



Design, synthesis, DNA assessment and molecular docking study of novel 2-(pyridin-2-ylimino)thiazolidin-4-one derivatives as potent antifungal agents

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ABSTRACT

A series of novel 2-imino-4-thiazolidinone derivatives **4a,b** was synthesized through reaction of unsymmetrical thioureas **3a,b** with chloroacetic acid. Condensation of **4a,b** with aromatic aldehydes **5a-e** yielded the corresponding 5-arylidene derivatives **6a-j**. In addition, the reaction of **4a,b** with 4-aryloxy-3-hydroxybenzaldehydes **8a-c** furnished the respective mono-aryloxy-4-thiazolidinones **10a-f**. All the newly synthesized compounds were confirmed by their elemental analysis and spectral data. The antifungal activity of the newly synthesized compounds was assessed and the compounds **6d**, **6e**, **6i**, **6j**, **9a,b** and **10a-f** revealed higher antifungal activity towards *Alternaria solani* than to the standard *Ridomil gold* plus. Moreover, the DNA toxicity of 4-thiazolidinone derivatives **6d**, **9a**, **10b**, **10c** and **10f** on the nucleic acid of *Alternaria solani* (KT354939) was performed and the results showed qualitatively more than 70% cleavage. Also, compounds **6i**, **6j**, **9b**, **10c** and **10f** were docked inside the active site of 1ZOY enzyme and suitable binding with the active site of amino acids, were displayed according to their bond lengths, angles and conformational energy.

1. Introduction

In spite of the progress in antimicrobial therapy, diseases caused by different types of bacteria and fungi are still a breakneck health problem. This is due to the continual development of resistance to the existing antimicrobial drugs. This led to an everlasting screening for new biologically active compounds with higher antimicrobial efficacy and lower cytotoxicity. *Alternaria solani* is considered as one of the well known examples of a pathogen, which early blight (EB), a disease infecting tomato plants. Foliar symptoms initially appear on the oldest leaves as small, brownish to black lesions. These leaf spots enlarge up to ½ in. (1.3 cm) in diameter in a characteristically concentric fashion. The area around the spot may become yellow, as may entire severely affected leaves (Fig. 1).

Early blight (EB) of the tomato plant is probably one of the most destructive disease of field crops. This is due to its property to infect rapidly after seed initiation. In addition, it destroys foliage and reduces yield typically by ~20% in some cases, it has been reported to reduce the yield by 70–80% [1]. Infection by the fungus is highly rapid at 28–30 °C in humid conditions. Various fungicides have been developed

to control EB, such as quinone outside inhibitors (QoIs, FRAC group 11), succinate dehydrogenase inhibitors (SDHIs, FRAC group 7) and others. SDHI fungicides target the succinate dehydrogenase complex, which blocks the citric acid cycle and the production of ATP required for fungal respiration. The succinate dehydrogenase (SDH) complex consists of a flavoprotein (SDH subunit A), an iron-sulphur protein (SDH subunit B) and two membrane-anchoring proteins (SDH subunits C and D). Succinate dehydrogenase inhibitors bind to a highly conserved active site composed of residues found in subunits B, C and D, where ubiquinone (coenzyme Q10) is reduced to ubiquinol [2]. Some commercial SDH fungicides were consisted of structurally diverse frameworks, including thifluzamide and boscalid which displayed broad spectrum in antifungal activity, as example thifluzamide has biological effect on basidiomycetes, particular for diseases caused by *Rhizoctonia* spp., on rice, wheat, papaya, corn, potato, tomato and sugar beet [3]. Also, boscalid has been used as a broad-spectrum fungicide against mainly on *Alternaria alternata* [4]. However, resistance against these fungicides in *Alternaria strains* [5] make it an advantageous and interesting task to discover more potent and effective compounds against *Alternaria strains* to prevent this disease. It is well known that 4-

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Fig. 1. Tomato early blight disease symptoms: (I) *Alternaria solani* spore; (II) Disease symptoms on tomato leaves; (III) concentric lesions on fruits.

thiazolidinone derivatives are one of the heterocyclic categories which play an important role in therapeutical chemistry, due to their variety in biological activity such as antiviral [6], anticancer [7], anti-inflammatory [8], antitumor [9], antimicrobial [10], anticonvulsant [11], antidepressant [12], antioxidant [13]. Moreover, 4-thiazolidinones are used as a potent antifungal activity [14–17] and as new class of anti-HIV-1 agents with marked reverse transcriptase (RT) inhibitory effects [18,19]. A variety of thiazolidinone based derivatives having different substituent around the core nucleus are being considered as potential antimicrobial agents [6,20–23]. Additionally, the combination of 4-thiazolidinones with other heterocyclic rings showed wide range of biological activities like thiazolo-linked pyrimidine and thiazolidinone-oxindoline base compounds have been used as a potent CA inhibitor [24,25]. Based on the above reports of interest biological activity of 4-thiazolidinones and in continuation of our interest in the synthesis of some new bioactive 4-thiazolidinone derivatives [26–35], we planned to synthesize some novel 2-imino-3-aryl-4-thiazolidinone derivatives which containing pyridine moiety as an effective compound against some types of fungi especially *Alternaria* strains.

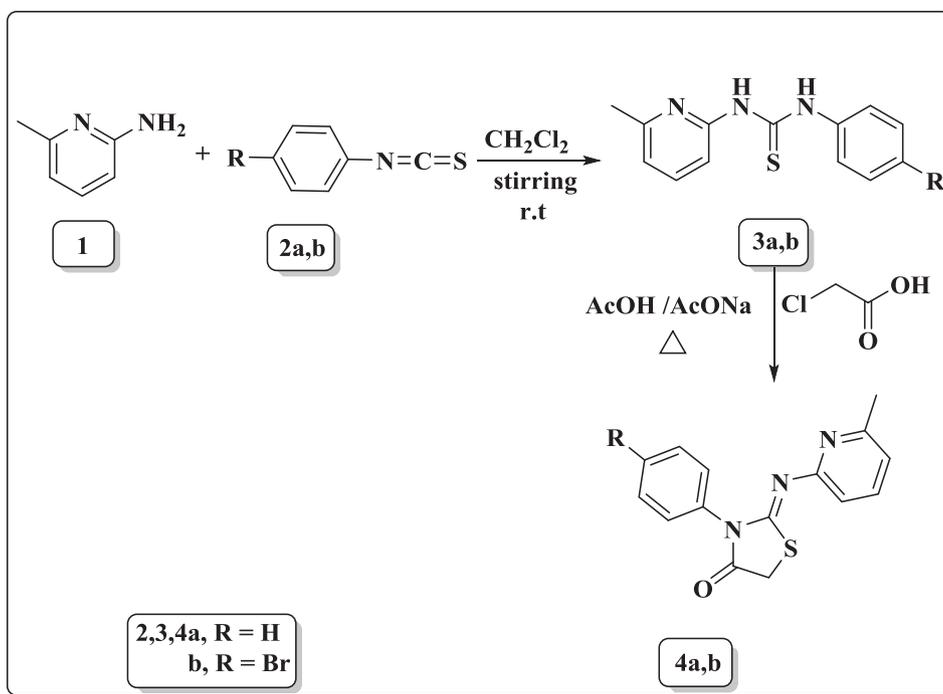
2. Results and discussion

2.1. Synthetic chemistry

Unsymmetrical 1,3-diarylthioureas **3a,b** as starting materials were prepared by the reaction of 2-amino-6-picoline (**1**) with isothiocyanate derivatives **2a,b** in dichloromethane under stirring at room temperature. The IR spectrum of the isolated product **3b** displayed a characteristic absorption bands at $\nu_{\max} = 3194$ and 3113 cm^{-1} corresponding to the two NH groups and another absorption band at $\nu_{\max} = 1256 \text{ cm}^{-1}$ for C=S stretch. Its ^1H NMR spectrum provided the position of methyl protons at δ 2.19 ppm, besides the two D_2O exchangeable signals appeared at $\delta = 10.76$ and 14.16 ppm, respectively assigned to the NH protons. Also, mass spectrum showed a corrected molecular ion peak at $m/z = 322$ (M^+ , 55.5%) beside some other abundant fragments, that established the molecular formula $C_{13}H_{12}BrN_3S$. Elemental analysis together with mass spectrum are in agreement with the proposed structure **3a,b**. Cyclization of compounds **3a,b** with chloroacetic acid in glacial acetic acid in the presence of anhydrous sodium acetate provided yellow crystals **4a,b**. The structure

of the isolated products **4a,b** was established by using ^1H NMR spectra which revealed a characteristic singlet signal at $\delta \sim 4.0$ ppm assigned to the methylene ($-\text{CH}_2-$) protons. Moreover, ^{13}C NMR for compound **4b** confirmed the proposed structure due to the appearance of a characterized signal at $\delta = 33.76$ ppm due to the active methylene carbon, besides the other expected signals due to the aromatic carbons. Analytical and spectral data of the prepared compounds **4a,b** were in agreement with the proposed structure. Mass spectrum of **4b** showed a corrected molecular ion peak at $m/z = 362$ (M^+ , 63.7%) which deduce the formula $C_{15}H_{12}BrN_3OS$ (see Schemes 1 and 2).

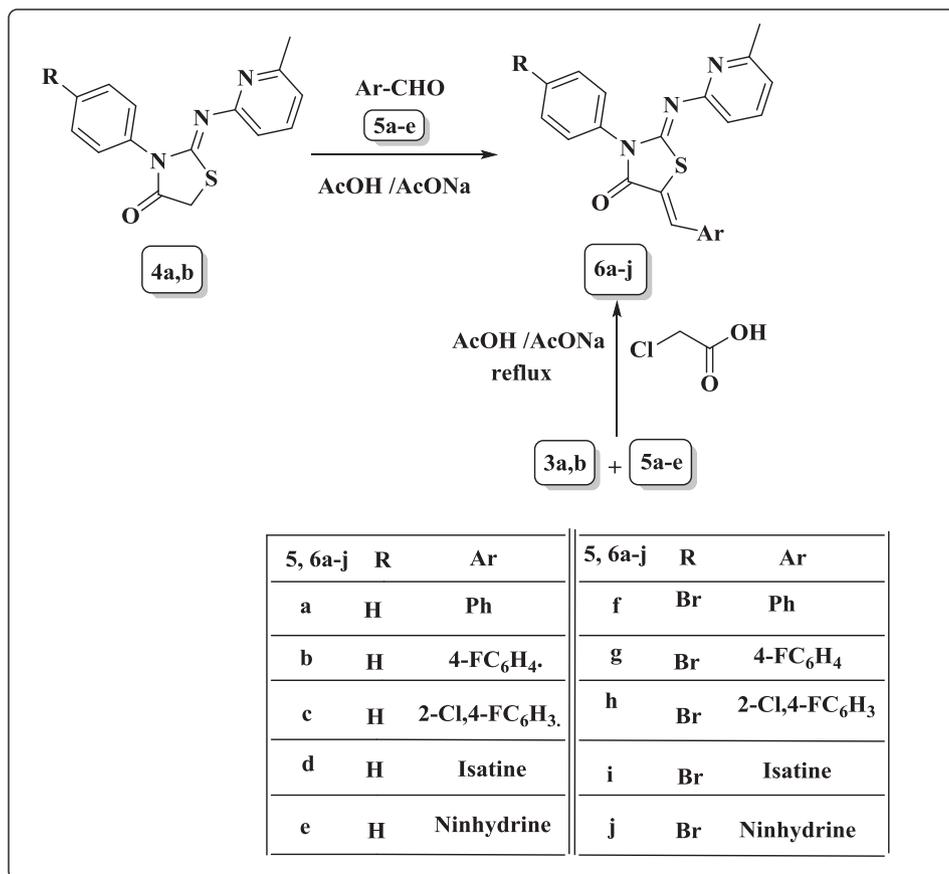
Condensation of 4-thiazolidinones **4a,b** with various aromatic aldehydes **5a-e** in refluxing glacial acetic acid in the presence of anhydrous sodium acetate afforded the respective arylidene-4-thiazolidinone derivatives **6a-j**. Structures of **6a-j** were inferred from their analytical and spectral data. Thus, IR spectrum of compound **6h** as example showed absorption band $\nu_{\max} = 1710 \text{ cm}^{-1}$ corresponding to the carbonyl group (C=O). Its ^1H NMR revealed a singlet signal at $\delta = 7.64$ ppm attributable to vinylic (CH=) proton in addition to the other expected signals corresponding to aromatic protons. Also, mass spectrum of **6h** showed a corrected molecular ion peaks at $m/z = 503$ (M^+ , 38.3%) and $m/z = 505$ ($M^+ + 2$, 11.5%) which is consistent with the formula $C_{22}H_{15}ClFN_3OS$. Moreover, compounds **6a-j** were synthesized as one pot synthesis via refluxing 1,3-unsymmetrical thioureas **3a,b** with aromatic aldehydes **5a-f** and chloroacetic acid in glacial acetic acid in the presence of anhydrous sodium acetate. Condensation of compounds **4a,b** with 4-aryloxy-3-hydroxybenzaldehydes **8a-c** which prepared from coupling reaction of 3-hydroxybenzaldehyde with aryldiazonium salts **7a-c** in ethanol containing sodium acetate at $0-5^\circ\text{C}$ afforded the isolated products **10a-f**. The structure of the synthesized compounds **10a-f** was determined by elemental analysis and spectral data. The IR spectrum of **10a** showed absorption band at $\nu_{\max} = 3408 \text{ cm}^{-1}$ corresponding to the hydroxyl group, in addition to the two absorption bands at $\nu_{\max} = 1710$ and 1661 cm^{-1} due to the carbonyl (C=O) and imino (C=N) groups, respectively. The ^1H NMR spectrum of the isolated product **10a** displayed a singlet signal exchangeable D_2O at $\delta = 9.83$ ppm assigned to the hydroxyl proton (OH). In addition to the expected protons for aryl and vinylic protons. Elemental analysis together with mass spectrum are confirmed the proposed structure of **10a**. Based on the above collective data for **10a**, the formation of diarylazo derivatives **11a-f** was ruled out.



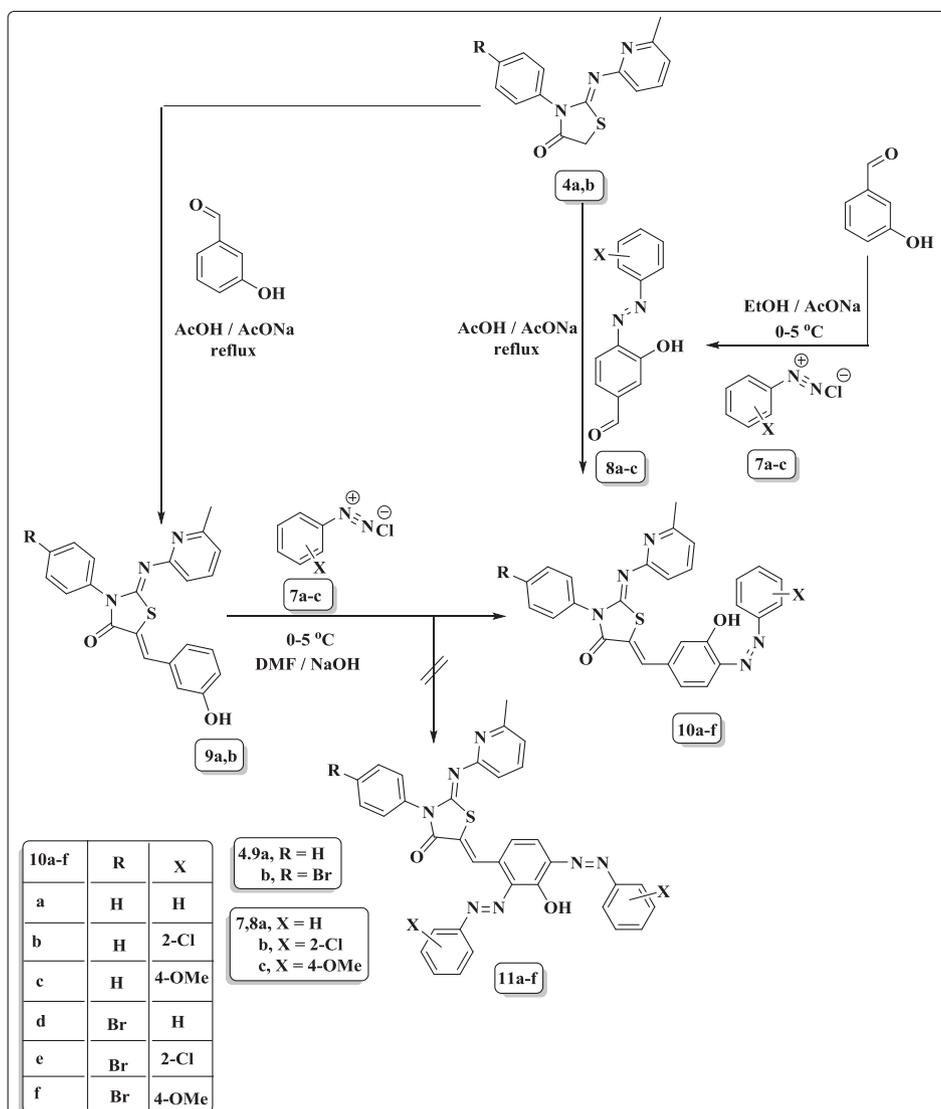
Scheme 1.

An alternative route for the formation of compounds **10a-f** chemically was observed, thus the condensation of compounds **4a,b** with 3-hydroxybenzaldehyde in glacial acetic acid containing anhydrous sodium acetate afforded the corresponding 5-arylidene derivatives **9a,b**. The

structure of the isolated products **9a,b** was determined by elemental analyses and spectral data. The IR spectrum of **9a** showed an absorption band at $\nu_{\text{max}} = 3408 \text{ cm}^{-1}$ corresponding to the hydroxyl group, in addition to an absorption band at $\nu_{\text{max}} = 1702 \text{ cm}^{-1}$ due to the



Scheme 2.



Scheme 3.

carbonyl (C=O) group. Its ^1H NMR spectrum displayed a singlet signal exchangeable D_2O at $\delta = 9.82$ ppm assigned to the hydroxyl proton (OH). In addition to the expected protons for aromatic and vinylic protons. Also, mass spectrum exhibited a molecular ion peak at $m/z = 387$ (M^+ , 99.1%) which was consistent the molecular formula $C_{22}H_{17}N_3O_2S$. Coupling of compounds **9a,b** with arenediazonium salts **7a-c** in *N,N*-dimethylformamide in the presence of sodium hydroxide at 0–5 °C afforded the corresponding arylazo derivatives which are identical in all aspects (TLC, melting point and mixed melting point) with compounds **10a-f** (Scheme 3).

2.2. Biological evaluation

2.2.1. Antifungal activity

The antifungal activity of the prepared compounds **4a,b**, **6a-j**, **9a,b** and **10a-f** against cultures of five fungal strains *Aspergillus niger* (KU681408), *Aspergillus flavus* (KU681428), *Alternaria solani* (KT354939), *Aspergillus ochraceus* (DQ087531) and *Macrophomina phaseolina* (AM410964) were investigated by agar dilution assay [36]. Where, the concentration of the compounds under investigation were prepared at 10 and 100 $\mu\text{g/ml}$. The results of in-vitro antifungal screening all the novel compounds which expressed in inhibition zone diameter are listed in Table 1. Both Ridomil gold plus and

Mancozeb used as positive antifungal control for *Alternaria solani*. Qualitatively, compounds **6d**, **6e**, **6i**, **6j**, **9a**, **9b**, **10a**, **10b** and **10c** gave higher potency than the positive control whereas **10d**, **10e** and **10f** represented moderate potency rather than compounds **4a**, **4b**, **6a**, **6b**, **6c**, **6f**, **6g** and **6h** revealed very lower activity.

2.2.2. Mechanism of action and the effect of the isolated compounds **6d**, **9a**, **10b**, **10c** and **10f** on the nucleic acid of the pathogenic fungus *Alternaria solani*

DNA damage study was performed according to the protocol of Kasibhatla and Gustava [37]. The fungal DNA amplified by PCR was treated with (100 $\mu\text{g/ml}$) from each of the synthesized compounds **6d**, **9a**, **10b**, **10c** and **10f**, also each sample was incubated for one hour. DNA without treatment served as a control. After treatment DNA was separated by electrophoresis using a 1% agarose gel at 75 V for 30 min. The gel was stained with ethidiumbromide and then subjected to UV irradiation to visualize the DNA bands.

2.2.3. Effect of 4-thiazolidinone derivatives **6d**, **9a**, **10b**, **10c** and **10f** on nucleic acid of *Alternaria solani*

In order to analyze the effect of 4-thiazolidinone derivatives **6d**, **9a**, **10b**, **10c** and **10f** on the nucleic acid of *Alternaria solani* (KT354939), we set up a gel electrophoresis analysis. The results showed the interact

Table 1
Antifungal activity for compounds **4a,b**, **6a-j**, **9a,b** and **10a-f**.

comp. fungi	<i>Aspergillus- Flavous</i>	<i>Aspergill- usniger</i>	<i>Aspergillus- ochraceous</i>	<i>Alternarisolani</i>	<i>Macrophomia- phasolina</i>
4a	NA	24	17	10	NA
4b	NA	15	27	19	NA
6a	19	20	24	25	10
6b	45	38	42	50	24
6c	40	44	49	51	40
6d	91	95	83	86	71
6e	93	93	84	88	83
6f	14	15	12	11	NA
6g	17	24	20	14	NA
6h	22	29	18	58	18
6i	94	89	90	95	87
6j	93	95	92	94	90
9a	90	94	94	90	84
9b	92	93	95	95	88
10a	88	86	91	93	87
10b	89	92	93	94	81
10c	95	92	94	95	95
10d	81	86	88	92	85
10e	81	86	82	89	84
10f	95	95	90	95	84
DMSO (negative control)	NA	NA	NA	NA	NA
Ridomil gold plus (positive control)				77	
Mancozeb (positive control)				70	
Activity color key	color				
Higher activity (more than 85%)	Red				
Moderate activity (between 70-84 %)	Green				
Low activity (less than 70 %)	Yellow				

DNA bands with the untreated DNA (control), where no significant damage occurred. In contrast, DNA treated with the selected 4-thiazolidinone derivatives showed substantial alteration in electrophoretic migration as shown in the Fig. 2. These results suggest that the antifungal effect of 4-thiazolidinone derivatives may occur by direct chemical damage to DNA. Thus, 4-thiazolidinones may act on the cell membrane of the pathogenic *Alternaria solani*, penetrating it, followed by damage and inhibition of DNA replication.

2.2.4. Structure-activity relationship (SAR) studies

Based on the results, mentioned in Table 1, it was found that the starting 2-imino-3-aryl-4-thiazolidinone derivatives **4a,b** showed noactivity against *Aspergillusflavus* and *Macrophominaphaseolina*. The results also showed low activity against the other type of antifungal as well as the 5-benzylidene-4-thiazolidinone **6a**, which bearing the phenyl group. Introducing the withdrawing groups in aryl group at position-5 like compounds **6b,c** which contain the fluoro and chloro groups improved the activity against *Aspergillusflavus* and *Macrophominaphaseolina* than the unsubstituted parent compound **6a**. On the

other hand, the 5-arylidene-3-aryl-4-thiazolidinones **6d,e** which bear the hetaryl ring at position-5 as istain and ninhydrin showed high efficacy towards all the fungus strains except the *Aspergillus ochraceous* fungi compared with positive antifungal control (Ridomil gold plus and Mancozeb) for *Alternaria solani*. Also, compounds **6i,j** revealed the high efficacy towards all the five fungus strains compared with positive antifungal control due to bearing the hetaryl moiety with the bromine atom as substituent in the phenyl ring. Moreover, the designed compounds 5-benzylidene-3-aryl-4-thiazolidinones **9a,b** which containing the hydroxyl group in the phenyl ring at position-5 appeared the high activity against the all types of fungus strain except the compound **9a** showed low activity against the *Macrophominaphaseolina* which unsubstituted phenyl. Additionally, compounds **10a,c** exposed potent antifungal activity against all types of fungus strain which may be attributed due to the presence of phenolic hydroxyl and arylazo groups in phenyl ring. Compounds **10b** and **10f** showed high activity against four type of fungus strain and showed a lower activity against *Macrophominaphaseolina*. Consistent with the previous results, compound **10d** appeared to have a high activity against four types of fungus and

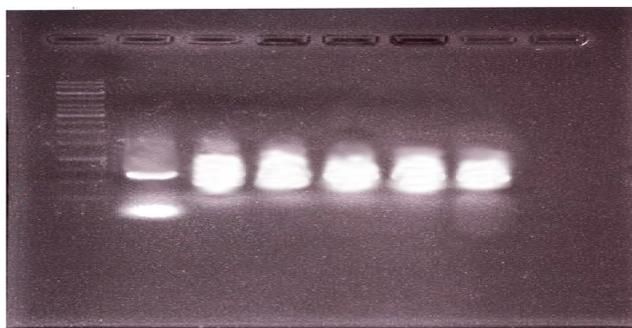


Fig. 2. Gel electrophoresis analysis of different synthetic compounds on DNA. After electrophoresis, the 1% agarose gel was stained with ethidium bromide. Lane 1, DNA size marker; lane 2, control DNA non-treated with compounds; lanes 3–7, DNA treated with compounds **6d**, **9a**, **10b**, **10c** and **10f**, respectively.

showed a low activity against *Aspergillusflavous*. Also, compound **10e** showed a high activity against the two types of fungus strain and low activity against the three types of fungus.

2.2.5. Molecular docking study

Compound **4a** binds into the active site of 1ZOY with minimum binding energy (ΔG) -8.8994 kcal/mol as shown in Table 2 and formed one hydrogen bond between carbonyl function and the residue amino acid SerD50 with bond length 2.19 Å (Fig. 3). Also, compound **6i** binds into the active site of 1ZOY with minimum binding energy (ΔG) -12.4351 kcal/mol (Table 2). The isatin ring in compound **6i** formed one hydrogen bond interaction between carbonyl group of 4-thiazolidinone and ArgD47 with bond length 1.99 Å. In addition to arene-arene interaction between ArgC46 and ArgD47 with pyridine and isatin ring, respectively of compound **6i** (Fig. 5). In addition, compound **6j** appeared one hydrogen bond between carbonyl function and ArgD47 with bond length 2.61 Å, beside to two arene-arene interaction between phenyl ring with ArgD47 and pyridine ring with ArgC46 (Fig. 7). Moreover, compound **9b** binds into the active site of 1ZOY with minimum binding energy (ΔG) -11.3364 kcal/mol and at the active site of 1ZOY, shows one hydrogen bond interaction between hydroxyl group in phenyl ring at position-5 and LysB239 with bond length 2.04 ,

Table 2

The protein-ligand interactions of compounds **4a**, **6i**, **6j**, **9b**, **10c** and **10f** with active site pocket 1ZOY.

Compound	S kcal/mol	Amino acids involved in the interaction	Length of hydrogen bonds and other interaction Å
4a	-8.8994	SerD50	H-bond acceptor 2.02 Å
6i	-12.4351	ArgD47 ArgD47 ArgC46	H-bond acceptor 1.99 Å Arene-arene interaction Arene-arene interaction
6j	-13.3429	ArgD47 ArgD47 ArgC46	H-bond acceptor 2.61 Å Arene-arene interaction Arene-arene interaction
9b	-11.3364	LysB239 ArgD47	H-bond acceptor 2.04 Å Arene-arene interaction
10c	-14.6964	LysB239 TrpD43 SerC53 ArgD47 HisB216	H-bond acceptor 2.30 Å H-bond acceptor 2.53 Å H-bond acceptor 1.68 Å Arene-arene interaction Arene-arene interaction
10f	$-16.5038.1753$	LysB239 SerC53 ArgD47 HisB216	H-bond acceptor 1.39 Å H-bond acceptor 1.64 Å Arene-arene interaction Arene-arene interaction

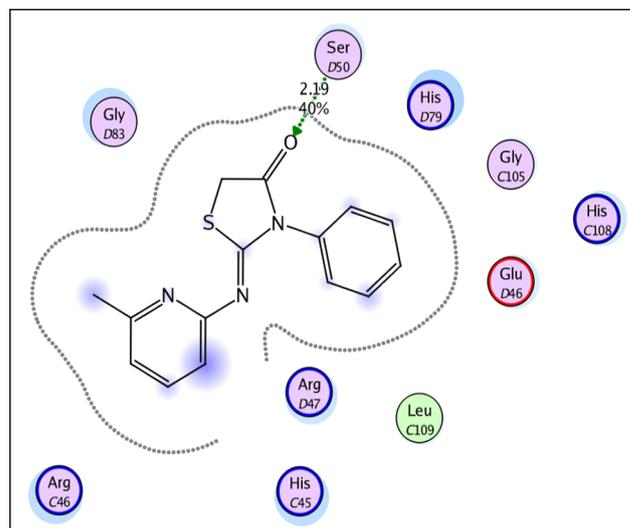


Fig. 3. 2D ligand interaction of the compound **4a** with the active site amino acids of 1ZOY.

whereas residue ArgD47 is involved in arene-arene interaction with one phenyl ring (Fig. 9). On the other hand, the 5-(3-hydroxy-4-[(4-methoxyphenyl)-diazanyl]benzylid-ene)-2-imino-3-phenylthiazolidin-4-one **10c** binds into the active site of 1ZOY with minimum binding energy (ΔG) -14.6964 kcal/mol and at the active site of 1ZOY, shows three hydrogen bond interaction between hydroxyl group in phenyl ring at position-5 and SerC53 with bond length 1.68 Å, the other two hydrogen bonds interaction between methoxy group which present in the aryl azo substituent and the residues two amino acids LysB239 and TrpD43 with bond length 2.30 and 3.53 Å, respectively. The last interaction between phenyl group of azo function with ArgD47 and HisD216, respectively via arene-arene interaction (Fig. 11). Compound **10f** shows two hydrogen bond between interactions methoxy group and LysB239 with bond length 1.93 Å and the other between nitrogen for imino group and serC53 with bond length 1.64 Å, also appeared two interactions as arene-arene stabilized by phenyl ring and HisB216 and ArgD47 (Fig. 13). However, molecular docking result showed a quite different orientation of compounds **6i**, **9b**, **10c** and **10f** at the active site of 1ZOY due to substituent changing at position-5 of 4-thiazolidinone ring. These results supported our binding and enzyme activity results, that these compounds offers sufficient number of interactions to 1ZOY especially compound number **10c**, resultantly formed a stable complex (see Figs. 4, 6, and 8). Also, these results are also in close agreement

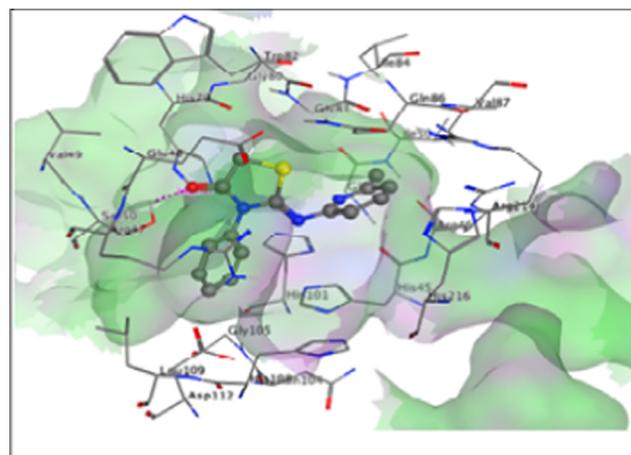


Fig. 4. 3D docking of compound **4a** with the active site pocket of the protein 1ZOY.

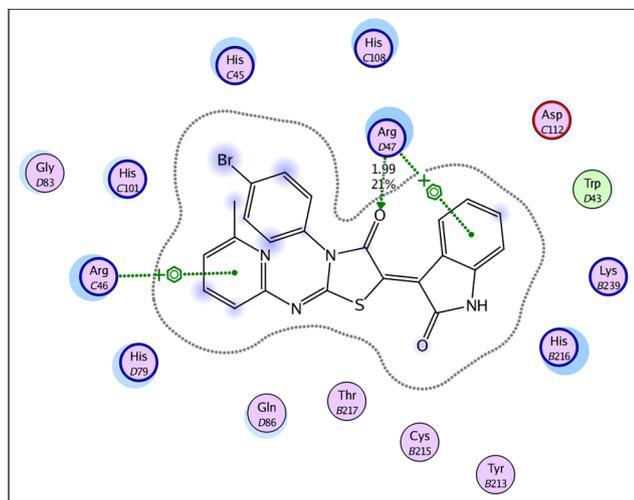


Fig. 5. 2D ligand interaction of the compound 6i with the active site amino acids of 1ZOY.

with the results of antifungal activity as shown in Table 1 (see Figs. 10 and 12–16).

3. Conclusion

A series of 4-thiazolidinone derivatives incorporated pyridine moiety was synthesized and evaluated for their antifungal activity. The antifungal activity of the prepared compounds 6d, 6e, 6i, 6j, 9a, 9b and 10a-f showed high potent inhibitory activity against *Alternaria solani* strain than Ridomil gold plus as the standard reference. One of the promising findings which can glorify the domain of developing these 4-thiazolidinones, was the interaction of 6d, 9a, 10b, 10c and 10f with *Alternaria solani* DNA Plasmid, the electrophoresis pattern obtained manifested specific DNA cleavage. Thus, DNA assessment accorded generous hint with supporting proof for describing the mechanism of inhibitory action of these 4-thiazolidinones. That can

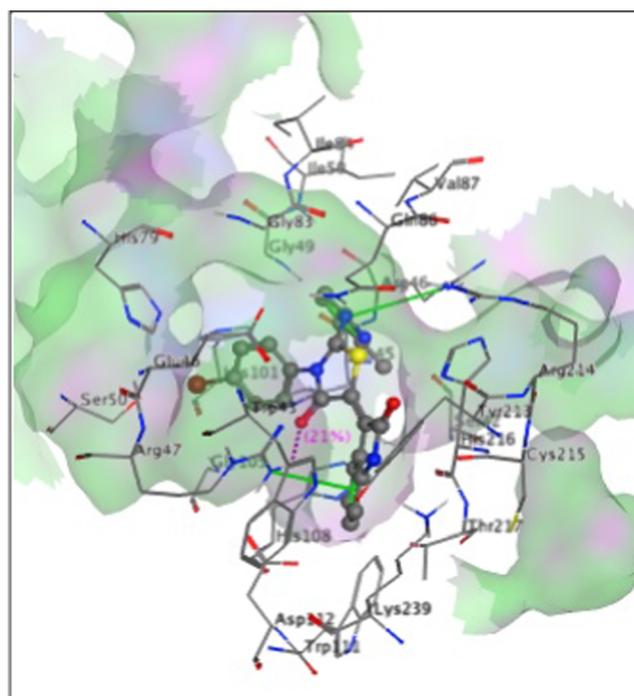


Fig. 6. 3D docking of compound 6i with the active site pocket of the protein 1ZOY.

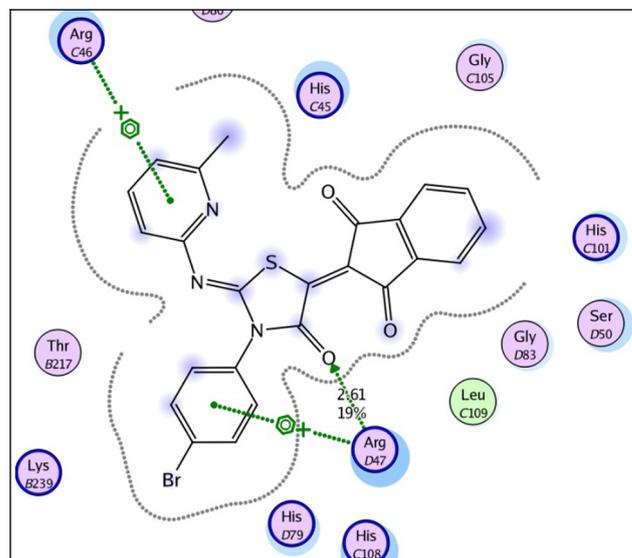


Fig. 7. 2D ligand interaction of the compound 6j with the active site amino acids of 1ZOY.

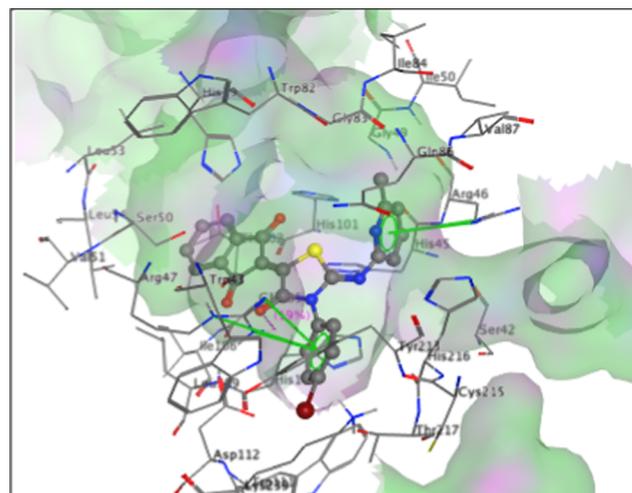


Fig. 8. 3D docking of compound 6j with the active site pocket of the protein 1ZOY.

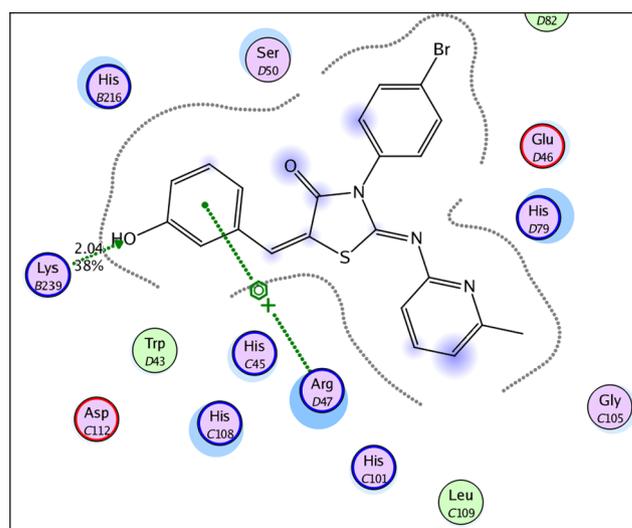


Fig. 9. 2D ligand interaction of the compound 9b with the active site amino acids of 1ZOY.

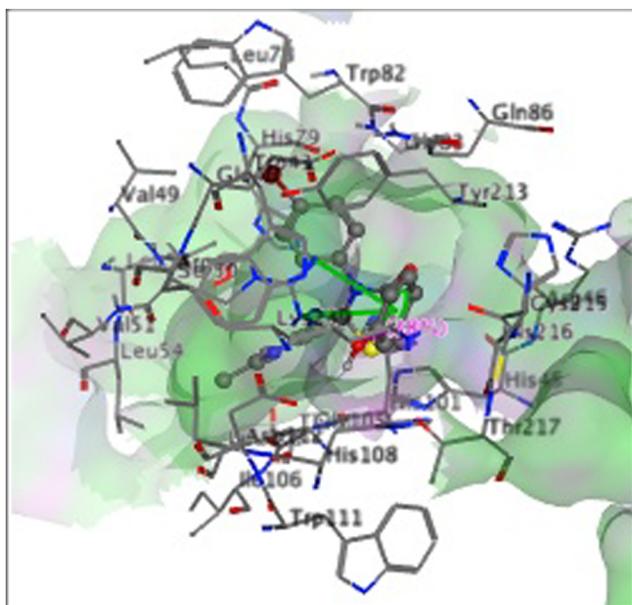


Fig. 10. 3D docking of compound 9b with the active site pocket of the protein 1ZOY.

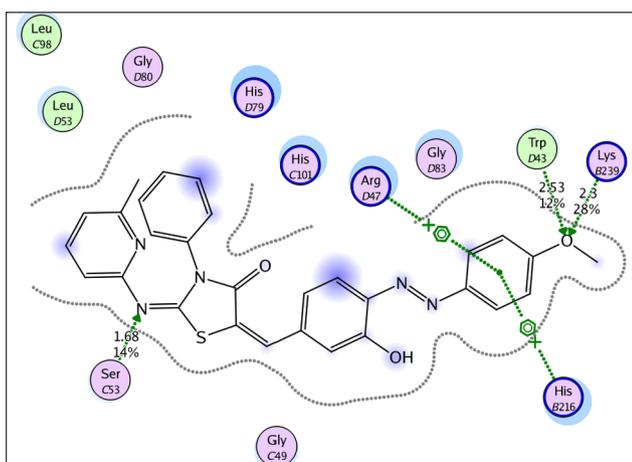


Fig. 11. 2D ligand interaction of the compound 10c with the active site amino acids of 1ZOY.

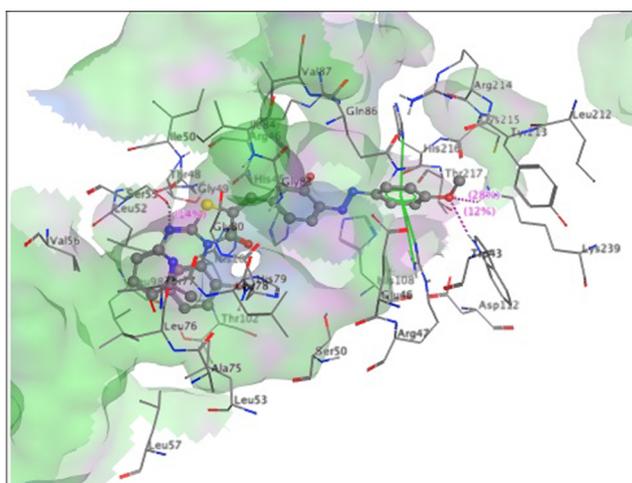


Fig. 12. 3D docking of compound 10c with the active site pocket of the protein.

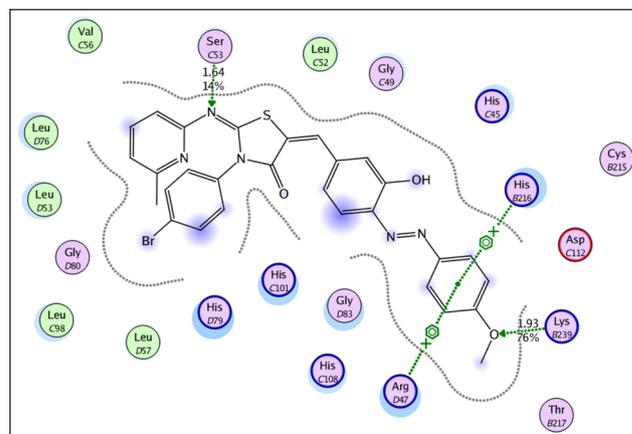


Fig. 13. 2D ligand interaction of the compound 10f with the active site amino acids of 1ZOY.

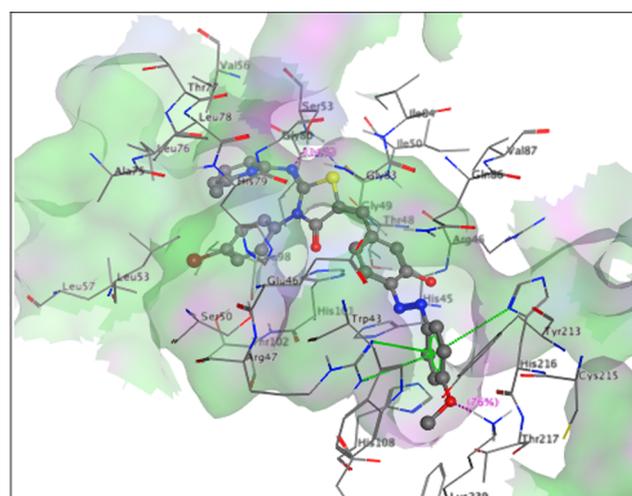


Fig. 14. 3D docking of compound 10f with the active site pocket of the protein.

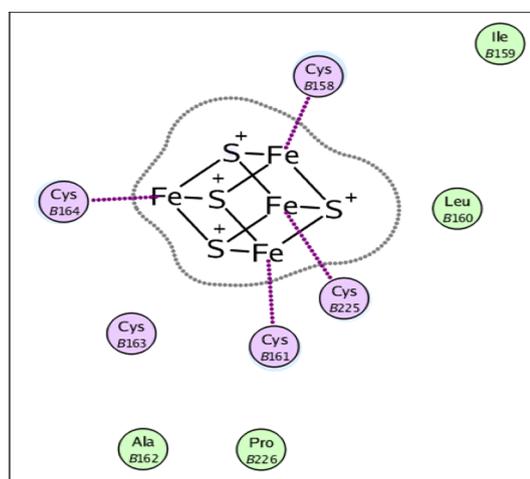


Fig. 15. The active site pocket of the protein 1ZOY.

enhance the future researches of 4-thiazolidinone and their potential to act as artificial nucleus mimics and metal-free nucleolytic agents. Qualitatively, theoretical molecular docking study consulted the data obtained from in vitro antifungal activity.

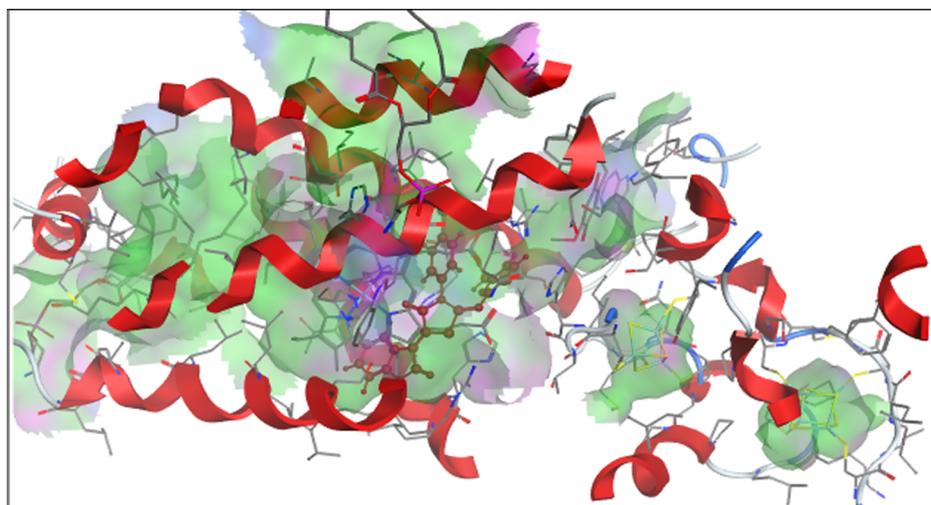


Fig. 16. 3D of the active site pocket of the protein 1ZOY.

4. Experimental section

All melting points are uncorrected and were taken on electrothermal capillary melting point apparatus (6100). IR spectra were recorded on a Perkin Elmer 1430 spectrophotometer. ^1H NMR and ^{13}C NMR spectroscopy were recorded in deuterated dimethylsulfoxide at mercury 400 and 100 MHz on a Varian Gemini NMR spectrophotometer using tetramethylsilane as internal reference and the results are expected as δ value, the main chemical warfare Laboratories, chemical warfare department, ministry of defense. Mass spectra were taken on a Shimadzu GMCS-GB1000 EX mass spectrophotometer at 70 eV in Micro analytical Center Laboratory, Cairo University. Elemental analysis carried out at the Microanalyses Center of Cairo University, Giza, Egypt and the results are within $\pm 0.4\%$ of the theoretical values. Antifungal activity and gel electrophoresis assay were carried out at the Agriculture Research Centre in Giza, Egypt.

5. Chemistry

5.1. General procedure for synthesis of 1-(6-methylpyridin-2-yl)-3-arylthioureas (**3a,b**)

A mixture of 2-amino-6-picoline **1** (0.01 mol) and phenyl isothiocyanate derivatives **2a,b** (0.01 mol) were stirred for 6 h in methylene chloride (15 ml) at room temperature. The precipitate so formed was collected, filtered off, and recrystallized from ethanol.

5.2. 1-(6-Methylpyridin-2-yl)-3-phenylthiourea (**3a**)

White crystals; yield; 70%; m.p. 186–188 °C; IR (KBr, cm^{-1}): 3189 (NH), 3109 (NH), 1247 (C=S); ^1H NMR (DMSO- d_6): δ = 2.21 (s, 3H, CH_3), 6.86 (d, 1H, J = 7.2 Hz, Ar), 7.0 (d, 1H, J = 8 Hz, Ar), 7.23–7.78 (m, 6H, Ar), 10.68 (s, 1H, NH), 13.94 (s, 1H, NH); Anal. Calcd for $\text{C}_{13}\text{H}_{13}\text{N}_3\text{S}$ (243.33): C, 64.17; H, 5.39; N, 17.27; S, 13.18. Found: C, 64.0; H, 5.58; N, 17.04; S, 13.39%.

5.3. 1-(4-Bromophenyl)-3-(6-methylpyridin-2-yl)thiourea (**3b**)

White crystals; yield 68.8%; m.p = 177–179 °C; IR (KBr, cm^{-1}): 3194 (NH), 3113 (NH), 1256 (C=S); ^1H NMR (DMSO- d_6): δ = 2.19 (s, 3H, CH_3), 6.92 (d, 1H, J = 7.6 Hz, Ar), 7.04 (d, 2H, J = 8 Hz, Ar), 7.16–7.77 (m, 4H, Ar), 10.76 (s, 1H, NH), 14.16 (s, 1H, NH); MS: m/z (%) = 323 ($\text{M}^+ + 1$, 12.5), 322 (M^+ , 55.5). Anal. Calcd for $\text{C}_{13}\text{H}_{12}\text{BrN}_3\text{S}$ (322.22): C, 48.46; H, 3.75; Br, 24.80; N, 13.04; S, 9.95. Found: C, 48.64; H, 3.91; N, 13.22; S, 9.78%.

5.4. General procedure of synthesis of 2-[(6-methylpyridin-2-yl)imino]-3-phenylthiazolidin-4-one **4a,b**

To a solution of each of **3a,b** (0.01 mol), chloroacetic acid (0.01 mol) was added and the mixture was heated under reflux in glacial acetic acid (15 ml) for 6 h containing anhydrous sodium acetate (0.01 mol), then the reaction mixture allowed to cool. The solid so formed, filtered off, washed with ethanol and recrystallized from ethanol-dioxane mixture.

5.5. 2-[(6-Methylpyridin-2-yl)imino]-3-phenylthiazolidin-4-one (**4a**)

Yellow crystals; yield 87%; m.p = 260–262 °C [lit. m.p = 262–264 °C] [38]. IR (KBr, cm^{-1}): 1722 (C=O); ^1H NMR (DMSO- d_6): δ = 2.40 (s, 3H, CH_3), 4.0 (s, 2H, CH_2), 6.67 (d, 1H, J = 7.6 Hz, Ar-H), 6.95 (d, 1H, J = 7.6 Hz, Ar), 7.32 (d, 2H, J = 7.6 Hz, Ar), 7.42–7.61 (m, 4H, Ar-H); MS: m/z (%) = 283 (M^+ , 99.9), 241 (95.3), 208 (50.8), 192 (97.0), 167 (17.3), 149 (85.7), 135 (35.6), 123 (37.8), 92 (86.2), 69 (99.9). Anal. Calcd. for $\text{C}_{15}\text{H}_{13}\text{N}_3\text{OS}$ (283.35): C, 63.58; H, 4.62; N, 14.83; S, 11.32. Found: C, 63.79; H, 4.80; N, 15.03; S, 11.65%.

5.6. 3-(4-Bromophenyl)-2-[(6-methylpyridin-2-yl)imino]thiazolidin-4-one (**4b**)

Yellow crystals; yield 76%; m.p = 250–252 °C; IR (KBr, cm^{-1}): 1717 (C=O); ^1H NMR (DMSO- d_6): δ = 2.44 (s, 3H, CH_3), 4.03 (s, 2H, CH_2), 6.67 (d, 1H, J = 7.6 Hz, Ar), 6.69 (d, 1H, J = 7.8 Hz, Ar), 6.95 (d, 2H, J = 7.8 Hz, Ar), 7.31–7.63 (m, 4H, Ar); ^{13}C NMR (DMSO- d_6): δ = 23.85, 33.76, 117.70, 119.64, 122.01, 131.34, 132.42, 135.59, 139.05, 155.76, 157.45, 159.65, 172.50. MS: m/z (%) = 363 ($\text{M}^+ + 1$, 79.3), 362 (M^+ , 63.7), 321 (71.4), 288 (23.6), 261 (20.3), 240 (97.5), 192 (99.9), 180 (22.0), 135 (28.8), 120 (34.1), 92 (99.7), 65 (74.6). Anal. Calcd for $\text{C}_{15}\text{H}_{12}\text{BrN}_3\text{OS}$ (362.24): C, 49.73; H, 3.34; Br, 22.06; N, 11.60; S, 8.85. Found: C, 49.51; H, 3.49; N, 11.83; S, 8.66%.

5.7. General procedures for synthesis of compounds **6a-j**

5.7.1. Method A

A mixture of aromatic aldehyde **5a-e** (0.01 mol) and compounds **4a,b** (0.01 mol) was refluxed in glacial acetic acid (15 ml) for 7 h in the presence of fused sodium acetate (0.01 mol). The precipitate obtained was filtered off, washed with water and recrystallized from *N,N*-dimethylformamide (DMF).

5.7.2. Method B

A mixture of diarylthioureas **3a,b** (0.01 mol), aromatic aldehyde **5a-e** (0.01 mol) and chloroacetic acid (0.01 mol) was heated under reflux in glacial acetic acid (15 ml) for 5 h in the presence of fused sodium acetate (0.01 mol). The precipitate obtained was filtered off, washed with water and recrystallized from *N,N*-dimethylformamide (DMF) to give **6a-j**.

5.7.2.1. 5-Benzylidene-2-[(6-methylpyridin-2-yl)imino]-3-phenylthiazolidin-4-one (**6a**). Yellow crystals; yield 60% (Method A); 66% (Method B); m.p = 274–276 °C. IR (KBr, cm^{-1}): 1707 (C=O). ^1H NMR (DMSO- d_6): δ = 2.49 (s, 3H, CH_3), 6.72 (d, 1H, J = 7.6 Hz, Ar), 6.97 (d, 1H, J = 7.4 Hz, Ar), 7.31 (s, 1H, CH Ar), 7.37–7.66 (m, 11H, Ar); Anal. Calcd for $\text{C}_{22}\text{H}_{17}\text{N}_3\text{OS}$ (371.45): C, 71.14; H, 4.61; N, 11.31; S, 8.63. Found: C, 71.32; H, 4.80; N, 11.52; S, 8.86%.

5.7.2.2. 5-(4-Fluorobenzylidene)-2-[(6-methylpyridin-2-yl)imino]-3-phenylthiazolidin-4-one (**6b**). Yellow crystals; yield 70% (Method A); 77% (Method B); m.p = 281–283 °C; IR (KBr, cm^{-1}) 1713 (C=O). ^1H NMR (DMSO- d_6) δ = 2.46 (s, 3H, CH_3), 6.77 (d, 2H, J = 8 Hz, Ar), 7.02 (d, 1H, J = 7.6 Hz, Ar), 7.42 (s, 1H, CH, Ar), 7.43–7.74 (m, 9H, Ar); Anal. Calcd for $\text{C}_{22}\text{H}_{16}\text{FN}_3\text{OS}$ (389.45): C, 67.85; H, 4.14; F, 4.88; N, 10.79; S, 8.23. Found: C, 67.64; H, 4.30; N, 10.60; S, 8.42%.

5.7.2.3. 5-(2-Chloro-4-fluorobenzylidene)-2-[(6-methylpyridin-2-yl)imino]-3-phenylthiazolidin-4-one (**6c**). Yellow crystals; yield 76% (Method A); 83% (Method B); m.p = 308–310 °C; IR (KBr, cm^{-1}): 1713 (C=O). ^1H NMR (DMSO- d_6) δ = 2.48 (s, 3H, CH_3), 6.73 (d, 2H, J = 8 Hz, Ar), 7.06 (d, 1H, J = 7.2 Hz, Ar), 7.43 (d, 1H, J = 7.2 Hz, Ar), 7.51–7.62 (m, 6H, Ar), 7.69 (d, 2H, J = 8.4 Hz, Ar); Anal. Calcd for $\text{C}_{22}\text{H}_{15}\text{ClFN}_3\text{OS}$ (423.89): C, 62.34; H, 3.57; Cl, 8.36; F, 4.48; N, 9.91; S, 7.56. Found: C, 62.50; H, 3.73; N, 9.73; S, 7.77%.

5.7.2.4. 2-[(6-Methylpyridin-2-yl)imino]-5-(2-oxoindolin-3-ylidene)-3-phenylthiazolidin-4-one (**6d**). Orange crystals, yield 75% (Method A); 80% (Method B), m.p = 292–294 °C; IR (KBr, cm^{-1}): 3431 (NH), 1690 (C=O); ^1H NMR (DMSO- d_6) δ = 2.43 (s, 3H, CH_3), 6.82–7.09 (m, 3H, Ar), 7.32–7.65 (m, 6H, Ar), 8.40 (s, 1H, Ar), 8.93 (d, 2H, J = 8 Hz, Ar), 11.56 (s, 1H, NH); Anal. Calcd for $\text{C}_{23}\text{H}_{16}\text{N}_4\text{O}_2\text{S}$ (412.46): C, 66.98; H, 3.91; N, 13.58; S, 7.77. Found: C, 66.80; H, 3.73; N, 13.41; S, 7.56%.

5.7.2.5. 2-[2-((5-Methylpyridin-2-yl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene]-1H-indene-1,3(2H)-dione (**6e**). Dark red crystals yield 72% (Method A); 79% (Method B); m.p > 300 °C. IR (KBr, cm^{-1}): 1685, 1689 (C=O). ^1H NMR (DMSO- d_6): δ = 2.49 (s, 3H, CH_3), 6.82 (d, 2H, J = 7.8 Hz, Ar), 7.38–7.87 (m, 10H, Ar); Anal. Calcd for $\text{C}_{24}\text{H}_{15}\text{N}_3\text{O}_3\text{S}$ (425.46): C, 67.75; H, 3.55; N, 9.88; S, 7.54. Found: C, 67.55; H, 3.72; N, 9.63; S, 7.36%.

5.7.2.6. 5-Benzylidene-3-(4-bromophenyl)-2-[(6-methylpyridin-2-yl)imino]-thiazolidin-4-one (**6f**). Pale yellow crystals; yield 60% (Method A); 66% (Method B); m.p = 270–272 °C. IR (KBr, cm^{-1}): 1710 (C=O). ^1H NMR (DMSO- d_6): δ = 2.47 (s, 3H, CH_3), 6.70 (d, 2H, J = 8 Hz, Ar), 6.95 (d, 1H, J = 7.2 Hz, Ar), 7.29 (s, 1H, CH, Ar), 7.35–7.64 (m, 9H, Ar); Anal. Calcd for: $\text{C}_{22}\text{H}_{16}\text{BrN}_3\text{OS}$ (450.35): C, 58.67; H, 3.58; Br, 17.74; N, 9.33; S, 7.12. Found: C, 58.88; H, 3.79; N, 9.55; S, 7.26%.

5.7.2.7. 3-(4-Bromophenyl)-5-(4-fluorobenzylidene)-2-[(6-methylpyridin-2-yl)imino]thiazolidin-4-one (**6g**). Pale yellow crystals; yield 69% (Method A); 75% (Method B); m.p = 273–275 °C; IR (KBr, cm^{-1}): 1716 (C=O). ^1H NMR (DMSO- d_6): δ = 2.45 (s, 3H, CH_3), 6.76 (d, 2H, J = 8 Hz, Ar), 7.01 (d, 1H, J = 7.6 Hz, Ar), 7.41 (s, 1H, CH, Ar), 7.42–7.73 (m, 8H, Ar); Anal. Calcd for $\text{C}_{22}\text{H}_{15}\text{BrFN}_3\text{OS}$ (468.34): C, 56.42; H, 3.23; Br, 17.06; F, 4.06; N, 8.97; S, 6.85. Found: C, 56.61; H, 3.43; N, 8.76; S, 6.66%.

5.7.2.8. 3-(4-Bromophenyl)-5-(2-chloro-4-fluorobenzylidene)-2-[(6-methylpyridin-2-yl)imino]thiazolidin-4-one (**6h**). Pale yellow crystals; yield 80% (Method A), 84% (Method B); m.p = 302–304 °C; IR (KBr, cm^{-1}): 1717 (C=O). ^1H NMR (DMSO- d_6): δ = 2.41 (s, 3H, CH_3), 6.81 (d, 2H, J = 8 Hz, Ar), 7.0 (d, 1H, J = 7.2 Hz, Ar), 7.44 (d, 2H, J = 9.2 Hz, Ar), 7.49–7.57 (m, 3H, Ar), 7.64 (s, 1H, CH), 7.73 (d, 2H, J = 8.4 Hz, Ar); m/z (%) = 503 (M^+ , 38.3), 505 ($\text{M}^+ + 2$, 11.5), 466 (98.8), 334 (73), 287 (43.7), 186 (69.4) 92 (65.3), 65 (27.8); Anal. Calcd for $\text{C}_{22}\text{H}_{14}\text{BrClFN}_3\text{OS}$ (502.79): C, 52.56; H, 2.81; Br, 15.89; Cl, 7.05; F, 3.78; N, 8.36; S, 6.38. Found: C, 52.75; H, 2.64; N, 8.52; S, 6.60%.

5.7.2.9. 3-[4-Bromophenyl]-2-[(5-methylpyridin-2-yl)imino]-5-(2-oxoindolin-3-ylidene)thiazolidin-4-one (**6i**). Orange crystals, yield 68% (Method A), 76% (Method B), m.p = 286–288 °C; IR (KBr, cm^{-1}): 3437 (NH), 1688 (C=O); ^1H NMR (DMSO- d_6): δ = 2.47 (s, 3H, CH_3), 6.90 (d, 2H, J = 8 Hz, Ar), 7.07–7.72 (m, 9H, Ar), 8.40 (s, 1H, Ar), 11.68 (s, 1H, NH); MS: (m/z) = 491 (M^+ , 18%), Anal. Calcd for $\text{C}_{23}\text{H}_{15}\text{BrN}_4\text{O}_2\text{S}$ (491.31): C, 56.22; H, 3.08; N, 11.40; S, 6.53. Found: C, 56.42; H, 3.27; N, 11.59; S, 6.73%.

5.7.2.10. 2-[3-(4-Bromophenyl)-2-[(5-methylpyridin-2-yl)imino]-4-oxothiazolidin-5-ylidene]-1H-indene-1,3(2H)-dione (**6j**). Dark red, yield 76%, m.p = 290–292 °C. IR (KBr, cm^{-1}): 1686 (C=O). ^1H NMR (DMSO- d_6): δ = 2.48 (s, 3H, CH_3), 6.81 (d, 1H, J = 7.8 Hz, Ar), 7.67–7.79 (m, 10H, Ar), 8.87 (s, 1H, Ar); Anal. Calcd for $\text{C}_{24}\text{H}_{14}\text{BrN}_3\text{O}_3\text{S}$ (504.36): C, 57.15; H, 2.80; Br, 15.84; N, 8.33; S, 6.36. Found: C, 57.33; H, 3.0; N, 8.56; S, 6.58%.

5.8. General procedure for synthesis of compounds **9a,b**

A mixture of each of **4a,b** (0.01 mol) and 3-hydroxybenzaldehyde (0.01 mol) was heated in glacial acetic acid (18 ml) in the presence of anhydrous sodium acetate (0.01 mol) for 7 h then left to cool. The solid so formed filtered off, washed with water and recrystallized from *N,N*-dimethylformamide (DMF).

5.8.1. 5-(3-Hydroxybenzylidene)-2-[(6-methylpyridin-2-yl)imino]-3-phenylthiazolidin-4-one (**9a**)

Yellow crystals; yield 89%; m.p = 281–283 °C; IR (KBr, cm^{-1}): 3408 (OH), 1702 (C=O), 1590 (C=C). ^1H NMR (DMSO- d_6): δ = 2.52 (s, 3H, CH_3), 6.76 (d, 2H, J = 8 Hz, Ar), 6.85 (d, 2H, J = 8 Hz, Ar), 7.01 (d, 1H, J = 7.6 Hz, Ar), 7.91 (s, 1H, CH, Ar), 6.99–7.64 (m, 7H, Ar), 9.82 (s, 1H, OH, exchangeable D_2O); MS: (m/z (%)) = 387 (M^+ , 99.1%), 283 (99.9), 240 (84.6), 192 (97.0), 69 (60.9); Anal. Calcd for: $\text{C}_{22}\text{H}_{17}\text{N}_3\text{O}_2\text{S}$ (387.45): C, 68.20; H, 4.42; N, 10.85; S, 8.28. Found: C, 68.39; H, 4.60; N, 11.07; S, 8.53%.

5.8.2. 4.4.2 3-(4-Bromophenyl)-5-(3-hydroxybenzylidene)-2-[(6-methylpyridin-2-yl)imino]thiazolidin-4-one (**9b**)

Yellow crystals; yield 87%; m.p = 270–272 °C; IR (KBr, cm^{-1}): 3413 (OH), 1707 (C=O), 1596 (C=C). ^1H NMR (DMSO- d_6): δ = 2.51 (s, 3H, CH_3), 6.75 (d, 2H, J = 8 Hz, Ar), 6.84 (d, 2H, J = 8 Hz, Ar), 7.0 (d, 1H, J = 7.6 Hz, Ar), 7.90–7.63 (m, 7H, Ar), 9.78 (s, 1H, OH, exchangeable D_2O); Anal. Calcd for: $\text{C}_{22}\text{H}_{16}\text{BrN}_3\text{O}_2\text{S}$ (466.35): C, 56.66; H, 3.46; Br, 17.13; N, 9.01; S, 6.88. Found: C, 56.84; H, 3.67; N, 9.23; S, 7.07%.

5.9. General procedure for synthesis of compounds **10a-f**

5.9.1. Method A

The appropriated amine (0.01 mol) was dissolved in hydrochloric acid (0.03 mol) then allowed to cool in ice bath. The prepared ice cold diazonium salts **7a-c** were added dropwise to a solution of each of **9a,b** (0.01 mol) and sodium hydroxide (0.01 mol) in DMF (4 ml). The mixture stirred with cooling two additional hours then the mixture poured

into crashed ice (10 ml), the colored solid so formed, filtered off, washed with water and recrystallized from ethanol-dioxane to give compounds **10a-f**.

5.9.2. Method B

A solution of conc. hydrochloric acid (0.01 mol) cooled at 0 °C was added to a solution of aniline (0.01 mol) and aqueous solution of NaNO₂ (0.01 mol) dropwise at 0–5 °C. The formed diazonium chloride was added on (0.01 mol) of 3-hydroxysalicylaldehyde in 5 ml of 0.4 g NaOH and 0.74 g of Na₂CO₃. The reaction stirred at 0 °C for 2 h, the solid so formed was filtered off and recrystallized from ethanol to give compounds **8a-c**. A mixture of compounds **8a-c** (0.01 mol) and **4a,b** (0.01) refluxed for 6 hr in glacial acetic acid (15 ml) in the presence of anhydrous sodium acetate (0.01 mol), the mixture allowed to cool at room temperature then the solid so formed and recrystallized from ethanol to produce **10a-f**.

5.9.2.1. 5-(3-Hydroxy-4-(phenyldiazonyl)benzylidene)-2-[(6-methylpyridin-2-yl)imino]-3-phenylthiazolidin-4-one (10a). Scarlet red crystals; yield 82% (method A), 70% (method B); m.p = 192–194 °C; IR (KBr, cm⁻¹): 3408 (OH), 1710 (C=O), 1661 (C=N). ¹H NMR (DMSO-d₆): δ = 2.43 (s, 3H, CH₃), 6.79 (d, 2H, J = 8 Hz, Ar), 6.88 (d, 2H, J = 8 Hz, Ar), 7.03 (d, 1H, J = 7.2 Hz, Ar), 7.10–7.66 (m, 11H, Ar), 7.93 (s, 1H, CH, Ar), 9.83 (s, 1H, OH, exchangeable D₂O); Anal. Calcd for C₂₈H₂₁N₅O₂S (491.56): C, 68.41; H, 4.31; N, 14.25; S, 6.52. Found: C, 68.60; H, 4.52; N, 14.49; S, 6.75%.

5.9.2.2. [(2-Chlorophenyl)diazonyl]-3-hydroxybenzylidene)-2-[(6-methylpyridin-2-yl)imino]-3-phenylthiazolidin-4-one (10b). Red crystals; yield 78% (method A), 70% (method B); m.p = 219–221 °C; IR (KBr, cm⁻¹): 3399 (OH), 1688 (C=O), 1653 (C=N). ¹H NMR (DMSO-d₆): δ = 2.42 (s, 3H, CH₃), 6.78 (d, 2H, J = 8 Hz, Ar), 6.87 (d, 2H, J = 8 Hz, Ar), 7.02 (d, 1H, J = 7.6 Hz, Ar), 7.09–7.65 (m, 10H, Ar), 7.91 (s, 1H, CH, Ar), 9.81 (s, 1H, OH, exchangeable D₂O); MS: m/z (%) = 526 (M⁺, 64.4%) Anal. Calcd for C₂₈H₂₀ClN₅O₂S (526.01): C, 63.93; H, 3.83; Cl, 6.74; N, 13.31; S, 6.10 Found: C, 63.71; H, 3.63; N, 13.08; S, 5.89%.

5.9.2.3. 5-(3-Hydroxy-4-[(4-methoxyphenyl)diazonyl]benzylidene)-2-[(6-methylpyridin-2-yl)imino]-3-phenylthiazolidin-4-one (10c). Red crystals; yield 83% (method A), 70% (method B); m.p = 234–236 °C; IR (KBr, cm⁻¹): 3411 (OH), 1713 (C=O), 1665 (C=N). ¹H NMR(DMSO-d₆): δ = 2.46 (s, 3H, CH₃), 3.82 (s, 3H, OCH₃), 6.82 (d, 2H, J = 8 Hz, Ar), 6.90 (d, 2H, J = 8 Hz, Ar), 7.06 (d, 1H, J = 7.2 Hz, Ar), 7.13–7.69 (m, 9H, Ar), 7.95 (s, 1H, CH, Ar), 9.86 (s, 1H, OH, exchangeable D₂O); Anal. Calcd for C₂₉H₂₃N₅O₃S (521.59): C, 66.78; H, 4.44; N, 13.43; S, 6.15. Found: C, 66.94; H, 4.64; N, 13.62; S, 6.34%.

5.9.2.4. 3-(4-Bromophenyl)-5-[3-hydroxy-4-(phenyldiazonyl)benzylidene)-2-[(6-methylpyridin-2-yl)imino]thiazolidin-4-one (10d). Scarlet red crystals; yield 69% (method A), 70% (method B); m.p = 182–184 °C; IR (KBr, cm⁻¹): 3412 (OH), 1714 (C=O), 1666 (C=N); ¹H NMR (DMSO-d₆): δ = 2.43 (s, 3H, CH₃), 6.78 (d, 2H, J = 8 Hz, Ar), 6.87 (d, 2H, J = 8 Hz, Ar), 7.02 (d, 1H, J = 7.6 Hz, Ar), 7.10–7.67 (m, 10H, Ar), 7.93 (s, 1H, CH, Ar), 9.83 (s, 1H, OH, exchangeable D₂O); Anal. Calcd for C₂₈H₂₀BrN₅O₂S (570.46): C, 58.95; H, 3.53; Br, 14.01; N, 12.28; S, 5.62. Found: C, 58.77; H, 3.71; N, 12.49; S, 5.80%.

5.9.2.5. 3-(4-Bromophenyl)-5-[4-(2-chlorophenyl)diazonyl]-3-hydroxybenzylidene)-2-[(6-methylpyridin-2-yl)imino]thiazolidin-4-one (10e). Red crystals; yield 83% (method A), 70% (method B); m.p = 207–209 °C; IR (KBr, cm⁻¹): 3407 (OH), 1699 (C=O), 1659 (C=N); ¹H NMR (DMSO-d₆): δ = 2.41 (s, 3H, CH₃), 6.77 (d, 2H, J = 8 Hz, Ar), 6.86 (d, 2H, J = 8.4 Hz, Ar), 7.01 (d, 1H, J = 7.6 Hz, Ar), 7.08–7.64 (m, 9H, Ar), 7.90 (s, 1H, CH, Ar), 9.79 (s, 1H, OH, exchangeable D₂O); Anal. Calcd for C₂₈H₁₉BrClN₅O₂S (604.90): C,

55.60; H, 3.17; Br, 13.21; Cl, 5.86; N, 11.58; S, 5.30. Found: C, 55.80; H, 3.37; N, 11.79; S, 5.52%.

5.9.2.6. 3-(4-Bromophenyl)-5-(3-hydroxy-4-[(4-methoxyphenyl)diazonyl]benzylidene)-2-[(6-methylpyridin-2-yl)imino]thiazolidin-4-one (10f). Red crystals; yield 71% (method A), 70% (method B); m.p = 215–217 °C; IR (KBr, cm⁻¹): 3415 (OH), 1717 (C=O), 1670 (C=N). ¹H NMR (DMSO-d₆): δ = 2.44 (s, 3H, CH₃), 3.80 (s, 3H, OCH₃), 6.81 (d, 2H, J = 8 Hz, Ar), 6.89 (d, 2H, J = 8.4 Hz, Ar), 7.05 (d, 1H, J = 7.2 Hz, Ar), 7.12–7.68 (m, 8H, Ar), 7.94 (s, 1H, CH, Ar), 9.85 (s, 1H, OH, exchangeable D₂O); Anal. Calcd for C₂₉H₂₂BrN₅O₃S (600.49): C, 58.00; H, 3.69; Br, 13.31; N, 11.66; S, 5.34. Found: C, 58.19; H, 3.90; N, 11.85; S, 5.50.

6. Antifungal activity

The tested organisms were sub-cultured for fungi. *Ridomil gold plus* and *Mancozeb* were used as a positive control and DMSO solvent as a negative control. The plates were done in duplicate and the average zone of inhibition was calculated. The fungal cultures were incubated at (25–30 °C) for 6–8 days. Antimicrobial activity was determined by measurement zone of inhibition. In-vitro antifungal screening of compounds **4a,b**, **6a-j**, **9a,b** and **10a-f** against cultures of five fungal strains (*Aspergillus Niger* ASP, *Aspergillus flavus*, *Alternaria solani*, *Aspergillus ochraceus*, *Macrophomina phaseolina*) *Ridomil gold plus* and *Mancozeb* were used as a positive control. DMSO solvent was used as a negative control to evaluate the potency of the tested compounds under the same conditions (Table 1). Nuclease activity experiments were accomplished by agarose gel electrophoresis. The illuminated gel was photographed by Alpha Innotech Corporation Instrument. Cleavage experiments of plasmid DNA (200 mg) by **6d**, **9a** and (100 μM) in (5 mM Tris-HCl/50 mM NaCl), buffer (pH 7.2) were carried out. The samples were incubated for 1 hat 37 °C. A loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol was added and electrophoresis was carried out at 60 V for one hour in Tris-HCl buffer using 1% agarose gel containing 1.0 μg/mL ethidiumbromide (EB)26.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioorg.2018.11.050>.

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