Bioorganic Chemistry 95 (2020) 103509

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Design and synthesis of novel cylopentapyrazoles bearing 1,2,3-thiadiazole moiety as potent antifungal agents

Betül Giray[a], Ayşe Esra Karadağ[b], Özgecan Şavluğ İpek[c,d], Hanife Pekel[c,e], Mustafa Güzel[c,f], Hatice Başpınar Küçük[g,*]

[a] Istanbul Medipol University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Kavacik Campus, Kavacik-Beykoz/Istanbul 34810, Turkey
[b] Istanbul Medipol University, School of Pharmacy, Department of Pharmacognosy, Beykoz, Istanbul 34810, Turkey
[c] Istanbul Medipol University, Regenerative and Restorative Medicine Research Center (REMER), Kavacik Campus, Kavacik-Beykoz/Istanbul 34810, Turkey
[d] Yildiz Technical University, Graduate School Of Natural And Applied Sciences, Department of Chemistry, Besiktas/Istanbul 34349, Turkey
[e] Istanbul Medipol University, Vocational School of Health Services, Pharmacy Services, Kavacik Campus, Kavacik-Beykoz/Istanbul 34810, Turkey
[f] Istanbul Medipol University, International School of Medicine, Department of Medical Pharmacology, Kavacik Campus, Kavacik-Beykoz/Istanbul 34810, Turkey
[g] Istanbul University-Cerrahpasa, Faculty of Engineering, Department of Chemistry, Organic Chemistry Division, Avcılar/Istanbul 34320, Turkey

ARTICLE INFO

Keywords:
Cylopentapyrazole
1,2,3-thiadiazole
Antifungal activity
Fungicide
Plant pathogen
Novel antifungal drug development
Docking
Molecular modelling studies

ABSTRACT

In drug-resistant phytopathogenic fungi, there has been extensive research on microbiological and antifungal drug development. In this study, a novel series of cylopentapyrazole bearing a 1,2,3-thiadiazole ring **2a-e** were designed and synthesized according to the principle of combination of bioactive structures. Thus, we have employed a [3 + 2] cycloaddition with 4-methyl-[1,2,3] thiadiazole-5-carboxylic acid hydrazones **1a-e** and cyclopentadiene ring. Novel synthesized compounds were identified with IR, ^{1}H and ^{13}C NMR, mass spectrometry and elemental analysis then, antifungal activities were assayed. Based on our study, a combination of the compounds **1a** and **2b** possess remarkable antifungal activity against *Botrytis cinerea* AHU 9424 with 100% inhibition. EC_{50} values were calculated by studying different doses in combinations with high inhibition rates. The combination of **1a** + **2b** has an EC_{50} value at 6.37 and 13.85 μg/ml concentrations against *B. cinerea* and *F. culmorum*, respectively. The combination of compound **1a** + **2b**, having a cylopentapyrazole ring on the 1,2,3-thiadiazole backbone, shows promising fungicidal activity and deserves further development. Additionally, the homology model of the CYP51 enzyme that belongs to *Fusarium moniliforme* was generated using CYP51B (PDB ID: 6CR2), and molecular docking was performed using this homology model for each compound. The results of this study clearly indicate that these novel compounds can be identified as promising lead compounds and potential fungicidal agents in future.

Introduction

Heterocycles have received notable attention in recent years for their agricultural and medicinal properties. In agricultural research, pyrazole derivatives are used as the active ingredients of insecticidal [1,2], acaricidal [3–5], fungucidal [6,7], and antiviral agents [8,9]. Pyrazole-based drugs are also effective in the treatment of atherosclerosis [10], inflammatory bowel syndrome [11], and Alzheimer's Disease (AD). 1,2,3-thiadiazole compounds have versatile biological activity, which possesses anti-inflammatory, antitumor, hypotensive, antibacterial, and antiallergic applications [12–14]. Some of the reported 1,2,3-thiadiazole compounds are considered as plant activators [15–17]. Additionally, these synthons have frequently used in organic synthesis [18] to develop druggable candidates.

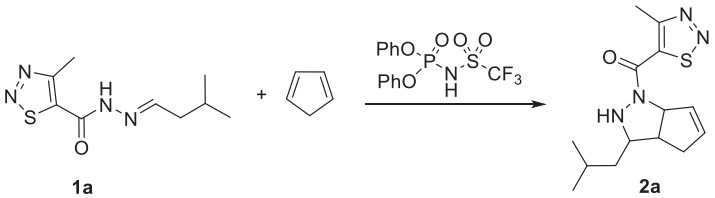
In exploring new bioactive compounds, we considered that a combination of cylopentapyrazole and 1,2,3-thiadiazole moieties would provide us novel entities with multiple biological activities. Pyrazolidines are conveniently synthesized with a [3 + 2] cycloaddition between hydrazones and alkenes [19–24]. Based on our literature search so far, we apply the [3 + 2] cycloaddition of 4-methyl-[1,2,3] thiadiazole-5-carboxylic acid hydrazones **1a-e** with cyclopentadiene to synthesize novel cyclopentapyrazoles with 1,2,3-thiadiazole moieties.

Plant diseases are very important factors in agricultural production and phytopathogenic fungi of different genera, infect countless crops. Particularly, some *Fusarium* species and *Botrytis cinerea* lead to very important plant diseases that cause economical losses in agriculture [25]. *Fusarium* and *Botrytis* species are especially pathogenic microorganisms against vegetables and fruits cultivated as food [26,27].

* Corresponding author.
E-mail address: baspinar@istanbul.edu.tr (H.B. Küçük).

https://doi.org/10.1016/j.bioorg.2019.103509
Received 16 September 2019; Received in revised form 28 November 2019; Accepted 16 December 2019
Available online 24 December 2019
0045-2068/

Table 1
Optimization of Reaction Conditions for the [3 + 2] cycloaddition reaction between hydrazone **1a** and cyclopentadiene.[a]



1a **2a**

Entry	Solvent	T (°C)	NTPA (mol%)	Time	Yield[b] (%)
1	CH_2Cl_2	Rt	5	24 h	48
2	CH_2Cl_2	Rt	10	24 h	53
3	CH_2Cl_2	Rt	20	24 h	59
4	$CHCl_3$	Rt	10	24 h	67
5	DCE	Rt	10	24 h	60
6	THF	Rt	10	24 h	20
7	Toluene	Rt	10	24 h	45
8	CH_2Cl_2	Reflux	10	12 h	62
9	$CHCl_3$	Reflux	10	12 h	75
10	CH_2Cl_2	Reflux	10	3 h	73
11	**$CHCl_3$**	Reflux	**10**	**3 h**	**84**

[a] Molar ratio of hydrazone **1a**/cylopentadiene was 1.0:2.0.
[b] Isolated yield.

Therefore, especially the antifungal agents investigated in this study aim to provide a solution to an important economic problem encountered in the production of vegetables and fruits. In recent years, the efficiency of fungicides traditionally used to control plant diseases has dramatically diminished. At the same time, improper use of conventional fungicides such as dicarboximides and benzimidazoles have caused an increase in drug resistance against fungal strains [28,29]. To overcome this, the discovery of new antifungal agents which can replace the current therapeutic strategies is therefore very important.

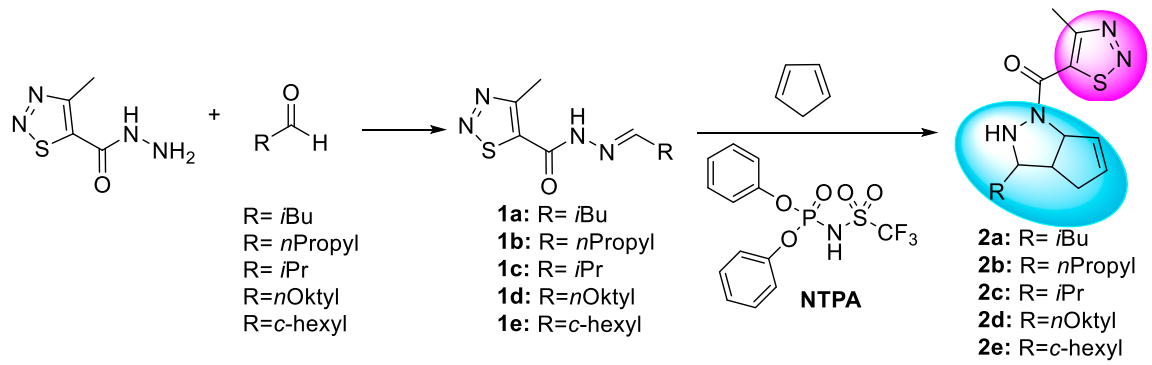
Since it has been known that pyrazoles and 1,2,3-thiadiazoles show decent activity against fungi, in this study we have investigated the antifungal activity of newly synthesized compounds **1a-e** and **2a-e** for *Fusarium moniliforme* NRRL 2374, *Fusarium culmorum* NRRL 3288, *Fusarium heterosporum* DSM 62719, *Botrytis cinerea* AHU 9424 strains, which are important plant pathogens. Furthermore, we determined their binding mode using homology model of CYP51 enzyme so as to explore their molecular interactions. The promising results of this study are highlighted below, which can further be thoroughly investigated in order to understand their mechanism as well as their toxicological behavior.

Results and discussion

Chemistry

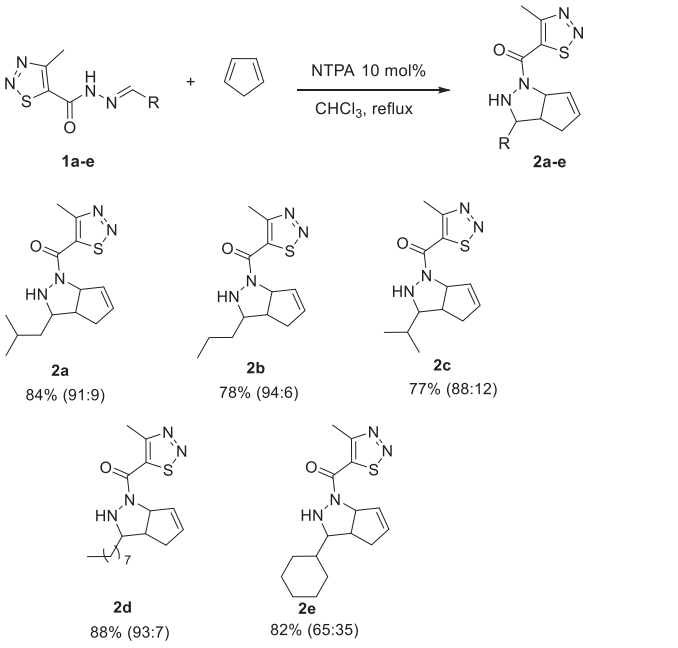
All the target compounds **1a-e** and **2a-e** were synthesized according to Scheme 1 [30–32]. The hydrazones **1a-e** were reacted with cyclopentadiene in the presence of *N*-triflylphosphoramide (NTPA) [33,34], the targeted compounds **2a-e** were obtained in 77–88% yields as a mixture of two diastereomers [35].

A model reaction was used to determine the optimal reaction conditions; we reacted the hydrazone **1a** with cyclopentadiene in a [3 + 2] cycloaddition setting. The catalyst loading, the role of different solvents, reaction times and reaction temperatures were studied and the results are shown in Table 1. Table 1, entry 1 shows that the reaction started with 5 mol% NTPA at room temperature with 42% yield. Entry 2 investigates the catalyst loading as 10 mol% and the product **2a** was obtained with a higher reaction yield (53%). When the catalyst loading was raised to 20%, the reaction yield had a slight increase (59%, Table 1, entry 3). Therefore the optimal catalyst loading was determined to be 10 mol% of NTPA as seen in Entry 2. Then, we wanted to see the effect of the solvent. The [3 + 2] cycloaddition of **1a** gave high yields when chlorinated solvents were used. Tetrahydrofuran and toluene gave very low yields (Table 1, entries 6 and 7). Chloroform was the best solvent among the chlorinated solvents (Table 1, entry 4). Another factor we investigated in the reaction was the temperature. Entries 8 and 9 show that the reactions were carried out at reflux conditions for 12 h, respectively. There was a slight improvement in the yield and side products were formed when we raise the reaction temperature to reflux conditions. It was a pleasant finding when we conducted the reaction time in much less time (3 h), which gives less



Scheme 1. The synthetic route for the target compounds.

Table 2
The substrate scope of [3 + 2] cycloaddition reaction between hydrazones **1a-e** and cyclopentadiene.



Ratio in parenthesis indicates ratio of diastereomers as determined by ^{1}H NMR.

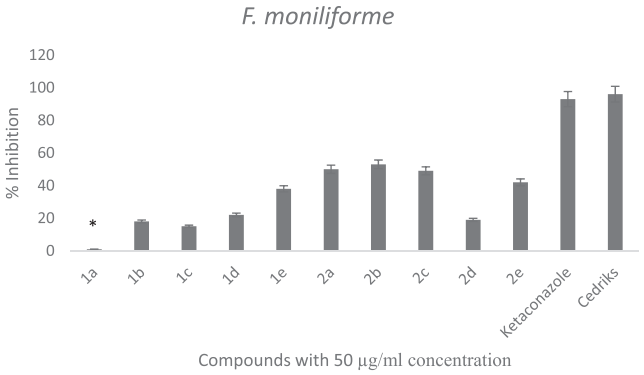


Fig. 1. Mycelial growth % inhibition of chemicals against *F. moniliforme.*

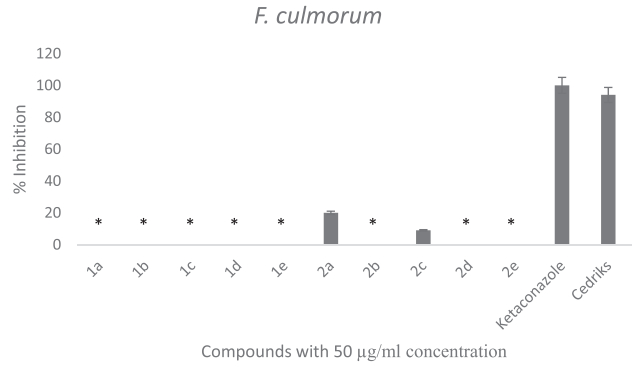


Fig. 2. Mycelial growth % inhibition of chemicals against *F. culmorum.*

Table 3
Antifungal activity of the compounds **1a-e**/**2a-e** and their combinations against *F. moniliforme, F. culmorum, F. heterosporum,* and *B. cinerea.*

		Inhibition (%)			
Compounds	Concentrations	*F. moniliforme* NRRL 2374	*F. culmorum* NRRL 3288	*F. heterosporum* DSM 62719	*Botrytis cinerea* AHU 9424
1a	50 µg/ml	-	-	33 ±3.4	-
1b	50 µg/ml	18 ± 2.9	-	-	-
1c	50 µg/ml	15 ±4.1	-	-	-
1d	50 µg/ml	22 ±3.2	-	-	-
1e	50 µg/ml	38 ±3.9	-	30 ±1.7	26 ±1.6
2a	50 µg/ml	50 ± 3.2	20 ±1.6	51 ± 2.2	26 ±1.1
2b	50 µg/ml	53 ± 2.7	-	52 ±3.1	25 ±1.4
2c	50 µg/ml	49 ±2	9 ±1	55 ±2.5	47 ±1.9
2d	50 µg/ml	19 ±3.6	-	27 ±2.3	-
2e	50 µg/ml	42 ±2.3	-	41 ±2	26 ±1.5
1a+2a	25 µg/ml + 25 µg/ml	73 ±4.1	75 ±1.8	25 ±1.4	75 ±1.8
1a+2c	25 µg/ml + 25 µg/ml	37 ±3.3	67 ±2.5	-	80 ±1.6
1a+2b	25 µg/ml + 25 µg/ml	48 ±2.6	74 ±3.1	-	100 ±1
2a+2c	25 µg/ml + 25 µg/ml	43 ± 3.7	44 ±3.8	36 ±1.9	61 ±2.3
2b+2e	25 µg/ml + 25 µg/ml	34 ±2.1	32 ±2.4	22 ±2	69 ±2.5
2c+2e	25 µg/ml + 25 µg/ml	46 ±2.9	42 ±1.9	31 ±2.3	55 ±2.1
1a+2a	12.5 µg/ml + 12.5 µg/ml	55±1.4	83±0.9	-	75±2.9
1a+2b	12.5 µg/ml + 12.5 µg/ml	39±1.1	77±1.7	-	73±2.4
1a+2c	12.5 µg/ml + 12.5 µg/ml	36±2.1	65±1.3	-	62±2.2
1a+2a	6.25 µg/ml + 6.25 µg/ml	34±1.9	75±2	-	73±1.8
1a+2b	6.25 µg/ml + 6.25 µg/ml	34±3.1	66±1.5	-	66±1.3
1a+2c	6.25 µg/ml + 6.25 µg/ml	30±1.4	63±2.4	-	55±2.1
Ketaconazole	**50 µg/ml**	**93±1**	**100±0.6**	**100±0.4**	**57±2**
Cedriks	**50 µg/ml**	**96±2**	**94 ±1**	**91±2**	**90±3**

*"–" implies no inhibition at the studied concentration.

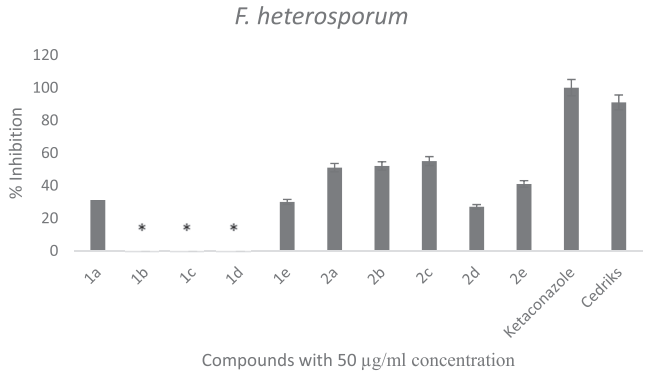


Fig. 3. Mycelial growth % inhibition of chemicals against *F. heterosporum.*

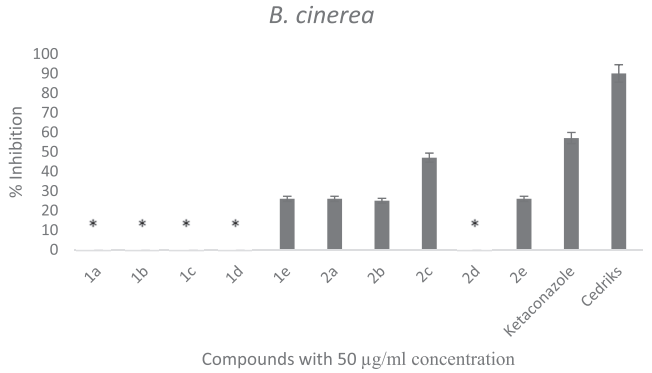


Fig. 4. Mycelial growth % inhibition of chemicals against *B. cinerea.*

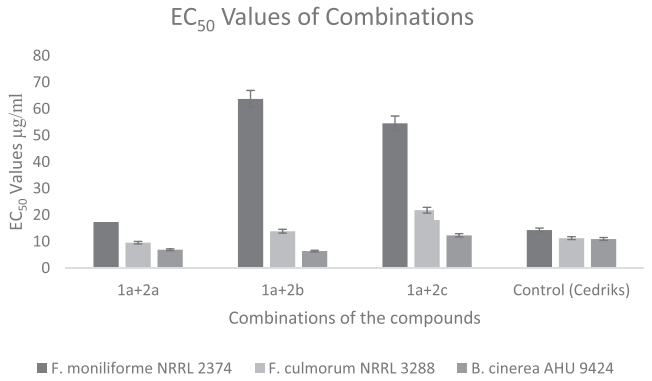


Fig. 5. EC_{50} values of combinations of most active compounds.

Table 4
EC_{50} values of combinations of most active compounds.

Compounds	*F. moniliforme* NRRL 2374	*F. culmorum* NRRL 3288	*B. cinerea* AHU 9424
1a + 2a	21.51 µg/ml	9.52 µg/ml	6.88 µg/ml
1a + 2b	63.72 µg/ml	13.85 µg/ml	6.37 µg/ml
1a + 2c	54.52 µg/ml	21.77 µg/ml	12.3 µg/ml
Control (Cedriks)	14.32 µg/ml	11.25 µg/ml	10.92 µg/ml

formation of undesired side product. The best performance was obtained with 10 mol% of NTPA catalyst loading, 3 h of reaction time in chloroform and reflux condition, giving 84% yield for **2a** (Table 1, entry 11). We also tried these conditions to the reactions involving reactive hydrazones.

We were interested in the scope of the reaction in order to fully understand its mechanism. Therefore, various hydrazones were synthesized involving the aldehydes under standard reaction conditions. Hydrazones **1a-e** derived from a saturated long-chain and branched aldehydes yielded the corresponding cycloadducts **2a-e** in good yields (77–88%) with diastereomeric ratios ranged from 65:35 to 94:6 as calculated by ^{1}H NMR (Table 2). It was found out that the hydrazones in our study were appropriate substrates for [3 + 2] cycloaddition. Surprisingly, when we investigated the [3 + 2] cycloaddition reaction with hydrazones derived from aromatic aldehydes and cyclopentadiene, we found that the reaction did not proceed well due to steric and electronic reasons. Thus it is imperative to use less bulky and aliphatic aldehydes to understand the mechanism which we are still investigating.

Antifungal activity

The antifungal effect of these novel compounds was tested with mycelial growth rate method against *Fusarium moniliforme* NRRL 2374, *Fusarium culmorum* NRRL 3288, *Fusarium heterosporum* DSM 62719, *Botrytis cinerea* AHU 9424. The provided data were studied in triplicates and the mean of the results was calculated by standard error. The results were compared to ketoconazole and Cedriks™ (Biological funguside = *Pseudomonas flourescens* strain) which are current antifungal drugs, as shown Table 3. The combination study of these novel molecules with several strains are depicted in Figs. 1–5 respectively.

As seen in Table 3, compound **2a** showed 51% and 52% mycelial growth inhibition against *F. moniliforme* and *F. heterosporum*, respectively. The compound **2b** showed an efficacy over 50% for the same strains. For **2c**, the inhibition against *F. heterosporum* strain was over 55%. After observing positive results then different combinations of these novel compounds were studied to see synergetic effects and resulted in greater and notable high inhibition values, demonstrating the evidence for a synergetic effect. Especially with the combination of **1a** + **2a** prominent antifungal activities against *F. moniliforme* and *B. cinerea* strains were observed 73% and 75%, respectively. Furthermore, the combination of **1a** + **2c** has a remarkable antifungal activity against *B. cinerea* with 80% inhibition, while **1a** + **2b combination impressively yielded 100% inhibition and completely inhibited the growth of *B. cinerea.*** These results indicate that synergetic effect of these novel cylopentylpyrazoles bearing 1,2,3-thiadiazole moiety may provide to produce new scaffold offering potential antifungal activity. Moreover, some representative compounds with good, ordinary antifungal activity were selected to run dose dependent studies as indicated in Table 3 and their EC_{50} values were then calculated by studying three different concentrations. As the results are summarized in Tables 3 and 4, the combinations of **1a** + **2a**, **1a** + **2b** and **1a** + **2c** showed prominent antifungal activities against three plant pathogens (*F. moniliforme, F. culmorum* and *B. cinerea*) with EC_{50} values between 6.37 µg/ml and 63.72 µg/ml. Among them, **1a** + **2a** and **1a** + **2b** combination showed noticeable activity against *B. cinerea* (EC_{50} values of 6.88 µg/ml, and 6.37 µg/ml respectively). Surprisingly this inhibition was even superior then the activity of the commercial fungicide Cedriks (10.12 µg/ml).

B. cinerea is also a fungus which is present in damp climates and subtropical regions. It survives on many plants as a facultative parasite and might cause diseases on the grapes, strawberries, squashes, and lettuces. Especially, it infects and harms wine grapes and causes a significant economic loss after harvesting the fruits. Therefore, it is extremely important to develop an effective fungicide agent against these plant pathogens and especially compounds. **1a** + **2a** and **1a** + **2c** can be used as possible lead compound combination for the development of potential agrochemicals. Also, compounds **1a** + **2a**, **1a** + **2b** and **1a** + **2c** showed ordinary activity against *F. culmorum* which is very important plant pathogen, with the EC_{50} values 9.52 µg/ml, 13.85 µg/ml, 21.77 µg/ml, respectively. Finally, **1a** + **2a** combination showed noteworthy broad-spectrum antifungal bioactivity against most of the tested fungi as indicated in Table 4.

Table 5
Binding energies of ketoconazole, **1e-a**, and **2a-e**.

Compounds	Binding energy (kcal/mol)
Ketoconazole	−10.12 ± 0.103
1a	−6.75 ± 0.05
1b	−6.38 ± 0.015
1c	−6.6 ± 0.024
1d	−7.36 ± 0.014
1e	−7.01 ± 0.021
2a	−8.64 ± 0.096
2b	−8.54 ± 0.017
2c	−8.86 ± 0.053
2d	−8.84 ± 0.032
2e	−9.05 ± 0.139

Homology modelling and docking studies

Lanosterol 14 alpha-demethylase, CYP51, is a member of highly conserved protein family that is ammeanable to the biosynthesis of ergosterol, which is a crucial component that regulates the cell membrane permeability of fungi [36]. So far, several inhibitors of these enzymes have been used in different pathological conditions. These inhibitors generally contain heterocyclic ring nitrogen atom that is coordinated to heme iron in order to inhibit the enzymatic activity [37–39]. From the light of the previous findings, our novel compounds may inhibit the action of CYP51 enzymes to block the fungal activity in the same manner. Although we have investigated four different fungi species *in vitro*, we have only evaluated the possible binding effects of our novel compounds in CYP51 of *Fusarium moniliforme* as a representative target due to the conserved structures of CYP51s. The homology model of CYP51 enzyme which belongs to *Fusarium moniliforme* was created using the 'SWISS-MODEL' tool [40]. Thereby, the X-ray crystal structure of 14-alpha sterol demethylase (CYP51B) from *Neosartorya fumigata* (PDB ID: 6CR2) [41] was utilized as a template with %70 sequence similarity in the homology modeling [42].

With this in mind, the crystal structure of LFV-bound CYP51B was redocked using 'AutoDock Vina' [43]. Root mean square deviation (rmsd) with respect to the crystal conformation and energy of the best pose were computed as 1.332 Å and −10.2 kcal/mol, respectively. Furthermore, the same ligand LFV was docked into the enzyme model. The rmsd value of the best pose was calculated 1.117 Å, and the binding energy value was predicted as −12 kcal/mol. Also, the best pose of ligand LFV from docking have similar interactions between ligand and protein in the binding cavity. On the other hand, based on the structural similarity between ligand LFV and ketoconazole, we decided to use this enzyme as a model.

Most of the crystal structures of the CYP51 enzymes in the PDB (Protein Data Bank) database are endowed with Fe coordinated nitrogen atom of the heterocyclic ring of ligand molecules [37–39]. Also, when an inhibitor molecule is bound to an active site of the enzyme, heme iron is found in Fe^{+3} form [44]. Distance between Fe^{+3} ion and nitrogen of ligand was calculated as 2.8 Å in the best pose, which has minimal rmsd value with respect to the conformation of ligand LFV. Henceforth, 2.8 Å was determined as a cutoff value in the selection of poses that were obtained from the molecular docking.

Novel compounds and ketoconazole have drawn using 2D Sketcher tool of Maestro [45] and optimized. Afterward, the maximum count of conformers was generated for each compound depending upon its number of rotatable bond. Indeed, utmost 100 conformers were minimized by means of 'ConfGen' [46]. Gasteiger charges and hydrogens were added to the model protein, and also ligands were prepared using AutoDockTools [47]. The x, y, z centers of grid box were determined according to the center of mass of the crystal structure of the ligand LFV, and the grid box dimensions were set as 30 × 22 × 22 $Å^3$. The flexible ligand docking studies were performed using 'AutoDock Vina' by using Lamarckian genetic algorithm [43].

Consequently, the comparison of binding energies is in line with gathered *in vitro* data that shows inhibition degrees of mycelial growth as shown in Fig. 1. Generally, the binding energies of compounds **1a-e** were lower than compounds **2a-e** as shown in Table 5. Particularly, binding poses of compound **1d** and compound **2e** were represented in Fig. 6.

According to the docking results, all the compounds interact with the similar residues, specifically as hydrophobic cavity residues (PHE212, PHE490, LEU489, ILE359, ALA291, TYR105, PHE217, LEU108, MET290, TYR119, PHE113) and polar (THR109, SER488, SER361, SER295) ones in the binding cavity. The interactions of compound **1d** and compound **2e** were demonstrated in Fig. 7, which are the best obtained poses in terms of binding energy. Moreover, when the best poses of compounds were superimposed, thiadiazole groups, which coordinate heme iron, were overlapped. Based on the same orientation of compounds with ketoconazole, our novel compounds may have a potential fungicidal effect.

Conclusions

In summary, a series of novel cylopentapyrazole bearing a 1,2,3-thiadiazole ring have been synthesized by a [3 + 2] cycloaddition reaction with 4-methyl-[1,2,3]thiadiazole-5-carboxylic acid hydrazones and cyclopentadiene with NTPA %10 mol as a catalyst then their

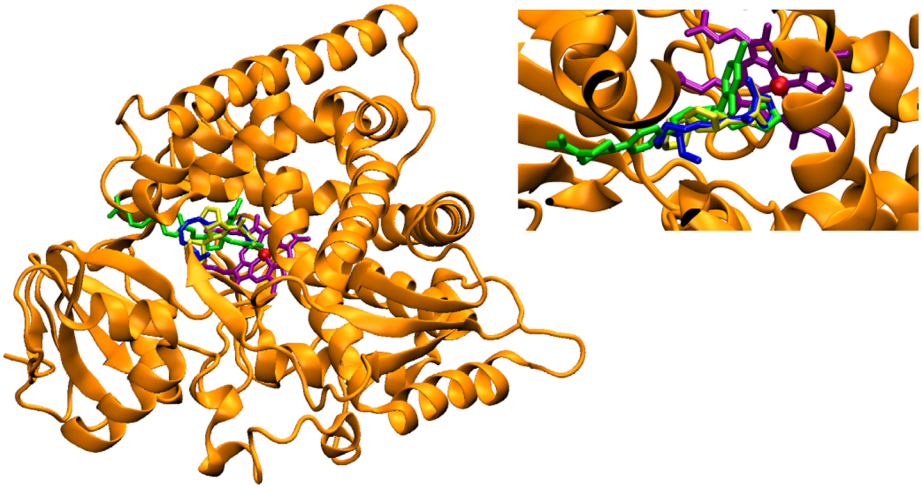


Fig. 6. Binding poses of ketoconazole (green), compound **1d** (blue), compound **2e** (yellow), heme (purple), and Fe^{3+} ion (red) in the binding cavity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

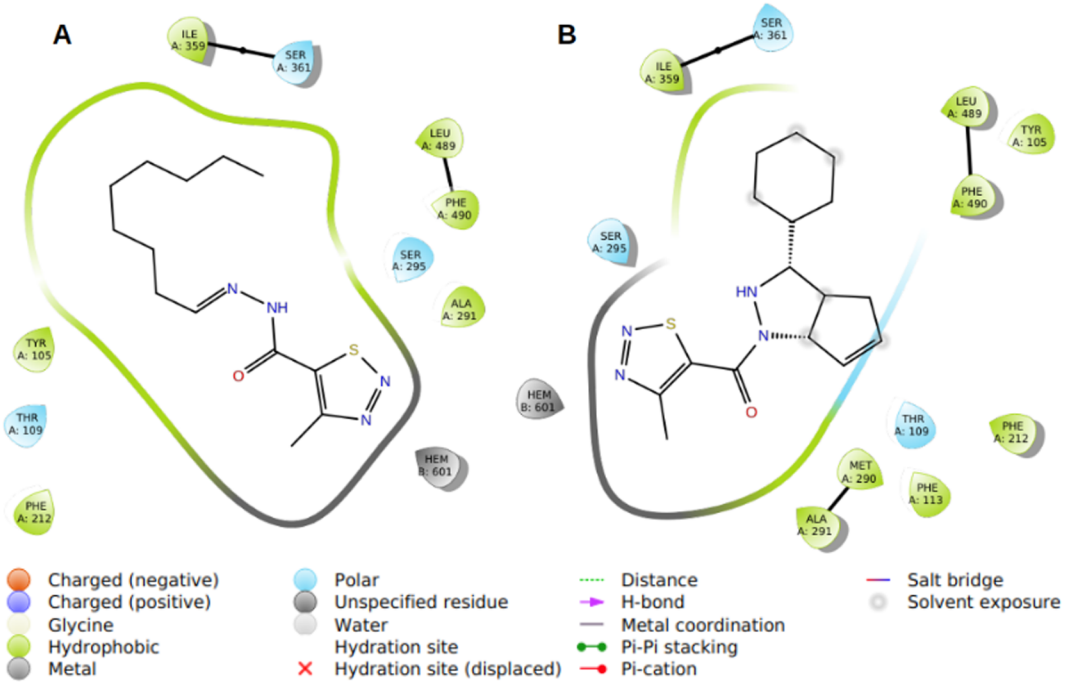


Fig. 7. The representation of the interactions between ligand and residues in the binding cavity from (A) compound **1d** and (B) compound **2e**.

antifungal activity was evaluated against some phytopathogenic fungi including *F. moniliforme, F. heterosporum, F. culmorum,* and *B. cinerea.* Tested trial combinations of these novel compounds (**1a + 2a, 1a + 2c and 1a + 2b)** showed promising antifungal activities particularly against *F. moniliforme* and *B. cinerea* with remarkable EC_{50} values. Moreover, molecular modeling studies also supported our results providing the interactions between our molecules and targeted protein. Therefore, *in silico* docking study also supports our experimental results and demonstrates that these compounds may have reasonable potential to display antifungal activity. In this study, the obtained results evidently may provide industrial advantages especially with combinations of the newly synthesized compounds that can be used as lead antifungal agents. Therefore, these results can help the agricultural economy in the world for the development of potential agrochemicals. More study needs to be conducted in order to demonstrate these class of compounds as anti-fungicide agents which will be reported in due course. Our ongoing efforts in order to increase the antifungal activity against these strains by developing more analogs will be highlighted in future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We sincerely thank Dr. Sukran Ozdatli Kurtulus for her invaluable assistance in biological assays, Melike Aybala Guzel for proofreading our manuscript.

Appendix A. Supplementary material

Experimental details, characterization data for all compounds and copies of ^{1}H NMR, ^{13}C NMR and MS spectra for all products were included in the supporting information. Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103509.

References

[1] B.-L. Wang, H.-W. Zhu, Y. Ma, et al., Synthesis, insecticidal activities, and SAR studies of novel pyridylpyrazole acid derivatives based on amide bridge modification of anthranilic diamide insecticides, J. Agric. Food Chem. 61 (23) (2013) 5483–5493, https://doi.org/10.1021/jf4012467.
[2] J. Wu, B.-A. Song, D.-Y. Hu, M. Yue, S. Yang, Design, synthesis and insecticidal activities of novel pyrazole amides containing hydrazone substructures, Pest Manag. Sci. 68 (5) (2012) 801–810, https://doi.org/10.1002/ps.2329.
[3] M. Li, C.-L. Liu, L. Li, et al., Design, synthesis and biological activities of new strobilurin derivatives containing substituted pyrazoles, Pest Manag. Sci. 66 (1) (2010) 107–112, https://doi.org/10.1002/ps.1837.
[4] T. Furuya, K. Machiya, S. Fujioka, M. Nakano, K. Inagaki, Development of a novel acaricide, pyflubumide, J. Pestic. Sci. 42 (3) (2017) 132–136, https://doi.org/10.1584/jpestics.J17-02.
[5] H. Song, Y. Liu, L. Xiong, Y. Li, N. Yang, Q. Wang, Design, synthesis, and insecticidal evaluation of new pyrazole derivatives containing imine, oxime ether, oxime ester, and dihydroisoxazoline groups based on the inhibitor binding pocket of respiratory complex I, J. Agric. Food Chem. 61 (37) (2013) 8730–8736, https://doi.org/10.1021/jf402719z.
[6] C.B. Vicentini, C. Romagnoli, E. Andreotti, D. Mares, Synthetic pyrazole derivatives as growth inhibitors of some phytopathogenic fungi, J. Agric. Food Chem. 55 (25) (2007) 10331–10338, https://doi.org/10.1021/jf072077d.
[7] Y. Li, H.-Q. Zhang, J. Liu, X.-P. Yang, Z.-J. Liu, Stereoselective synthesis and antifungal activities of (*E*)-α-(Methoxyimino)benzeneacetate derivatives containing 1,3,5-substituted pyrazole ring, J. Agric. Food Chem. 54 (10) (2006) 3636–3640, https://doi.org/10.1021/jf060074f.
[8] G. Ouyang, X.-J. Cai, Z. Chen, et al., Synthesis and antiviral activities of pyrazole derivatives containing an oxime moiety, J. Agric. Food Chem. 56 (21) (2008) 10160–10167, https://doi.org/10.1021/jf802489e.
[9] S.-R. Shih, T.-Y. Chu, G. Reddy, et al., Pyrazole compound BPR1P0034 with potent and selective anti-influenza virus activity, J. Biomed. Sci. 17 (1) (2010) 13, https://doi.org/10.1186/1423-0127-17-13.
[10] X. Liu, X. Huang, W. Lin, et al., New aromatic substituted pyrazoles as selective inhibitors of human adipocyte fatty acid-binding protein, Bioorg. Med. Chem. Lett. 21 (10) (2011) 2949–2952, https://doi.org/10.1016/J.BMCL.2011.03.063.
[11] P. Lan, Z.-J. Huang, J.-R. Sun, et al., 3D-QSAR and molecular docking studies on fused pyrazoles as p38α mitogen-activated protein kinase inhibitors, Int. J. Mol. Sci. 11 (9) (2010) 3357–3374, https://doi.org/10.3390/ijms11093357.
[12] P. Zhan, X. Liu, Y. Cao, Y. Wang, C. Pannecouque, E. De Clercq, 1,2,3-Thiadiazole thioacetanilides as a novel class of potent HIV-1 non-nucleoside reverse transcriptase inhibitors, Bioorg. Med. Chem. Lett. 18 (20) (2008) 5368–5371, https://doi.org/10.1016/J.BMCL.2008.09.055.
[13] Orazio A. Attanasi †, Lucia De Crescentini †, Gianfranco Favi †, et al. Expeditious synthesis of new 1,2,3-thiadiazoles and 1,2,3-selenadiazoles from 1,2-diaza-1,3-butadienes via Hurd−Mori-type reactions, 2003. doi: 10.1021/JO0264832.
[14] A.H. Mandour, T.H. El-Shihi, A.-L. Nehad, Z.E. El-bazza, Synthesis and biological evaluation of 1,2,3-thia and selenadiazoles-4-derivatives, Phosphor. Sulfur Silicon Relat Elem. 113 (1–4) (1996) 155–163, https://doi.org/10.1080/10426509608046386.

[15] Yufang Xu †, Zhengjiang Zhao †, Xuhong Qian *,†, Zhigang Qian ‡, Wenhong Tian † and, Jianjiang Zhong* ‡. Novel, unnatural benzo-1,2,3-thiadiazole-7-carboxylate elicitors of taxoid biosynthesis, 2006. doi: 10.1021/JF0618574.

[16] P. Stanetty, M. Kremslehner, H. Völlenkle, A new type of plant activator: synthesis of thieno[2,3-d][1,2,3]thiadiazole-6-carboxylic acid derivatives via Hurd-Mori cyclization, J. Chem. Soc. Perkin Trans. 1 (5) (1998) 853–856, https://doi.org/10.1039/a708375k.

[17] Z. Fan, X. Liu, F. Liu, L. Bao, Y. Zhang, Zhiwu-Baohu-Xuebao Jikan = Acta Phytophylacica Sinica, vol. 32, Zhongguo Zhiwu Baohu Xuehui (2005).

[18] Y.Y. Morzherin, T.V. Glukhareva, V.A. Bakulev, Rearrangements and transformations of 1,2,3-thiadiazoles in organic synthesis (Review), Chem. Heterocycl. Compd. 39 (6) (2003) 679–706, https://doi.org/10.1023/A: 1025689208261.

[19] M. Rueping, M.S. Maji, H.B. Küçük, I. Atodiresei, Asymmetric Brønsted acid catalyzed cycloadditions-efficient enantioselective synthesis of pyrazolidines, pyrazolines, and 1,3-diamines from *N*-acyl hyrazones and alkenes, Angew. Chem. Int. Ed. 51 (51) (2012) 12864–12868, https://doi.org/10.1002/anie.201205813.

[20] X. Hong, H.B. Küçük, M.S. Maji, Y.-F. Yang, M. Rueping, K.N. Houk, Mechanism and selectivity of *N*-triflylphosphoramide catalyzed (3^+ + 2) cycloaddition between hydrazones and alkenes, J. Am. Chem. Soc. 136 (39) (2014) 13769–13780, https://doi.org/10.1021/ja506660c.

[21] E. Frank, Z. Mucsi, I. Zupkó, et al., Efficient approach to androstene-fused arylpyrazolines as potent antiproliferative agents. experimental and theoretical studies of substituent effects on BF_3-catalyzed intramolecular [3 + 2] cycloadditions of olefinic phenylhydrazones, J. Am. Chem. Soc. 131 (11) (2009) 3894–3904, https://doi.org/10.1021/ja808636e.

[22] A. Zamfir, S. Schenker, W. Bauer, T. Clark, S.B. Tsogoeva, Silicon lewis acid catalyzed [3+2] cycloaddition reactions of hydrazones/cyclopentadiene: mild access to pyrazolidine derivatives, Europ. J. Org. Chem. 2011 (20–21) (2011) 3706–3709, https://doi.org/10.1002/ejoc.201100206.

[23] Seiji Shirakawa, Pamela J. Lombardi, J.L. Leighton*. A simple and general chiral silicon lewis acid for asymmetric synthesis: highly enantioselective [3 + 2] acylhydrazone − enol ether cycloadditions, 2005. doi: 10.1021/JA052307 + .

[24] H. Xie, J. Zhu, Z. Chen, S. Li, Y. Wu, Reaction of a trifluoromethylated N-monosubstituted hydrazone with α, β-ethenyl ketones: a novel synthesis of substituted pyrazolidines and pyrazolines, Synthesis (Stuttg) 2011 (17) (2011) 2767–2774, https://doi.org/10.1055/s-0030-1260127.

[25] C. Chen, L. Long, F. Zhang, et al., Antifungal activity, main active components and mechanism of Curcuma longa extract against Fusarium graminearum. Sarrocco S, ed, PLoS One 13 (3) (2018) e0194284, https://doi.org/10.1371/journal.pone.0194284.

[26] C. Booth, The genus Fusarium, Genus Fusarium (1971).

[27] M. Staats, P. van Baarlen, J.A.L. van Kan, Molecular phylogeny of the plant pathogenic genus botrytis and the evolution of host specificity, Mol. Biol. Evol. 22 (2) (2004) 333–346, https://doi.org/10.1093/molbev/msi020.

[28] Universitatea de Stiinte Agronomice si Medicină Veterinară Bucuresti, S. Facultatea de Biotehnologii, M. Butu, P. Petrache, A. Butu, C.P. Cornea, Scientific Bulletin. Series F, Biotechnologies, vol. 18. University of Agricultural Sciences and Veterinary Medicine, Faculty of Biotechnology, 2014.

[29] L. He, Y. Liu, A. Mustapha, M. Lin, Antifungal activity of zinc oxide nanoparticles against Botrytis cinerea and Penicillium expansum, Microbiol. Res. 166 (3) (2011) 207–215, https://doi.org/10.1016/J.MICRES.2010.03.003.

[30] W.-L. Dong, Z.-X. Liu, X.-H. Liu, Z.-M. Li, W.-G. Zhao, Synthesis and antiviral activity of new acrylamide derivatives containing 1,2,3-thiadiazole as inhibitors of hepatitis B virus replication, Eur. J. Med. Chem. 45 (5) (2010) 1919–1926, https://doi.org/10.1016/J.EJMECH.2010.01.032.

[31] J. Mu, Z. Zhai, C. Tan, et al., Synthesis and herbicidal activity of 1,2,4-triazole derivatives containing a pyrazole moiety, J. Heterocycl. Chem. 56 (3) (2019) 968–971, https://doi.org/10.1002/jhet.3476.

[32] J. Barrot, B. Elissalde, G. Roques, C. Descamps Impr., *Europe, Europes: Espaces En Recomposition*, Vuibert, 1995.

[33] H.B. Küçük, Practical synthesis of 2,5-disubstituted 1,3-dioxolane-4-ones and highly diastereoselective cis-2,5-disubstituted 1,3-dioxolane-4-ones from α-hydroxy acids catalyzed by N-triflylphosphoramide, Tetrahedron Lett. 56 (41) (2015) 5583–5586, https://doi.org/10.1016/J.TETLET.2015.08.046.

[34] T. Yıldız, H.B. Küçük, An organocatalytic method for the synthesis of some novel xanthene derivatives by the intramolecular Friedel-Crafts reaction, RSC Adv. 7 (27) (2017) 16644–16649, https://doi.org/10.1039/C6RA27094H.

[35] H.B. Küçük, B. Giray, A.E. Karadag, O.S. Ipek, N. Guzel, Original Heteroaryl-pyrazole derivative molecules and their uses as antifungal agents 2019/06570.

[36] J. Zhang, L. Li, Q. Lv, L. Yan, Y. Wang, Y. Jiang, The fungal CYP51s: their functions, structures, related drug resistance, and inhibitors, Front. Microbiol. 10 (April) (2019), https://doi.org/10.3389/fmicb.2019.00691.

[37] N. Strushkevich, S.A. Usanov, H.W. Park, Structural basis of human CYP51 inhibition by antifungal azoles, J. Mol. Biol. 397 (4) (2010) 1067–1078, https://doi.org/10.1016/j.jmb.2010.01.075.

[38] A. Debnath, C.M. Calvet, G. Jennings, et al., CYP51 is an essential drug target for the treatment of primary amoebic meningoencephalitis (PAM), PLoS Negl. Trop Dis. 11 (12) (2017) e0006104, , https://doi.org/10.1371/journal.pntd.0006104.

[39] A.A. Sagatova, M.V. Keniya, R.K. Wilson, B.C. Monk, J.D.A. Tyndall, Structural insights into binding of the antifungal drug fluconazole to Saccharomyces cerevisiae lanosterol 14α-demethylase, Antimicrob. Agents Chemother. 59 (8) (2015) 4982–4989, https://doi.org/10.1128/AAC.00925-15.

[40] A. Waterhouse, M. Bertoni, S. Bienert, et al., SWISS-MODEL: homology modelling of protein structures and complexes, Nucl. Acids Res. 46 (W1) (2018) W296–W303, https://doi.org/10.1093/nar/gky427.

[41] L. Friggeri, T.Y. Hargrove, Z. Wawrzak, et al., Sterol 14α-demethylase structure-based design of VNI ((R)- N-(1-(2,4-dichlorophenyl)-2-(1 H-imidazol-1-yl)ethyl)-4-(5-phenyl-1,3,4-oxadiazol-2-yl)benzamide)) derivatives to target fungal infections: synthesis, biological evaluation, and crystallographic analysis, J. Med. Chem. 61 (13) (2018) 5679–5691, https://doi.org/10.1021/acs.jmedchem.8b00641.

[42] F. Madeira, Y.M. Park, J. Lee, et al., The EMBL-EBI search and sequence analysis tools APIs in 2019, Nucl. Acids Res. 47 (W1) (2019) W636–W641, https://doi.org/10.1093/nar/gkz268.

[43] O. Trott, A. Olson, Autodock vina: improving the speed and accuracy of docking, J. Comput. Chem. 31 (2) (2010) 455–461, https://doi.org/10.1002/jcc.21334. AutoDock.

[44] T.Y. Hargrove, K. Kim, M. de Nazaré Correia Soeiro, et al., CYP51 structures and structure-based development of novel, pathogen-specific inhibitory scaffolds, Int. J. Parasitol. Drugs Drug Resist. 2 (2012) 178–186, https://doi.org/10.1016/j.ijpddr.2012.06.001.

[45] Schrödinger. Maestro | Schrödinger, Schrödinger Release 2018-1, 2018.

[46] K.S. Watts, P. Dalal, R.B. Murphy, W. Sherman, R.A. Friesner, J.C. Shelley, ConfGen: a conformational search method for efficient generation of bioactive conformers, J. Chem. Inf. Model 50 (4) (2010) 534–546, https://doi.org/10.1021/ci100015j.

[47] G.M. Morris, R. Huey, W. Lindstrom, et al., Reference-36 docking simulation.pdf, J. Comput. Chem. 16 (2009) 2785–2791, https://doi.org/10.1002/jcc.21256.