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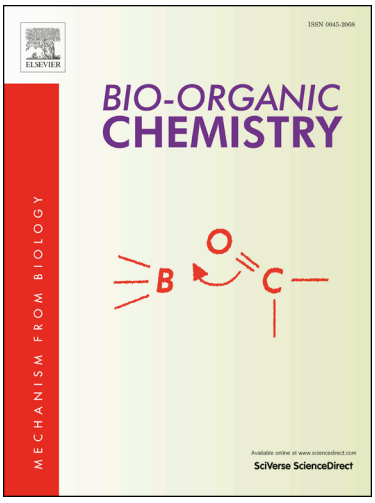

Design, synthesis, DNA assessment and molecular docking study of novel 2-(pyridin-2-ylimino)thiazolidin-4-one derivatives as potent antifungal agents

Nadia Hanafy Metwally, Ibrahim Taha Radwan, Walaa Salah El-Serwy, Mohamed Ahmed Mohamed

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

Nadia Hanafy Metwally[1]*, Ibrahim Taha Radwan[2], Walaa Salah El-Serwy[3]

and Mohamed Ahmed Mohamed[4]

[1]*Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt 12613.*

[2]*Basic Science Department, Faculty of Oral and Dental Medicine, Future University, New Cairo, Egypt.*

[3]*Therapeutical Chemistry Department, National Research Center, Giza, Egypt 12622.*

[4]*Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. 12619.*

E-mail address:nhmmohamed@yahoo.com

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-arylazo-3-hydroxybenzaldehydes **8a-c** furnished the respective mono-arylazo-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**revealedhigher 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 1ZOYenzyme and suitable binding with the active site of amino acids,were displayedaccording to their bond lengths, angles and conformational energy.

KEYWORDS: Iminoarylthiazolidinones, Arylidene iminoarylthiazolidinones, Antifungal activity, *Alternaria solani*, DNA toxicity, Docking study.

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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 inch (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).

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

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 in fest 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

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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 atternata* [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-thiazolidinone derivatives are one of the heterocyclic categories which play an important role in therapeutical chemistry, due to their variety in biological activitysuch as antiviral [6], anticancer [7], anti-inflammatory [8], antitumor [9], antimicrobial [10], anticonvulsant [11], antidepressant [12], antioxidant [13]. Moreover, 4-thiazolidinonesare used as apotent 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 [20-24]. Additionally, the combination of 4-thiazolidinones with other heterocyclic rings showed wide range of biological activities likethiazolo-linked pyrimidine and thiazolidinone-oxoindoline base compounds have been used as a potent CA inhibitor [25,26].Based on the above reports of interest biological activity of 4-thiazolidinoes and in continuation of our interest in the synthesis of some new bioactive 4-thiazolidinone derivatives [27-36], we planned to synthesize some novel2-imino-3-aryl-4-thiazolidinone derivatives which containing pyridine moiety as an effective compound against some types of fungi especially *Alternaria strains*.

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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 υ_{max} = 3194 and 3113 cm^{-1} corresponding to the two NH groups and another absorption band at υ_{max} = 1256 cm^{-1} for C=S stretch. Its ^{1}H NMR spectrum provided the position of methyl protons at δ 2.19 ppm, besides the two D_2Oexchangeable signals appeared at δ = 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 ^{1}H NMR spectra which revealed a characteristic singlet signal at δ ~ 4.0 ppm assigned to the methylene ($-CH_2-$) protons. Moreover, ^{13}C NMR for compound **4b** confirmed the proposed structure due to the appearance of a characterized signal at δ = 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 $C_{15}H_{12}BrN_3OS$.

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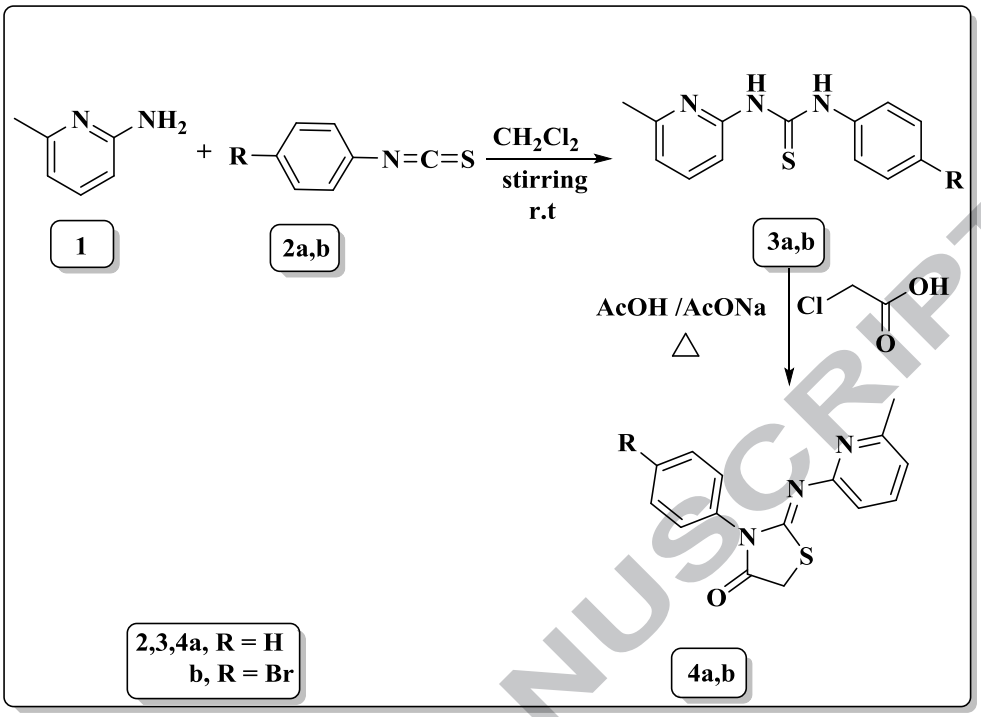

Scheme 1

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 υ_{max} = 1710 cm^{-1} corresponding to the carbonyl group (C=O). Its ^{1}H NMR revealed a singlet signal at δ = 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-arylazo-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 ^{o}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

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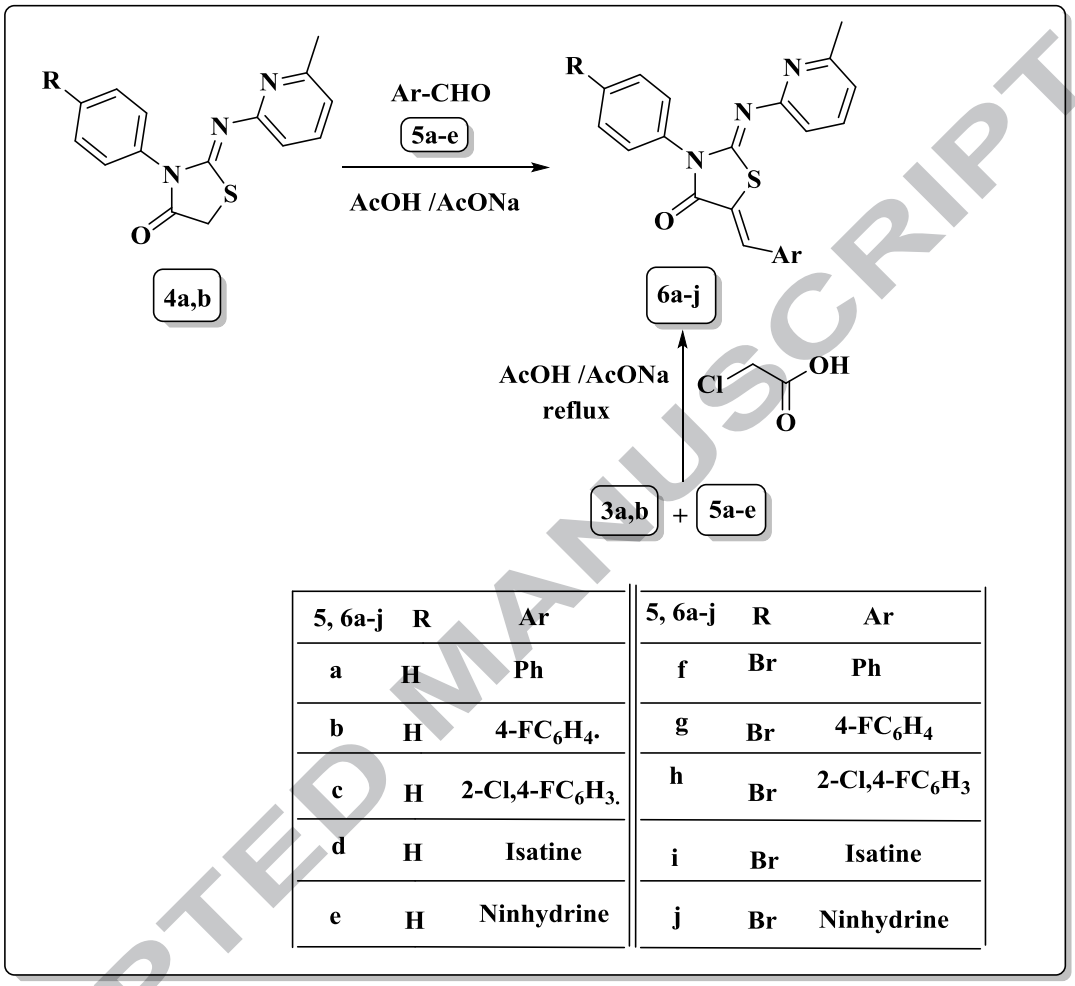
absorption band at υ_{max} = 3408 cm^{-1} corresponding to the hydroxyl group, in addition to the two absorption bands at υ_{max} = 1710 and 1661 cm^{-1} due to the carbonyl (C=O)



5, 6a-j	R	Ar
a	H	Ph
b	H	4-FC_6H_4.
c	H	2-Cl,4-FC_6H_3.
d	H	Isatine
e	H	Ninhydrine

5, 6a-j	R	Ar
f	Br	Ph
g	Br	4-FC_6H_4
h	Br	2-Cl,4-FC_6H_3
i	Br	Isatine
j	Br	Ninhydrine

Scheme 2

and imino (C=N) groups, respectively. The ^{1}H NMR spectrum of the isolated product **10a** displayed a singlet signal exchangeable D_2O at δ = 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. 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 υ_{max} = 3408 cm^{-1} corresponding to the hydroxyl group, in addition to an absorption band at υ_{max} = 1702cm^{-1} due to the

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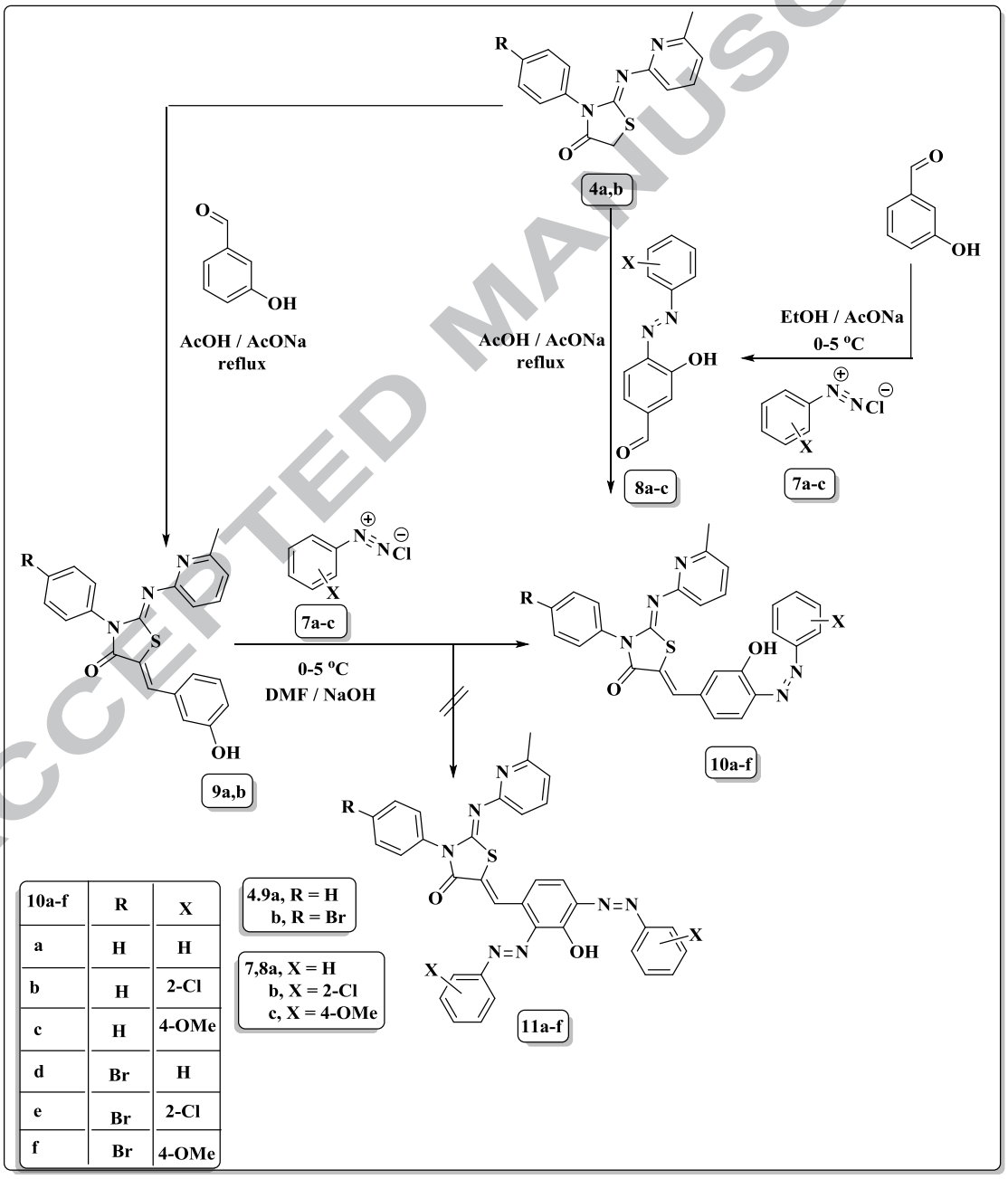
carbonyl (C=O) group. Its ^{1}H NMR spectrum displayed a singlet signal exchangeable *D_2O* at δ = 9.82 ppm assigned to the hydroxyl proton (OH). In addition to the expected protons for aromatic and vinylic protons. Also, mass spectrum exhibited a molecular ion peak at m/z = 387 (M^+, 99.1%) which was 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^{o}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).



10a-f	R	X
a	H	H
b	H	2-Cl
c	H	4-OMe
d	Br	H
e	Br	2-Cl
f	Br	4-OMe

Scheme 3

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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 *Aspergillusniger* (KU681408), *Aspergillus flavus*(KU681428), *Alternaria solani*(KT354939), *Aspergillus ochraceous* (DQ087531) and *Macrophominaphaseolina* (AM410964) were investigated by agar dilution assay [37].Where, the concentration of the compounds under investigation were prepared at10 and 100 µg/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 controlwhereas **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 *Alternariasolani*.

DNA damage study was performed according to the protocol of Kasibhatla and Gustava [38]. The fungal DNA amplified by PCR was treated with (100 µg/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 % agarosegel 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 *Alternariasolani*

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 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

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electrophoretic migration as shown in the figure 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.

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

comp. / fungi	*Aspergillus-Flavous*	*Aspergill-usniger*	*Aspergillus-ochraceous*	*Alternarisolani*	*Macrophomia-phasolina*
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 controle				**70**	

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Activity color key	color
Higher activity (more than 85%)	
Moderate activity (between 70-84 %)	
Low activity (less than 70 %)	

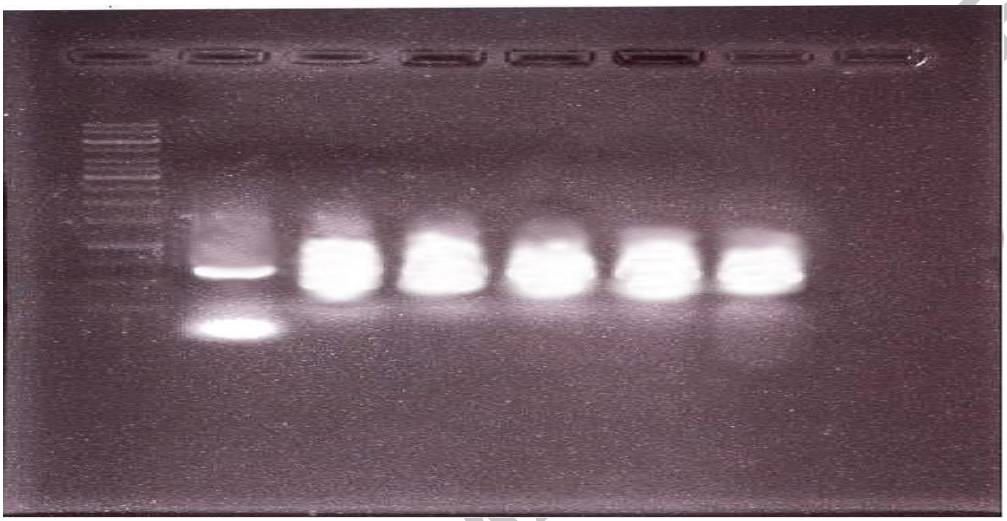

Fig. 2. Gel electrophoresis analysis of different synthetic compounds on DNA. After electrophoresis, the 1 % agarose gel was stained with ethidium bromide. Lane 1, DNA size marker; lane 2, control DNA non-treated with compounds; lanes 3 to 7, DNA treated with compounds **6d**, **9a**, **10b**, **10c**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

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 fluro 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 strainsexcept the *Aspergillusochraceous*fungi compared with positive antifungal control (Ridomil gold plus and Mancozeb) for *Alternaria*

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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 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 Ser*D*50 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 Arg*D*47 with bond length 1.99 Å. in addition to arene-arene interaction between Arg*C*46 and Arg*D*47 with pyridine and isatin ring, respectively of compound **6i** (Figure 5). In addition, compound **6j** appeared one hydrogen bond between carbonyl function and Arg*D*47 with bond length 2.61 Å, beside to two arene-arene interaction between phenyl ring with Arg*D*47 and pyridine ring with Arg*C*46 (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 onehydrogen bond interaction between hydroxyl group in phenyl ring at position-5 and Lys*B*239 with bond length 2.04,whereas residue Arg*D*47 is involved in arene-arene interaction with one phenyl ring (Figure 9). On the other hand, the 5-(3-hydroxy-4-[(4-

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methoxyphenyl)-diazenyl)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 Ser*C*53 with bond length 1.68Å, the other two hydrogen bonds interaction between methoxy group which present in the aryl azo substituent and the residues two amino acids Lys*B*239 and Trp*D*43 with bond length 2.30 and 3.53Å, respectively. The last interaction between phenyl group of azo function with Arg*D*47 and His*D*216,respectively *via* arene-arene interaction (Fig. 11). Compound **10f** shows two hydrogen bond between interactions methoxy group and Lys*B*239 with bond length 1.93Å and the other between nitrogen for imino group and ser*C*53 with bond length 1.64Å, also appeared two interactions as arene-arene stabilized by phenyl ring and His*B*216 and Arg*D*47 (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 1**0c**, resultantly formed a stable complex. Also, these results are also in close agreement with the results of antifungal activity as shown in Table 1.

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	Ser*D*50	H-bond acceptor 2.02 Å
6i	-12.4351	Arg*D*47 Arg*D*47 Arg*C*46	H-bond acceptor 1.99 Å Arene-arene interaction Arene-arene interaction
6j	-13.3429	Arg*D*47 Arg*D*47 Arg*C*46	H-bond acceptor 2.61 Å Arene-arene interaction Arene-arene interaction
9b	-11.3364	Lys*B*239 Arg*D*47	H-bond acceptor 2.04 ÅArene-arene interaction

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10c	-14.6964	Lys*B*239 Trp*D*43 Ser*C*53 Arg*D*47 His*B*216	H-bond acceptor2.30 Å H-bond acceptor2.53 Å H-bond acceptor 1.68 Å Arene-arene interaction Arene-arene interaction
10f	-16.5038.1753	Lys*B*239 Ser*C*53 Arg*D*47 His*B*216	H-bond acceptor 1.39Å H-bond acceptor 1.64 A^{o} Arene-arene interaction Arene-arene interaction

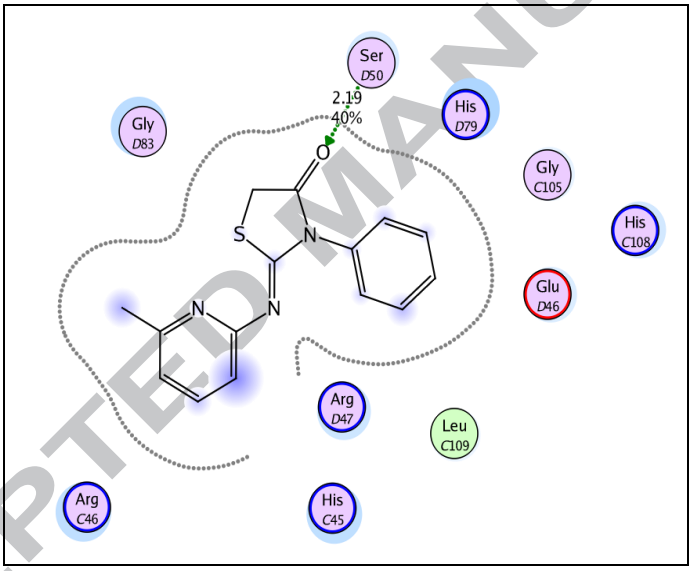

Fig. 3. 2D ligand interaction of the compound **4a** with the active site amino acids of 1ZOY.

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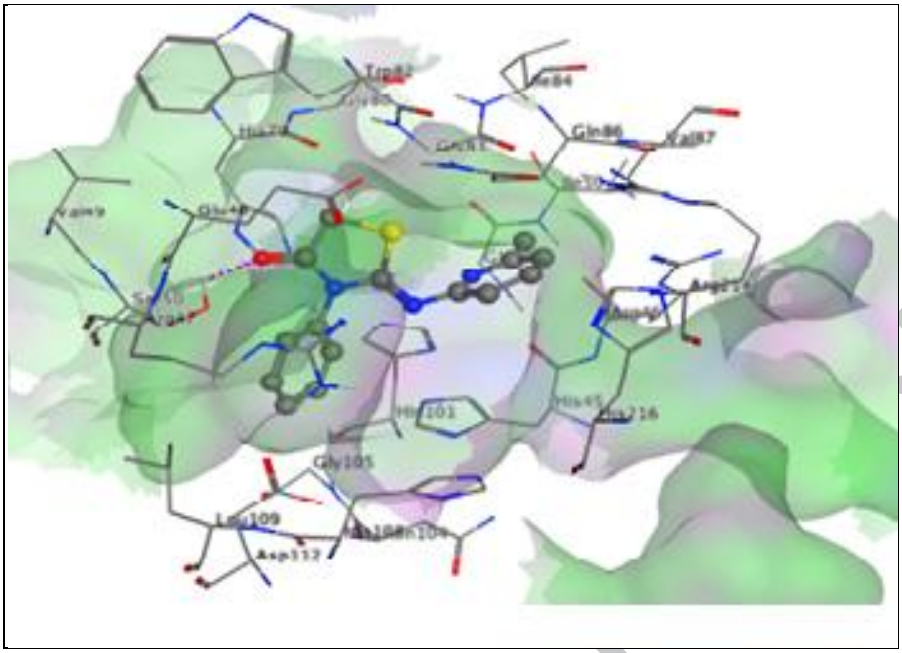

Fig. 4. 3Ddocking of compound **4a** with the active site pocket of the protein1ZOY.

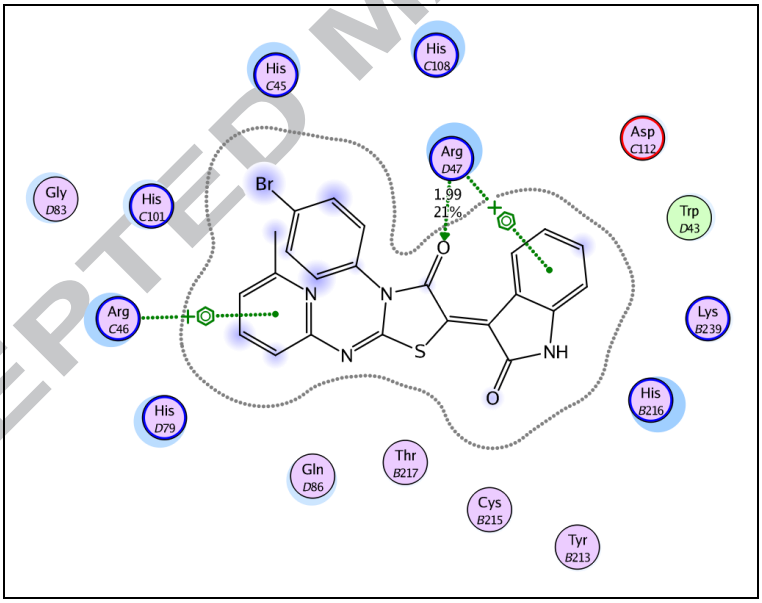

Fig. 5. 2D ligand interaction of the compound **6i** with the active site amino acids of 1ZOY.

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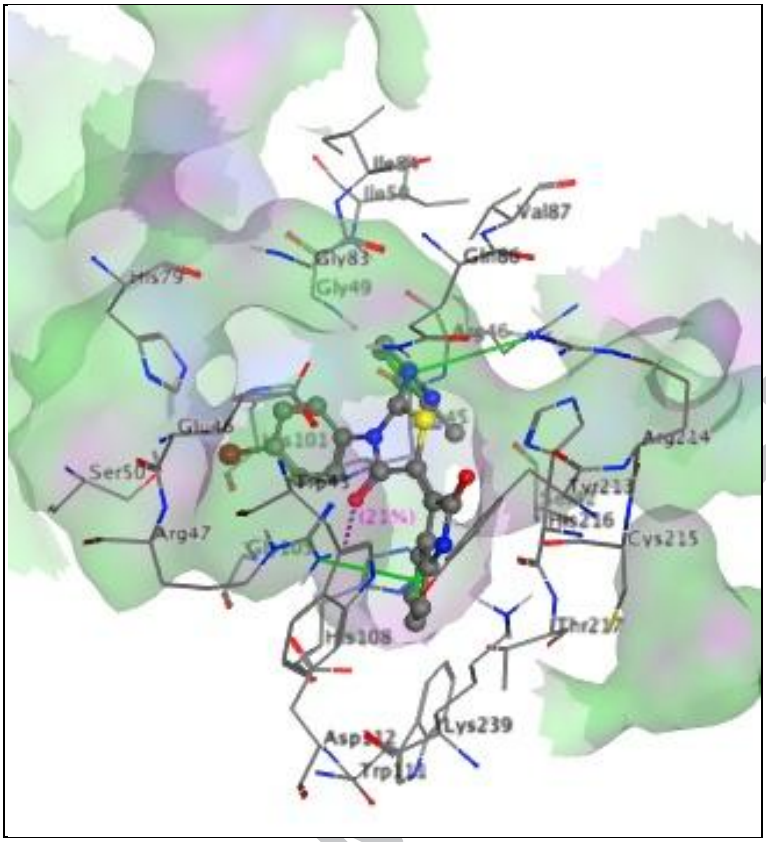

Fig. 6. 3D docking of compound **6i** with the active site pocket of the protein 1ZOY.

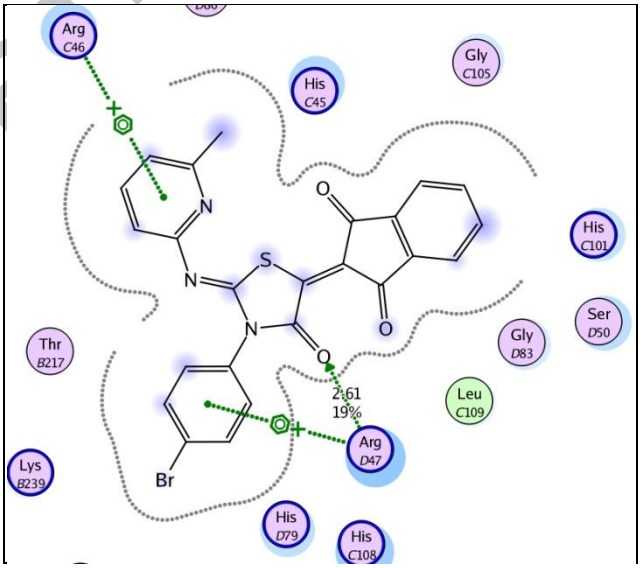

Fig. 7. 2D ligand interaction of the compound **6j** with the active site amino acids of 1ZOY.

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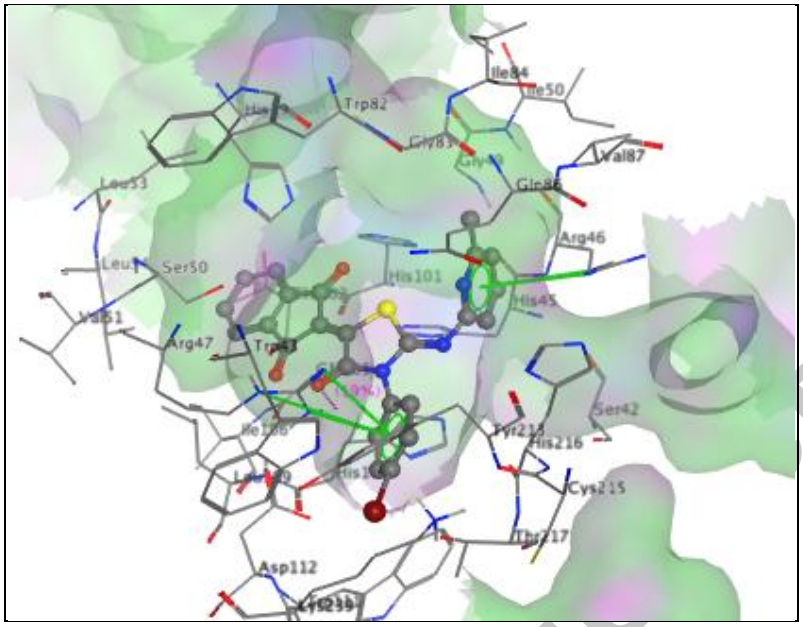

Fig. 8. 3D docking of compound **6j** with the active site pocket of the protein 1ZOY.

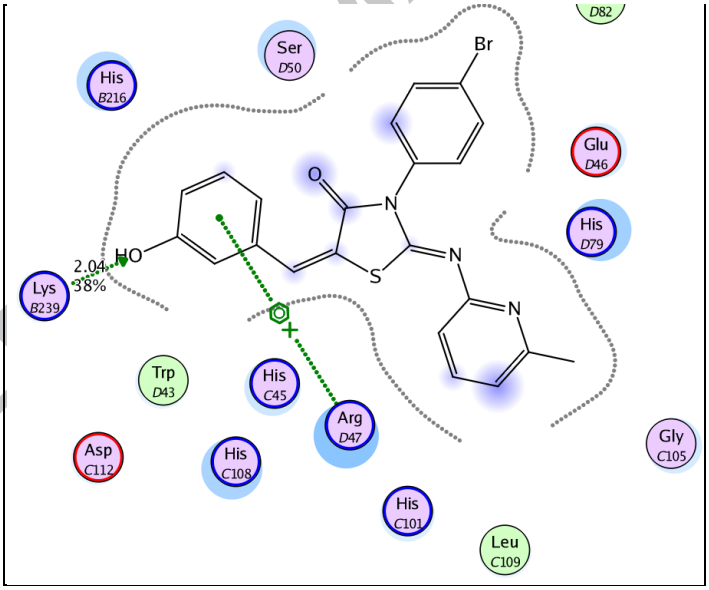

Fig. 9. 2D ligand interaction of the compound **9b** with the active site amino acids of 1ZOY.

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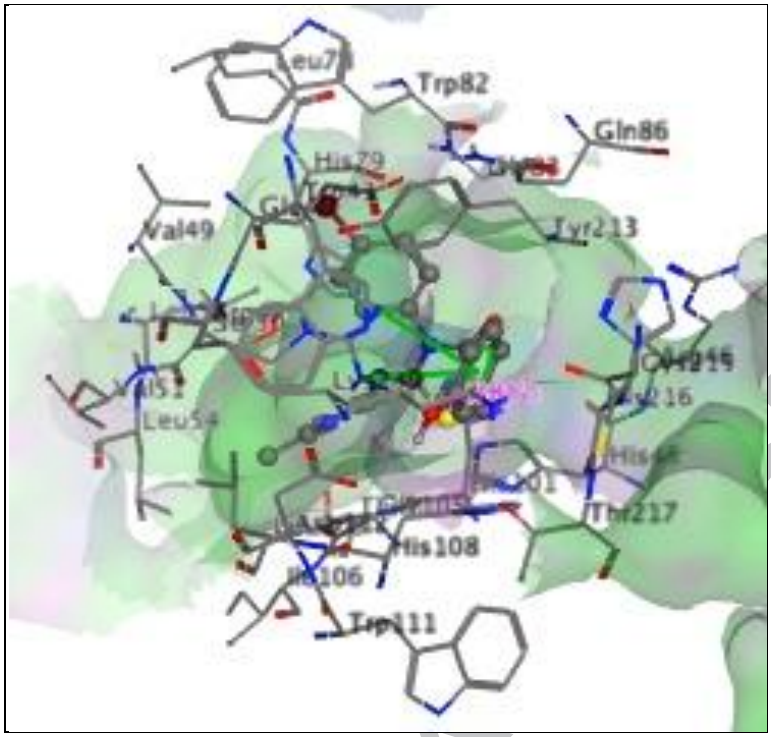

Fig. 10. 3D docking of compound **9b** with the active site pocket of the protein 1ZOY

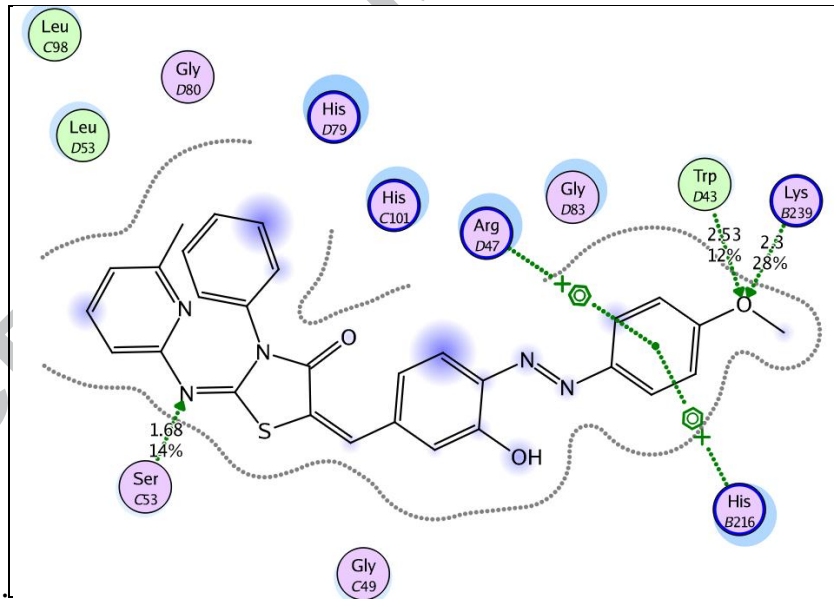

Fig. 11. 2D ligand interaction of the compound **10c** with the active site amino acids of 1ZOY.

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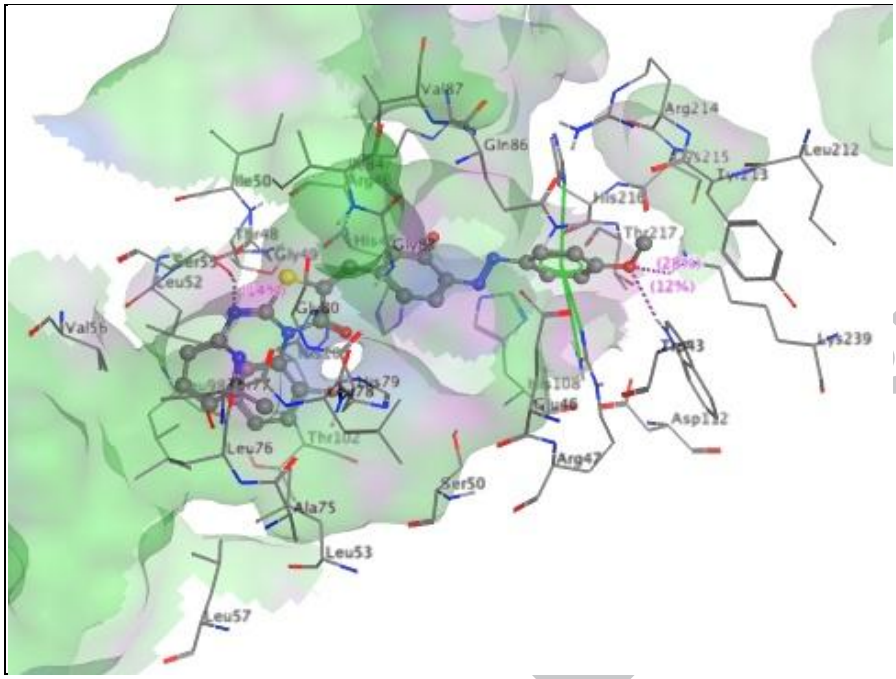

Fig. 12. 3D docking of compound **10c** with the active site pocket of the protein

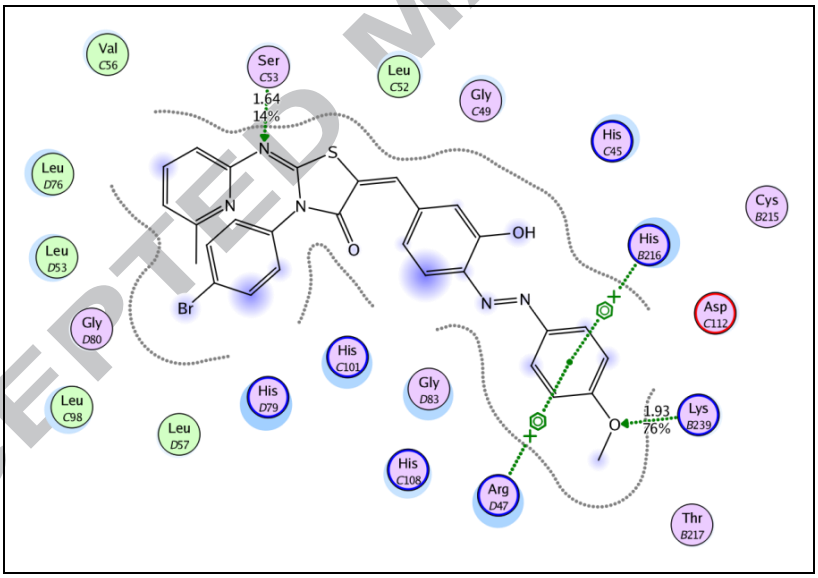

Fig. 13. 2D ligand interaction of the compound **10f** with the active site amino acids of 1ZOY.

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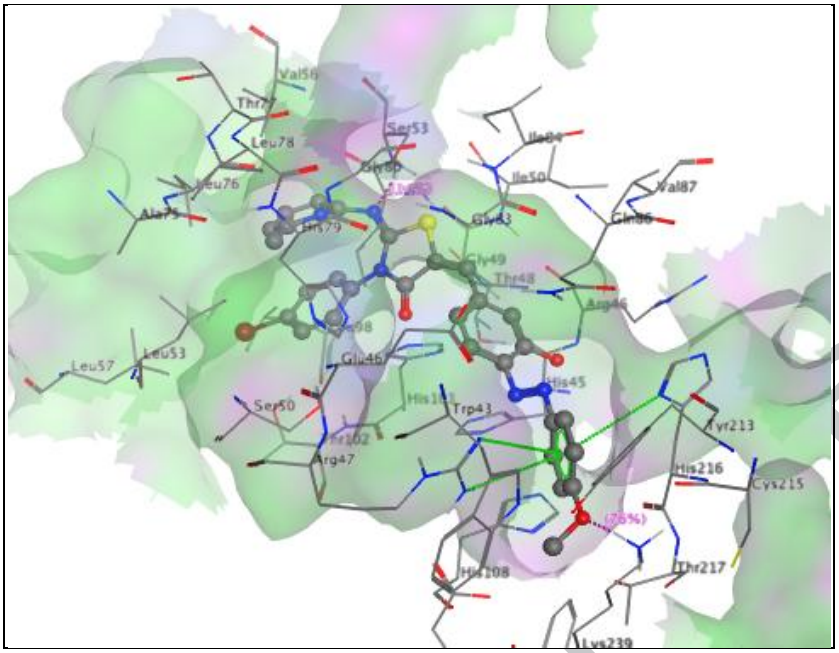

Fig. 14. 3D docking of compound **10f** with the active site pocket of the protein

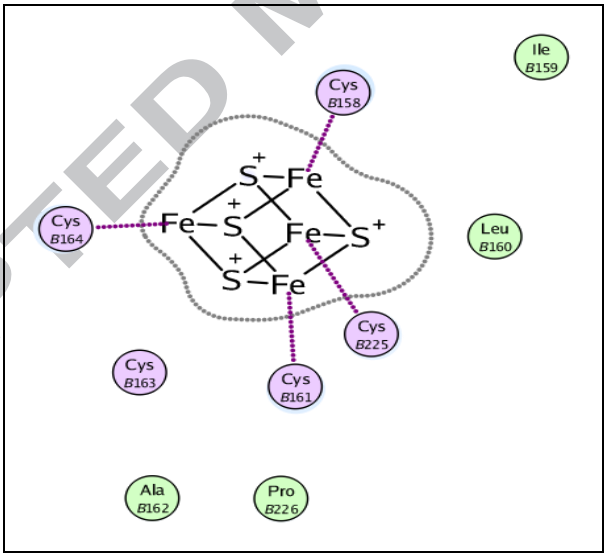

Fig. 15. The active site pocket of the protein 1ZOY.

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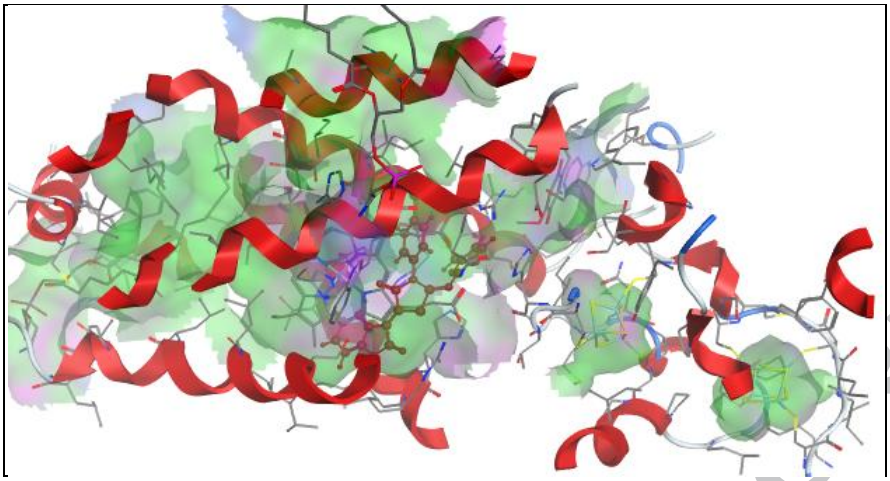

Fig. 15. The active site pocket of the protein1ZOY.

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

3. Experimental section

All melting points are uncorrected and were taken on electro-thermal capillary melting point apparatus (6100). IR spectra were recorded on a Perkin Elmer 1430 spectrophotometer. ^{1}HNMR and ^{13}CNMR spectroscopy were recorded in deuterateddimethylsulfoxide at mercury 400 and 100 MHz on a Varian Gemini NMR spectrophotometer usingtetramethylsilane 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 Shimaduze GMCS-

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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 ±0.4% of the theoretical values.Antifungal activityand gel electrophoresis assay were carried out at the Agriculture Research Centre in Giza, Egypt.

4. Chemistry

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 hours in methylene chloride (15 ml) at room temperature. The precipitate so formed was collected, filtered off, and recrystallized from ethanol.

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); ^{1}H 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 $C_{13}H_{13}N_3S$ (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%.

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

White crystals; yield 68.8%; m.p = 177-179 ^{o}C; IR (KBr, cm^{-1}): 3194 (NH), 3113 (NH), 1256 (C=S); ^{1}H 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 (M^++1, 12.5), 322 (M^+, 55.5), Anal. Calcd for $C_{13}H_{12}BrN_3S$ (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%.

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

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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 hours 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.

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

Yellow crystals; yield 87%; m.p = 260-262 ^{o}C [lit. m.p = 262-264 ^{o}C] [39]. IR (KBr, cm^{-1}): 1722 (C=O); ^{1}H 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 $C_{15}H_{13}N_3OS$ (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%

3-(4-Bromophenyl)-2-[(6-methylpyridin-2-yl)imino]thiazolidin-4-one (4b). Yellow crystals; yield 76%; m.p = 250-252 ^{o}C; IR (KBr, cm^{-1}): 1717 (C=O); ^{1}H 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 (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 $C_{15}H_{12}BrN_3OS$ (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%.

General procedures for synthesis of compounds 6a-j

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 hours in the presence of fused sodium acetate (0.01mol). The precipitate obtained was filtered off, washed with water and recrystallized from *N*,*N*-dimethylformamide (DMF).

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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 hours 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-Benzylidene-2-[(6-methylpyridin-2-yl)imino]-3-phenyltiazolidin-4-one (6a.)

Yellow crystals; yield 60% (Method A); 66% (Method B); m.p = 274-276 ^{o}C. IR (KBr, cm^{-1}): 1707 (C=O). ^{1}H 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 $C_{22}H_{17}N_3OS$ (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-(4-Fluorobenzylidene)-2-[(6-methylpyridin-2-yl)imino]-3-phenylthiazoli-din-4-one (6b).

Yellow crystals; yield 70% (Method A); 77% (Method B); m.p = 281-283 ^{o}C; IR (KBr, cm^{-1}) 1713 (C=O). ^{1}H 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 $C_{22}H_{16}FN_3OS$ (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-(2-Chloro-4-fluorobenzylidene)-2-[(6-methylpyridin-2-yl)imino]-3-phen-ylthiazolidin-4-one (6c).

Yellow crystals; yield 76% (Method A); 83% (Method B); m.p = 308-310 ^{o}C; IR (KBr, cm^{-1}): 1713 (C=O). ^{1}H 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 $C_{22}H_{15}ClFN_3OS$ (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%.

2-[(6-Methylpyridin-2-yl)imino]-5-(2-oxoindolin-3-ylidene)-3-phenylthiazo-lidin-4-one(6d).

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Orange crystals, yield 75% (Method A); 80% (Method B), m.p = 292-294^{o}C; IR (KBr, cm^{-1}): 3431 (NH), 1690 (C=O); ^{1}H 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 $C_{23}H_{16}N_4O_2S$ (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%.

2-[2-((5-Methylpyridin-2-yl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene]-1H-indene-1,3(2H)-dione (6e).

Dark redcrystals yield 72% (Method A); 79% (Method B);m.p>300 ^{o}C.IR (KBr, cm^{-1}): 1685, 1689 (C=O). ^{1}H 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 $C_{24}H_{15}N_3O_3S$ (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-Benzylidene-3-(4-bromophenyl)-2-[(6-methylpyridin-2-yl)imino]-thiazoli-din-4-one (6f).

Pale yellow crystals; yield 60% (Method A); 66% (Method B); m.p = 270-272 ^{o}C. IR (KBr, cm^{-1}): 1710 (C=O). ^{1}H 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: $C_{22}H_{16}BrN_3OS$ (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%.

3-(4-Bromophenyl)-5-(4-fluorobenzylidene)-2-[(6-methylpyridin-2-yl)-mino]thiazolidin-4-one (6g).

Pale yellow crystals; yield 69% (Method A); 75% (Method B); m.p = 273-275 ^{o}C; IR (KBr, cm^{-1}): 1716 (C=O). ^{1}H 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 $C_{22}H_{15}BrFN_3OS$ (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%.

3-(4-Bromophenyl)-5-(2-chloro-4-fluorobenzylidene)-2-[(6-methyl-pyridin-2-yl)imino]thiazolidin-4-one (6h).

Pale yellow crystals; yield 80% (Method A), 84% (Method B); m.p = 302-304 ^{o}C; IR (KBr, cm^{-1}): 1717 (C=O). ^{1}H NMR (DMSO-d_6): δ = 2.41 (s, 3H, CH_3), 6.81 (d, 2H, *J*

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= 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 (M^++2, 11.5), 466 (98.8), 334 (73), 287 (43.7), 186 (69.4) 92 (65.3), 65 (27.8);Anal. Calcd for $C_{22}H_{14}BrClFN_3OS$ (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%.

3-[4-Bromophenyl)-2-((5-methylpyridin-2-yl)imino]-5-(2-oxoindolin-3-ylid-ene)thiazolidin-4-one (6i).

Orange crystals, yield 68% (Method A),76%(Method B), m.p = 286-288^{o}C;IR (KBr, cm^{-1}): 3437 (NH), 1688 (C=O);^{1}H 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 $C_{23}H_{15}BrN_4O_2S$ (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 %.

2-[3-(4-Bromophenyl)-2-((5-methylpyridin-2-yl)imino]-4-oxothiazolidin-5-ylidene)-1*H*-indene-1,3(2*H*)-dione (6j).Dark red, yield 76%, m.p = 290-292^{o}C. IR (KBr, cm^{-1}): 1686 (C=O). ^{1}H 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 $C_{24}H_{14}BrN_3O_3S$ (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%.

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 hours then left to cool. The solid so formed filtered off, washed with water and recrystallized from *N,N*-dimethylformamide (DMF).

5-(3-Hydroxybenzylidene)-2-[(6-methylpyridin-2-yl)imino]-3-phenylthia-zolidin-4-one (9a).

Yellow crystals; yield 89%; m.p = 281-283 ^{o}C; IR (KBr, cm^{-1}): 3408 (OH), 1702 (C=O), 1590 (C=C). ^{1}H 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^+,

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99.1%), 283 (99.9), 240 (84.6), 192 (97.0), 69 (60.9); Anal. Calcd for: $C_{22}H_{17}N_3O_2S$ (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%.

*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 ^{o}C; IR (KBr, cm^{-1}): 3413 (OH), 1707 (C=O), 1596 (C=C); ^{1}H 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: $C_{22}H_{16}BrN_3O_2S$ (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%.

General procedure for synthesis of compounds 10a-f

Method A

The appropriated amine (0.01mol) 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.**

Method B

A solution of conc. hydrochloric acid (0.01mol) cooled at 0 ^{o}C was added to a solution of aniline (0.01mol) and aqueous solution of $NaNO_2$ (0.01mol) dropwise at 0-5^{o}C. The formed diazonium chloride was added on (0.01mol) of 3-hydroxysalicyaldehyde in 5ml of 0.4g NaOH and 0.74g of Na_2CO_3. The reaction stirred at 0 ^{o}C for 2hr, the solid so formed was filtered off and recrystallized from ethanol to give compounds **8a-c**.A mixture of compounds **8a-c** (0.01mol) 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**.

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5-(3-Hydroxy-4-(phenyldiazenyl)benzylidene)-2-[(6-methylpyrid-in-2-yl)-imino]-3-phenylthiazolidin-4-one (10a).

Scarlet red crystals; yield 82% (method A), 70% (method B); m.p = 192-194^{o}C; IR (KBr, cm^{-1}): 3408 (OH), 1710 (C=O), 1661 (C=N). ^{1}H NMR (DMSO-d_6): δ = 2.43 (s, 3H, CH_3), 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 *D_2O*); Anal. Calcd for $C_{28}H_{21}N_5O_2S$ (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%.

[(2-Chlorophenyl)diazenyl]-3-hydroxybenzylidene)-2-[(6-methy-lpyridin-2-yl)imino]-3-phenylthiazolidin-4-one (10b).

Red crystals; yield 78% (method A),70% (method B);m.p = 219-221^{o}C; IR (KBr, cm^{-1}): 3399 (OH), 1688 (C=O), 1653 (C=N). ^{1}H NMR (DMSO-d_6): δ = 2.42 (s, 3H, CH_3), 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 *D_2O*); MS: m/z (%) = 526 (M^+, 64.4%) Anal.Calcd for $C_{28}H_{20}ClN_5O_2S$ (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-(3-Hydroxy-4-[(4-methoxyphenyl)diazenyl)benzylidene]-2-[(6-methyl-pyridin-2-yl)imino]-3-phenylthiazolidin-4-one (10c).

Red crystals; yield 83% (method A),70% (method B); m.p = 234-236 ^{o}C; IR (KBr, cm^{-1}): 3411 (OH), 1713 (C=O), 1665 (C=N). ^{1}H NMR(DMSO-d_6); δ = 2.46 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 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 *D_2O*);Anal. Calcd for $C_{29}H_{23}N_5O_3S$ (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%.

3-(4-Bromophenyl)-5-[3-hydroxy-4-(phenyldiazenyl)benzylidene]-2-[(6-methyl-pyridin-2-yl)imino]thiazolidin-4-one (10d).

Scarlet red crystals; yield 69% (method A), 70% (method B); m.p = 182-184 ^{o}C; IR (KBr, cm^{-1}): 3412 (OH), 1714 (C=O), 1666 (C=N); ^{1}H NMR (DMSO-d_6): δ = 2.43 (s,

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3H, CH_3), 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 D_2O); Anal. Calcd for $C_{28}H_{20}BrN_5O_2S$ (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%.

3-(4-Bromophenyl)-5-[4-(2-chlorophenyl)diazenyl]-3-hydroxy-benzylid-ene)-2-[(6-methylpyridin-2-yl)imino]thiazolidin-4-one (10e).

Red crystals; yield 83% (method A),70% (method B); m.p = 207-209 ^oC; IR (KBr, cm^{-1}): 3407 (OH), 1699 (C=O), 1659 (C=N); ^1H NMR (DMSO-d_6): δ = 2.41 (s, 3H, CH_3), 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 D_2O); Anal. Calcd for $C_{28}H_{19}BrClN_5O_2S$ (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%.

3-(4-Bromophenyl)-5-(3-hydroxy-4-((4-methoxyphenyl)diazenyl)-benzylid-ene)-2-((6-methylpyridin-2-yl)imino)thiazolidin-4-one (10f).

Red crystals; yield 71% (method A), 70% (method B); m.p = 215-217 ^oC; IR (KBr, cm^{-1}): 3415 (OH), 1717 (C=O), 1670 (C=N). ^1H NMR (DMSO-d_6); δ = 2.44 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 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 D_2O); Anal. Calcd for $C_{29}H_{22}BrN_5O_3S$ (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.

5. 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**againstcultures of five fungal strains (*Aspergillus Niger* ASP, *Aspergillusflavus, Alternaria solani, Aspergillus ochraceus, Macrophominaphaseolina*) *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

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tested compounds under the same conditions (Table 1). Nuclease activity experiments were accomplished by agarosegel 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 (5mM 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 carriedout at 60 V for one hour in Tris-HCl buffer using 1% agarose gel containing 1.0 μg/mL ethidiumbromide (EB)26.

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Highlights

Pyridine-thiazolidinone derivatives were synthesized.
Compounds were evaluated for antifungal activity.
Compounds **6d**, **6e**, **6i**, **6j**, **9a,b** and **10a-f** revealed higher antifungal activity towards *Alternaria solani* than to the standard *Ridomil gold* plus.
Molecular docking study was performed on 1ZOY.

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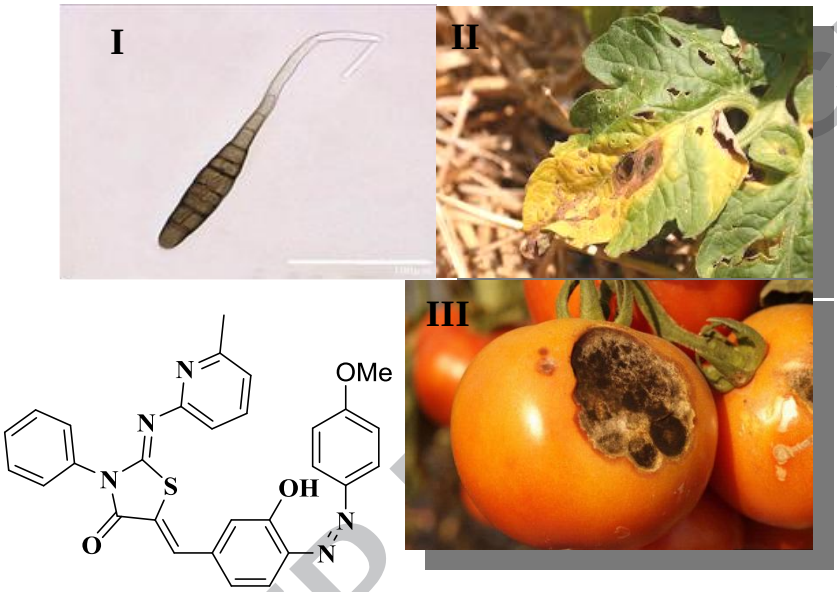
Design, synthesis, DNA assessment and molecular docking study of novel 2-(pyridin-2-ylimino)thiazolidin-4-one derivatives as potent antifungal agents

Nadia Hanafy Metwally[1]*, Ibrahim Taha Radwan[2], Walaa Salah El-Serwy[3] and Mohamed Ahmed Mohamed[4]



Compound **10c** has a high potent activity against *Alternaria Solani*