Original Article

Novel substituted 5-arylidene-1, 3-thiazolidin-4-one analogues: Synthesis, optimization, anticancer screening and docking studies

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Abstract

We have developed novel series of 5-arylidene-1, 3-thiazolidin-4-one analogues using diethylamine as catalyst. Diethylamine was found suitable in synthesizing variety of title compounds in 62-85% yield. *In-vitro* anticancer activity of synthesized analogues was evaluated using human breast (MCF7), lung (Hop62) and hepatic (HepG2) cell lines using SRB assay. Amongst the synthesized compounds 9b, 9e, 9f are excellent in inhibiting growth of cancer cell lines with GI50 value <10µg/ml in comparison with Adriamycin standard. We have also performed docking studies using SHP2 in complex with inhibitor (PDB ID: 2Shp) but results were non-supportive, very weak. We found no correlation between anticancer studies and docking studies.

Keywords: 5-arylidene-1, 3-thiazolidin-4-one, Diethylamine, SRB assay, *In-vitro* anticancer, 2Shp.

1. INTRODUCTION

4-Thiazolidinone has vibrant pharmacophoric activities ranging from antibacterial, antiviral, anticancer, anticonvulsant, and anticancer [1-5]. Numerous reports are available in literature synthesizing thiazolidinone and analogues. Many protocols make the use of $ZnCl_2$ [6,7], solid acid catalyst, activated fly ash, microwaves and involve the condensation, cyclization of aldehydes, amine and thioglycolic acid [8,9]. Literature reports for base catalyzed synthesis of thiazolidinone analogues are also available. Knoevenagel condensation for synthesis of 5-substituted thiazolidine-4-one analogues using piperidine [10], sodium acetate [11] and baker's yeast [12] are some of the new approaches reported in literature. Diethylamine is a simple aliphatic amine utilized for the synthesis of coumarins by Knoevenagel condensation with high yield. The major difference between basicity of diethylamine and piperidine is due to presence of alkyl groups on amino nitrogen. It is suggested that this structural change improves overall kinetics and efficiency of the reaction as the nonbonded electron pair is more available for the catalytic action [13].

Various potent and selective SHP2 inhibitors have been identified in the past years. **Fig. 1** shows the chemical structures of some such potent inhibitors. Two categories of SHP2 inhibitors are reported viz. Type 1: interacting with PTP catalytic domain, Type 2: these are allosteric inhibitors interacting with region outside of catalytic domain with higher selectivity [14, 15]. Many of these inhibitors are in various stages of clinical trials ranging from preclinical to phase 3. Phenylhydrazanopyrazolone sulfonates (PHPS1, GS49) Quinoline hydrazines (NSC 87877), Oxindole derivatives (NSC17199, SPI 112) are developed in recent years as SHP2 inhibitors. The acidic groups of these developed molecules like sulfonates, hydrazine and heterocyclic rings interact with Cys-459, Arg-465, Lys-280, Asn-281 at the catalytic site of SHP2 [16]. Various thiazolidinone analogues with benzimidazole, benzthiazoles, thiazole nucleus have also reported to have SHP2 inhibitory activity [17]. It was observed that very limited attempts have been made in field of type II SHP2 inhibitors. Due to many similarities between other kinases and SHP2, inhibitors of SHP2 can be combined synergistically. 1, 3-thiazolidin-4-one is a scaffold of interest to scientific world due to its varied biological activities [3, 18, 19, 20, 21]. Thiazolidinone moiety with =NHN have shown its importance in biological activity. The structure of the co-crystallized ligand of SHP2 as well as the structures of compounds synthesized in the present work are depicted in **Fig. 2**.

Figure 1: Reported SHP2 Inhibitors

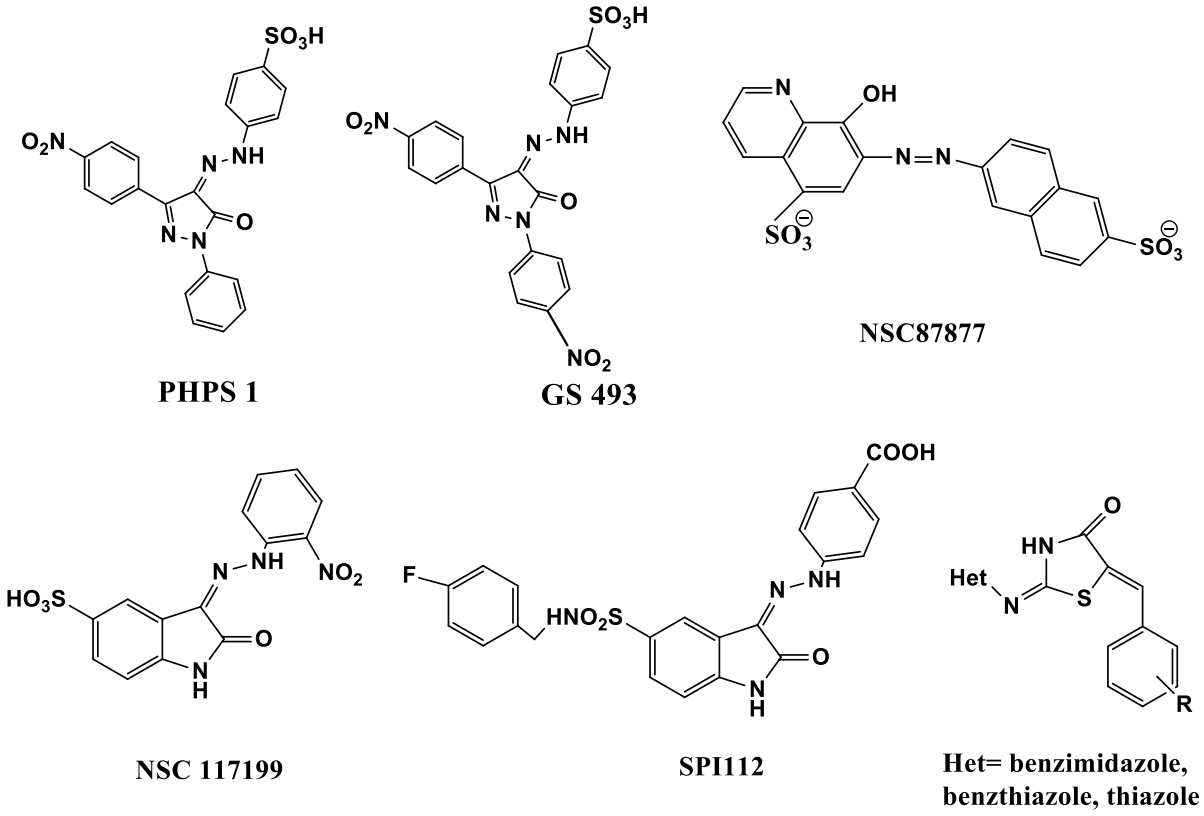
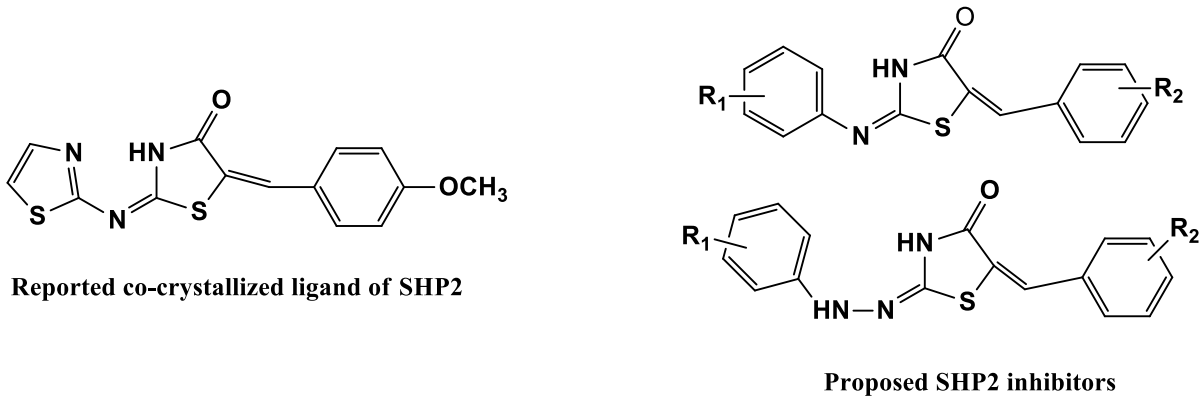


Figure 2: Reported co-crystallized SHP2 inhibitor and proposed SHP2 inhibitors



We are reporting an optimized synthesis of 5-substituted thiazolidine-4-one analogues using diethylamine/PEG-400 followed by comprehensive SAR study. Considering the importance of acidic moieties, as reported in the literature, we decided to maintain acidic functionalities of =NNH and =NH in all analogues. Anticancer potential of all the synthesized compounds was also evaluated by SRB assay. We have also carried out *in-silico* studies of highly active analogue by using co-crystallized SHP2 inhibitor complex (PDB ID: 2Shp) as a native ligand.

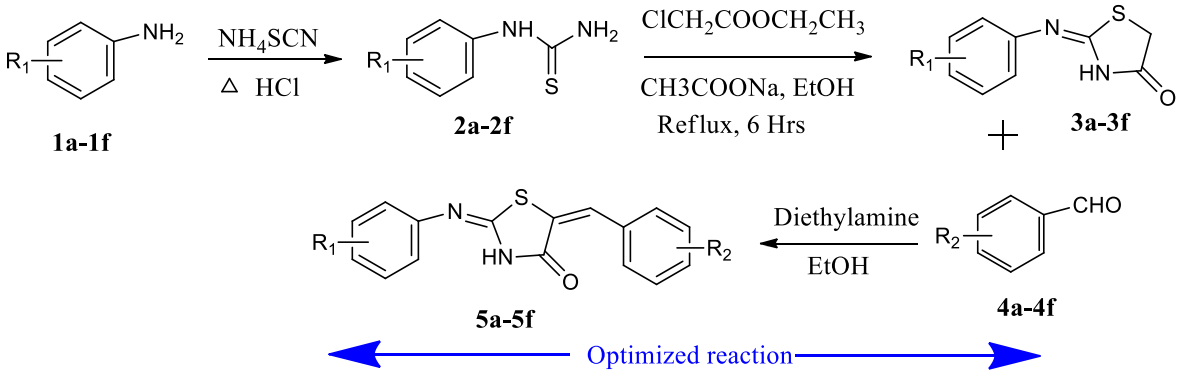
2. MATERIALS AND METHODS

All the reagents and substrates were used as received in their commercially available form and purchased from Bhavichem, Mumbai, India and from S. D. Fine chemicals limited, India. All the solvents were used as received without further purification. Progress of all the reactions were monitored by thin-layer chromatography on silica gel plates (GF 254) and visualized with

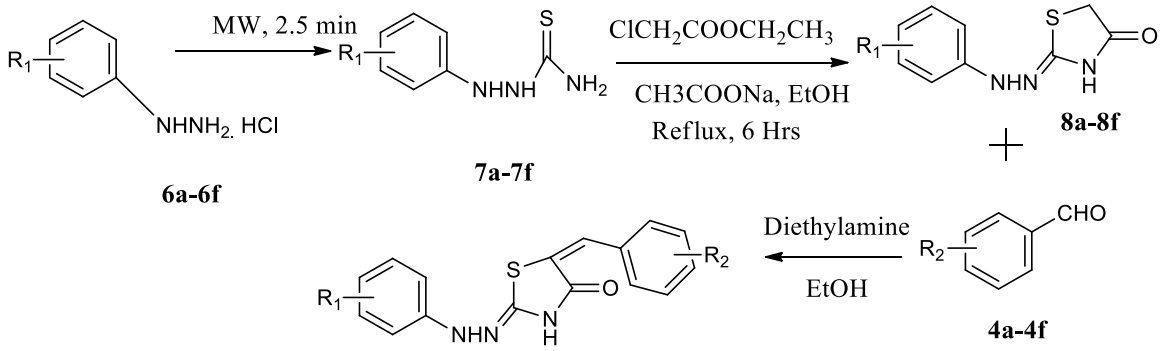
UV light. Melting points were uncorrected and recorded on DBK Prog. melting point apparatus. IR spectra were recorded in KBr pellets on Shimadzu IR Affinity-1 FTIR spectrophotometer. ^{1}H NMR, ^{13}C NMR spectra were recorded in $CDCl_3$, DMSO-d6, D_2O (if applicable) on a Bruker Avance II 400 MHz spectrometer with tetramethylsilane (TMS) as an internal reference at SAIF, Panjab University, Chandigarh, India and at IIT, Bombay, India. The chemical shifts are given in δ (ppm) referenced to the respective solvent peak and coupling constants are reported in Hz. Mass spectra were recorded on Agilent MSD at NMIMS University, Mumbai, India. Almost all compounds were characterized by FTIR, ^{1}H NMR, ^{13}C NMR and HRMS. The anticancer activity was carried out at ACTREC, Navi Mumbai, India.

The compounds were synthesized by the **scheme 1** and **2** as depicted below.

Scheme 1: Synthesis of **5a-5f**



Scheme 2: Synthesis of **9a-9f**



2.1 General procedure for the preparation of substituted phenylthiourea (2a-2f)

In a single neck, dry, 25ml round bottom flask substituted aniline (**1a-1f**, 1eq.), saturated solution of NH_4SCN (1 eq.) and 6N HCl (10 mL) were placed. The reaction mixture was heated to boiling and it was continued for 12 hrs. until precipitate formed and then poured into cold water (50 mL). The precipitated substituted phenylthiourea was filtered, recrystallized from ethanol to obtain pure compound (90%-85%) and characterized by melting point and FTIR. The results are found to be similar with literature reported values.[22,23,24,25]

2.2 General procedures for the synthesis of substituted 2-(arylimino)-1, 3-thiazolidin-4-one (3a-3f)

To a stirred mixture of substituted phenylthiourea **2a-2f** (1eq.), anhydrous sodium acetate (5 eq.) in ethanol (20 ml), ethyl chloroacetate (2 eq.) was added and mixture was heated at 60^{O} C for 6 hrs. The precipitate formed after cooling was filtered using vacuum, washed with ethanol (10 mL). The filtrate was concentrated using rotary evaporator and extracted using ethyl acetate (3 X 30 mL). The organic layer was concentrated using rotary evaporator to obtain more yield. The crude product was then recrystallized using ethyl acetate and used without purification. (Yield: 70%-75%). [22] The compounds were characterized

by melting point, ^{1}H NMR and ^{13}C NMR. (Figure S2-S47).

2.3 General procedure for the optimization of synthesis of substituted 5-arylidine-2-(arylimino)-1,3-thiazolidin-4-one (5a-5f)

In the model reaction, mixture of **3a**, substituted benzaldehyde **4a** in ethanol, diethylamine or PEG 400 was heated. To derive the optimal reaction parameters such as amount of diethylamine or PEG 400 required, time, temperature and influence of solvent; model reaction was performed using different variation. After completion of the reaction (TLC), mixture was cooled to room temperature and filtered using vacuum. The product was washed with petroleum ether (20 mL) and ethanol (20 mL). The product was then extracted with ethyl acetate (3 X 30 mL). The organic layer was washed with brine and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using dichloromethane: ethyl acetate (80:20) as eluting solvent. The compounds were characterized by melting point, ^{1}H NMR, ^{13}C NMR and mass spectrometer. [22] (Figure S2-S47).

2.4 General procedure for preparation of substituted 2-phenylhydrazinecarbothiomide (7a-7f)

Substituted phenylhydrazine **6a-6f** (2 eq.) was mixed thoroughly with thiourea (1 eq.) in a 5 mL beaker. Then the beaker was placed in conventional microwave oven until mixture became liquid. To remove unreacted thiourea, water (10 ml) was added and reaction mixture was filtered. The crude product was then recrystallized by water-ethanol and characterized by melting point and FTIR. The results are found to be similar with literature reported values. [26 27, 28, 29] (Figure S2-S47).

2.5 General procedure for preparation of substituted 1, 3-thiazolidine-2,4-dione-2-(phenylhydrazone) 8a-8f

Compounds **8a-8f** were synthesized from **7a-7f** (1 eq.), anhydrous sodium acetate (5 eq.) in ethanol (20 ml), ethyl chloroacetate (2 eq.) by same procedure as mentioned in section 2.2. All the compounds were characterized by melting point, ^{1}H NMR and ^{13}C NMR. (Figure S2-S47).

2.6 General procedure for synthesis of substituted 5-arylidene-1,3-thiazolidine-2,4-dione-2-(phenylhydrazone) 9a-9f

All the compounds **9a-9f** were synthesized from substituted benzaldehydes **4a-4f**, diethylamine and **8a-8f** by exactly same optimized procedure as mentioned in section 2.3. All the compounds were characterized by melting point, ^{1}H NMR and ^{13}C NMR and mass spectrometer. (Figure S2-S47).

2.7 Anticancer screening studies: SRB Assay [30]

Anticancer activity of synthesized compounds **5a-5f** and **9a-9f** was done by Sulphorhodamine B (SRB) assay at ACTREC, Mumbai, India by using human breast cancer (MCF 7), Human lung cancer (Hop 62) and human hepatoma (Hep G2) cell lines. The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For screening experiment, cells were inoculated into 96 well microtiter plates in 100 µl. After inoculation, the microtiter plates were incubated at 37 °C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 hrs. prior to the addition of experimental drugs. After 24 hrs. one 96 well plate containing $5x10^3$ cells/well was fixed *in situ* with TCA to represent a measurement of the cell population (Tz) at the time of drug addition. Experimental drugs were initially solubilized in dimethyl sulfoxide at 100 mg/ml and diluted to 1 mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to 10µg/ml, 20 µg/ml, 40 µg/ml and 80 µg/ml with complete medium. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentrations i.e.10µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml. Adriamycin was used as standard.

2.8 General procedure for molecular docking studies

The docking studies were performed using Glide module (version 5.1, Schrödinger, LLC, NY) installed on Linux workstation. Protein preparation was done using the Protein Preparation Wizard of Maestro software at pH 7.4. SHP2 in complex with

inhibitor (PDB ID: 2Shp) was treated to add missing hydrogens, proper bond orders were assigned and water molecules more than 5 Å from the heterogeneous groups were deleted. The H bonds were optimized using sample orientations. All the polar hydrogens were displayed. Finally, the protein structure was minimized to the default Root Mean Square Deviation (RMSD) value of 0.0733. The inhibitor structure was minimized using the OPLS 2005 force field. Ligand preparation was done using Ligprep module using default setup.

The receptor grid was generated using specific residues Asp181, Phe182 important for the catalytic active site to participate in catalysis and substrate binding was defined, and the co-crystallized ligand was differentiated from the active site of receptor. The atoms were scaled by van der Waals radii of 1.0 Å with the partial atomic charge less than 0.25 defaults. The active site was defined as an enclosing box at the centroid of the workspace ligand as selected in the receptor folder. The ligands similar in size to the workspace ligand were allowed to dock into the active site. No constraints either positional, H bonding, or hydrophobic were defined. Ligand docking was performed using OPLS 2005 force field with standard protocol.

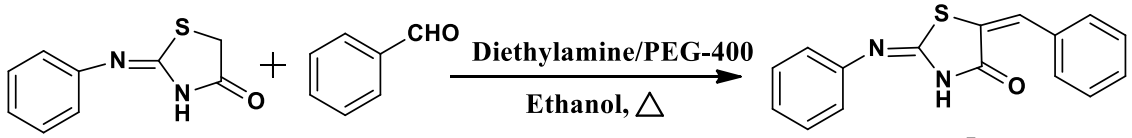
The docking results were viewed using pose-viewer. The final ligand binding poses were ranked according to a computer model score that encompasses the grid score, proprietary GLIDE score, and the internal energy strain. The score function of Glide or Glide score, a modified and expanded version of ChemScore, was used for binding affinity prediction and ligand ranking. The detailed description of the study is available in supplementary section of this article.

3. RESULTS AND DISCUSSION

3.1 Chemistry

Compounds **4a-f** were prepared by condensation of substituted aromatic aldehydes and substituted-2-aryl-thiazolidine -4-ones **(3a-f)**. Refluxing the mixture of ethylchloroacetate, and substituted phenyl thiourea **(2a-f)** in presence of sodium acetate yielded **3a-f** by literature reported procedure, where **2a-f** are the results of reaction between substituted aniline **1a-f** and ammonium thiocyanate in acidic medium. Compounds **8a-f** are synthesized by literature reported procedure of condensation of substituted 1, 3-thiazolidine-2,4-dione-2-phenyl hydrazone **7a-f** with base. Refluxing the substituted phenyl hydrazines **6a-f** and ammonium thiocyanate in ethanol by reported procedure gave substituted-2-phenylhydrazine carbothioamide **7a-f**, which on further treatment with ethylchloroacetate, sodium acetate yielded **8a-f**.

Table 1: Optimization of synthesis of **(5a)**[a] using diethylamine or PEG-400



3a 4a 5a

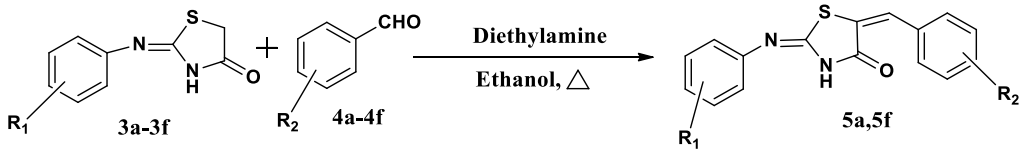
Entry	Amount of diethylamine[b] (mmol)	Amount of PEG-400[b] (mmol)	Solvent	Time (Hrs.)	Temp (°C)	% Yield[c]
1	0.1	--	Ethanol	6	Reflux	50
2	0.5	--	Ethanol	6	Reflux	83
3	0.5		Ethanol	4	Reflux	60
4	1.0	--	Ethanol	6	Reflux	84
5	2.0	--	Ethanol	6	Reflux	84
6	2.5	--	Ethanol	6	Reflux	85
7	--	0.1	Ethanol	6	Reflux	42

8	--	0.5	Ethanol	6	Reflux	60
9	0.5	--	Methanol	6	Reflux	80
10	0.5	--	Benzene	6	Reflux	55
11	0.5	--	1,4-Dioxane	6	100	22
12	0.5	--	THF	6	100	45

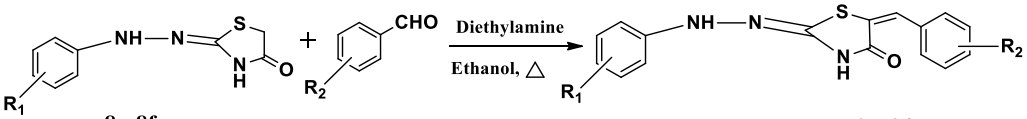
[a] Reaction conditions: 1 eq. of 2-phenylimino-thiazolidine-4- one **(3a)**, 1 eq. of benzaldehyde **(4a)** in presence of various solvents (10 ml) heated at different temperatures for the indicated time period. [b]The molar equivalents of diethylamine or PEG-400 used with respect to **3a**. [c]Isolated yield of **5a**.

The synthesis of novel compound **5a** was optimized using the model reaction **(Table 1)**. It was observed that the use 0.5 mmol of diethylamine yielded 83% of **5a,** whereas reaction catalyzed by PEG- 400 yielded 60% (Table 1**,** entry 2, 8). The influence of solvent was also evaluated for model reaction. The reaction was performed using hydrocarbon, halogenated hydrocarbon, ethereal and polar protic and aprotic solvents. The best results were obtained in ethanol (83%, Table 1, entry 2), methanol (80 %, Table 1, entry 9). Based on Pfizer solvent selection guidelines [29] ethanol was preferred over methanol.

Table 2: Diethylamine catalyzed synthesis of **(5a-5f)** and **(9a-9f)**[a]



Code	R_1	R_2	Yield (%)[b]
5a	H	H	83
5b	H	3-OMe, 4-OH	81
5c	H	4-OH	62
5d	H	4-$N(CH_3)_2$	82
5e	3,5-diNO_2	H	84
5f	3,5- diNO_2	4-$N(CH_3)_2$	79



9a	H	H	72
9b	H	3-OMe, 4-OH	85
9c	H	4-OH	87
9d	H	4-$N(CH_3)_2$	78
9e	3,5-diNO_2	H	81

9f	3,5- diNO_2	4-$N(CH_3)_2$	78

[a]Reaction conditions: 1 mmol. **(3a-3f or 8a-8f)**, 1 mmol. of benzaldehyde **(4a-4f)** and diethylamine (0.5 mmol.) in ethanol (10 ml) refluxed for 6 hrs. [b]Isolated yield of **5a-5f and 9a-9f**.

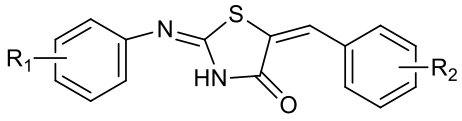
Further, various substituted benzaldehydes **4a-4f** were reacted with **3a-3f** and **8a-8f**, using diethylamine by optimized reaction conditions to form **5a-5f** and **9a-9f** respectively. The results are summarized in **Table 2**. It was observed that diethylamine is suitable catalyst in synthesizing **5a-5f** and **9a-9f** series of compounds.

The structures of **5a-f** and **9a-f** were characterized by ^{1}H NMR, ^{13}C NMR, and mass spectroscopic analysis. In ^{1}H NMR spectra, the aromatic protons resonated at δ 7.34-7.47 ppm. Resonance signals at δ11.35 confirmed the presence of –NH. Multiplet between δ 7.14-7.18 confirmed the presence of aromatic ring protons linked at 5th position of thiazolidine-4-one ring, whereas proton of this junctional carbon resonated at δ 7.65. In ^{13}C NMR spectra, aromatic carbon resonated between δ 126 - 149 ppm with many overlaps in these regions, while the ketone C=O resonated around δ 180-185 ppm. (Supplementary Information).

3.2 Biological screening

Compounds were tested for their anticancer potential using Sulphorhodamine B (SRB) assay using human breast cancer cell lines MCF7, human lung cancer cell lines Hop62 and human liver cancer cell lines HepG2 by the procedure mentioned. Adriamycin (ADR) was used as a standard. The synthesized compounds exhibited interesting activity with GI50 value ranging from >80 - <10 µg/ml. against the cancer cell lines as seen in **Table 3**. The results of the anticancer activity revealed that nature and type of aromatic ring is important for anti-cancer activity, also difference in activity depends upon substitution on aromatic ring. Compound **9b**, **9e** and **9f** with GI50 value of <10 µg/ml have shown anticancer activity equal to that of Adriamycin on MCF7 cell lines. **9b** also found to be effective in controlling % growth rate of Hop62 cell lines with GI50 value equal to Adriamycin (<10 µg/ml). In the hydrazone series of compounds unsubstituted hydrazone **9a** shown the moderate activity compared to standard. The presence of substituent on A and/or B phenyl ring has major influence on anticancer activity. Amongst the compounds **9a-9d** where phenyl ring 'A' is unsubstituted **9c**, **9d** found to have less potential in controlling the growth of MCF7 and Hop62 cell lines this can be attributed to presence of electron releasing group like hydroxy **(9c)** and -NMe_2 **(9d)** at *para* position of ring B phenyl. Whereas compounds with unsubstituted/substituted ring 'B' such as **9e-9f**, presence of para substituted electron releasing group like -NMe_2 **(9f)** along with disubstituted electron withdrawing groups at *ortho*, *para* position of A ring phenyl improves the anticancer activity. Both the compounds **9e** and **9f** are effective in controlling the % growth of MCF 7 cell lines at when compared with standard and GI50 value of <10 µg/ml µg/ml. All compounds of this series have shown similar behavior in controlling the % growth of Hop62

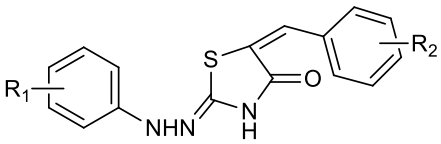
Table 3. Anticancer evaluation using Sulforhodamine Blue Assay (SRB)



A ring **B ring**

Code No.	**R_1**	**R_2**	**GI50 (µg/ml)**		
			MCF7	**Hop62**	**HepG2**
5a	H	H	>80	>80	>80
5b	H	3-OMe, 4-OH	<10	<10	<10
5c	H	4-OH	36.8	39.2	35.6
5d	H	4-$N(CH_3)_2$	39.2	25.3	34.5
5e	3,5-diNO_2	H	25	27.3	20

5f	3,5- diNO$_2$	4-N(CH$_3$)$_2$	26	33.5	18.5



A ring **B ring**

Code No.	**R$_1$**	**R$_2$**	**GI50 (µg/ml)**		
			MCF7	**Hop62**	**HepG2**
9a	H	H	12	21	50.6
9b	H	3-OMe, 4-OH	<10	<10	75.4
9c	H	4-OH	16.8	38.2	60.4
9d	H	4-N(CH$_3$)$_2$	19.2	21.3	>80
9e	3,5-diNO$_2$	H	<10	19.3	67.2
9f	3,5- diNO$_2$	4-N(CH$_3$)$_2$	<10	14.9	65.6
Adriamycin	--	--	<10	<10	<10

cell lines and % control growth varies by the order disubstituted aromatic 'A' ring with electron withdrawing groups & electron donating groups in ring 'B' (**9e, 9f**) > unsubstituted aromatic A and B ring (**9a**)> A ring and B ring with electron donating groups (**9b, 9c**), where **9e,9f** refers to highly effective and **9c** is least effective. This underlines the need of mono (para)-, di (ortho, para)- substituted aromatic 'A' ring linked to thiazolidinone backbone with -NHN= group along with para substituted electron releasing group containing 'B' ring at 5th position. In case of 2-arylimino-thiazolidine-4-ones **5a-f** only compound **5c** with electron donating substituents (3'OCH$_3$ and 4'OH) on B ring shows the activity on Hop62 cell lines all other compounds are less successful in controlling the growth of Hop62 and MCF7 cell lines. This underlines the role of lipophilicity, H-bonding capacity, type and size of substituent on aromatic A ring, presence of -NHN= linkage at 2nd position of thiazolidine-4-one and substituted B ring at 5th position. **Fig. 3-5** represent the results of cell line studies.

Figure 3: Results of anticancer activity using Human Breast Cancer cell lnes MCF7. Each point represents the mean ± SD of triplicate measurements.

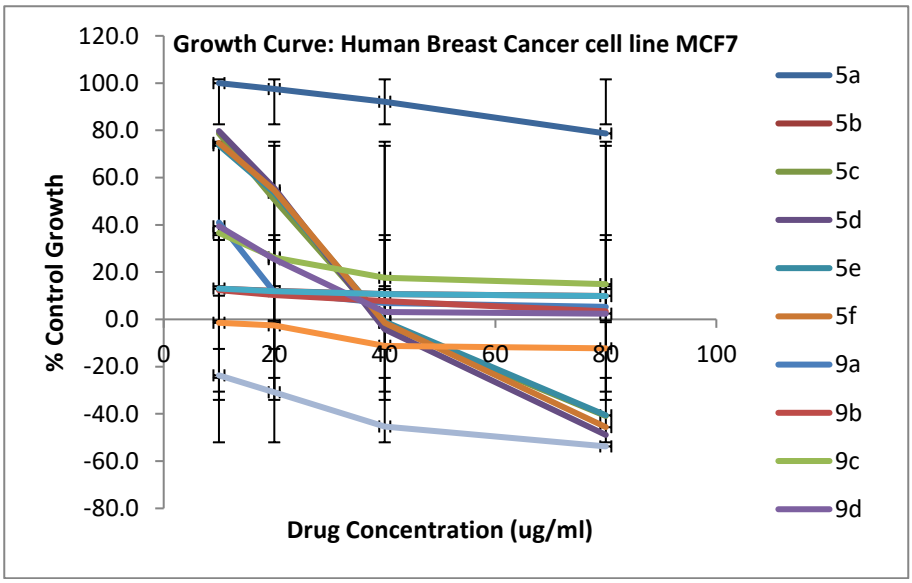


Figure 4: Results of anticancer activity using Human Lung Cancer cell lines Hop62. Each point represents the mean ± SD of triplicate measurements

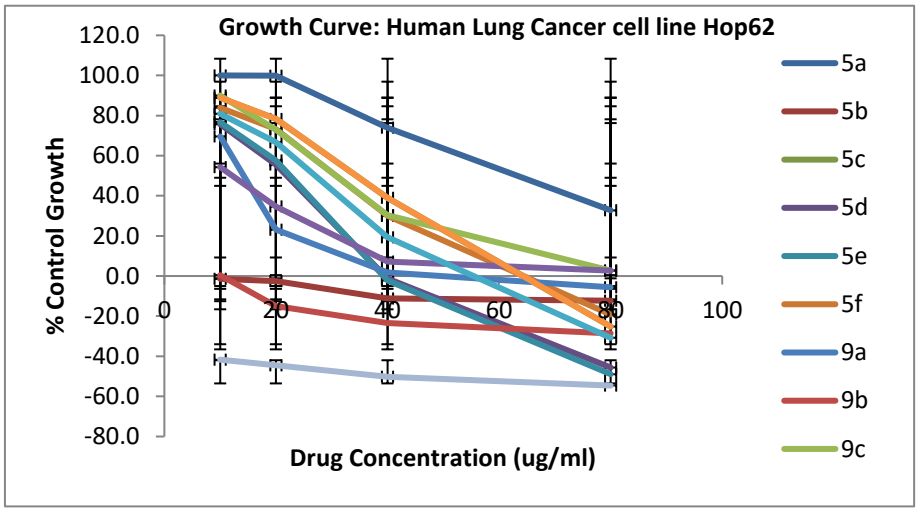
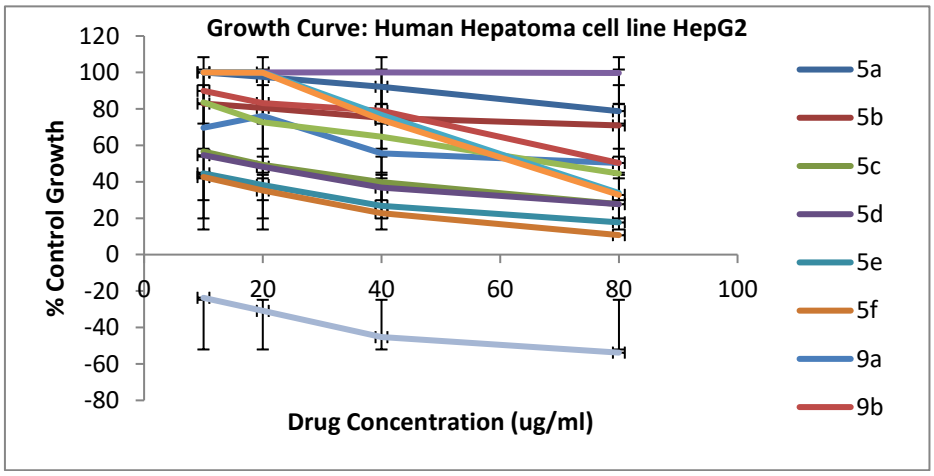
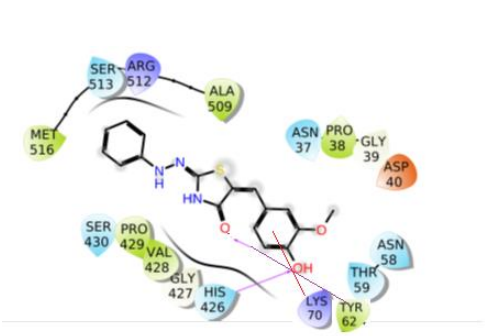


Figure 5: Results of anticancer activity using Human Hepatoma cell lines HepG2. Each point represents the mean ± SD of triplicate measurements

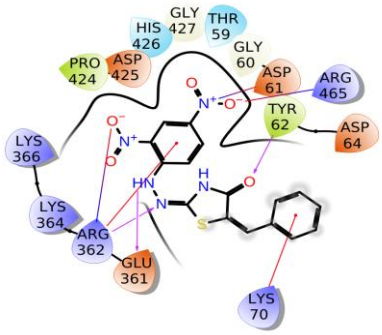


3.3 Molecular Docking studies

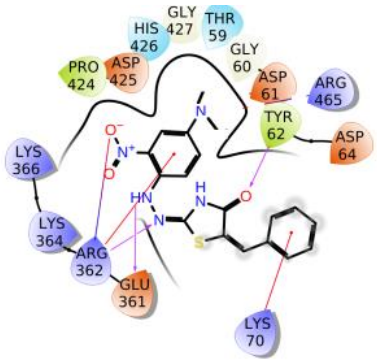
Figure 6: Details of interactions of compounds **9b (a), 9e (b), 9f (c) and native (d)** with 2Shp active site. Only interacting residues were labelled. Where, hydrogen bonding interactions are shown in pink arrows, π- cation interactions are shown in red lines, solvent effect is shown as grey shaded area and solvent bridge is indicated by purple lines



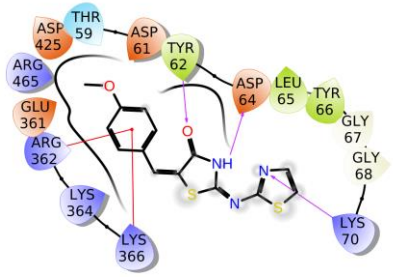
(a)



(b)

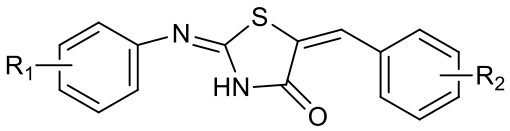


(c)



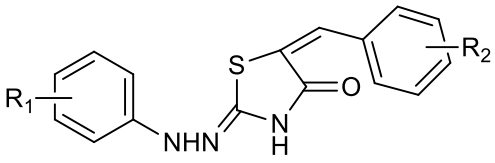
(d)

Table 4: Designed molecules with their docking score



A ring **B ring**

Code No.	R_1	R_2	Docking score
5a	H	H	-3.688
5b	H	3-OMe, 4-OH	-3.243
5c	H	4-OH	-3.314
5d	H	4-$N(CH_3)_2$	-3.478
5e	3,5-diNO_2	H	-4.192
5f	3,5- diNO_2	4-$N(CH_3)_2$	-4.382



A ring **B ring**

Code No.	R_1	R_2	Docking Score
9a	H	H	-3.253
9b	H	3-OMe, 4-OH	-3.848
9c	H	4-OH	-3.247
9d	H	4-$N(CH_3)_2$	-2.966

9e	3,5-diNO_2	H	-3.963
9f	3,5- diNO_2	4-$N(CH_3)_2$	-3.368
Native	--	--	-4.082

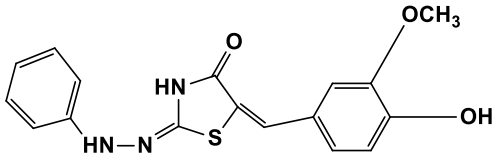
Redocking of the co-crystallized ligand was the first step in docking so as to ensure that most of the important interactions with the protein are reproduced. We noticed that the docking score of the co-crystallized ligand was on the lower side although it was able to reproduce all the interactions. The RMSD value of the co-crystallized pose and the docked pose was < 1.0 thereby validating the docking protocol. The major interactions observed with the co-crystallized ligand include Hydrogen bonding interactions with Tyr62, Asp64 and Lys70 and π-cation interactions with Arg362 and Lys 366.

Among the synthesized compounds, Compound **9e** was able to reproduce most of the interactions like the co-crystallized ligand. Hydrogen bonding interaction of the carbonyl group on the thiazole ring with Tyr62 is retained in all the active compounds whereas the interaction with Lys70 is altered to π-cation interaction in the synthesized compounds. The replacement of the sp2 hybrid carbon (-CH=) with NH-N= has brought about an additional Hydrogen bonding interaction with Glu361 in the second series **(Fig. 6a-d, Table 4)**.

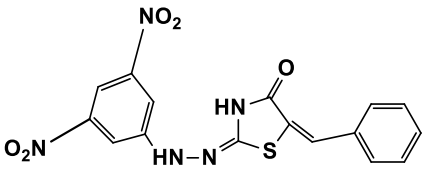
3. CONCLUSION

In conclusion, we have successfully synthesized novel N-substituted 2-arylimino-5-arylidene-thiazolidin-4-one and 5-arylidene-1,3-thiazolidine-2,4- diones using diethylamine as catalyst. Synthesis of the compounds was carried out by using literature reported simple and efficient protocols. The detailed SAR studies of substituted thiazolidine-4-one and substituted 1, 3-thiazolidine-2,4-diones led us to the discovery of **9b**, **9e**, **9f** (**Fig. 7**) with GI50 value of <10 μg/ml on MCF 7 cell lines, this activity is equal to Adriamycin standard. **9b** is excellent in controlling the growth of HOP62 cell line which also indicated by their GI50 value of <10 μg/ml. None of the compound was found effective in controlling % growth of HepG2 cell lines. This indicates that all the compounds have cytotoxic potential in terms of human cancer cell lines only. This supports the fact that anticancer activity of compound is sole outcome of structure and no systemic toxicity has been observed. The docking study undertaken failed to support the hypothesis that the compounds are SHP2 inhibitors. Docking scores are very weak and preliminary to reach to the conclusion of their SHP2 inhibitory potential. We found no correlation between anticancer activity screening and docking of the compounds.

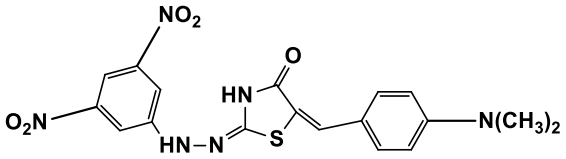
Figure 7: Structures of most active analogues **9b**, **9e**, **9f**



9b



9e



9f

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY INFORMATION

Additional supplementary information including figure, table, and spectra may be found online in the supplementary information tab for this article.

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