0.1mol), was added phosphorus oxychloride(0.012mol) drop-wise and the temperature was maintained below 10^{0}C. To the mixture, an ice-cold solution of phenyl hydrazine (0.01mol) was added in lots wise with stirring under ice cold condition. After the completion of the addition, the reaction mixture was stirred and refluxed at60-70^{0}C for 6hr. Solution was cooled and poured into crushed ice with stirring and neutralized with aq.$NaHCO_3$ solution. The solid product obtained was filtered under suction, dried and recrystallized from methanol.

General procedure for synthesis of 3-(Substituted aryl)-1-phenyl-1*H*-pyrazolyl-2,4-thiazolidinediones (3a-h)

A mixture of 3-(Substituted aryl)-1-phenyl-1*H*-pyrazol-4-carbaldehyde 2a (0.5g, 2mmol) and 1, 3-thiazolidine-2,4-dione (0.4g, 2mmol) in glacial acetic acid (20ml) and 2-3 drops of piperidine was refluxed at 120-130^{0}C for 3-4hr. A solid would separatefrom the reaction mixture within 15-20min and the refluxing was continued for 3-4hrto complete the reaction. The reaction mixture was then cooled to room temperature, filtered and washed with ethanol to give the pure product.

Spectral analysis

3a.5-[(1,3-diphenyl-1H-pyrazol-4-yl) methylidene]-1,3-thiazolidine-2,4-dione: $C_{19}H_{13}N_3O_2S$, IR (KBr, cm^{-1}): 3448.49(N-H str), 3058.89 (Ar C-H str), 1735,1685(C=O str),1593.09 (C=N str**)**. ^{1}H-NMR (DMSO-d_6): δ 12.6 s (1H,NH ofthiazolidinedione), 8.7 s(1H, pyrazole), 7.4-8.1 (10H, Ar-H&1H, HC=C-thiazolidinedione). EI-MS (m/z):347.50(M$^+$), 370.50(M+Na)$^+$.

3b. 5-{[3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl] methylidene}-1,3-thiazolidine-2,4-dione: $C_{19}H_{12}ClN_3O_2S$, IR (KBr, cm^{-1}):3124.47(N-H str), 3024.16 (Ar C-H str), 1758.95,1685.67(C=O str), 1593.09 (C=N str), 758.04(C-Cl str). ^{1}H-NMR (DMSO-d_6): δ 12.6 s(1H, NH of thiazolidinedione), 8.7 s (1H, pyrazole), 7.4-8.0(9H, Ar-H &1H, HC=C-thiazolidinedione).EI-MS m/z:381.50 (M$^+$), 404.80(M+Na)$^+$.

3c. 5-{[3-(4-methylphenyl)-1-phenyl-1H-pyrazol-4-yl] methylidene}-1,3-thiazolidine-2,4-dione:

$C_{20}H_{15}N_3O_2S$, IR (KBr cm^{-1}):3120.61(N-Hstr), 3004.69(Ar C-H str),2781.16 (C-H str ofCH_3), 1735.81,1685.57(C=O str), 1604.65(C=N str).^{1}H-NMR (DMSO-d_6) : δ 12.5 s (1H,NH of thiazolidinedione), 8.6 s (1H, pyrazole), 7.3-8.1 (9H,Ar-H&1H, HC=C-thiazolidinedione),2.3-2.5 (3H,CH_3). EI-MS m/z:361.4 (M^+), 384.4 $(M+Na)^+$.

3d. 5-{[3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl]methylidene}-1,3-thiazolidine-2,4-dione:

$C_{20}H_{15}N_3O_3S$, IR (KBr cm^{-1}):3329 (N-H str),3067.57 (Ar C-H str), 2876.1 (C-H str of CH_3), 1743,1685(C=O str), 1612.38 (C=N str), 1244.97 (aryl-O str), 1178.43, 1017.38 (CH_3-O str).^{1}H-NMR (DMSO-d_6): δ 12.5 s (1H,NH of thiazolidinedione), 8.6 s(1H, pyrazole), 7.3-8.1 (9H,Ar-H&1H, HC=C-thiazolidinedione),2.3-2.5 (3H,CH_3).EI-MS m/z:377.41(M^+).

3e. 5-{[3-(2-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl]methylidene}-1,3-thiazolidine-2,4-dione:

$C_{20}H_{15}N_3O_3S$, IR (KBr cm^{-1}):3328 (N-H str), 3120.45 (Ar C-H str), 2869.23 (C-H str of CH_3), 1742,1682(C=O str), 1611.88 (C=N str), 1240.87 (aryl-O str), 1176.22, 1012.68 (CH_3-O str). ^{1}H-NMR (DMSO-d_6): δ 12.5 s (1H,NH of thiazolidinedione), 8.6 s (1H, pyrazole, C=CH), 7.3-8.1 (9H,Ar-H&1H, HC=C-thiazolidinedione),2.3-2.5 (3H,CH_3). EI-MS m/z:377.41(M^+).

3f. 5-{[3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl]methylidene}-1,3-thiazolidine-2,4-dione:

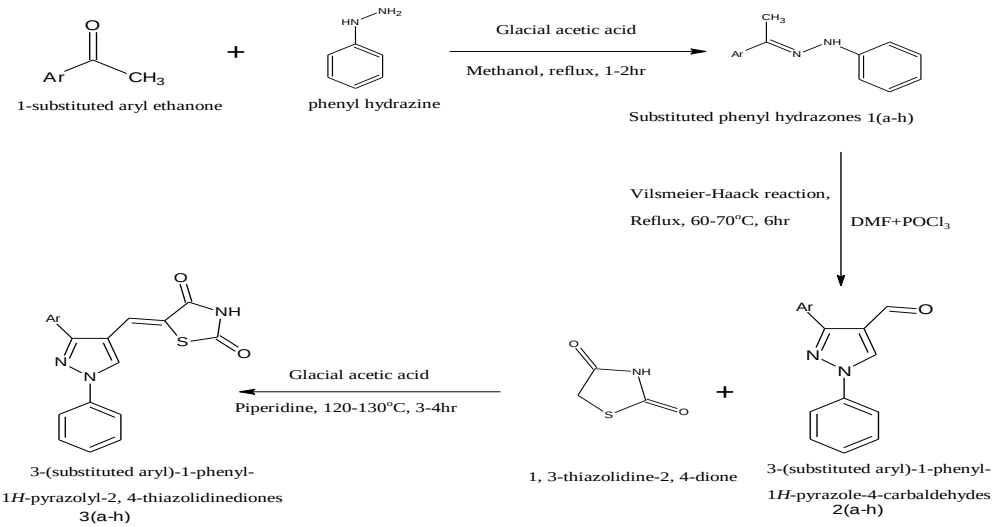
$C_{19}H_{13}N_3O_3S$, IR (KBr cm^{-1}):3348 (N-H str), 3340.48 (O-H str), 3109.04 (Ar C-H str), 1729,1690(C=O str), 1596.95 (C=N str). ^{1}H-NMR (DMSO-d_6) : δ 12.5 s (1H,NH of thiazolidinedione), 8.6 s (1H, pyrazole), 7.3-8.1 (9H, Ar-H&1H, HC=C thiazolidinedione), 5.35 s (1H, OH). EI-MS m/z:363.38(M^+).

3g.5-{[3-(2-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl]methylidene}-1,3-thiazolidine-2,4-dione:

$C_{19}H_{13}N_3O_3S$, IR (KBr cm^{-1}):3348(N-H str), 3342.45 (O-H str), 3103.06 (Ar C-H str), 1729, 1688(C=O str), 1595.82 (C=N str). ^{1}H-NMR (DMSO-d_6) : δ 12.5 s (1H,NH of thiazolidinedione), 8.6s (1H, pyrazole), 7.3-8.1 (9H Ar-H &1H, HC=C-thiazolidinedione), 5.25 s (1H, OH). EI-MS m/z:363.38(M^+).

3h. 5-{[3-(naphthalen-1-yl)-1-phenyl-1H-pyrazol-4-yl] methylidene}-1,3-thiazolidine-2,4-dione:

$C_{23}H_{15}N_3O_2S$, IR (KBr cm^{-1}): 3337 (N-H str), 3047.32 (Ar C-H str), 1735,1596(C=O str), 1596.95 (C=N str). ^{1}H-NMR (DMSO-d_6) : δ 12.5 s (1H,NH of thiazolidinedione), 8.4 s (1H, pyrazole), 7.3-8.1 (12H,Ar-H& 1H, HC=C-thiazolidinedione). EI-MS m/z: 397.5(M^+).



Compd.	a	b	c	d	e	f	g	h
Ar	C_6H_5-	4-Cl-C_6H_4-	4-CH_3-C_6H_4-	4-OCH_3-C_6H_4-	2-OCH_3-C_6H_4-	4-OH- C_6H_4-	2-OH- C_6H_4-	Napthyl-

Scheme 1 Synthetic pathway of 3-(substituted aryl)-1-phenyl-1*H*-pyrazolyl-2, 4-thiazolidinedione derivatives

Cytotoxic assay

The cytotoxic assay of the test compounds was evaluated in vitro against a panel of three cell lines namely, a human lung cancer cell line (A549), a human breast cancer cell line (MCF-7) and a human prostate cancer cell line (DU145) by MTT micro cultured tetrazolium assay method[23]. Doxorubicin was used as the reference drug for the evaluation of cytotoxic activity. Cells were harvested from the logarithmic phase cultures and re-suspended in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum(FBS). The cell counts were adjusted and equal number of cells were plated into each well of 96-well culture plates and allowed to grow overnight at 37°Cin presence of 5% CO_2. The cells were treated with test substances at various concentrations as indicated, for 72 hours. In vehicle control culture wells, a maximum of 0.5% CO_2 DMSO was added. Culture medium was renewed at every 24 hours with fresh culture medium supplemented with test substances. Thereafter, 0.5mg/ml of MTT reagent was added to each well and the micro plate was incubated further for 4 hours at 37°C in presence of 5% CO_2. Finally, the cells were solubilized by adding solubilizing solution and allowed to incubate at 37°C overnight. After complete solubilization of the formazan crystals, the absorbance was read at 540nm in a micro plate reader (Bio-Rad, USA). The results (mean OD+SD) obtained from quadruplicate wells were used in calculation to determine the cytotoxicity(50% of inhibitory concentration, IC_{50}) of the test compounds.

Results and discussion

Chemistry

The synthetic methodology followed to obtain the target compounds is outlined under Scheme 1. 2, 4-thiazolidinedione was prepared as per the procedure mentioned in literature [24]. Cyclization of different phenyl hydrazones was done by Vilsmeier-Haack reaction

to give 1-phenyl-3-substituted phenyl-1*H*-pyrazole-4-carbaldehydes (2a-h), which on condensation with 2, 4-thiazolidinedione in glacial acetic acidand catalytic amounts of piperidine afforded title compounds(3a-h) in reasonable yields. The compounds were characterized on the basis of physical and spectral data.

Cytotoxic activity

The compounds were tested against three cell lines using MTT[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] based cytotoxic assy. The results are tabulated below.

Table1: IC_{50} values of the synthesized compounds against the three different cancer cell lines

S.No.	Compound	IC_{50} values of different cell lines in µM Conc.		
		A549	MCF-7	DU145
1	3a	21.25	23.65	52.65
2	3b	04.46	01.63	05.27
3	3c	05.12	09.16	43.71
4	3d	61.54	15.23	06.51
5	3e	62.37	49.42	42.23
6	3f	06.25	45.82	31.67
7	3g	52.25	4.46	06.32
8	3h	06.83	4.44	59.29
9	Doxil	07.92	08.12	07.22

CONCLUSION

In the present investigation, a total of eight new thiazolidinedione incorporated pyrazole derivatives were synthesized by employing hybridization approach and the structures of the synthesized compounds were confirmed on the basis of FTIR, Mass and ^{1}H NMR spectral data. All compounds were screened against three different cancer cell lines,a human lung cancer cell line (A549), a human breast cancer cell line (MCF-7) and a human prostate cancer cell line (DU145) using MTT assay method. Among all the derivatives, compound 3b possessing p-chloro substitution showed highest activity against all the three cell lines with IC_{50} values 4.63, 1.32 and 5.25µg respectively. Compounds 3c and 3h were found to be effective against both lung and breast cancer cell lines with IC_{50} values ranging from 4.44 to 9.16µg.

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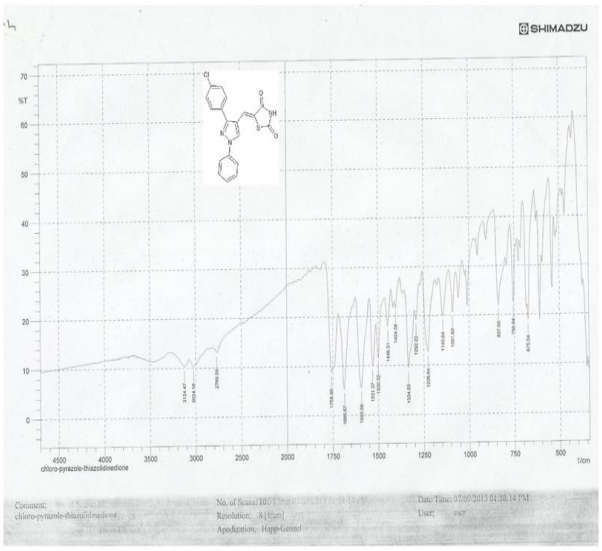

Fig 1: IR Spectrum of 5-{[3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl] methylidene}-1,3-thiazolidine-2,4-dione (3b)

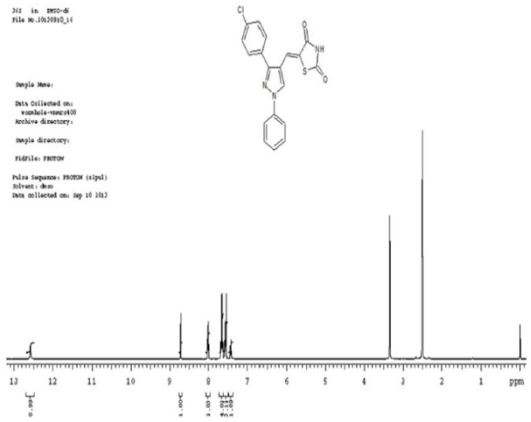

Fig. 2: ^{1}H NMR Spectrum of 5-{[3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl] methylidene}-1,3-thiazolidine-2,4-dione (3b)

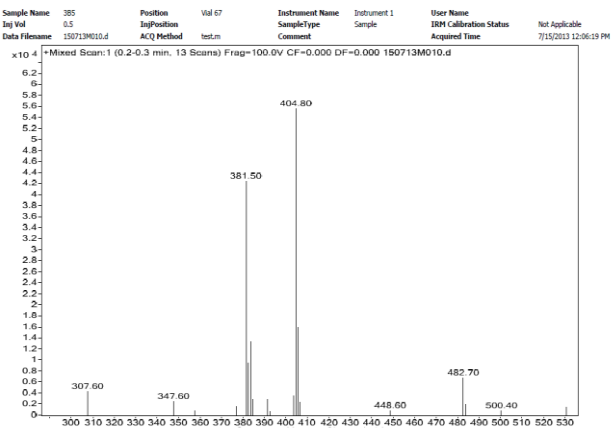

Fig. 3: EI-Mass Spectrum of 5-{[3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl] methylidene}-1,3-thiazolidine-2,4-dione (3b)

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