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VARIOUS TECHNOLOGICAL PROCESSES

Green Synthesis of Thiazol-2-ylthiazolidin-4-ones as Potential Antifungals[1]

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Abstract—MORE (Microwave oriented reaction enhancement) green methodology was utilized to synthesize benzothiazol/thiazol-2-ylthiazolidin-4-ones and relatively assayed for their antifungal activity *w.r.t* precursors against three agriculturally important phytopathogenic fungi *viz. Colletotrichum falcatum, Pyricularia grisea* and *Ustilago hordei.* Thiazol-2-ylthiazolidin-4-ones displayed better inhibition of growth of fungi than their precursor's thiazol-2-amines, with some compounds showing results comparable to their standard fungicides endorsing the effectiveness of chemical hybridization of thiazoles/benzothiazoles with thiazolidin-4-ones in a single molecule. *In silico* toxicity of all the compounds was found to be equivalent to the standard fungicides. Lipinski parameters were used to rationalize structure activity relation using statistical analysis software.

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INTRODUCTION

Inspite of the introduction of a variety of pesticidal agents, in multiple unrelated chemical classes, resistance continues to emerge [1]. So, it is imperative to find novel chemical solution to eradicate this fungal menace especially at the time of emergency. Sulphur-nitrogen heterocycles *viz.* thiazoles, benzothiazoles, isothiazole, thiadiazoles groups are of special attention with the view that they belong to the category of low or unknown resistance behaviour and

they are among the category of low risk pesticides (FRAC code list [2]). 2-substituted-1,3-thiazoles among them are an important scaffolds of biological interest and have number of characteristic pharmacological features which enhances bioactivity profile, such as, relative stability, their ease to use as starting materials, built in biocidal unit, enhanced lipid solubility with hydrophilicity and easy metabolism [3]. They, exclusively, inflict many biological activities like anti-inflammatory [4], anticancer [5], analgesic [6], anti-HIV [7], antitumor [8] along with anti-fungal [9], pesticidal [10], and antiprotozoal [11]. Change of the structure of substituent group at C-2 position commonly resulted in the change of its bioactivity [12]. Combination of thiazoles with other heterocyclics is a well-known approach to design new drug like molecules which allows achieving new pharmacological profile with different mode of action and lowered toxicity [13].

Thiazolidin-4-one nucleus exhibits diverse pharmacological activities like antifungal [10], antituberculor [14], anti-HIV [15], analgesic, anti-inflammatory [16], ulcerogenic activity, etc. These observations served as an impetus for the extension of investigation in the field of synthesis of 4-thiazolidinone derivatives in the hope of discovering compounds with good pharmacological properties.

In continuation with our work to find novel hybrid molecules with higher antifungal potential [17], the present study involves them microwave oriented synthesis of thiazol/benzothiazol-2-ylthiazolidin-4-ones [18, 19] and to evaluate *in vitro* antifungal potential against various phytopathogenic fungi *viz. C. falcatum, P. grisea* and *U. hordei*. The compounds were checked for their *in silico* toxicity using Toxtree software programme. Drug like indexes *viz.* Lipinski parameters [20] were evaluated to establish structure-activity relationship (SAR) of the synthesized compounds using SAS statistical analysis programme.

[1] The text was submitted by the authors in English.

MATERIAL AND METHODS

Chemistry

Melting points were determined on electrical melting point apparatus and are uncorrected. The products were splendidly purified and the purity of all the compounds was checked on a silica gel-G plates and visualization was done using iodine lamp. All solvents and reagents were purchased from Sigma-Aldrich Company. Solvents used were of analytical reagent grade and purified by simple distillation. Elemental analyses were done on Vario EL, CHNOS elemental analyzer. The IR spectra were recorded on a Perkin Elmer FT-IR spectrometer using KBr disc. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance II 400 MHz spectrometer in $CDCl_3$ and DMSO-d_6 using TMS as an internal standard. Mass spectra were recorded on Perkin Elmer Clarus 500 Mass Spectrometer. The microwave assisted procedures were carried out in domestic microwave at 180 W.

General method for the synthesis of 1,3-benzothiazol-2-amines (1-3). Arylamine (0.1 mol) and potassium thiocyanate (0.4 mol) were dissolved in glacial acetic acid (40 mL) and cooled. Bromine (0.04 mol) mixed with glacial acetic acid (24 mL) was added from dropping funnel at such a rate that temperature does not rise beyond 5–6°C. After all bromine had been added, the solution was stirred with glass rod for an additional 2 h at low temperature. The mixture was allowed to stand overnight, during which period orange-yellow precipitates get settled at the bottom. Water was added and slurry formed was heated at 850°C on steam bath for 20 min and filtered hot. The filtrate was cooled and neutralised with ammonia solution to pH 6. Yellow precipitates obtained were collected, washed with water and recrystallized from benzene to get crystals of 1,3-benzothiazol-2-amine and its derivatives **1-3** [21, 22]**.**

1,3-Benzothiazol-2-amine (1). Orange red crystals; IR (KBr): 3430, 3100, 3010, 1350, 900 cm^{-1}; ^{1}H NMR (DMSO, 400 MHz) δ, ppm: 5.3 (2H, s, NH_2), 6.6–7.3 (4H, m, Ar C–H). ^{13}C NMR (DMSO, 400 MHz) δ, ppm: 166.3, 130.8, 153.2, 121.8, 118.3, 124.5, 125.3.

6-Fluoro-1,3-benzothiazol-2-amine (2). Off white crystals; IR (KBr), cm^{-1}: 3452, 3080, 3010, 1350, 1215, 900; ^{1}H NMR (DMSO, 400 MHz) δ, ppm: 5.4 (2H, s, NH_2), 6.5–7.4 (3H, m, Ar C–H). ^{13}C NMR (DMSO, 400 MHz) δ, ppm: 166.3, 131.6, 148.8, 158.5, 108.0, 117.8, 113.9.

6-Nitro-1,3-benzothiazol-2-amine 3. Yellow crystals; IR (KBr), cm^{-1}: 3460, 3100, 3010, 1558, 1350, 900; ^{1}H NMR (DMSO, 400 MHz) δ, ppm: 5.7 (2H, s, NH_2), 7.2–8.1 (3H, m, Ar C–H). ^{13}C NMR (DMSO, 400 MHz) δ, ppm: 166.3, 131.3, 159.3, 144.3, 119.1, 117.3, 121.3.

General procedure for the synthesis of 4-arylthiazol-2-amines (4–6). Thiourea (0.2 mol) and iodine (0.2 mol) were triturated and mixed with acetophenone/its derivatives (0.1 mol). The mixture was heated on a water bath, with constant shaking, for 8 h. The solid obtained was triturated with diethyl ether to remove unreacted acetophenone. Excess of ether was distilled off. The crude product was dissolved in hot water and filtered to remove sulphone formed during the course of reaction. The product was precipitated by adding ammonia solution to the filtrate. The precipitates were washed with water and crystallized from methanol to obtain 4-phenylthiazol-2-amine and its derivatives **4-6** [23, 24].

4-Phenylthiazol-2-amine (4). Off white crystals; IR (KBr), cm^{-1}: 3420, 3240, 1500, 1460, 715; ^{1}H NMR (DMSO, 400 MHz) δ, ppm: 6.74 (1H, s, NH_2), 6.75 (1H, s, thiazole C–H), 7.2–7.8 (5H, m, Ar C–H). ^{13}C NMR (DMSO, 400 MHz) δ, ppm: 168.8, 101.9, 150.2, 133.0, 127.5, 127.5, 129.2, 129.2, 128.7.

4-(4-Chlorophenyl)thiazol-2-amine (5). Off white crystals; IR (KBr), cm^{-1}: 3392, 3276, 1473, 1400, 731, 667; ^{1}H NMR (DMSO, 400 MHz): δ 6.71 (1H, s, NH_2), δ 6.74 (1H, s, thiazole C–H), δ 7.4–7.9 (4H, m, Ar C–H). ^{13}C NMR (DMSO, 400 MHz): 168.8, 101.9, 150.2, 134.3, 131.1, 129.3, 128.9, 129.3, 128.9.

4-*p*-Tolylthiazol-2-amine (6). Light yellow crystals; IR (KBr), cm^{-1}: 3405, 3245, 1500, 1460, 717; ^{1}H NMR (DMSO, 400 MHz) δ, ppm: 6.74 (1H, s, NH_2), 6.75 (1H, s, thiazole C–H), 7.2–7.6 (4H, m, Ar C–H), 2.34 (3H, s, $–CH_3$). ^{13}C NMR (DMSO, 400 MHz) δ, ppm: 168.8, 101.9, 150.2, 130.0, 131.7, 125.7, 129.5, 125.7, 129.5, 21.3.

General procedure for the microwave oriented synthesis of thiazol-2-ylthiazolidinones (1a-6a). Benzothiazol/thiazol-2-amines **(1–6)** (0.01 mol) and vanillin (0.01 mol) were dissolved in minimum amount of ethanol. The reaction mixture was subjected to microwave irradiation at 180 W for 5 min. The formation of Schiff base as intermediate was checked by TLC at regular intervals. The mercaptoacetic acid (0.02 mol) was added in same beaker after the formation of Schiff base. The reaction mixture was again irradiated at 180 W for 5 min. Completion of reaction was checked by using TLC with hexane:ethyl acetate (1 : 1) solvent system. After completion, the solvent was evaporated

and the crude product was digested with sodium bicarbonate solution. The product was crystallised using methanol to afford pure 3-(benzo[d]thiazol-2-yl)-2-(3-hydroxy-4-methoxyphenyl)thiazolidin-4-ones **1a–3a** and 2-(3-hydroxy-4-methoxyphenyl)-3-(4-phenylthiazol-2-yl)thiazolidines **4a–6a.**

3-(Benzo[*d*]thiazol-2-yl)-2-(3-hydroxy-4-methoxyphenyl)thiazolidin-4-one (1a). Off white crystals; IR (KBr), cm^{-1}: 3446, 2815, 1650, 1575, 1500, 1460, 1026. ^1H NMR (DMSO, 400 MHz) δ, ppm: 5.0 (1H, s, Ar–CH), 3.1 (2H, d.d, S-CH_2), 3.4 (3H, s, –OCH_3), 6.3–7.9 (7H, m, Ar C–H), 9.0 (1H, s, –OH).^{13}C NMR (DMSO, 400 MHz) δ, ppm: 164.6, 65.6, 130.8, 33.5, 171.2, 149.2, 148.5, 147.0, 121.8, 118.3, 132.9, 115.4, 112.7, 122.3, 124.5, 125.3, 56.1.

3-(5-Fluorobenzo[*d*]thiazol-2-yl)-2-(3-hydroxy-4-methoxyphenyl)thiazolidin-4-one (2a) Off white crystals; IR (KBr), cm^{-1}: 3450, 2817, 1649, 1575, 1500, 1460, 1262, 1026; ^1H NMR (DMSO, 400 MHz) δ, ppm: 5.2 (1H, s, Ar–CH), 3.2 (2H, d.d, S-CH_2), 3.36 (3H, s, –OCH_3), 6.7–8.0 (6H, m, Ar C–H), 9.1 (1H, s, –OH). ^{13}C NMR (DMSO, 400 MHz) δ, ppm: 164.6, 65.6, 26.4, 33.5, 171.2, 150.6, 157.8, 148.5, 147.0, 123.4, 109.1, 132.9, 114.6, 115.4, 112.7, 122.3, 56.1.

2-(3-Hydroxy-4-methoxyphenyl)-3-(5-nitrobenzo-[*d*]thiazol-2-yl)thiazolidin-4-one (3a). Yellow crystals; IR, cm^{-1} (KBr): 3460, 3026, 1745, 1575, 1565, 1500, 1460, 1027; ^1H NMR (DMSO, 400 MHz) δ, ppm: 5.7 (1H, s, Ar-CH), 3.3 (2H, d.d, S-CH_2), 3.45 (3H, s, –OCH_3), 7.3–8.7 (6H, m, Ar C–H), 9.1 (s, –OH). ^{13}C NMR (DMSO, 400 MHz): 164.6, 65.6, 136.9, 33.5, 171.1, 149.9, 148.5, 147.0, 146.2, 122.7, 117.3, 132.9, 115.4, 112.7, 119.2, 122.3, 56.1.

2-(3-Hydroxy-4-methoxyphenyl)-3-(4-phenylthiazol-2-yl)thiazolidin-4-one (4a). Light yellow crystals; IR (KBr), cm^{-1}: 3430, 1717, 1575, 1500, 1460, 1045; ^1H NMR (DMSO, 400 MHz) δ, ppm: 5.5 (1H, s, Ar–CH), 3.0 (2H, d.d, S-CH_2), 3.25 (3H, s, –OCH_3), 6.7–7.8 (9H, m, Ar C–H), 9.04 (1H, s, –OH). ^{13}C NMR (DMSO, 400 MHz) δ, ppm: 160.3, 65.6, 105.0, 33.5, 171.2, 150.2, 148.5, 147.0, 132.9, 133.0, 115.4, 112.7, 122.3, 127.5, 127.5, 129.2, 129.2, 128.7, 56.1.

3-(4-(4-Chlorophenyl)thiazol-2-yl)-2-(3-hydroxy-4-methoxyphenyl)thiazolidin-4-one (5a). White crystals; IR (KBr), cm^{-1} : 3435, 1720, 1560, 1445, 1405, 1045, 670; ^1H NMR (DMSO, 400 MHz) δ, ppm: 5.7 (1H, s, Ar–CH), 3.15 (2H, d.d, S-CH_2), 3.3 (3H, s, –OCH_3), 6.7–7.9 (8H, m, Ar C–H), 9.0 (1H, s, –OH). ^{13}C NMR (DMSO, 400 MHz) δ, ppm: 160.3, 65.6, 105.0, 33.5, 171.2, 150.2, 134.3, 148.5, 147.0, 132.9, 131.1, 129.3, 115.4, 112.7, 122.3, 128.9, 129.3, 128.9, 56.1.

2-(3-Hydroxy-4-methoxyphenyl)-3-(4-*p*-tolyl-thiazol-2-yl)thiazolidin-4-one (6a). Light yellow crystals; IR (KBr), cm^{-1}: 3437, 1719, 1575, 1500, 1460, 1045; ^1H NMR (DMSO, 400 MHz) δ, ppm: 5.3 (1H, s, Ar–CH), 3.0 (2H, d.d, S-CH_2), 3.2 (3H, s, –OCH_3), 6.7–7.4 (8H, m, Ar C–H), δ 8.8 (1H, s, –OH), δ 2.35 (3H, s, –CH_3). 13C NMR (DMSO, 400 MHz): 160.3, 65.6, 105.0, 33.5, 171.2, 150.2, 148.5, 147.0, 132.9, 130.0, 131.7, 115.4, 112.7, 122.3, 125.7, 129.5, 125.7, 129.5, 56.1, 21.3.

Antifungal Evaluation

The *in vitro* antifungal activity of the compounds were performed by Poisoned food technique [25, 26] against two phytopathogenic fungi *viz C. falcatum* and *P. grisea* and by Spore germination inhibition technique [27] against *U. hordei* in comparison with the standard fungicides Bavistin 50 WP (methyl benzimidazol-2-ylcarbamate), Raxil [(RS)-1-(4-Chlorophenyl)-4,4-dimethyl-3-(1*H*,1,2,4-triazol-1-ylmethyl)pentan-3-ol] and Tilt{1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1*H*-1,2,4-triazole}. The isolates of phytopathogenic fungi were provided by the Plant Pathology Department of the Punjab Agricultural University and the standards Bavistin, Tilt, and Raxil which served as the positive control were obtained from their respective manufacturers.

Stock solutions. Stock solution of the test compounds and standard fungicide were prepared by dissolving each chemical (20 mg) in 1 mL of Tween 20 (Polyoxyethylenesorbitan) volume was made to 10 mL with sterilized distilled water. Stock solutions of 2000 μg mL^{-1}, were kept in refrigerator till further use. Serial dilutions were done to 1000, 500, 250, 100, 50, 25, and 10 μg mL^{-1}, respectively.

Poisoned food technique. Antifungal activity of test compounds against Phytopathogenic fungi *C. falcatum* and *P. grisea* was tested by means of poison food technique. PDA (Potato dextrose agar) media (99 mL) was taken in the round bottom flasks, each compound (1 mL, different concentrations) was added to different flasks and the contents were mixed thoroughly. The

contents of the flask were poured aseptically into the petriplates. Test compound was, however, replaced by an equal amount of tween 20 only in the control set. After the media solidified, one inoculum disc of mycelium of the test fungus was aseptically placed/inoculated to each petriplate and incubated at 24 ± 1°C. The average diameter of fungal colonies was measured on the 7th day after inoculation. EC_{50} values were calculated by using PDO software programme.

For *P. grisea* CDA media was taken instead of PDA and same procedure was followed [28].

Spore germination inhibition method. Antifungal activity of test compounds against phytopathogenic fungus, *U. hordei* was tested by means of Spore germination inhibition technique. The spores of *U. hordei* were taken and used as such without culturing for testing. The spore suspension was made by addition of sterilized distilled water in the infected smuts of barley. The suspension was filtered through three layers of sterilized cheese cloth under aseptic conditions in order to remove agar bits and mycelium. Haemocytometer was used to get spore suspension (1×10^6 spore mL^{-1}). Screening of the test compounds against *U. hordei* involved floating of fungal spores on the surface of test solution in cavity slides. Small droplets (0.02 mL) of the test solution and spore suspension in equal amount were seeded in the cavity slides. These slides were kept in petriplates lined with moist filter paper and incubated for 72 h at 25 ± 1°C. The slides were checked for germination and per cent spore germination inhibition was determined from which EC_{50} values were calculated [29].

In Silico Analysis

In silico molecular modification was the most important preliminary step in the molecular designing of novel biomolecules. In the present study different proposed derivatives were screened for different biological and physicochemical properties by using different software.

Toxicity analysis. Toxtree v2.6.6 is an open-source software application that places chemicals into categories and predicts various kinds of toxic effect by applying various decision tree approaches. Toxtree was developed by IdeaConsult Ltd. (Sofia, Bulgaria) under the terms of an ECB contract. The software is made freely available by ECB as a service to scientific researchers and anyone with an interest in the application of computer-based estimation methods in the assessment of chemical toxicity. The new module with the revised list of SAS includes also structure–activity relationships (SAR) models that enable the toxicity evaluations for a number of chemical classes to be fine-tuned.

In order to find out the toxic hazards of all the synthesized compounds, two dimensional models of the compounds were first converted into its simplified molecular-input line-entry system

(SMILES format). Then simply putting the SMILES code into the Chemical identifier row available in the Toxtree software we can easily get the toxic characters.

Lipinski parameter. Molinspiration, a web based software, was used to obtain parameters of Lipinski's parameters. These parameters were formulated by Christopher A. Lipinski in 1997 [30]. This analysis involved two computer assisted steps: (a) To draw geometrically optimized structure of the molecule followed by its conversion into SMILES (Simplified Molecular-Input Line-Entry System), (b) calculation of molecular descriptors. The structures of the molecules were drawn by ChemOffice 2006 and saved as .mol file formats. The .mol file formats were then converted into SMILES format using Online SMILES Translator and Structure File Generator.

The various drugs like descriptors chosen are log *P*, where *P* is the partition coefficient in *n*-octanol/water system. It reflects the overall lipophilicity of a molecule, a property of major importance in biochemical applications. The molecular weight, that defines the effect of size of the molecule on its biological activity, the topological polar surface area (TPSA), another physiochemical property describing the polarity of the molecules that gives the correlation with passive molecular transport through membranes [31]. The number of hydrogen donors and hydrogen acceptors helps in determines the upper limits for the biomolecules to be able to penetrate through biomembranes.

Statistical Analysis

The SAS 9.3.1 statistical software was used for analysis of the results recorded for antifungal evaluation. The results recorded in triplicates were subjected to one way analysis of variance (ANOVA) followed by post hoc Duncan's test to confirm its effective demarcation

from control set. The paired student's test was applied to compare the parent compounds with their thiazolidinone derivatives. The correlation analysis was performed to confirm a relationship between the calculated Lipinski parameters and the resultant EC_{50} values.

The relationship between EC_{50} values and Lipinski parameters was assessed using a dynamic regression model. Multiple regression analysis involving finding the best fit of a dependent variable (antifungal activity, EC_{50}) to a linear combination of independent variables (molecular descriptors) by the least square method was used. This is formally expressed as follows:

$$Y = a_0 + a_1x_1 + a_2x_2 + a_2x_2 + a_4x_4 \ldots + a_nx_n,$$

where, Y is related to antifungal activity of a compound, $x_1, x_2, x_3 \ldots, x_n$ are the molecular descriptor values related to the compounds and $a_0, a_1, a_2, a_3, \ldots, a_n$ are the regression coefficients determined by the least square analysis. This equation is developed for each compound in the QSAR study. $P < 0.05$, i.e., statistical significance at 5% level of significance was chosen as criterion for compilation of all the results.

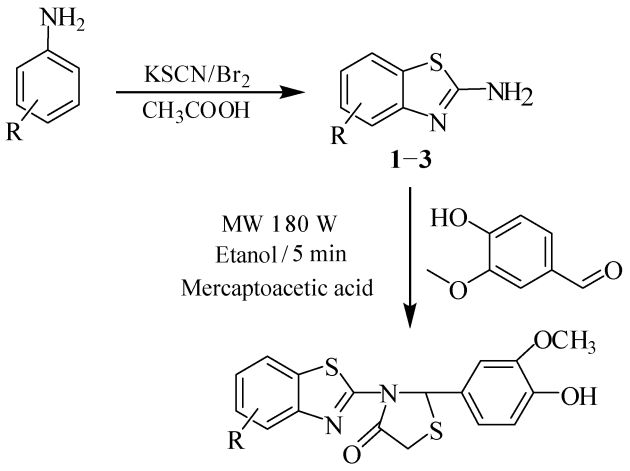
RESULTS AND DISCUSSION

Chemistry

The compounds described in this paper were synthesized by the multistep reaction protocol. The synthetic strategies adopted for the synthesis of 2-substituted benzo fused thiazoles *viz.* 1,3-benzothiazol-2-amines **(1–3)** and 3-(benzo[d]thiazol-2-yl)-2-phenylthiazolidin-4-ones **(1a–3a)** are given in Scheme 1 and the synthetic route followed for the synthesis of 2- arylthiazoles *viz.* 4-arylthiazol-2-amines **(4–6)** and 2-phenyl-3-(4-arylthiazol-2-yl)thiazolidin-4-ones **(4a–6a)** are given in Scheme 2**.** The Microwave procedure adopted for the synthesis of thiazol-2yl thiazolidin-4-ones reduced the long reaction hours taken in conventional refluxing methods to just few minutes [13].

Purity of the compounds was checked by TLC and melting points were determined. The structural assignments of the synthesized compounds were based on their elemental analysis and spectral studies *viz.* IR, NMR and Mass spectrometry. The physical characteristics, mass spectrometric peaks and elemental analysis of synthesized thiazol-2-amines **(1–6)** and thiazol-2-ylthiazolidin-4-ones **(1a–6a)** are outlined in Table 1.

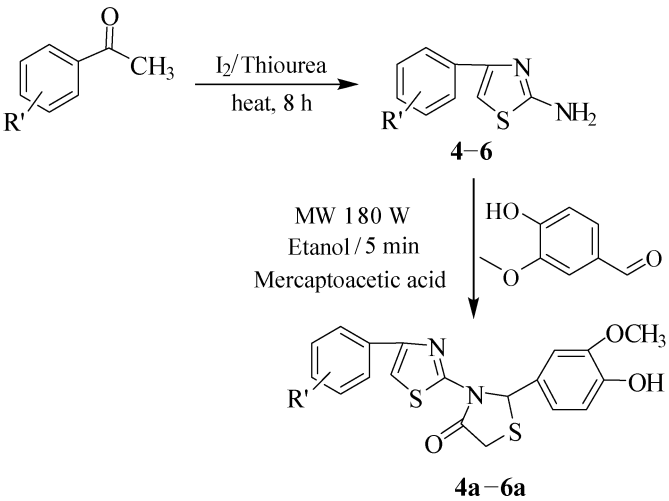
Scheme 1.



1a–3a

Compd. no.	–R
1	–H
2	–F
3	$–NO_2$
1a	–H
2a	–F
3a	$–NO_2$

Scheme 2.



4a–6a

Compd. no.	–R
4	–H
5	–Cl
6	$–CH_3$
4a	–H
5a	–Cl
6a	$–CH_3$

Table 1. Physical characteristics, elemental analysis, and molecular formula of thiazoles **1–6** and thiazolidinones **1a–6a**

Compd. no.	mp[a], °C	Yield, %	Elemental analysis calculated (found), %			Molecular formula
			C	H	N	
1	130	55	55.97 (55.89)	4.03 (4.02)	18.65 (18.74)	$C_7H_6N_2S$
2	185	84	49.99 (49.95)	3.00 (3.04)	16.66 (16.61)	$C_7H_5FN_2S$
3	165	74	43.07 (43.01)	2.58 (2.64)	21.53 (21.55)	$C_7H_5N_3O_2S$
4	147	88	61.34 (61.42)	4.58 (4.55)	15.90 (15.93)	$C_9H_8N_2S$
5	169	58	51.31 (51.35)	3.35 (3.30)	13.30 (13.34)	$C_9H_7N_2SCl$
6	134	52	63.13 (63.03)	5.30 (5.34)	14.72 (14.78)	$C_{10}H_{10}N_2S$
1a	150	72	56.96 (56.94)	3.94 (3.98)	7.82 (7.81)	$C_{17}H_{14}N_2O_3S_2$
2a	170	60	54.24 (54.22)	3.48 (3.46)	7.44 (7.40)	$C_{17}H_{13}FN_2O_3S_2$
3a	160	75	50.61 (50.63)	3.25 (3.20)	10.42 (10.35)	$C_{17}H_{13}N_3O_5S_2$
4a	145	66	59.35 (59.19)	4.19 (4.25)	7.29 (7.18)	$C_{19}H_{16}N_2O_3S_2$
5a	175	55	54.47 (54.26)	3.61 (3.54)	6.69 (6.63)	$C_{19}H_{15}FN_2O_3S_2$
6a	160	70	60.28 (60.14)	4.55 (4.63)	7.03 (7.12)	$C_{20}H_{18}N_2O_3S_2$

[a] The mp were determined on electric melting point apparatus and are uncorrected.

Table 2. Antifungal potential of 2-substituted thiazoles

Compd. no.	EC_{50} values, μmol mL^{-1}			log *P*	Molecular weight	TPSA	H donors no.	H acceptors no.
	C. falcatum	*P. grisea*	*U. hordei*					
1	1.33	1.16	1.06	1.98	150.03	38.91	2	2
2	0.54	1.96	1.25	2.12	168.02	38.91	2	2
3	1.69	1.28	1.28	1.91	195.01	84.74	5	2
4	0.99	1.13	0.97	2.15	176.04	38.91	2	2
5	0.95	1.52	1.21	2.83	210.00	38.91	2	2
6	1.34	1.57	1.47	2.60	190.06	38.91	2	2
1a	0.50	0.45	0.56	3.21	358.04	62.66	5	1
2a	0.48	0.95	0.67	3.35	376.04	62.66	5	1
3a	0.99	0.74	0.78	3.15	403.03	108.49	8	1
4a	0.39	0.46	0.47	3.38	384.06	62.66	5	1
5a	0.61	0.52	0.54	4.06	418.02	62.66	5	1
6a	0.65	0.63	0.64	3.83	398.08	62.66	5	1
Bavistin[a]	0.392	–	–	1.46	191.19	67.02	5	2
Raxil[a]	–	–	0.08	3.59	307.82	50.95	4	1
Tilt[a]	–	0.146	–	3.64	342.43	49.19	5	0

[a] Standard fungicide against *C. falcatum, P. grisea*and *U. hordei*

Antifungal Assays

All the synthesized compounds **1–6** and **1a–6a** were screened for their *in vitro* antifungal potential against various phytopathogenic fungi and EC_{50} values were calculated (Table 2). These data are the mean of three replicate tests performed with each antifungal compound. The results of antifungal evaluation of the synthesized compounds along with standard fungicides used *viz.* Bavistin, Raxil and Tilt against various phytopathogenic fungi, are reported in Table 2.

For the sake of comparison of fungitoxicity of the molecules with standard fungicides, it was appropriate to express the results in terms of mM. Investigation of antifungal screening revealed that most of the synthesized compounds showed promising inhibition of germination against all the test fungi.

All the synthesized compounds had shown EC_{50} values of less than 1.96 mM against all the test fungi. Against *C. falcatum,* the EC_{50} value of all the test compounds were found to be less than 1.69 M. Thiazol-2-amines **1–6** were found to be less potent than thiazol-2-ylthiazolidin-4-ones **1a–6a**. 2-(3-hydroxy-4-methoxyphenyl)-3-(4-phenylthiazol-2-yl)thiazolidin-4-one **(4a)** inflicted appreciable fungitoxicity with EC_{50} of 0.39 mM which is comparable to standard fungicide Bavistin (EC_{50} value 0.23 mM). This was followed by compounds **2a** and **1a** showing moderate potential with EC_{50} values of 0.48 mM and 0.50 mM, respectively.

Against *P. grisea,* similar trend of greater potential of thiazol-2ylthiazolidin-4-one derivatives was observed. The unsubstituted thiazol-2-amines and thiazol-2-ylthiazolidin-4-ones**1a** and **4a** were found to be more effective than their substituted derivatives. The most potent among the series was 3-(benzo[d]thiazol-2-yl)-2-(3-hydroxy-4-methoxyphenyl)thiazolidin-4-one (**1a**) with the EC_{50} value of 0.45 mM followed by 2-(3-hydroxy-4-methoxyphenyl)-3-(4-phenylthiazol-2-yl)thiazolidin-4-one (**4a**) with EC_{50} value of 0.46 mM.

Against *U. hordei,* synthesized compounds were found to be moderately effective with EC_{50} values of less than 1.47 mM. Compound **4a** was found to be most potent with the EC_{50} value of 0.47 mM followed by compounds **5a** and **1a** with EC_{50} value of 0.52 and 0.56 mM.

The overall results indicated that most of the hybrids revealed fungitoxicity better than their parent analogues against all the test fungi and this fact was supported by the significant *p*-values i.e. less tham 0.05, for pooled test calculated using statistical analysis programme. Thus, the synergistic effect of thiazolidin-4-one and thiazole moieties in a single molecule had proved.

In Silico Analysis

Toxtree Analysis. Estimation of toxic hazards by Cramer rules was carried out using Toxtree v.2.6.6 software [32], showed that compounds belong to class III level of toxicity, which was same as that of the toxicity level of standards fungicides used.

SAR Analysis. Table 2 shows all the synthesized compounds had followed the rules of Lipinski filtration. The molecular weights of all the compounds along with the standards were below 500 and log *P* values lower than 5. The number of hydrogen bond acceptors and donors were also less than 5 and at par with the standard compounds except for the nitro substituted derivatives.

Regression Analysis

The best fit multiple regression equation and cross validation were carried out to analyze the structure activity relationship of the Lipinski parameters with observed fungitoxicity and the results are given below.

For *C. falcatum,*

$$EC_{50} = 5.607 - 1.495\log P - 0.022\mathrm{MW} + 0.108\mathrm{TPSA} - 1.138H_{don} + 0.03H_{acc},$$

For *P. grisea,*

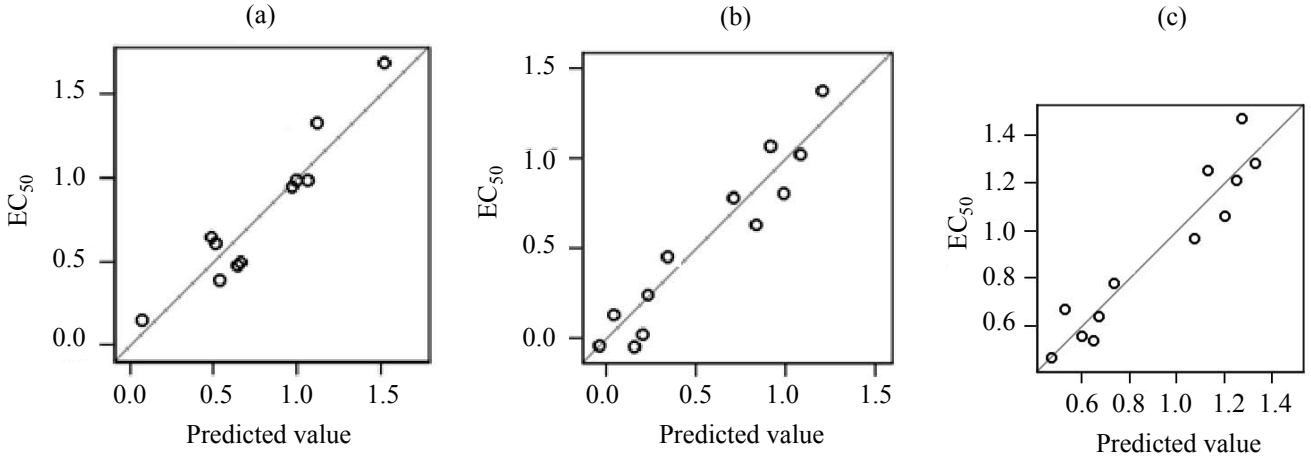
$$EC_{50} = 2.668 - 0.174\log P - 0.005\mathrm{MW} + 0.104\mathrm{TPSA} - 1.55H_{don} + 1.235H_{acc},$$

For *U. hordei,*

$$EC_{50} = 0.739 - 0.745\log P - 0.01\mathrm{MW} + 0.0045\mathrm{TPSA} - 0.136H_{don} + 0.001H_{acc},$$

The calculated *p*-values for all the three equations were significant having *R*-square values 98.23, 96.83, and 90.3%, respectively. The plot of the observed and calculated values of the effective concentration of compounds against all the test fungi using the best fit equation is shown in the figure.

The log *P* values were found to be negatively correlated with the EC_{50} values, revealing that the fungitoxicity of the compounds increases with increase in log *P* values



Plot of the observed and predicted values of the EC_{50} values against (a) *C. falcatum,* (b) *P. grisea,* and (c) *U. hordei.*

i.e. the higher lipophilicity is required for the compounds to be more effective. The TPSA values had not found to be following any pattern but their intermediate values were considered to be much supportive. The molecular weight also follows the negative correlation, with high value supporting the high fungitoxicity of the synthesized compounds. Cross validation method applied to the data set also proved the consonance of theoretical and actual fungitoxicity results.

CONCLUSIONS

The outstanding results for synthesized hybrid compounds against phytopathogenic fungi *viz. C. falcatum, P. grisea,* and *U. hordei* which are at par with the standard fungicides, deserve further investigation of the concept of lead hybridization to clarify the mode of action at molecular level, responsible for the activity observed. And the milder reaction conditions, simple workup, and good yields are the other significant advantages of this procedure in synthesis of these potential biologically active compounds further compliment the results.

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