Research Square

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Microwave-Assisted One Pot Three-Component Synthesis of Novel Bioactive Thiazolyl-pyridazinediones As Potential Antimicrobial Agents against Antibiotic-Resistant Bacteria

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Abstract

Novel 2-thiazolyl-pyridazinedione derivatives were prepared via multicomponent synthesis under microwave irradiation as ecofriendly energy source and using the eco-friendly naturally occurring chitosan basic catalyst with high/efficient yields and short reaction time. All the prepared compounds were fully characterized by spectroscopic methods, and their in-vitro biological activities were investigated. The obtained results were compared with those of standard antibacterial/antifungal agents. DFT calculations and molecular docking studies were used to investigate the electronic properties and molecular interactions with specific microbial receptors.

1. Introduction

In recent years a substantial number of pyridazine derivatives containing different moieties and/or substituents have demonstrated anti-inflammatory/analgesic, antipyretics, antiplatelet, anticancer, antidiabetic, antihypertensive, antidepressant/anxiolytic, anticonvulsant, antifungal, antibacterial, antitubercular, anti-bronchial asthma, antiallergic and other anticipated biological properties [1-8]. Moreover, thiazoles are considered an important class of heterocyclic compounds, found in many potent biologically active molecules such as sulfathiazole (an antimicrobial drug), Ritonavir (an antiretroviral drug), Abafungin (an antifungal drug) and Tiazofurin (an antineoplastic drug).

Over the years, it has been noted that fascinating biological activities have been associated with thiazole derivatives [9,10]. Recently, thiazoles have a wide variety of uses in drug discovery as new inhibitors of bacterial DNA gyrase B and for the treatment of schizophrenia, hypnotics, viral, allergies, inflammation, and more recently, for the treatment of pain, as anti-thrombotic fibrinogen receptor antagonists [11-16].

Multi-component reactions (MCRs) are one-pot processes that form a single product with at least three components, combining most or all of the starting materials [17-20]. In recent years, the great interest in these MCRs has been directed towards the production of combinatorial chemistry methods, due to their high efficiency and the simplicity of these reactions compared to multi-stage methods. In addition, the use of microwave irradiation MCRs in the synthesis of heterocyclic compounds increased the reaction rate and enhanced regioselectivity [21-24].

The catalyst is an important species in organic synthesis to minimize the reaction time and obtain the desired high yield product. Chitosan is a copolymer that includes acetylglucoseamine and glucoseamine units, a biocompatible and biodegradable naturally occurring polysaccharide. In heterocyclic syntheses, chitosan is regarded as an important heterogeneous basic biocatalyst (due to the presence of amino groups)[25-30]. With this in mind, we present here in continuation of our reported earlier work on the synthesis of new bioactive heterocycles [31-37], an effective synthesis of novel 1-thiazolyl-pyridazinedione derivatives as an eco-friendly energy source in a multicomponent synthesis under microwave irradiation and using the eco-friendly chitosan catalyst.

2. Results And Discussion

2.1 Synthesis

In continuation of our previous work to synthesize bioactive heterocyclic compounds under mild conditions [38-43]. Herein we wish to report mild and efficient procedures for the synthesis of some novel thiazolyl-pyridazinedione *via* the three-component reaction of maleic anhydride **1**, thiosemicarbazide **2** and the appropriate 2-oxo-N-arylpropanehydrazonoyl chlorides **3a-f** in ethanol in the presence of chitosan under microwave irradiation at 500 W and 150 °C for 4-8 min. as monitored by TLC (Scheme 1).

The elemental analyses and spectral data (^{1}H-NMR, MS and IR) confirmed the structure of products **6a-f**. For example, in addition to the predicted signals for the aromatic protons and the two doublet signals for the CH=CH protons, the ^{1}H NMR spectra exhibited singlet signals at δ ~2.56 ppm (CH_3) and one D_2O exchangeable peaks at δ ~10.71ppm related to NH proton. Because of the two carbonyl groups and the NH group, the IR spectra of product **7** showed three absorption bands in the regions ~ 1654, 1668 and 3435 cm^{-1} in each case. For each compound, the mass spectrum showed a molecular ion peak, which is compatible with the respective molecular weight.

Alternatively, Compound **6a** was synthesized reaction of 2-oxo-N-phenylpropanehydrazonoyl chloride (**3a**) in ethanol containing catalytic amount of chitosan under MWI with carbthioamide **7** (prepared separately through condensation of maleic anhydride **1** with thiosemicarbazide **2**). The obtained product was found to be identical to **6a** in all regards (mp., TLC and IR spectrum) which provides additional evidence to all **6a-f** structures.

In an identical way, when the three-component reaction of maleic anhydride **1**, thiosemicarbazide **2** and the appropriate ethyl (*N*-arylhydrazono)-chloroacetates **8a-e** under the same reaction condition, it yielded in each case a single product, namely, 1-(4-oxo-5-(2-arylhydrazono)-4,5-dihydrothiazol-2-yl)-1,2-dihydropyridazine-3,6-diones **10a-e** (Scheme 2). The structure of compounds **10a-e** was proved based on spectral data and elemental analyses (see Experimental part). Compound **12** proved based on spectral data and elemental analyses (see Experimental part). Coupling of thiazolone **12** with PhN_2Cl in pyridine yielded the respective product identical in all respects with **10a**.

We find from literature studies that compounds with more than one thiazole ring unit also demonstrate high biological activity [44-47]. Bleomycin, an anticancer inhibitor, comprising the 2,4'-bis thiazole system and Myxothiazol, is an inhibitor of the mitochondrial cytochrome bc1 complex. From the above results, we thought it was useful to synthesize a heterocyclic ring system that carries a bi-thiazole moiety and a pyridazine ring in single compound. This aim was achieved *via* the reaction of *bis*-hydrazonoyl chlorides **13a** and **13b** with two moles of maleimide **1** and two moles of thiosemicarbazide **2** under microwave irradiation in presence of chitosan to afford the respective *bis*-thiazoles **14** and **15** in good yield. The structure of compounds **14** and **15** was proved based on spectral data and elemental analyses (Experimental part).

2.2. XTT assay results

XTT assay was used to find the lowest concentrations of the investigated compounds that have an inhibitory action on cell metabolism/viability of *S. aureus*, *P. aeruginosa* and *C. albicans*. These concentrations are known as *"minimum inhibitory concentration"* (MIC). The results of the XTT assay are summarized in **Table 1.** As can be seen from the table, compound **7d** demonstrates a distinguished antibacterial (*S. aureus*, MIC: 0.12 – 0.49 mg/mL) and antifungal (*C. albicans*, MIC: 15.63 mg/mL) activities in terms of MIC when compared to the standard counterparts (vancomycin and amphotericin B).

Table 1. Minimum inhibitory concentration (MIC, µg/mL) of the compounds under investigation against sensitive and resistant microorganisms using XTT assay

Sample	Minimum inhibitory concentration (µg/mL)					
	Gram positive bacteria		Gram negative bacteria		Fungus	
	Sensitive *Staphylococcus aureus* ATCC 25923	*Methicillin-resistant Staphylococcus aureus (MRSA)* ATCC-BAA-1720	Sensitive *Pseudomonas aeruginosa* ATCC10145	Penicillins and Cephalosporins-resistant *Pseudomonas aeruginosa* ATCC BAA-2108	Azole-Sensitive *Candida albicans* ATCC 18804	Azole-resistant *Candida albicans* ATCC 10231
7a	1.95	3.9	15.63	1.95	NA	NA
7b	0.98	1.95	3.9	15.63	1.95	NA
7c	7.81	7.81	NA	NA	62.5	NA
7d	0.12	0.49	NA	NA	0.24	15.63
7e	1.95	1.95	7.81	62.5	31.25	NA
7f	31.25	15.63	1.95	1.95	15.63	NA
11a	1.95	7.81	3.9	7.81	3.9	15.63
11b	7.81	1.95	15.63	NA	15.63	NA
11c	0.49	1.95	NA	NA	NA	NA
11d	0.12	0.49	0.24	1.95	0.12	3.9
11e	0.49	15.63	0.98	3.9	7.81	7.81
15	7.81	7.81	NA	NA	250	NA
16	0.49	0.12	NA	0.49	0.12	3.9
Vancomycin	0.24	0.98	0.49	3.9	ND	ND
Amphotericin B	ND	ND	ND	ND	0.24	1.95

3.3. Molecular modeling

The geometries of the synthesized molecules that showed the best biological activities in the XTT assay (**7d**, **7e**, **11c** and **11d**) were studied at the B3LYP/6-311G level of theory (**Figure 1**). The results revealed that the studied molecules are almost planar. In all of the studied molecules, the highest occupied molecular orbitals (HOMO) are localized on the substituted phenyl and thiazole rings, while the lowest unoccupied molecular orbitals (LUMO) are localized on the pyridazine-3,6-dione rings.

Table 2. Quantum chemical parameters of compounds **7d, 7e, 11c** and **11d.**

Parameter	7d	7e	11c	11d
E_t (eV)	-40.188	-49.578	-50.556	-63.062
E_{LUMO} (eV)	-3.13	-3.43	-3.65	-3.72
E_{HOMO} (eV)	-6.01	-6.47	-6.58	-6.77
ΔE (eV)	2.87	3.03	2.93	3.06
Ionization energy (eV)	6.01	6.47	6.58	6.77
Electron affinity (eV)	3.13	3.43	3.65	3.72
Mulliken electronegativity	4.57	4.95	5.11	5.25
Softness	0.695	0.659	0.683	0.654
Hardness	1.437	1.517	1.464	1.529
Chemical potential (eV/mol)	-4.57	-4.95	-5.11	-5.25
Electrophilicity index	7.27	8.07	8.93	9.00

The quantum chemical parameters of the selected molecules are shown in **Table 2**. It was found that the energy gaps between HOMO and LUMO are in the range from -3.13 to -3.72 eV with 7d having the smallest energy gap.

Molecular docking was used to study the ligand-receptor interactions that may result in the obtained biological activities of the studied molecules. Based on an extensive literature review, it is well known that thiazole derivatives have excellent antimicrobial activities toward *Staphylococcus aureus* and *Candida albicans*. Accordingly, the investigated compounds showed high antimicrobial activities against these two microorganisms. Moreover, the compounds under study demonstrated good antimicrobial activity against *Pseudomonas aeruginosa*. Therefore, we have selected the most suitable receptors belonging to the organisms mentioned above for molecular docking studies.

Secreted Aspartic Proteinase (SAP) has an important role as a virulence factor during disseminated/mucosal infections of *C. albicans*. This receptor is thought to be responsible for the attachment/invasion of the fungus, and hence it has an important role in its pathogenicity. Accordingly, SAPs can offer suitable target receptors for drug intervention for candidiasis [48].

Staphylococcus aureus is a well-known gram-positive bacterium that causes wound infections that may lead to staphylococcal scalded skin syndrome (a cutaneous reaction to a staphylococcal exotoxin that is absorbed into the bloodstream) [49]. Enoyl-[acyl-carrier-protein] reductase (FabI) is one of the key components of the FAS II system (a group of fatty acid synthases used by most of bacteria and plants to catalyze fatty acid synthesis). This enzyme is also crucial for other types of bacteria such as *Pseudomonas aeruginosa*. Accordingly, SAP and FabI were selected for docking with the studied molecules to relate the *in-silico* results with those obtained from the experimental antibacterial tests.

The structures of the studied receptors are shown in **Figure 2** and **Figure 3**. Molecular docking indicated that the best ligands for SAP2 of *C. albicans*, FabI of *S. aureus* and FabI of *P. aeruginosa* are compounds **7d, 7d** and **11c**, respectively. The calculated docking scores were found to be -8.808, -8.618 and -7.271 kcal/mol for SAP2 of *C. albicans*/**7d**, FabI of *S. aureus*/**7d** and FabI of *P. aeruginosa*/**11c**, respectively.

3. Materials And Methods

3.1. General Experimental Procedures

From common sellable sources, all chemicals and reagents have been procured. By normal steps, all the solvents have been filtered and dried. All reactions have been observed in the silica gel GF254 plate with thin-layer chromatography (TLC). On a Shimadzu GCeMS-QP1000 EX mass spectrometer at 70 eV, mass spectrums were recorded. With an Electrothermal IA 9000 series digital melting point apparatus, melting points were determined. On the PyeUnicamSP 3300 infrared spectrophotometer, IR spectra were recorded on potassium bromide discs. On the VarianMercury 400 MHz spectrophotometer in DMSO-d_6, the ^{1}H-NMR and ^{13}C-NMR spectra were measured using TMS as internal. Using a German-made ElementarVario LIII CHNS analyzer, elemental analysis was calculated.

Synthesis of thiazole derivatives 6a-f, and 10a-e

A mixture of maleic anhydride **1** (0.098 g, 1 mmol) and thiosemicarbazide **2** (0.092g, 1 mmol) glacial acetic acid (0.5 mL) in EtOH (15mL) was irradiated under microwave oven at 500 W and 150 °C for 2 min. Then the appropriate hydrazonoyl halides **3a-f** or **8a-e** and chitosan (0.1 g) were added and the irradiation was continued until all the starting material was consumed (4–8 min. as monitored by TLC). The hot solution was filtered and excess solvent was removed under lower pressures to eliminate chitosan. The solid product was filtered, and crystalized from the appropriate solvent to give pure products **6a-f** and **10a-e,** respectively. Below are the physical features and spectral data of the products obtained.

1-(4-Methyl-5-(phenyldiazenyl)thiazol-2-yl)-1,2-dihydropyridazine-3,6-dione) (6a)

Red solid; m.p. 213-215 °C (Dioxane); IR (KBr): *v* 3435 (NH), 3049, 2926 (C-H), 1668, 1654 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.56 (s, 3H, CH_3), 6.24 (d, *J* = 12Hz, 1H, CH=CH), 6.66 (d, *J* = 12Hz, 1H, CH=CH), 7.01-7.56 (m, 5H, Ar-H), 10.62 (br s, 1H, NH); MS m/z (%) 313 (M^+, 12), 250 (7), 233 (18), 149 (23), 133 (30), 128 (41), 113 (60), 98 (39), 73 (100), 65 (41), 55 (91). Anal. Calcd. for $C_{14}H_{11}N_5O_2S$ (313.33): C, 53.67; H, 3.54; N, 22.35. Found C, 53.55; H, 3.35; N, 22.14%.

1-(4-Methyl-5-(p-tolyldiazenyl)thiazol-2-yl)-1,2-dihydropyridazine-3,6-dione) (6b)

Red solid; m.p. 187-189 °C (EtOH); IR (KBr): *v* 3429 (NH), 3027, 2921 (C-H), 1690, 1654 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.36 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 6.23 (d, *J* = 12Hz, 1H, CH=CH), 6.52 (d, *J* = 12Hz, 1H, CH=CH), 7.15-7.31 (m, 4H, Ar-H), 10.66 (br s, 1H, NH); MS m/z (%) 327 (M^+, 5), 270 (14), 199 (16), 159 (77), 133 (9), 106 (76), 91 (100), 77 (43), 57 (33). Anal. Calcd. for $C_{15}H_{13}N_5O_2S$ (327.36): C, 55.04; H, 4.00; N, 21.39. Found C, 55.35; H, 3.70; N, 21.18%.

1-(4-Methyl-5-(m-tolyldiazenyl)thiazol-2-yl)-1,2-dihydropyridazine-3,6-dione) (6c)

Red solid; m.p. 206-208 °C (EtOH); IR (KBr): *v* 3433 (NH), 3011, 2923 (C-H), 1683, 1669 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.34 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 6.23 (d, *J* = 12Hz, 1H, CH=CH), 6.48 (d, *J* = 12Hz, 1H, CH=CH), 7.06-7.64 (m, 4H, Ar-H), 10.93 (br s, 1H, NH); MS m/z (%) 327 (M^+, 8), 222 (48), 129 (21), 91 (58), 77 (36), 63 (100). Anal.Calcd.for $C_{15}H_{13}N_5O_2S$ (327.36): C, 55.04; H, 4.00; N, 21.39. Found C, 55.25; H, 3.79; N, 21.17%.

1-(5-((4-Methoxyphenyl)diazenyl)-4-methylthiazol-2-yl)-1,2-dihydropyridazine-3,6-dione (6d). Red solid; m.p. 177-178 °C (EtOH); IR (KBr): *v* 3423 (NH), 3022, 2924 (C-H), 1676, 1659 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.66 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 6.23 (d, *J* = 12Hz, 1H, CH=CH), 6.62 (d, *J* = 12Hz, 1H, CH=CH), 7.03-7.86 (m, 4H, Ar-H), 10.87 (br s, 1H,

NH); MS m/z (%) 343 (M^+, 3), 313 (6), 199 (5), 129 (11), 108 (15), 97 (27), 73 (40), 57 (100). Anal.Calcd.for $C_{15}H_{13}N_5O_3S$ (343.07): C, 52.47; H, 3.82; N, 20.40. Found C, 52.48; H, 3.65; N, 20.23%.

1-(5-((4-Chlorophenyl)diazenyl)-4-methylthiazol-2-yl)-1,2-dihydropyridazine-3,6-dione (6e)

Red solid; m.p. 237-239 °C (DMF); IR (KBr): *v* 3433 (NH), 3042, 2925 (C-H), 1671, 1657 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.56 (s, 3H, CH_3), 6.27 (d, *J* = 12Hz, 1H, CH=CH), 6.64 (d, *J* = 12Hz, 1H, CH=CH), 7.26-8.12 (m, 4H, Ar-H), 10.84 (br s, 1H, NH); MS m/z (%) 397 (M^+, 12), 283 (4), 267 (22), 185 (4), 152 (8), 129 (26), 111 (60), 99 (66), 86 (61), 57 (100). Anal.Calcd.for $C_{14}H_{10}ClN_5O_2S$ (397.04): C, 48.35; H, 2.90; N, 20.14. Found C, 48.75; H, 2.74; N, 19.98%.

1-(5-((4-Bromophenyl)diazenyl)-4-methylthiazol-2-yl)-1,2-dihydropyridazine-3,6-dione (6f)

Red solid; m.p. 225-227 °C (DMF); IR (KBr): *v* 3434 (NH), 3032, 2923 (C-H), 1685, 1660 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.43 (s, 3H, CH_3), 6.12 (d, *J* = 12Hz, 1H, CH=CH), 6.46 (d, *J* = 12Hz, 1H, CH=CH), 7.39-8.20 (m, 4H, Ar-H), 11.23 (br s, 1H, NH); MS m/z (%) 392 (M^+, 2), 325 (53), 274 (11), 171 (25), 129 (15), 91 (57), 86 (89), 73 (64), 57 (100). Anal.Calcd. for $C_{14}H_{10}BrN_5O_2S$ (392.23): C, 42.87; H, 2.57; N, 17.86. Found C, 43.21; H, 2.25; N, 17.55%.

1-(4-Oxo-5-(2-phenylhydrazineylidene)-4,5-dihydrothiazol-2-yl)-1,2-dihydropyridazine-3,6-dione (10a). Yellow solid; m.p. 168-170 °C (EtOH); IR (KBr): *v* 3429, 3178 (2NH), 3040, 2975 (C-H), 1706, 1680, 1653 (3C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 6.20 (d, *J* = 12Hz, 1H, CH=CH), 6.41 (d, *J* = 12Hz, 1H, CH=CH), 7.04-7.82 (m, 5H, Ar-H), 10.75, 11.00 (2br s, 2H, 2NH); MS m/z (%) 315 (M^+, 7), 307 (100), 279 (22), 150 (14), 104 (10), 92 (67), 77 (35), 65 (29). Anal.Calcd. for $C_{13}H_9N_5O_3S$ (315.31): C, 49.52; H, 2.88; N, 22.21. Found C, 49.70; H, 2.57; N, 21.88%.

1-(4-Oxo-5-(2-(p-tolyl)hydrazineylidene)-4,5-dihydrothiazol-2-yl)-1,2-dihydropyridazine-3,6-dione (10b). Yellow solid; m.p. 179-181 °C (EtOH); IR (KBr): *v* 3431, 3278 (2NH), 3030, 2979 (C-H), 1705, 1679, 1629 (3C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ (s, 3H, CH_3), δ 6.23 (d, *J* = 12Hz, 1H, CH=CH), 6.45 (d, *J* = 12Hz, 1H, CH=CH), 7.40-8.00 (m, 4H, Ar-H), 10.54, 10.79 (2 br s, 2H, 2NH); MS m/z (%) 329 (M^+, 7), 263 (12), 155 (18), 125 (4), 111 (10), 101 (16), 97 (15), 86 (100), 58 (46). Anal. Calcd. for $C_{14}H_{11}N_5O_3S$ (329.33): C, 51.06; H, 3.37; N, 21.27. Found C, 51.35; H, 3.06; N, 21.03%.

1-(5-(2-(4-Chlorophenyl)hydrazineylidene)-4-oxo-4,5-dihydrothiazol-2-yl)-1,2-dihydropyridazine-3,6-dione (10c). Yellow solid; m.p. 157-159 °C (EtOH); IR (KBr): *v* 3431, 3219 (2NH), 3039, 2989 (C-H), 1705, 1657, 1629 (3C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 6.30 (d, *J* = 12Hz, 1H, CH=CH), 6.54 (d, *J* = 12Hz, 1H, CH=CH), 6.93-7.58 (m, 4H, Ar-H), 9.96, 12.68 (2 br s, 2H, 2NH); MS m/z (%) 351 (M^++2, 2), 349 (M^+, 7), 310 (5), 239 (5), 152 (10), 125 (36), 111 (31), 83 (39), 69 (58), 57 (100). Anal.Calcd. for $C_{13}H_8ClN_5O_3S$ (349.75): C, 44.64; H, 2.31; N, 20.02. Found C, 44.93; H, 2.01; N, 19.70%.

1-(5-(2-(2,4-Dichlorophenyl)hydrazineylidene)-4-oxo-4,5-dihydrothiazol-2-yl)-1,2-dihydropyridazine-3,6-dione (10d). Yellow solid; m.p. 168-170 °C (EtOH); IR (KBr): *v* 3383, 3219 (2NH), 3039, 2983 (C-H), 1696, 1657, 1641 (3C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 6.30 (d, *J* = 12Hz, 1H, CH=CH), 6.54 (d, *J* = 12Hz, 1H, CH=CH), 6.93-7.58 (m, 3H, Ar-H), 9.96, 12.68 (2 br s, 2H, 2NH); MS m/z (%) 382 (M^+, 3), 375 (100), 349 (12), 347 (18), 218 (5), 160 (21), 133 (21), 112 (9), 82 (12). Anal.Calcd. for $C_{13}H_7Cl_2N_5O_3S$ (382.19): C, 40.64; H, 1.84; N, 18.23. Found C, 40.93; H, 1.55; N, 18.00%.

1-(5-(2-(4-Nitrophenyl)hydrazineylidene)-4-oxo-4,5-dihydrothiazol-2-yl)-1,2-dihydropyridazine-3,6-dione (10e). Yellow solid; m.p. 179-181 °C (EtOH); IR (KBr): *v* 3426, 3178 (2NH), 3030, 2922 (C-H), 1703, 1649, 1632 (3C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.26 (s, 3H, CH_3), 6.27 (d, *J* = 12Hz, 1H, CH=CH), 6.51 (d, *J* = 12Hz, 1H, CH=CH), 6.98-7.97 (m, 4H, Ar-H), 10.54, 10.77 (2 br s, 2H, 2NH); MS m/z (%) 360 (M^+, 12), 328 (9), 259 (4), 221 (3), 180 (4), 152 (4), 129 (12), 113 (40),

97 (27), 87 (38), 71 (48), 59 (100). Anal. Calcd. for $C_{13}H_8N_6O_5S$ (360.30): C, 43.34; H, 2.24; N, 23.33. Found C, 43.42; H, 2.09; N, 23.15%.

Alternate synthesis of thiazole derivative 6a

i) Synthesis of 3,6-dioxo-3,6-dihydropyridazine-1(2*H*)-carbothioamide (7).

To a solution of maleic anhydride **1** (0.098 g, 1 mmol), thiosemicarbazide **2** (0.092g, 1 mmol) in ethanol (15 mL), an equivalent amount of glacial acetic acid (0.5 mL) was added. The reaction mixture was heated in a microwave oven at 500 W and 150 °C for 2 min. as monitored by TLC. The reaction mixture was triturated with methanol and the product separated was filtered, washed with methanol, dried and recrystallized from ethanol to give pure carbothioamide derivative **7** as white solid; m.p. 309-311 °C; IR (KBr): *v* 3387-3314, 3258 (NH_2 and NH), 3149, 2963 (C-H), 1687, 1634 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 6.19 (d, *J* = 12Hz, 1H, CH=CH), 6.44 (d, *J* = 12Hz, 1H, CH=CH), 9.30 (br s, 2H, NH_2), 10.46 (br s, 1H, NH); MS *m/z* (%): 171 (M^+), 132 (19), 107 (80), 87 (53), 57 (100). Anal. Calcd. for $C_5H_5N_3O_2S$ (171.17): C, 35.08; H, 2.94; N, 24.55. Found C, 35.01; H, 2.84; N, 24.49%.

Reaction of 7 with 3a

A mixture of 2-oxo-N-phenylpropane hydrazonoyl chloride **3a** (0.196g, mmol) and carbothioamide **7** (0.171 g, l mmol) in EtOH (15 mL) / chitosan (0.1 g) was heated in a microwave oven for 5 min. as monitored by TLC → gave product identical in all respects with compounds **6a**.

Alternate synthesis of thiazole derivative 10a

i) 1-(4-Oxo-4,5-dihydrothiazol-2-yl)-1,2-dihydropyridazine-3,6-dione (12).

A solution of maleic anhydride **1** (0.098 g, 1 mmol), thiosemicarbazide **2** (0.092g, 1 mmol), and ethyl 2-bromoacetate **11** (0.0165 g, 1 mmol) in ethanol (15 mL) / chitosan (0.1 g) was heated in microwave oven for 4 min. to give thiazolone **12** as yellow solid; m.p. 157-159 °C (EtOH); IR (KBr): *v* 3438 (NH), 3168, 2987 (C-H), 1708, 1650, 1648 (3C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 3.82 (s, 2H, CH_2), 6.22 (d, *J* = 12Hz, 1H, CH=CH), 6.53 (d, *J* = 12Hz, 1H, CH=CH), 10.25 (s, 1H, NH); MS m/z (%) 211 (M^+, 16), 149 (19), 117 (63), 92 (66), 57 (100). Anal.Calcd. for $C_7H_5N_3O_3S$ (211.01): C, 39.81; H, 2.39; N, 19.90. Found C, 40.21; H, 2.17; N, 19.64%.

ii) Coupling of 12

Benzenediazonium chloride solution [prepared as usual by diazotizing aniline (1 mmol) in hydrochloric acid (1 ml, 6 M) with sodium nitrite (0.07 g, 1 mmol) in 10 ml water] was added portionwise to a cold solution of **12** (0.211g, 1 mmol) in 10 mL pyridine. The yellow solid which so formed was filtered and then recrystallized from EtOH to give **10a**.

Synthesis of bis-thiazole 14 and bis-thiazolone 15

A mixture of maleic anhydride **1** (0.196 g, 2 mmol) and thiosemicarbazide **2** (0.184g, 2 mmol) in ethanol (20 mL) / glacial acetic acid (1mL) was heated in microwave oven for 2 min. Then the appropriate bis-hydrazonoyl halides **13a,b** (1mmol for each) and chitosan (0.2 g) were added, the reaction mixture was further heated for 8 min., gave products **14** and **15,** respectively.

1,1'-(([1,1'-Biphenyl]-4,4'-diylbis(diazene-2,1-diyl))bis(4-methylthiazole-5,2-diyl))bis(1,2-dihydropyridazine-3,6-dione) (14). Yellow solid; m.p. 168-170 °C (EtOH); IR (KBr): *v* 3428 (NH), 2922 (C-H), 1699, 1659 (2C=O) cm^{-1}; ^{1}H-NMR (DMSO-

d_6): δ 2.58 (s, 6H, CH_3), 6.27 (d, J = 12Hz, 2H, CH=CH), 6.63 (d, J = 12Hz, 2H, CH=CH), 7.43 (s, 8H, Ar-H), 10.64 (br s, 2H, 2NH); MS m/z (%) 624 (M^+, 22), 373 (13), 341 (10), 299 (2), 271 (21), 112 (20), 98 (39), 86 (67), 69 (45), 54 (100). Anal.Calcd. for $C_{28}H_{20}N_{10}O_4S_2$ (624.11): C, 53.84; H, 3.23; N, 22.42. Found C, 54.04; H, 3.09; N, 22.27%.

1-(5-(2-(4'-(2-(2-(3,6-Dioxo-3,6-dihydropyridazin-1(2*H*)-yl)-4-oxothiazol-5(4*H*)-ylidene)hydrazinyl)-[1,1'-biphenyl]-4-yl)hydrazineylidene)-4-oxo-4,5-dihydrothiazol-2-yl)-1,2-dihydropyridazine-3,6-dione (15). Yellow solid; m.p. 179-181 °C (EtOH); IR (KBr): ν 3422, 3032 (2NH), 2978, 2930 (C-H), 1683, 1655, 1636 (3C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 6.29 (d, J = 12Hz, 2H, CH=CH), 6.53 (d, J = 12Hz, 2H, CH=CH), 7.52 (m, 8H, Ar-H), 10.37, 10.79 (2 br s, 4H, 2NH); MS m/z (%) 628 (M^+, 4), 367 (31), 334 (24), 313 (19), 294 (49), 236 (25), 184 (63), 139 (66), 97 (36), 71 (49), 55 (100). Anal.Calcd. for $C_{26}H_{16}N_{10}O_6S_2$ (628.60): C, 49.68; H, 2.57; N, 22.28. Found C, 49.59; H, 2.48; N, 22.10%.

3.2 In-vitro XTT assay

XTT assay, a non-radioactive colorimetric assay system, is usually used for measuring cell viability, proliferation and cytotoxicity through the measurement of cellular metabolic activity. This test depends on the reduction of a yellow tetrazolium salt (XTT dye) to an orange formazan dye by metabolically active cells. The minimal inhibitory concentration (MIC) values, which represent the lowest concentrations of samples or standard drugs (Vancomycine for bacteria and Amphotricine B for fungi) that completely inhibit the microbial growth. MICs were determined using the microdilution method. The bacterial inoculum was prepared and the suspensions modified to 10^6 CFU/mL. The examined samples and the standard drugs were prepared in dimethyl sulfoxide (DMSO), accompanied by two double dilutions in a 96-well plate. Every microplate well included 40 µL of the growth medium, 10 µL of the inoculum and 50 µL of the investigated compounds diluted at final concentrations (1000-0,12 µg/mL), and DMSO was used as a negative monitor. The plates were incubated at 37 °C for 24 hours. Thereafter, 40 µL of tetrazolium salt was applied. The plates were incubated in dark for 1 h at 37 °C, after which colorimetric change in the XTT reduction assay was measured using a µL plate reader at 492 nm. The MIC was detected as the lowest concentration capable of causing the largest color change compared to the negative control [50].

3.3 In-silico studies

The electronic properties of the synthesized derivatives that demonstrated the best biological activities in the in-vitro XTT assay were investigated with density functional theory calculations. The calculations were carried out with the aid of Gaussian 09 [51]. The geometry of the studied molecules was fully optimized using B3LYP/6-311G functional and the obtained molecular orbitals were visualized.

Molecular docking was used to investigate the interaction of the best biologically active molecules with the microbial receptors. We selected the most probable bacterial/fungal proteins that can be affected by the synthesized thiazole ligands based on the results previously reported in the literature. Molecular docking was carried out with the aid of the Molecular Operating Environment (MOE) 2014 software [52]. The geometry-optimized compounds that demonstrated the lowest MIC values in the XTT assay were selected and docked with the corresponding receptors. High-resolution 3D molecular structures of the receptors Secreted Aspartic Proteinase (SAP2; *C. albicans*; PDB ID: 1EAG), Enoyl-acyl Carrier Protein Reductase (fabI; *S. aureus*; PDB ID: 3GR6) and Enoyl-acyl Carrier Protein Reductase (FabI; *P. aerugiosa*; PDB ID: 4NR0) were obtained from the Protein Data Bank (PDB).

4. Conclusions

In summary, we have developed a new green methodology and synthesized several novel 1-thiazolylpyridazine derivatives by microwave irradiation in high, efficient yields and short reaction time. Also, the antimicrobial activities of the candidate lead molecules were tested against *S. aureus*, *P. aeruginosa* and *C. albicans* using the XTT assay and compounds with the highest activity in terms of MIC were docked with the corresponding microorganisms receptors. The results depict that compound **7d** shows comparable biological activities to these of the standard antibacterial/antifungal drugs in case of *S. aureus* and *C. albicans*. In addition, compound **11d** demonstrated the highest activity against *P. aeruginosa*.

Declarations

Author contributions

S.A.M. and S.M.G. conceived the experiment(s), M.M.E., A.S.A., A.S.A.D. and Z.A.M. conducted the experiment(s), S.M.G., A.S.A., A.S.A.D. and Z.A.M. analysed the results. All authors reviewed the manuscript.

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Competing interests

The authors declare no competing interests.

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