

SYNTHESIS, CHARACTERIZATION, AND ANTICANCER ACTIVITY OF SOME NOVEL ACRIDINE DERIVATIVESSWETHA PADIGELA¹, BHAGAVAN RAJU RM², RAJENDRA PRASAD VVS^{3*}¹Department of Pharmaceutical Chemistry, Vishnu Institute of Pharmaceutical Educational and Research, Narsapur, Telangana, India.²Department of Pharmaceutical Chemistry, Sri Venkateshwara College of Pharmacy, Hyderabad, Telangana, India. ³Center for Molecular Cancer Research, Vishnu Institute of Pharmaceutical Educational and Research, Narsapur, Telangana, India. Email: rajendraprasad.vvs@vipera.ac.in

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ABSTRACT**Objective:** The objective of the study was to synthesize and evaluate the anticancer activity of some novel acridine derivatives.**Methods:** The present works involve condensation of acridine and various 2, 4-Thiazolidine-2,4-dione derivatives (2a–2h) with chloroacetyl chloride to give a novel acridine derivatives (5a–5l), respectively.**Results:** All the newly synthesized molecules (5a–5l) were characterized by FTIR, ¹H-NMR, and mass spectral analysis along with physical data. The biological potentials of the new synthesized compounds are evaluated for their *in vitro* anticancer activity by MTT assay.**Conclusion:** The synthesized compounds 5a, 5f, and 5h exhibited good anticancer activity against MCF-7 and SKV03 cancer cell lines at a concentration of 0.5 mg/mL⁻¹.**Keywords:** Acridine, 2, 4-Thiazolidinedione, Substituted aldehydes, Chloroacetyl chloride, Anticancer activity, MCF-7, SKV03 cells.© 2020 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2020.v13i6.35794>**INTRODUCTION**

Acridine was first isolated by Carl Grabe and Heinrich Caro in Germany in 1870 from high boiling fraction of coal tar [1]. The antimicrobial property [2] of acridine was discovered Ehrlich and Benda in 1917. Bernthsen reported the primary synthesis of acridine, in which diphenylamine was reacted with benzoic acid using zinc chloride and high temperatures. The synthesis of acridine and its derivatives has attracted considerable attention from untreated and medicinal chemists for many natural life, as a number of natural source have been report to have this heterocyclic nucleus. Chemically, acridine is also known by the names of dibenzopyridine, 2,3,5,6-dibenzopyridine, and 10-azaanthracene. It has an irritating odor and crystallizes in colorless to light yellow needles with melting point of 110°C and boiling point of 346°C.

Acridine is a class of heterocyclic compounds which merits special attention because it belongs to a group of substances with activity in medicinal chemistry. This tricyclic nucleus derivatives are associated with anti-inflammatory [3,4], anticancer [5], antimicrobial [6], antitubercular [7,8], antiparasitic [9], antimalarial [10,11], antiviral [12,13], and fungicidal activities [14]. The basic in nature of pyridine, quinoline, and acridine is more or less similar compounds which possess no benzene ring, one benzene ring, and two benzene rings, respectively. Acridone is the one of the heterocyclic compounds with a tricyclic ring having nitrogen at 10th positions and keto group at 9th positions with the formula C₁₃H₉N. Acridines are substituted derivative of the parent ring. It is a planar molecule so as to be structurally related to anthracene by means of one of the central CH groups replaced by nitrogen.

In view of the facts mentioned above and the wide applications of acridine molecule and its derivatives in medicinal chemistry, an attempt has been made to synthesize novel 3-(2-(9-oxoacridin-10(9H)-yl) acetyl)-5-(benzylidene) thiazolidine-2,4-dione moiety as new anticancer agents.

METHODS

The synthesized compound was screened for sterile and anticancer activities. Fourier transform IR spectrometer (model Shimadzu 8700) in

the range of 400–4000 cm⁻¹ using KBr pellets and values are report in cm⁻¹ and the spectra were interpreted. ¹H-NMR spectra were recorded on DPX-200 MHz NMR spectrometer using DMSO-d₆ and chemical shift (δ) is reported in parts per million downfield from internal reference tetramethylsilane and the spectra were interpreted. Mass spectra were record on mass spectrophotometer (model Shimadzu) by LC-MS and the spectra were interpreted. Pre-coated silica gel G plates were used to monitor the progress of reaction as well as to check the purity of the compound: n-Hexane: ethyl acetate (7:3) [15-17].

General procedures*Step 1: Preparation of N-phenyl anthracitic acid*

In a 500 ml round-bottomed flask are placed a combination of o-Chlorobenzoic acid (20 g, 0.128 mol), Aniline (11.8 ml, 0.128 mol) and Copper metallic (0.5 g). To this solution 100 ml of amyl alcohol is delivered with constant stirring. To this mixture, dry potassium carbonate (20 g) was slowly added with stirring and the reaction mixture was allowed to reflux for 6 h in a light liquid paraffin oil bath at 135–140°C. Then the amyl alcohol was removed by using steam distillation and combination poured into two 2 L of hot water and acidified with targeted hydrochloric acid. The bluish-black precipitate formed was filtered, washed with hot water, and collected. The crude acid was dissolved in aqueous 10% sodium hydroxide solution, boiled in the presence of activated charcoal, and filtered. On acidification of the filtrates with concentrated hydrochloric acid, light yellowish precipitate was obtained, which was washed with hot water. The crude acid was recrystallized from aqueous methanol to give a light yellow solid.

Step 2: Preparation of acridin-9-one

N-Phenyl anthranilic acid (18 g, 0.084 mol) was taken in a 500 ml of round bottom flask to which polyphosphoric acid (180 g, 0.5327 mol) was added, shaken well, and refluxed on a water bath at 100°C for 3 h. Appearance of yellow color indicated the completion of reaction. Then, it was poured into 2 L of hot water and made alkaline by 25% ammonia solution. The yellow precipitate formed was filtered, washed with hot water, and collected. The crude acridin-9(10H)-one was recrystallized from acetic acid.

Step 3: General procedure for thiazolidine-2, 4-dione

The equimolar quantity (1:1) of chloroacetic acid (56.4 g, 0.6 mol) in 60 ml of irrigate be added to the answer of thiourea (45.6 g, 0.6 mol) in 60 ml of water. The mixture be enthused for 15 min and precipitates were obtain after cooling. After that 60 ml of concentrated hydrochloric acid was added slowly by using a dropping funnel. Once the mixture got transformed to solution shape, it was refluxed for 8–10 h at 100–110°C. On cooling, the contents of the flask solidified to a come together of white needles. The manufactured goods were filtered and washed with water to take away traces of hydrochloric acid and dried. It was purified by recrystallization as of ethyl alcohol. Yield: 85%; m.p.: 123–125°C.

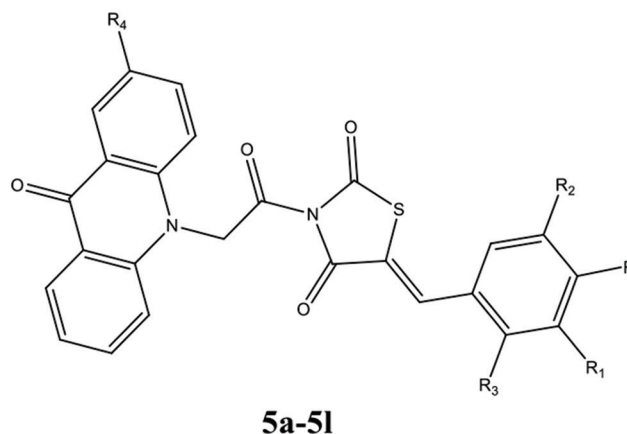
Step 4: General procedure for the synthesis of 5-substituted-1,3-thiazolidine-2,4-dione (4a-4h)

A combination of 2,4-thiazolidinedione 1 (1.17 g, 0.01 mol), substitute benzaldehyde (0.01 mol), glacial acetic acid (25 mL), and fused sodium acetate (0.18 g) be refluxed for 1 h with occasional shaking. Cool, then, the reaction mixture be cooled to room temperature and it was poured in water (250 mL), the product obtained was filtered, wash with water, alcohol, and ether and was recrystallized with glacial acetic acid.

Step 5: 3-(2-(9-oxoacridin-10(9H)-yl) acetyl)-5-(benzylidene) thiazolidine-2,4-dione

A mixture of acridone (0.01 mol), 5-substituted-1,3-thiazolidine-2,4-dione (0.01 mol), and chloroacetyl chloride (0.01 mol) in DMF (30 ml)

was refluxed for 3–4 h. Progress of reaction was monitor by TLC using n-Hexane: acetone (7:3). After the completion of response, the content was added to cold water. The solid product was obtained and filtered, dried, and purified by crystallization from ethanol (Table 1).

**5a. (Z)-3-(2-(9-Oxoacridin-10(9H)-yl) acetyl)-5-(N,N-dimethylaminobenzylidene)thiazolidine-2,4-dione**

M.P 250–252°C; Mol. formula: C₂₇H₂₁N₃O₄S, yield 76%, 3038 (C-H Str, Ar), 2917, 2874 (C-H Str, Aliphatic), 2309 (C-S-C Str), 1716, 1702 (C=O Str, thiazolidine-dione), 1523 (C-CH Str), 1429 (C=C Str, Ar), 1102 (C-N Str). ¹H

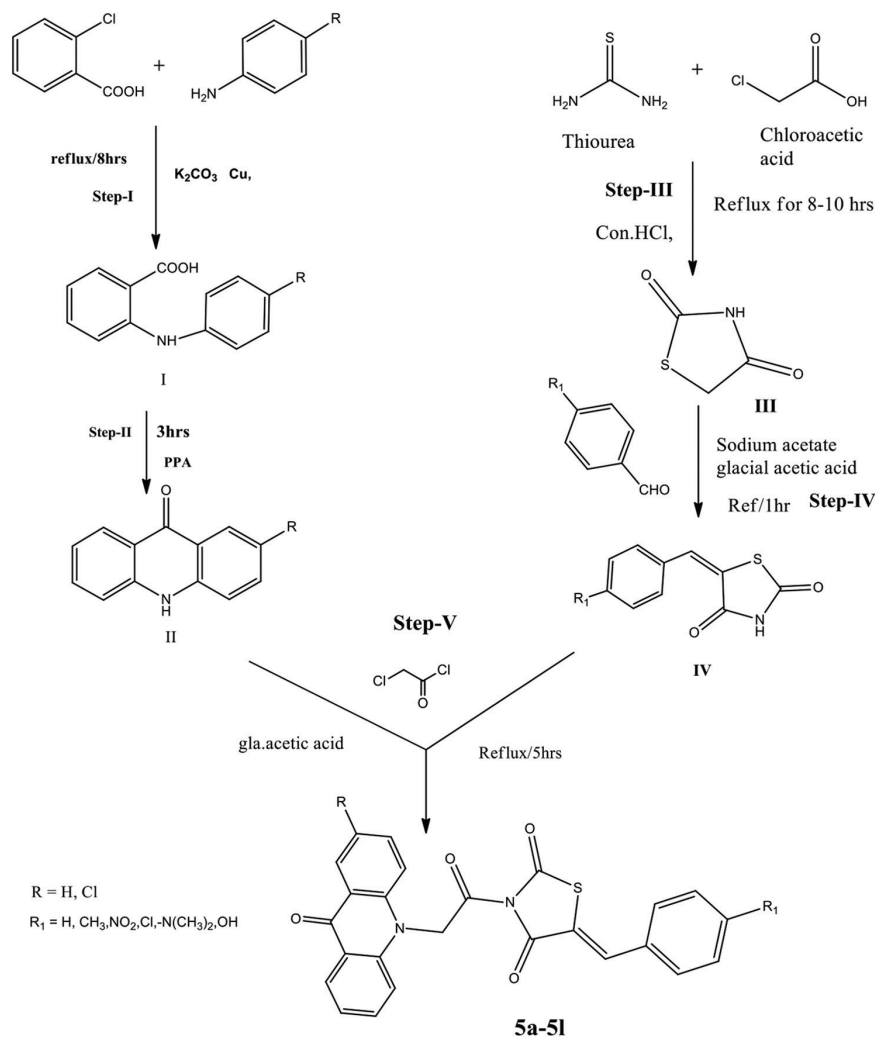


Table 1: Physical data of compounds 5a-5l

S. No.	R	R ₁	R ₂	R ₃	R ₄	Mol. for	M.P(°C)	% yield	Rf
5a	-N(CH ₃) ₂	H	H	H	H	C ₂₇ H ₂₁ N ₃ O ₄ S	250–252	76	0.73
5b	-OCH ₃	-OCH ₃	-OCH ₃	H	H	C ₂₈ H ₂₂ N ₃ O ₄ S	297–299	71	0.68
5c	H	H	H	H	H	C ₂₅ H ₁₅ N ₃ O ₄ SCl	>300°C	64	0.76
5d	Cl	H	H	H	H	C ₂₅ H ₁₅ N ₃ O ₄ SCl	287–289	72	0.59
5e	H	H	H	Cl	H	C ₂₆ H ₁₈ N ₃ O ₄ S	261–263	68	0.73
5f	CH ₃	H	H	H	H	C ₂₆ H ₁₈ N ₃ O ₄ S	269–271	72	0.77
5g	H	NO ₂	H	H	H	C ₂₅ H ₁₅ N ₃ O ₄ S	280–283	68	0.73
5h	-OCH ₃	-OCH ₃	H	H	H	C ₂₇ H ₂₀ N ₃ O ₄ S	238–240	74	0.67
5i	NO ₂	H	H	H	H	C ₂₅ H ₁₅ N ₃ O ₆ S	243–246	66	0.82
5j	-NH-C ₂ H ₅	H	H	H	H	C ₂₇ H ₂₁ N ₃ O ₄ S	211–213	70	0.59
5k	-N(CH ₃) ₂	H	H	H	CH ₃	C ₂₈ H ₂₃ N ₃ O ₄ S	263–266	74	0.62
5l	-OCH ₃	-OCH ₃	-OCH ₃	H	CH ₃	C ₂₉ H ₂₄ N ₃ O ₆ S	205–207	69	0.75

NMR (DMSO) $\delta\delta$ ppm: 8.11 (s, 1H, benzylidene), 7.89–7.80 (d, 2H, Ar-H), 7.79–7.76 (d, 2H, Ar-H), 7.69–7.67 (d, 2H, Ar-H), 7.66–7.57 (t, 2H, Ar-H), 7.56–7.53 (t, 2H, Ar-H), 7.53–7.11 (d, 2H, Ar-H), 4.18–4.16 (s, 2H, -CH₂-), 1.94–1.81 (s, 6H, -N(CH₃)₂); mass (LC-MS): m/z 483(M), 484(M + 1, 100%).

5b. (Z)-3-(2-(9-Oxoacridin-10(9H)-yl)acetyl)-5-(benzylidene)thiazolidine-2,4-dione

M.P. 297–299°C; Mol. formula: C₂₈H₂₂N₃O₇S, yield 71%, 3023 (C-H Str, Ar), 2897, 2945, 2805 (C-H Str, Aliphatic), 2300 (C-S-C Str), 1713, 1690 (C=O Str, thiazolidine-dione), 1503 (C=CH Str), 1423 (C=C Str, Ar), 1104 (C-N Str). ¹H NMR (DMSO) $\delta\delta$ ppm: 8.35 (s, 1H, benzylidene), 8.06–8.04 (d, 2H, Ar-H), 7.94–7.92 (d, 2H, Ar-H), 7.82–7.821 (d, 2H, Ar-H), 7.77–7.75 (t, 2H, Ar-H), 7.17–7.14 (t, 2H, Ar-H), 6.88–6.86 (t, 3H, Ar-H), 4.29–4.28 (s, 2H, -CH₂-); mass (LC-MS): m/z 440 (M), 441(M + 1, 100%).

5c. (Z)-3-(2-(9-Oxoacridin-10(9H)-yl)acetyl)-5-(4-chlorobenzylidene)thiazolidine-2,4-dione

M.P. >300°C; Mol. formula: C₂₅H₁₅N₃O₄SCl, yield 64 %, IR (ν cm⁻¹): 3096 (C-H Str, Ar), 2960, 2905, 2895 (C-H Str, Aliphatic), 2324 (C-S-C Str), 1720, 1698 (C=O Str, thiazolidine-dione), 1526 (C=CH Str), 1454 (C=C Str, Ar), 1108 (C-N Str), 855 (C-Cl Str, Ar). ¹H NMR (DMSO) $\delta\delta$ ppm: 8.45 (s, 1H, benzylidene), 8.37–8.31 (d, 2H, Ar-H), 8.15–8.09 (d, 2H, Ar-H), 8.05–8.01 (d, 2H, Ar-H), 7.94–7.89 (d, 2H, Ar-H), 7.84–7.78 (t, 2H, Ar-H), 7.75–7.09 (t, 2H, Ar-H), 4.506–4.504 (s, 2H, -CH₂-); mass (LC-MS): m/z 474 (M), 475 (M + 1, 100%), 476 (M + 2).

5d. (Z)-3-(2-(9-Oxoacridin-10(9H)-yl) acetyl)-5-(2-chlorobenzylidene) thiazolidine-2,4-dione

M.P. 287–289°C; Mol. formula: C₂₅H₁₅N₃O₄SCl, yield 72%. IR (ν cm⁻¹): 3003 (C-H Str, Ar), 2945, 2930, 2868 (C-H Str, Aliphatic), 2304 (C-S-C Str), 1704, 1686 (C=O Str, thiazolidine-dione), 1506 (C=CH Str), 1443 (C=C Str, Ar), 1115 (C-N Str), 798 (C-Cl Str, Ar). ¹H NMR (DMSO) $\delta\delta$ ppm: 8.36 (s, 1H, benzylidene), 8.29–8.14 (d, 2H, Ar-H), 7.88–7.84 (d, 2H, Ar-H), 7.79–7.77 (d, 2H, Ar-H), 7.69–7.67 (t, 2H, Ar-H), 7.55–7.45 (t, 2H, Ar-H), 7.41–7.10 (t, 2H, Ar-H), 4.29–4.25 (s, 2H, -CH₂-); mass (LC-MS): m/z 474 (M), 475 (M + 1, 100%), 476 (M + 2, 30%).

5e. (Z)-3-(2-(9-Oxoacridin-10(9H)-yl)acetyl)-5-(4-methylbenzylidene)thiazolidine-2,4-dione

M.P. M.P. 261–263°C; Mol. formula: C₂₆H₁₈N₃O₄S, yield 68%. IR (ν cm⁻¹): 3038 (C-H Str, Ar), 2932, 2911, 2873 (C-H Str, Aliphatic), 2311 (C-S-C Str), 1716, 1671 (C=O Str, thiazolidine-dione), 1520 (C=CH Str), 1465 (C=C Str, Ar), 1107 (C-N Str). ¹H NMR (DMSO) $\delta\delta$ ppm: 8.37 (s, 1H, benzylidene), 8.31–8.10 (d, 2H, Ar-H), 7.88–7.84 (t, 2H, Ar-H), 7.68–7.63 (t, 2H, Ar-H), 7.58–7.57 (d, 2H, Ar-H), 7.55–7.51 (d, 2H, Ar-H), 7.14–7.13 (d, 2H, Ar-H), 4.15–4.13 (s, 2H, -CH₂-), 1.87–1.83 (s, 3H, -CH₃); mass (LC-MS): m/z 454(M), 455(M + 1, 100%).

Pharmacological activity: Anticancer activity [20]

Cell viability was evaluated by the MTT assay with three independent experiments with six concentrations of compounds in triplicates. Cells were trypsinized and perform the trypan blue assay to know viable cells

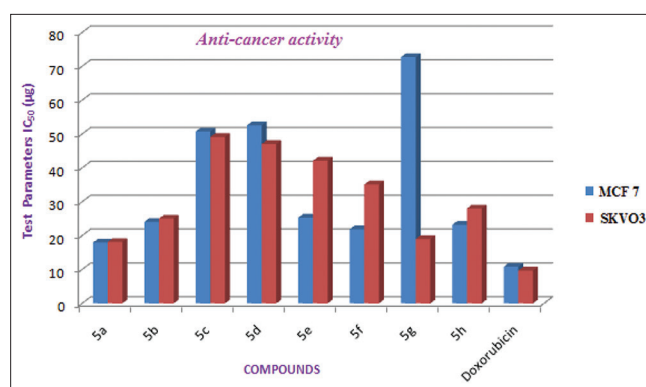


Fig. 1: Graphical representation of novel acridine derivatives on MCF-7 and SKVO3 cells

in cell suspension. Cells were counted by hemocytometer and seeded at density of 5.0×10^3 cells/well in 100 μ l media in 96-well plate culture medium and incubated overnight at 37°C. After incubation, take off the old media and add fresh media 100 μ l with different concentrations of test compound in labeled wells in 96 plates. After 48 h, discard the drug solution and add the fresh medic with MTT solution (0.5 mg/mL⁻¹) be added to each well and plates were incubated at 37°C for 3 h. At the end of incubation time, precipitates are formed as a result of the decrease of the MTT salt to chromophore Formosan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader.

The percentage growth inhibition was calculated using the following formula and concentration of test drug essential to slow down cell growth by 50% values is generated from the dose- response curves for each cell line by with origin software.

$$\% \text{ Inhibition} = \frac{100(\text{Control} - \text{Treatment})}{\text{Control}}$$

RESULTS AND DISCUSSION

The present work involves the reaction between condensation of acridine and various 2, 4-Thiazolidine-2,4-dione derivatives (2a–2h) with chloroacetyl chloride to give a novel acridine derivatives (5a–5l), respectively. The synthesized compounds were screened for antimicrobial and anticancer activities. The structures of all the newly synthesized compounds were characterized as 5a–5l on the basis of satisfactory analytical and spectral data including IR, LC-MASS, and ¹H-NMR data.

Anticancer activity

Novel acridine derivatives (5a–5l) were evaluated for cytotoxicity against human breast cancer cells (MCF7) and human ovarian

