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Synthesis and characterization of derivatives including thiazolidine-2,4-dione/1-*H*- imidazole and evaluation of antimicrobial, antioxidant, and cytotoxic properties of new synthetic heterocyclic compounds

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Abstract

Several new derivatives of thiazolidine-2,4-dione and 1-*H*-imidazole were prepared using imidazole aldehydes **6a–6f** in ethanol as a solvent. Products **7a–7f** were obtained in reasonable yields and great purity. The antioxidant activity for finish products was evaluated by DPPH radical scavenging activity and showed relatively good activity against ascorbic acid. Compounds **7d**, **7e**, and **7f** had the highest antioxidant activity. Compound **7c** showed the lowest amount of IC50 versus ascorbic acid. The antimicrobial activity of these compounds against gram-positive bacteria including *Bacillus anthracis (B. anthracis) and Staphylococcus aureus (S. aureus)* and gram-negative bacteria including *Escherichia coli (E. coli) and Pseudomonas aeruginosa (P. aeruginosa)* bacteria was evaluated by the inhibition zone diameter assay method, and the compounds showed moderate to low antibacterial activity. The toxicity properties of all synthesized compounds against cisplatin were investigated. Most of the compounds showed good activity against the positive control group, and the toxicity of compound **7b** was higher than that of other compounds.

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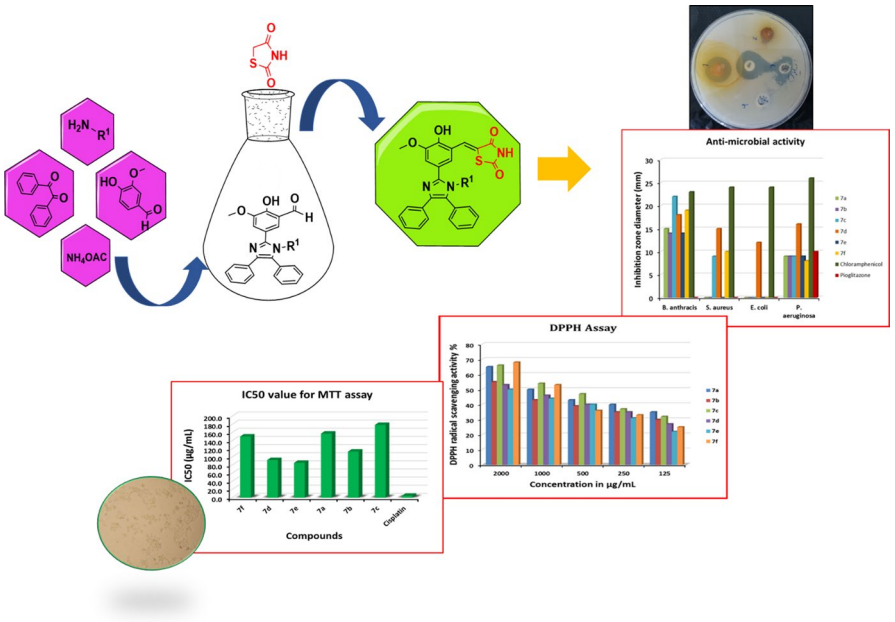
Graphic abstract



Keywords 1-*H*-imidazole · Antimicrobial activities · Antioxidant activities · DPPH radical · IC50 · Toxicity · Cisplatin

Introduction

In recent years, the entry of antibiotics into the pharmaceutical industry to fight infectious diseases seemed to be sufficient and appropriate for treatment, but with the indiscriminate and arbitrary use of antibiotics, bacteria resistance has increased and has become a public health problem [1, 2]. The search for new antimicrobial agents with strong antibacterial properties, reduced toxicity, and lower side effects has prompted researchers to investigate [3–8].

Accordingly, heterocyclic compounds have received much attention. Heterocyclic compounds play an essential role in medicinal chemistry and are central to synthetic organic chemistry and have the highest practical and theoretical importance. These compounds containing heteroatoms show significant changes in the cyclic molecular structure due to the non-common electron pairs and the negative electron difference between the heteroatoms and the carbon. Heterocyclic ring systems with special shifts in the right positions have emerged as powerful scaffolds for biological estimation. In this context, heterocyclic systems containing sulfur and nitrogen exhibit a variety of biological and pharmacological activities, in part due to their many similarities between natural and synthetic molecules and known potentials.

In the environment, heterocyclic compounds are widely distributed, and many heterocyclic compounds synthesized in laboratories have been used successfully as clinical agents. Compounds containing aromatic polycyclic heterocycles are active in various pharmaceutical fields. Among the most important and basic living compounds containing heterocyclic groups that are based on aromatic heterocyclic systems, we can name DNA and RNA [9–14].

Thiazolidine-2,4-dione (**TZD**) is a five-membered heterocyclic ring containing sulfur and nitrogen, and its nucleus has two carbonyl moieties on the second and fourth positions, and it is widely found in nature in various forms. **TZD** ring has attracted a great deal of attention because of their diverse biological activities. The main drugs with the **TZD** ring are found in the structure of diabetes drugs that are prescribed to control blood sugar in patients. Clinical drugs include pioglitazone **1**, rosiglitazone **2**, troglitazone **3**, and rivoglitazone **4** [15]. Some well-known clinical **TZD** drugs are mentioned in Fig. 1. Synthesized compounds containing a **TZD** ring have been used for the treatment of such antimalarial, antimicrobial, anti-viral, antioxidant, anti-convulsant, anti-HIV, anti-tubercular, anticancer, and anti-inflammatory agent [16–20].

Also, another five-membered heterocyclic ring that has high therapeutic properties today and has been considered by chemists is imidazole (**IMD**). **IMD** has increased the range of recovery of various drugs in clinical trials [21]. Some clinical drugs with the **IMD** ring are shown in Fig. 1. Among the medicinal properties of compounds containing **IMD**, we can mention antimicrobial, antibacterial, antifungal, anticancer, and antioxidant [22–26].

The two rings of **IMD** and **TZD** and their derivatives, due to their excellent ability against bacteria and also their remarkable antioxidant properties, have been widely considered for the construction of new heterocycles [27–30]. One of the well-known clinical drugs used to treat diabetes, which includes both rings, is

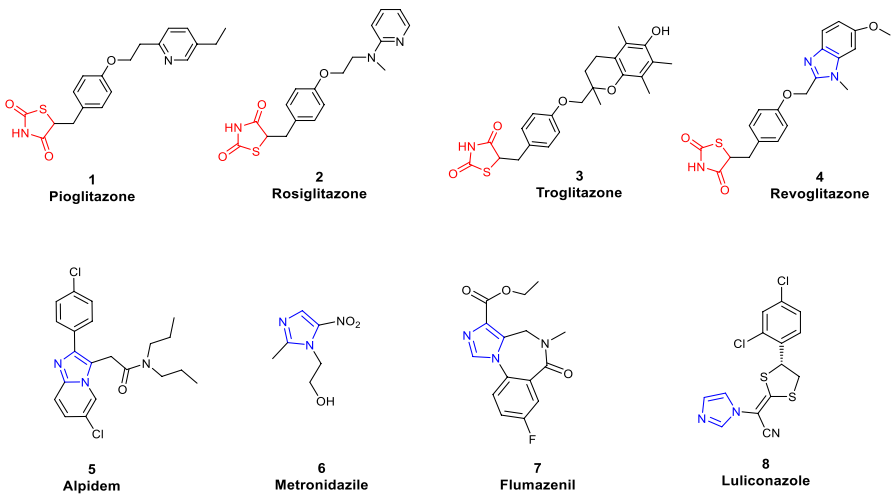


Fig. 1 Clinical drugs containing **TZD** and **IMD** ring

rivoglitazone **4**, shown in Fig. 1. Accordingly, in this study, we created new synthetic compounds by combining two rings of **IMD** and **TZD** and investigated the antibacterial activity of these compounds against gram-positive bacteria including *Bacillus anthracis (B. anthracis) and Staphylococcus aureus (S. aureus)* and gram-negative bacteria including *Escherichia coli (E. coli) & Pseudomonas aeruginosa (P. aeruginosa)* bacteria by inhibition zone diameter assay method, and antioxidant activity was evaluated by DPPH radical scavenging properties of the newly synthesized compounds.

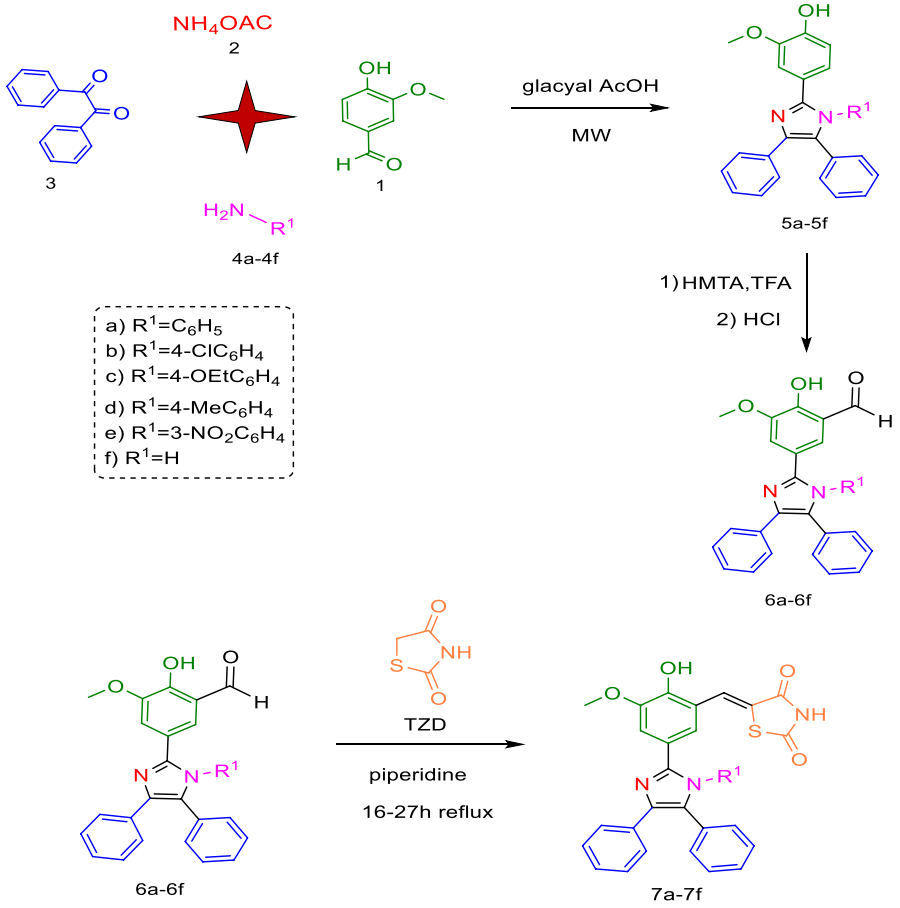
Results and discussion

Chemistry

In this project, synthesize new compounds containing **TZD** and imidazole (**IMD**), which is based on the use of IMD-aldehydes **6a–6f** and **TZD**, and finally, the target products (4,5-triphenyl-1*H*-imidazol-2-yl)benzylidene)thiazolidine-2,4-dione) **7a–s7f** are completed. The synthesis steps of the finishing compounds **7a–7f** are shown in Scheme 1, and the final structure compounds are depicted in Table 1. Our results show that high-yield products are obtained and easily purified using non-chromatographic methods. The advantages of this method are simple adjustment and product separation and reproducibility, use of cheap and minimal solvents.

Initially, hydroxyl-IMD **5a–5f** was synthesized through a four-multi-component reaction among benzyl **3**, vanillin **1**, aniline derivatives **4a–4f** and NH_4OAc **2** in the presence of glacial AcOH as catalyst and solvent under microwave irradiation during a short reaction time (4–6 min) [31, 32]. As shown in Scheme 2, AcOH increases the electrophilic strength of the carbonyl group of vanillin **1**. Imine **8** is then formed during a condensation reaction from an ammoniacal nucleophilic attack derived from NH_4OAc **2** to carbonyl and the removal of a H_2O molecule. Subsequently, a nuclear attack of aniline **4a** results in the formation of an intermediate of **9**. Intermediate **9** during a compression reaction with benzil **3** forms an intermediate **11** via an intermediate **10** by removing a molecule of H_2O in an acidic medium. The stable aromatic product **5a** is then produced.

Then, to synthesize IMD-aldehyde **6a–6f**, the premade hydroxyl-IMD **5a–5f** was participated in the Duff reaction refluxed by using HMTA (hexamethylenetetramine) and TFA (trifluoroacetic acid)), after which the medium is acidified and the temperature reaches 100 °C for additional 12-h reflux. The **IMD**-aldehyde **6a–6f** product was obtained with very good yields [33]. The proposed mechanism for the synthesis of compounds **6a–6f** shown in Scheme 3 is that, first, hexamethylenetetramine (HMTA) **12** is protonated in the presence of trifluoroacetic acid (TFA), because angular pressure causes rearrangement and production of iminium ion **14** through ion **13**. Intermediate 14 is then attacked by an ortho-carbon of the phenolic compound **5** through a nucleophilic substitution reaction, producing a ketone intermediate **15**. Intermediate **15** is rearranged in the presence of TFA ion (CF_3COO^-) to form a more stable phenolic compound **16**. Further protonation of the intermediate **16** and rearrangement leads to the formation of iminium ion **18** via fragmentation of



Scheme 1 The general method for the syntheses of compounds (IMD-TZD) **7a–7f**

17, which produces an intermediate **19** during a hydride transfer. Finally, iminium ion **19** is hydrolyzed in the presence of an aqueous solution of HCl to formulate the product **6**. Also, the rest of the structure related to HMTA is sequentially hydrolyzed to ammonia and formaldehyde during these steps (Scheme 3).

To synthesize (4,5-diphenyl-1*H*-imidazol-2-yl)benzylidene)thiazolidine-2,4-dione) **7a–7f**, the **TZD** ring according to the literature was premade. Mix the thiourea and chloroacetic acid in water and HCl and reflux for 12 h, and the reaction product with high purity after recrystallization was achieved [34]. **IMD**-aldehyde **6a–6f** and **TZD** were dissolved in EtOH; then piperidine is added as a catalyst, and the mixture was refluxed for 36 h and then acidified and refined with Conc. AcOH and then recrystallized to obtain highly purified products. All steps are depicted in Scheme 1, and the proposed mechanism for the synthesis of **7a–7f** from **6a–6f** via piperidine took place through intermediates **20–25** as shown in Scheme 4. The structure, molecular formula, MW, and MP of the final products **7a–7f** are shown in Table 1.

Table 1 Physical properties and yield % **7a–7f**

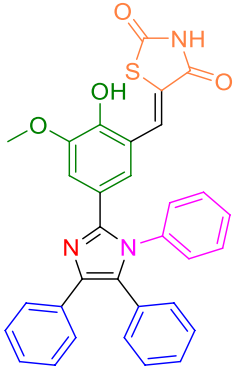
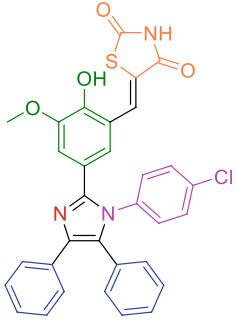
Product	Structure	Molecular formula (Mol.Wt.)	Yield (%)	M.P (°C)	Picture
7a		$C_{32}H_{23}N_3O_4S$ 545.61	82	232.59	
7b		$C_{32}H_{22}ClN_3O_4S$ 580.06	78	255.34	

Table 1 (continued)

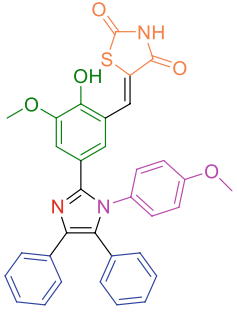
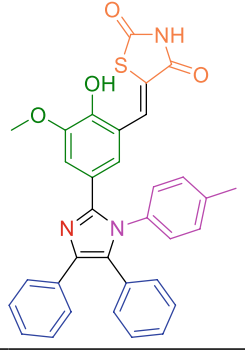
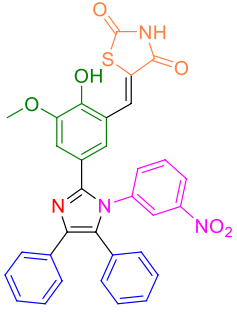
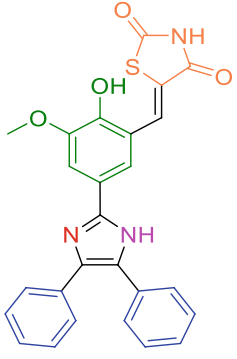
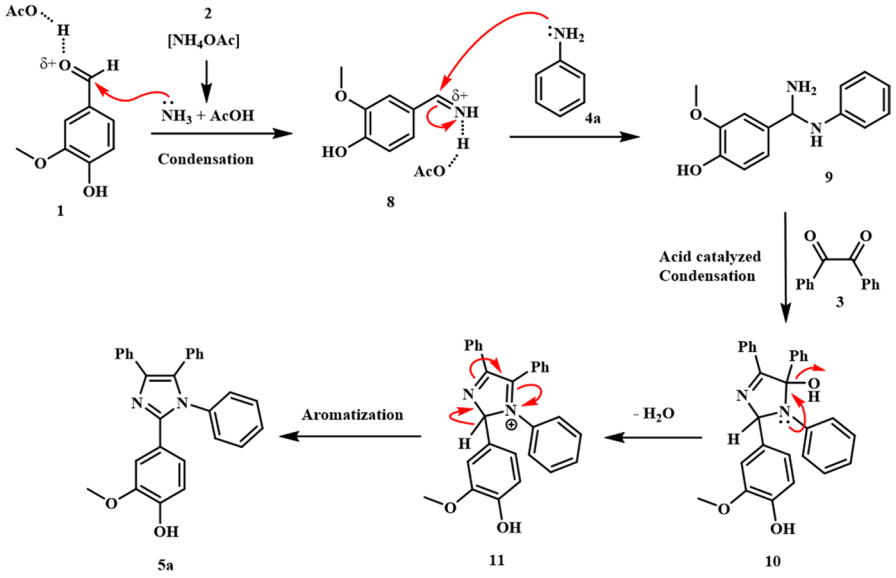
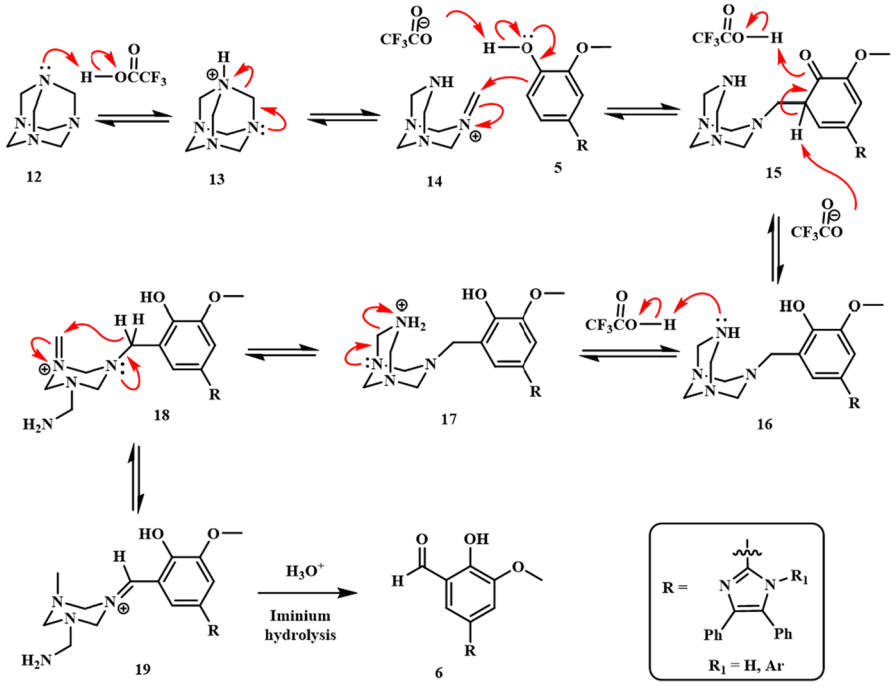
Product	Structure	Molecular formula (Mol.Wt.)	Yield (%)	M.P (°C)	Picture
7c		$C_{34}H_{27}N_3O_5S$ 589.67	85	161.88	
7d		$C_{33}H_{25}N_3O_4S$ 559.64	83	252.69	

Table 1 (continued)

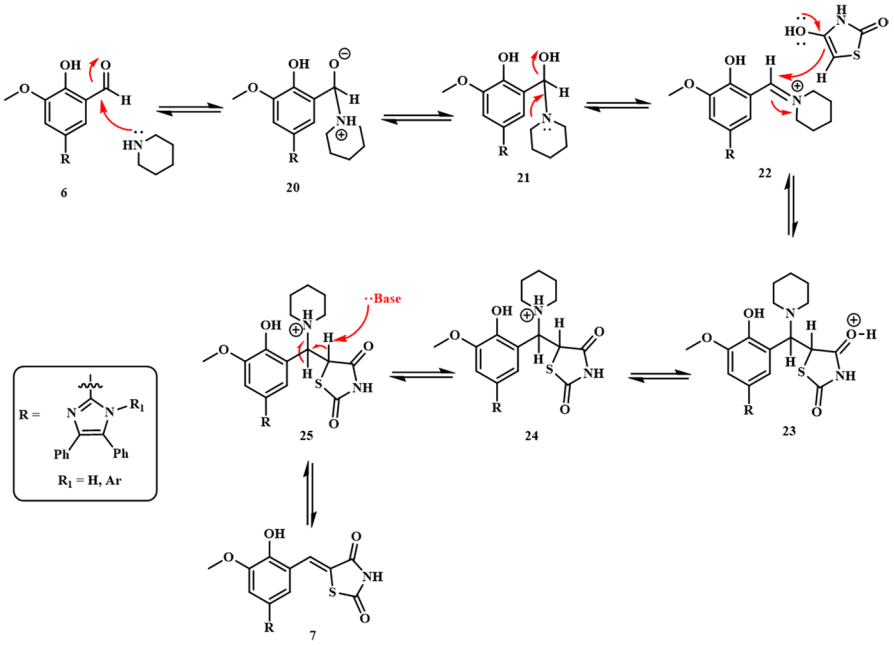
Product	Structure	Molecular formula (Mol.Wt.)	Yield (%)	M.P (°C)	Picture
7e		$C_{32}H_{22}N_4O_6S$ 590.61	80	240.77	
7f		$C_{26}H_{19}N_3O_4S$ 469.52	75	200.13	



Scheme 2 The proposed mechanism for the synthesis of compounds **5a–5f**



Scheme 3 The proposed mechanism for the synthesis of compounds **6a–6f** via HMTA and TFA



Scheme 4 The proposed mechanism for the synthesis of compounds **7a–7f**

The structures of IMD-aldehydes **6a–6f** and (4,5-diphenyl-1*H*-imidazol-2-yl)benzylidene)thiazolidine-2,4-dione) (**IMD-TZD**) **7a–7f** were determined by FT-IR, ^{1}H NMR, and ^{13}C NMR spectra. In the FT-IR spectrum, of IMD-aldehyde derivatives, **6a–6f** a carbonyl stretching band in the range of 1600–1700, was observed, while, in the final product **7a–7f**, two carbonyl stretching bands at the range of 1600–1750, the N–H stretching vibration bands at 3393–3417 and the C=CH band at 1500–1600 were perceived, which confirmed the presence of the thiazolidine-2,4-dione moiety in the structure of the desired products.

In ^{1}H NMR spectra of **6a–6f** in $CDCl_3$, the presence of a single peak in the range 9.70–9.82 ppm confirms the presence of aldehyde O=C–H. Also, the presence of a single peak region of about 11.10–11.32 ppm is related to the O–H signal. The OCH_3 signal appears individually in the range of 3.60–3.85 ppm. Other aromatic protons appeared in the expected range at 7.00–7.68 ppm. The products (IMD-TZD) **7a–7f** are dissolved in DMSO, as the olefin signal C=CH is expected to appear in the range 7.90–8.10 ppm according to the literature [16] and the N–H single in the range 10.2–10.81 ppm. The O–H signal appears to be in the range of 12.50–12.77 ppm individually and confirms that the **TZD** moiety has replaced the aldehyde. In ^{13}C NMR spectra, of **6a–6f** in $CDCl_3$ as a solvent, the H–C=O signal appeared at 196.07–196.63 ppm. The OCH_3 signal is in the range of 55.92–56.22 and other aromatic carbons appeared at 114.14–158.79 ppm. The product (**IMD-TZD**) **7a–7f** in DMSO provided the expected number and types of carbons. The C=O peak in the **TZD** moiety appears in different signals due to its binding to other atoms. The NH-C=O–C of the **TZD** ring appeared at 167.15–167.31 ppm, and the

NH–C = O–S appeared at 167.48–167.96 ppm. The signal at 55.87–56.14 is attributed to OCH_3, and other aromatic carbons appeared at 114.14–158.79 ppm. Elemental analysis was used to find the percentage of elements used in the synthesized compounds.

Biological activities

Antimicrobial activity section

The antimicrobial activity of the newly synthesized compounds **7a–7f** against the target bacteria was evaluated qualitatively and quantitatively in the presence or absence of inhibition zone diameter. Four bacterial strains examined by the cup plate method were used for this method, from gram-positive bacteria including *Bacillus anthracis (B. anthracis) and Staphylococcus aureus (S. aureus)* and gram-negative bacteria including *Escherichia coli (E. coli) and Pseudomonas aeruginosa (P. aeruginosa)*. Samples were prepared at a Conc. (5.0 mg/ml) in DMSO, and chloramphenicol was used as a standard antibiotic. Also, pioglitazone along with new compounds was studied as a pharmacological compound with **TZD** ring antimicrobial activity ring. The display results are shown in Table 2.

According to the results observed in Table 2 and Fig. 2, which shows the comparison of the antimicrobial activity of synthesized compounds **7a–7f** in comparison with chloramphenicol and pioglitazone, it can seem that all the investigated derivatives displayed moderate to good activity. As shown in Fig. 2, newly synthesized compound **7c** is showed an inhibitory effect like that of chloramphenicol in bacteria *(B. anthracis)* and can use as a standard.

In contrast to bacteria *(S. aureus)*, only compounds **7c**, **7d,** and **7f** exhibit activity that is moderately active, with activity **7d** being good. The only compound that is active against bacteria *(E. coli)* is compound **7d**. All products are active against

Table 2 Antimicrobial activity of the newly synthesized compounds (IMD-TZD) **7a–7f**

Compound	Antimicrobial activity (inhibition zone diameter (mm))			
	Gram-positive		Gram-negative	
	B. anthracis	*S. aureus*	*E. coli*	*P. aeruginosa*
7a	15	–	–	9
7b	14	–	–	9
7c	22	9	–	9
7d	18	15	12	16
7e	14	–	–	9
7f	19	10	–	8
Chloramphenicol	23	24	24	26
Pioglitazone	–	–	–	10

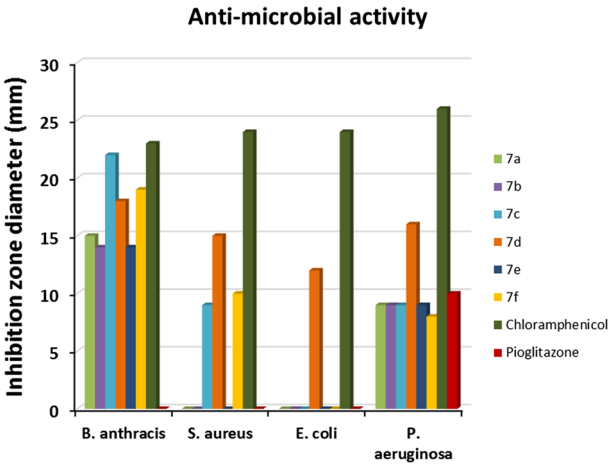


Fig. 2 Antimicrobial activity inhibition zone (mm) of synthesized compounds (IMD-TZD) **7a–7f**

bacteria *(P. aeruginosa)*, even pioglitazone, which was less active than other bacteria, and compound **7d** has better performance.

Antioxidant activity section

Since the main mechanism of antioxidant action in compounds is radical-scavenging, many methods have been developed in which the antioxidant activity is evaluated by the scavenging of synthetic radicals in polar organic solvents such as MeOH at r. t. In this study, the DPPH method was selected to evaluate the antioxidant activity of new compounds because it is one of the most effective methods for evaluating the Conc. of radical-scavenging materials active by a chain-breaking mechanism. Also, the DPPH radical is a stable free radical, and the DPPH radical-scavenging activity was determined by the change in color from purple to colorless and decrease in absorbance at 517 nm, due to reduction by the antioxidant (AH) or reaction with a radical species to form a stable DPPH-H molecule, as follows (1) [35, 36]:

$$\mathrm{DPPH}^{\bullet} + \mathrm{H}^{\bullet} \rightarrow \mathrm{DPPH} - \mathrm{H} \quad (1)$$

By comparing structural information and antioxidant activity, it was observed that DPPH radical scavenging antioxidant activity depends on two parameters: the functional groups on the aromatic ring and the position of functional groups on the ring. Antioxidants donate hydrogen atoms to become stable free radicals. The degree of stability and antioxidant potential is in direct relationship with the range of delocalization. The antioxidant activities of compounds (**IMD-TZD**) **7a–7f** were screened at Conc. of 2000, 1000, 500, 250, 125 µg/mL at 517 nm for DPPH assay, respectively Fig. 3.

Also, IC50 values (the Conc. of compounds to scavenge 50% of DPPH) were calculated by plotting radical scavenging activity against Conc. and obtaining a line equation in Fig. 4 Ascorbic acid was used as a standard in Table 3 [35].

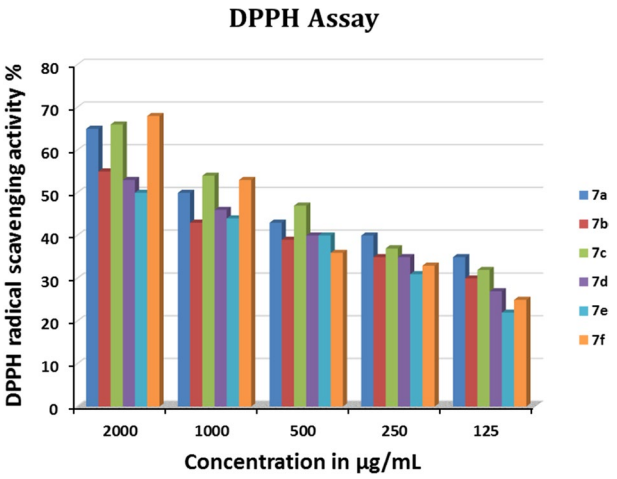


Fig. 3 DPPH radical scavenging activity in different Conc. for the synthesis compounds **7a–7f**

Fig. 4 IC_{50} values of synthesis compounds (IMD-TZD) **7a–7f** by DPPH

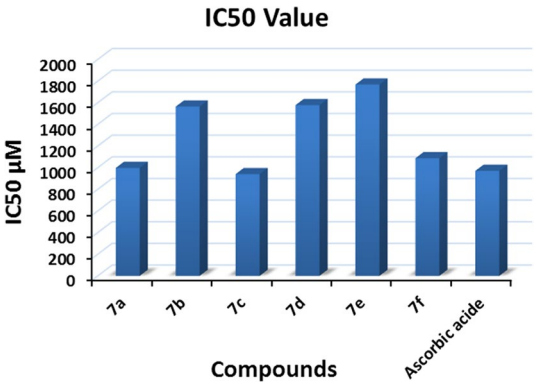


Table 3 IC50 values for DPPH radical scavenging activity of synthesis compounds **7a–7f**

Compound	DPPH assay IC50(µM)
7a	998
7b	1564
7c	940
7d	1578
7e	1768
7f	1087
Ascorbic acid	971

As is depicted in Fig. 3, all of the investigated compounds demonstrated considerable free radical scavenging activities. Compounds **7b**, **7d**, **7e,** and **7f** showed higher antioxidant activity at lower Conc. (500–125 µg/mL) in comparison with others and

were dose-dependent other compounds showed moderate activity. Also, IC50 values of products (**IMD-TZD**) **7a–7f** were calculated in as shown Fig. 4. The IC50 values were in the range of 940–1768 µM for the DPPH assay in Table 3. Product **7c** was much more active than ascorbic acid, and products **7b**, **7d** and **7e** were much weak than standard in the DPPH assay.

Products **7a** and **7f** showed moderate activity than ascorbic acid. The IC50 value of product **7c** may be due to the presence of a *p*-OEt functional group in the structure of this compound.

Cytotoxic activity section

One way to treat cancer is to use strong chemical drugs or chemotherapy. Today, several chemical drugs have been used and researched to treat cancer. Finding new drugs that can fight cancer cells encouraged us to study the anticancer properties of compounds **7a–7f**. Accordingly, we investigated the cytotoxic effects of **7a–7f** on human breast cancer cell line proliferation (MCF-7) in the present work. Cells were treated with different concentrations of the **7a–7f** and cisplatin as a positive control group in different amounts ranging from 0 to 200 µg/mL for 24 h. Cells were incubated with **7a–7f,** and cell viability was measured 18 h later by MTT assay. Inhibition of cell proliferation in each treatment is reported as a percentage of the number of cells treated concerning the untreated control cells (Fig. 5).

Figure 6 shows the IC50 values of **7a–7f** and cisplatin against the (MCF-7) cells. The IC50 of cisplatin, as the positive control group, under all identical conditions, was approximately 4.61 µg/mL, respectively. MTT assay results showed that **7b** had higher cytotoxicity than other compounds. However, all IC50 values showed approximately reasonable activity for the compounds. Among the synthesis compounds in this test, **7b**, **7d** and **7e** showed the best anti-growth activity with IC50 of 113.3, 92.4 and 85.6 µg/mL, respectively.

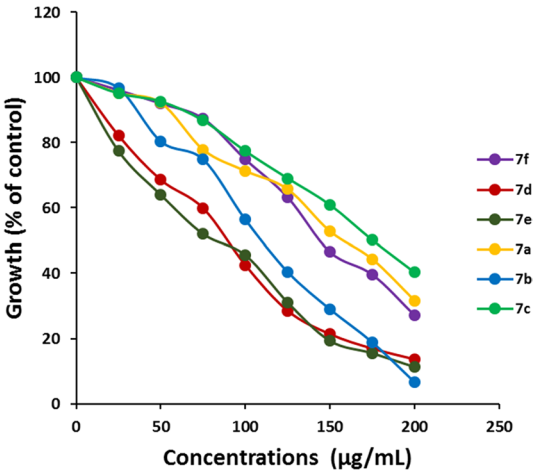


Fig. 5 The anti-growth activity of compounds **7a–7f** against (MCF-7) cells using MTT assay

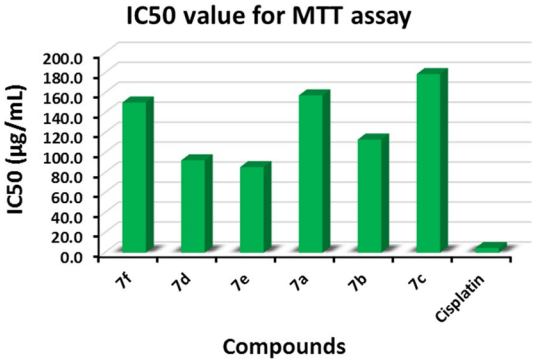


Fig. 6 IC50 values of derivatives **7a–7f** and cisplatin against the (MCF-7) cells

Examining the obtained results, it seems that the presence of chloride and nitrous oxide groups in the structure of the compounds increases their toxicity and increases their activity in inhibiting (MCF-7) cell growth. On this account, these compounds show poorer performance against (MCF-7) cells compared to cisplatin as a positive control group. The toxicity of other compounds in comparison with cisplatin drug was found to be in order of: **7b** > **7e** > **7d** > **7f** > **7a** > **7c**. Figure 7 shows a view of (MCF-7) cells versus composition **7b** and the effect of cisplatin as a positive control group on the cells.

TZD ring has an important role in antioxidant activity [37, 38]. As it is depicted in Scheme 5, the N–H of the **TZD** ring can readily donate hydrogen radical to DPPH radical and form a radical of the test compound [39].

The new radical species makes it possible to create different and stable radical species through the **TZD** ring and the phenol ring and the **IMD** ring in the structure of the compounds. In the phenol ring, the formation of the enol-ketone group causes more radical stability, and it also seems that other parts of the product structure also affect the radical activity. The most important effective

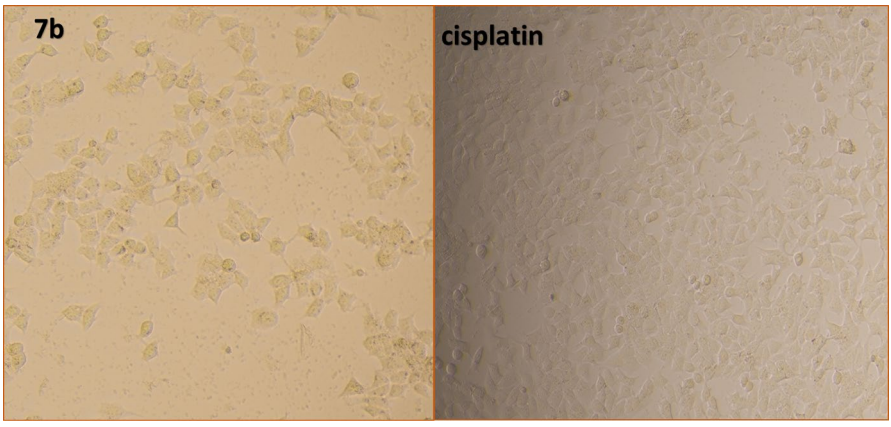
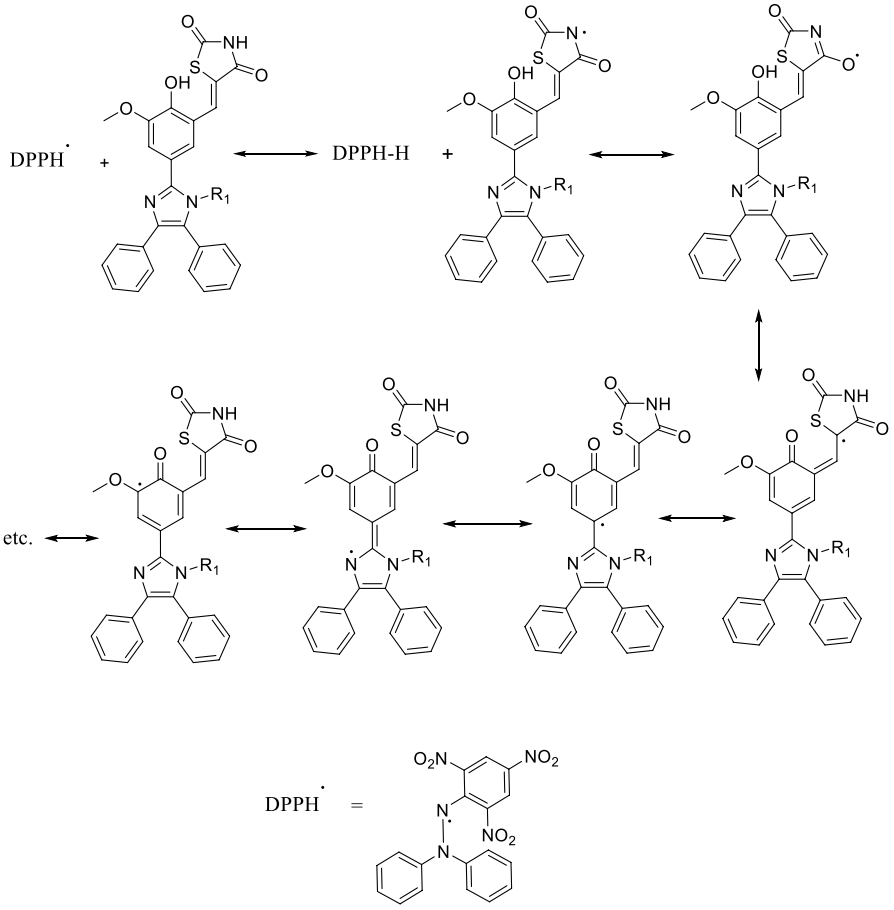


Fig. 7 Cellular death of (MCF-7) cells against for compound 7b and cisplatin as a positive control



Scheme 5 Proposed mechanism of radical scavenging activity

part can be attributed to the groups attached to the **IMD** ring that can increase the antioxidant power. The high activity of compound **7e** is probably due to the NO_2 group, which leads to radical instability, as shown in Scheme 5.

Experimental section

Material

The chemical reagents and solvents used in synthesis were purchased from Merck, Fluka, and Aldrich. The purity of the synthesized compounds was checked by thin-layer chromatography (TLC) using Merck silica gel 60F254 aluminum sheet.

Instrumentation

Melting points were determined on the 91100 s electric device, and are uncorrected. The infrared (IR) spectra were recorded on a Shimadzu FT-IR-8400 spectrometer in the region of 400–4000 cm^{-1}in the KBr disk. A microwave oven (ETHOS 1600, Milestone) with a power of 600 W specially designed for organic synthesis was used. ^{1}H NMR and ^{13}C NMR spectra were measured with Bruker 500, 400 MHz spectrometers in DMSO-d_6 and $CDCl_3$ as solvents. All chemical shifts were reported as (ppm) values. The Mass spectra were recorded by (Agilent Technology-5973), mass spectrometer operating at an ionization potential of 70 eV.

General procedure for the synthesis of (1,2,4,5-tetraphenyl-1*H*-imidazole)derivatives 5a–5f

The hydroxyl-substituted imidazole derivatives **5a–5f** were synthesized through one-pot four-component reactions. A mix of aniline derivatives **4a–4f** (1.1 mmol), benzyl **3** (1 mmol, 0.210 g), vanillin **1** (1 mmol), and NH_4OAc **2** (1 mmol) in glacial AcOH (2.5 mL) was solved and heated in a stir when it was microwaved (230 W) for 4–6 min in a heat-resistant container.

The process of forming the final product **5a–5f** can be examined by TLC. After the reaction, (12 mL) of distilled water was added to the stirring solution and the pH of the solution becomes quenched with NH_4OH. Then, the precipitates were collected by filtration and recrystallized, and finally, the products **5a–5f** were obtained with good yields. The structures of the prepared compounds were confirmed by analyzing their spectral characteristics by FT-IR, $^{1}HNMR$, $^{13}CNMR$, and determination of the substrates' purity and reaction monitoring were accompanied by thin-layer chromatography (TLC) [31, 40].

Spectra data for the synthesized products of 5a–5f

2-methoxy-4-(1,4,5-triphenyl-1H-imidazol-2-yl)phenol (5a) White solid; yield: 66%; m.p.: 208.82 °C; FT-IR (KBr, υ/cm^{-1}): 3449 (O–H stretch); 3054 (aromatic C–H stretch); 1644, 1602 (C = N stretch); 1537, 1489 (C = C stretch); 1273 (C–O stretch); 1228 (C–N stretch); 764, 697 (aromatic C–H out of plane bend). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.29 (s, 1H), 7.50 (dd, J = 8.2, 1.4 Hz, 2H), 7.38–7.34 (m, 3H), 7.33–7.24 (m, 9H), 7.18 (tt, J = 7.2, 1.7 Hz, 1H), 6.88 (dd, J = 8.2, 2.1 Hz, 1H), 6.85 (d, J = 2.1 Hz, 1H), 6.69 (d, J = 8.2 Hz, 1H), 3.52 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6)δ: 147.4, 147.3, 146.8, 137.5, 136.9, 135.1, 131.6, 131.1, 131.1, 129.6, 129.4, 129.0, 128.9, 128.8, 128.6, 126.9, 126.8, 121.9 (2C), 115.7, 112.8, 55.6. HRMS-ESI (*m/z*) [M^+] Calcd. for $C_{28}H_{22}N_2O_2$ 418.1683, found 418.1676. Elemental analysis Calcd. for $C_{28}H_{22}N_2O_2$ (%): C, 80.36; H, 5.30; N, 6.69; O, 7.65 Found: C, 80.45; H, 5.20; N, 6.67; O, 7.68.

4-(1-(4-chlorophenyl)-4,5-diphenyl-1H-imidazol-2-yl)-2-methoxyphenol (5b) White solid; yield: 72%; m.p.: 201.36 °C; FT-IR (KBr, υ/cm^{-1}): 3449 (O–H stretch); 1637 (C = N stretch); 1535, 1488 (C = C stretch); 1430, 1384; 1274 (C–O stretch); 1232 (C–N

stretch); 778, 698 (aromatic C–H out of plane bend). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.33 (s, 1H), 7.50 (dd, J = 8.6, 1.4 Hz, 2H), 7.43 (d, J = 8.6 Hz, 2H), 7.36–7.33 (m, 3H), 7.30 (d, J = 8.6 Hz, 2H), 7.28–7.24 (m, 4H), 7.19 (tt, J = 7.4, 1.7 Hz, 1H), 6.89 (d, J = 2 Hz, 1H), 6.83 (dd, J = 8.4, 2.2 Hz, 1H), 6.72 (d, J = 8.4 Hz, 1H), 3.59 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6)δ: 147.5, 147.5, 147.0, 137.0, 136.4, 134.9, 133.6, 131.6, 131.2, 131.0, 130.9, 129.6, 129.1, 129.0, 128.6, 126.9, 126.9, 122.1, 121.7, 115.7, 113.0, 55.7. HRMS-ESI (m/z) [M$^+$] Calcd. for $C_{28}H_{21}ClN_2O_2$ 452.1292, found 452.1288. Elemental analysis Calcd. for $C_{28}H_{21}ClN_2O_2$ (%): C, 74.25; H, 4.67; Cl, 7.83; N, 6.18; O, 7.06 Found: C, 74.21; H, 4.69; Cl, 7.80; N, 6.21; O, 7.09.

4-(1-(4-ethoxyphenyl)-4,5-diphenyl-1H-imidazol-2-yl)-2-methoxyphenol (5c) Taupe solid; yield: 73%; m.p.: 219.45 °C; FT-IR (KBr, υ/cm^{-1}): 3417 (O–H stretch); 3055 (aromatic C-H stretch); 2980 (aliphatic C-H stretch); 1605 (C = N stretch); 1510, 1480 (C = C stretch); 1432; 1240 (C–O stretch); 832, 775, 696 (aromatic C–H out of plane bend). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.28 (s, 1H), 7.49 (dd, J = 8.2, 1.4 Hz, 2H), 7.34–7.30 (m, 3H), 7.27–7.23 (m, 4H), 7.20–7.15 (m, 3H), 6.91–6.86 (m, 4H), 6.70 (d, J = 8.4 Hz, 1H), 3.98 (q, J = 7 Hz, 2H), 3.57 (s, 3H), 1.30 (t, J = 7.1,2.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 158.7, 147.4, 147.3, 147.0, 136.7, 135.2, 131.6, 131.4, 131.2, 130.5, 130.0, 128.9, 128.7, 128.6, 126.8, 126.7, 122.1, 121.8, 115.6, 115.0, 112.8, 63.8, 55.7, 15.0. HRMS-ESI (m/z) [M$^+$] Calcd. for $C_{30}H_{26}N_2O_3$ 462.1943, found 462.1936. Elemental analysis Calcd. for $C_{30}H_{26}N_2O_3$ (%): C, 77.90; H, 5.67; N, 6.06; O, 10.38 Found: C, 78.00; H, 5.62; N, 6.07; O, 10.32.

4-(4,5-diphenyl-1-(p-tolyl)-1H-imidazol-2-yl)-2-methoxyphenol (5d) White crystal solid; yield: 77%; m.p.: 222.61 °C; FT-IR (KBr, υ/cm^{-1}): 3457 (O–H stretch); 3033 (aromatic C–H stretch); 1603 (C = N stretch); 1516, 1481 (C = C stretch); 1434, 1383; 1264 (C–O stretch); 1230 (C–N stretch); 777, 700 (aromatic C-H out of plane bend). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.27 (s, 1H), 7.49 (dd, J = 8.8, 1.4 Hz, 2H), 7.33–7.30 (m, 3H), 7.27–7.23 (m, 4H), 7.20–7.12 (m, 5H), 6.88 (dd, J = 8, 2 Hz, 1H), 6.86 (d, J = 2 Hz, 1H), 6.70 (d, J = 8 Hz, 1H), 3.54 (s, 3H), 2.28 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 147.3, 147.2 (d, J = 8.0 Hz), 146.9, 138.5, 136.9, 135.1, 134.9, 131.6, 131.2 (2C), 130.1, 129.1, 128.9, 128.8, 128.6, 126.9, 126.8, 122.1 (d, J = 4.0 Hz), 121.9, 115.6 (d, J = 9.0 Hz), 112.8, 55.6, 21.1. HRMS-ESI (m/z) [M] Calcd. for $C_{29}H_{24}N_2O_2$ 432.1838, found 432.1833. Elemental analysis Calcd. for $C_{29}H_{24}N_2O_2$ (%): C, 80.53; H, 5.59; N, 6.48; O, 7.40 Found: C, 80.60; H, 5.62; N, 6.37; O, 7.41.

2-methoxy-4-(1-(3-nitrophenyl)-4,5-diphenyl-1H-imidazol-2-yl)phenol (5e) Yellow solid; yield: 70%; m.p.: 231.38 °C; FT-IR (KBr, υ/cm^{-1}): 3427 (O–H), 3053 (aromatic C–H stretch), 1924 (aliphatic C–H stretch), 1602 (C = N stretch), 1530 (NO_2 asymmetric stretch), 1486 (C = C stretch), 1445, 1350 (NO_2 symmetric stretch), 1291 (C–O stretch), 1223 (C-N stretch), 779, 695 (aromatic C–H out-of-plane bend). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.45 (s, 1H), 7.68 (d, J = 1.6 Hz, 1H), 7.62 (dd, J = 8.4, 1.6 Hz, 2H), 7.41 (d, J = 1.6 Hz, 1H), 7.32–7.22 (m, 7H), 7.20–7.16 (m, 4H), 7.02 (d, J = 8.4 Hz, 2H), 3.80 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 147.5, 147.5, 147.0,

137.1, 136.8, 134.9, 132.6, 131.6, 131.5, 130.9, 130.8, 129.1, 129.0, 128.6, 126.9, 126.9, 122.1, 122.1, 121.7, 115.7, 113.0, 55.7. HRMS-ESI (*m/z*) [M^+] Calcd. for $C_{28}H_{21}N_3O_4$ 463.1589, found 463.1582. Elemental analysis Calcd. for $C_{28}H_{21}N_3O_4$ (%): C, 72.56; H, 4.57; N, 9.07; O, 13.81 Found: C, 72.60; H, 4.63; N, 8.99; O, 13.78.

4-(4,5-diphenyl-1H-imidazol-2-yl)-2-methoxyphenol (5f) Color less crystal solid; yield: 94%; m.p.: 261.21 °C; FT-IR (KBr, υ/cm^{-1}): 3087 (aromatic C–H stretch); 1654, 1607 (C = N stretch); 1499 (C = C stretch); 1274 (C–O stretch); 1228 (C-N stretch); 767, 697 (aromatic C–H out of plane bend). The structure of the product was confirmed by FT-IR spectroscopy and comparison of the observed melting temperature with the reported melting temperature ([41] 260 °C).HRMS-ESI (*m/z*) [M^+] Calcd. for $C_{22}H_{18}N_2O_2$ 342.1435, found 342.1422. Elemental analysis Calcd. for $C_{22}H_{18}N_2O_2$ (%): C, 77.17; H, 5.30; N, 8.18; O, 9.35 Found: C, 77.13; H, 5.33; N, 8.21; O, 9.33.

General procedure for the synthesis of aldehyde imidazole derivatives 6a–6f

Hexamethylenetetramine (HMTA) (4 mmol) was added to the solution dissolved **5a–5f** (3.75 mmol) in trifluoroacetic acid (TFA) (7 mL), and the resulting solution was refluxed for 12 h at 70 °C. Then 6 mL of HCl 3 M was added to it and refluxed again for 30 min. The solution was cooled to r. t. and quenched with NH_4OH. Then, the precipitates were collected by filtration and washed with the AcOH solution, and the imidazole aldehydes **6a–6f** were obtained with good results.

Spectra data for the synthesized products of 6a–6f

2-Hydroxy-3-methoxy-5-(1,4,5-triphenyl-1H-imidazol-2-yl) benzaldehyde (6a) Tan solid; yield: 76%; m.p: 176.31 °C; FT-IR (KBr, υ/cm^{-1}): 3382 (O–H); 3051 (aromatic C–H stretching); 1670 (aldehyde C = O); 1599 (C = N stretch); 1477, 1430, (C = C stretch); 1278, 1075 (C–O stretch); 1204 (C–N stretch); 769, 697 (aromatic C–H out of plane bending). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 11.18 (br, 1H), 9.74 (s, 1H), 7.60 (dd, *J* = 8.6, 1.5 Hz, 2H), 7.37 (d, *J* = 2.0 Hz, 1H), 7.36–7.32 (m, 3H), 7.31–7.22 (m, 6H), 7.15 (dd, *J* = 8.0, 1.5 Hz, 2H), 7.13–7.11 (m, 3H), 3.65 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$)δ: 196.56, 151.59, 147.82, 145.32, 138.16, 136.92, 133.84, 131.07, 131.01, 130.08, 129.44, 128.72, 128.50, 128.45, 128.26, 128.21, 127.51, 126.93, 125.22, 122.07, 120.26, 117.89, 55.99. HRMS-ESI (*m/z*) [M^+] Calcd. For $C_{29}H_{22}N_2O_3$ 446.1681, found 446.1673. Elemental analysis Calcd. For $C_{29}H_{22}N_2O_3$ (%): C, 78.01; H, 4.97; N, 6.27; O, 10.75 Found: C, 78.05; H, 4.91; N, 6.23; O, 10.81.

5-(1-(4-Chlorophenyl)-4,5-diphenyl-1H-imidazol-2-yl)-2-hydroxy-3-methoxybenzaldehyde (6b) Dark yellow solid; yield: 75%; m.p.: 197.74 °C; FT-IR (KBr, υ/cm^{-1}): 3415 (O–H); 3063 (aromatic C-H stretching); 2844 (aliphatic C–H); 1656 (aldehyde C = O); 1600 (C = N stretch); 1492, 1469,1431 (C = C stretch); 1260, 1092 (C–O stretch); 1177 (C–N stretch); 962, 744, 719, 696 (aromatic C–H out

of plane bending). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 11.23 (br, 1H), 9.77 (s, 1H), 7.60 (d, J = 7.3 Hz, 2H), 7.32–7.23 (m, 9H), 7.16 (d, J = 6.8 Hz, 2H), 7.12 (s, 1H), 7.05 (d, J = 8.2 Hz, 2H), 3.71 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$)δ: 196.42, 151.73, 147.98, 145.34, 138.54, 135.57, 134.62, 133.94, 131.05, 130.82, 130.03, 129.68, 129.61, 128.65, 128.40, 128.28, 127.33, 126.94, 125.05, 122.18, 120.28, 117.82, 56.05. HRMS-ESI (*m*/*z*) [M^+] Calcd. For $C_{29}H_{21}ClN_2O_3$ 480.1298, found 480.1291. Elemental analysis Calcd. For $C_{29}H_{21}ClN_2O_3$ (%): C, 72.42; H, 4.40; Cl, 7.37; N, 5.82; O, 9.98 Found: C, 72.46; H, 4.39; Cl, 7.35; N, 5.89; O, 9.91.

5-(1-(4-Ethoxyphenyl)-4,5-diphenyl-1H-imidazol-2-yl)-2-hydroxy-3-methoxybenzaldehyde (6c) Pale yellow solid; yield: 76%; m.p.: 114.68 °C; FT-IR (KBr, υ/cm^{-1}): 3411 (O–H); 3051 (aromatic C–H stretching); 2975, 2935 (aliphatic C–H); 1661 (aldehyde C = O); 1606 (C = N stretch); 1511, 1476, 1441 (C = C stretch); 1244, 1073 (C–O stretch); 1168 (C–N stretch); 723, 697 (aromatic C-H out of plane bending). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 9.75 (s, 1H), 7.59 (dd, J = 8.5, 1.5 Hz, 2H), 7.36 (d, J = 2.0 Hz, 1H), 7.30–7.23 (m, 6H), 7.21 (d, J = 2.0 Hz, 1H), 7.16 (dd, J = 7.9, 1.7 Hz, 2H), 7.02 (d, J = 8.9 Hz, 2H), 6.82 (d, J = 8.9 Hz, 2H), 4.00 (t, J = 7.0 Hz, 2H), 3.71 (s, 3H), 1.42 (t, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 196.63, 158.90, 151.55, 147.83, 145.46, 137.91, 133.90, 131.26, 131.08, 130.20, 129.49, 129.31, 128.43, 128.24, 128.14, 127.48, 126.87, 125.17, 122.20, 120.23, 117.98, 114.97, 63.78, 56.06, 14.64. HRMS-ESI (*m*/*z*) [M^+] Calcd. For $C_{31}H_{26}N_2O_4$ 490.1958, found 490.1966. Elemental analysis Calcd. For $C_{31}H_{26}N_2O_4$ (%): C, 75.90; H, 5.34; N, 5.71; O, 13.05 Found: C, 75.86; H, 5.37; N, 5.77; O, 13.00.

5-(4,5-Diphenyl-1-(p-tolyl)-1H-imidazol-2-yl)-2-hydroxy-3-methoxybenzaldehyde (6d) Brown-green solid; yield: 70%; m.p.: 181.94 °C; FT-IR (KBr, υ/cm^{-1}): 3415 (O–H); 3043 (aromatic C–H stretching); 2850 (aliphatic C–H); 1675 (aldehyde C = O); 1602 (C = N stretch); 1511, 1476, 1434 (C = C stretch); 1280, 1078 (C–O stretch); 1205 (C–N stretch); 751, 695 (aromatic C–H out of plane bending). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 11.21 (br, 1H), 9.76 (s, 1H), 7.61 (dd, J = 8.6, 1.4 Hz, 2H), 7.39 (d, J = 2.0 Hz, 1H), 7.31–7.29 (m, 3H), 7.27–7.21 (m, 3H), 7.17 (dd, J = 7.8, 1.6 Hz, 2H), 7.14–7.12 (m, 3H), 7.00 (d, J = 8.2 Hz, 2H), 3.66 (s, 3H), 2.36 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$)δ: 196.63, 151.44, 147.73, 145.34, 138.70, 138.22, 134.44, 134.24, 131.08, 131.07, 130.40, 129.97, 128.40, 128.23 (2C), 128.07, 127.36, 126.75, 125.10, 122.59, 120.24, 117.88, 55.92, 21.12. HRMS-ESI (*m*/*z*) [M^+] Calcd. For $C_{30}H_{24}N_2O_3$ 460.1875, found 460.1901. Elemental analysis Calcd. For $C_{30}H_{24}N_2O_3$ (%): C, 78.24; H, 5.25; N, 6.08; O, 10.42 Found: C, 78.30; H, 5.28; N, 6.12; O, 10.30.

2-Hydroxy-3-methoxy-5-(1-(3-nitrophenyl)-4,5-diphenyl-1H-imidazol-2-yl)benzaldehyde (6e) Olive solid; yield: 69%; m.p.: 205.31 °C; FT-IR (KBr, υ/cm^{-1}): 3421 (O–H); 3074 (aromatic C–H stretching); 1654 (aldehyde C = O); 1601 (C = N stretch); 1532, 1348 (NO_2 symmetric stretching); 1466, 1446, 1393 (C = C stretch); 1256, 1072 (C-O stretch); 1212 (C–N stretch); 962, 759, 700 (aromatic

C–H out of plane bending). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 11.18 (br, 1H), 9.73 (s, 1H), 8.20 (ddd, J = 8.1, 2.1, 1.1 Hz, 1H), 7.97 (t, J = 2.1 Hz, 1H), 7.61 (dd, J = 8.5, 1.4 Hz, 2H), 7.52 (t, J = 8.1 Hz, 1H), 7.44 (ddd, J = 8.1, 2.1, 1.1 Hz, 1H), 7.34–7.28 (m, 5 H), 7.26 (tt, J = 7.2, 2.6 Hz, 1H), 7.21 (s, 2H), 7.17 (dd, J = 7.8, 1.5 Hz, 2H), 3.75 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 196.07, 152.07, 148.37, 148.30, 145.52, 138.82, 138.16, 134.32, 133.55, 131.10, 130.75, 130.24, 129.59, 128.90, 128.82, 128.34, 127.29, 127.16, 125.00, 123.44, 123.33, 121.69, 120.28, 117.94, 56.22. HRMS-ESI (*m*/*z*) [M^+] Calcd. For $C_{29}H_{21}N_3O_5$ 491.1562, found 491.1575. Elemental analysis Calcd. For $C_{29}H_{21}N_3O_5$ (%): C, 70.87; H, 4.31; N, 8.55; O, 16.28 Found: C, 70.92; H, 4.28; N, 8.60; O, 16.20.

5-(4,5-Diphenyl-1H-imidazol-2-yl)-2-hydroxy-3-methoxy benzaldehyde (6f) Dark khaki solid; yield: 85%, m.p.: 167.82 °C; FT-IR (KBr, υ/cm^{-1}): 3304 (N–H); 3054 (aromatic C–H stretching); 1648 (aldehyde C = O); 1468, 1445, 1397 (C = C stretch); 1261, 1092 (C–O stretch); 1205 (C–N stretch); 955, 760, 695 (aromatic C–H out of plane bending). The product was too insoluble in any appropriate solvent for analysis by NMR spectroscopy. HRMS-ESI (*m*/*z*) [M^+] Calcd. For $C_{23}H_{18}N_2O_3$ 370.1384, found 370.1391. Elemental analysis Calcd. For $C_{23}H_{18}N_2O_3$ (%): C, 74.58; H, 4.90; N, 7.56; O, 12.96 Found: C, 74.52; H, 4.93; N, 7.60; O, 12.95.

General procedure for the synthesis of thiazolidine-2,4-diones derivatives 7a–7f

To a mixture of TZD (0.6 mmol), 2-hydroxy-3-methoxy-5-(1,4,5-triphenyl-1*H*-imidazol-2-yl)benzaldehyde (6a, 0.6 mmol) in EtOH (5 mL) was added amount of piperidine as catalytic; it was refluxed for 18–24 h. Then, the reaction mixture was poured into cold H_2O and AcOH was added dropwise until the solution became acidic. After that, it was stirred for 30 min and the precipitates of the product were filtered, washed with H_2O and recrystallized with EtOH, to obtain pure (Z)-5-(2-hydroxy-3-methoxy-5-(1,4,5-triphenyl-1*H*-imidazol-2-yl)benzylidene)thiazolidine-2,4-dione derivative **7a** as yellow solids. All the other compounds **7b–7f** were obtained in a similar reaction procedure of **7a** in moderate to high yields (Scheme 1 and Table 1).

Spectra data for the synthesized products of 7a–7f

(Z)-5-(2-hydroxy-3-methoxy-5-(1,4,5-triphenyl-1H-imidazol-2-yl)benzylidene)thiazolidine-2,4-dione (7a) Yellow solid; yield: 82%; m.p.: 232.59 °C; FT-IR (KBr) ν cm^{-1}: 3411 (N–H); 3059 (aromatic C–H stretching); 2933 (aliphatic C–H); 1740, 1703 (C = O); 1559 (C = N stretch); 1490, 1426 (C = C stretch); 1273, 1072 (C–O stretch); 1159 (C–N stretch); 767, 695 (aromatic C-H out of plane bend). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.57 (s, 1H), 10.49 (s,1H), 7.93 (s, 1H,), 7.52–7.50 (d, J = 7.8 Hz, 2H), 7.44–7.34 (ddd, J = 8.3 Hz, 11H), 7.30–7.28 (d, J = 8.3 Hz, 2H), 7.20 (s, 1H), 7.13 (s, 1H), 3.71 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 167.83, 167.21, 148.19, 147.58, 144.64, 134.97, 131.37, 131.17, 129.53, 129.40, 129.25, 128.63, 128.50, 128.11, 128.08, 127.15, 125.54, 123.64, 120.93, 120.23, 116.87, 114.40, and 56.02. ^{13}C NMR-DEPT (135): CH (131.14, 130.54, 129.43,

128.62, 128.31, 126.91, 126.48, 125.79) and CH_3 (55.78). HRMS-ESI (*mlz*) [M^+] Calcd. For $C_{32}H_{23}N_3O_4S$ 545.1412, found 545.1396. Exact mass [M^+] (*m/z*) = 346, 340(100), 252, 169, 128, 97. Elemental analysis Calcd. For $C_{32}H_{23}N_3O_4S$ (%): C, 70.44; H, 4.25; N, 7.70; O, 11.73; S, 5.88 Found: C, 70.54; H, 4.20; N, 7.67; O, 11.70; S, 5.89.

(Z)-5-(5-(1-(4-chlorophenyl)-4,5-diphenyl-1H-imidazol-2-yl)-2-hydroxy-3-methoxybenzylidene)thiazolidine-2,4-dione (7b) Pale yellow solid; yield: 78%; m.p.: 255.34 °C; FT-IR (KBr) ν cm^{-1}: 3407 (N–H); 3058 (aromatic C–H stretching); 2955 (aliphatic C–H); 1743, 1706 (C = O); 1607 (C = N stretch); 1491, 1430 (C = C stretch); 1269, 1076 (C–O stretch); 1156 (C–N stretch); 1011 (C–Cl); 834,776, 725, 699 (aromatic C–H out of plane bend). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.53 (s, 1H), 10.24 (s, 1H,), 7.94 (s, 1H), 7.49–7.48 (d, J = 8.4 Hz, 2H), 7.43–7.41 (d, J = 8.4 Hz, 2H), 7.36–7.34 (m, 5H,), 7.28–7.21 (m, 5H), 7.13 (s, 1H), 6.99 (s, 1H), 3.73 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 167.75, 167.31, 152.81, 150.11, 148.90, 147.57, 133.65, 131.54, 131.19, 130.59, 129.67, 129.45, 128.76, 128.64, 128.30, 126.91, 126.53, 125.97, 123.03, 122.61, 119.96, and 55.87. ^{13}C NMR-DEPT (135): CH (131.15, 129.55, 129.29, 128.65, 127.11, 125.39) and CH_3 (55.95). HRMS-ESI (*mlz*) [M^+] Calcd. For $C_{32}H_{22}ClN_3O_4S$ 579.1023, found 579.1041. Exact mass [M^+] (*m/z*) = 329, 317, 219, 195, 177, 168(100), 152, 130, 94. Elemental analysis Calcd. For $C_{32}H_{22}ClN_3O_4S$ (%): C, 66.26; H, 3.82; Cl, 6.11; N, 7.24; O, 11.03; S, 5.53 Found: C, 66.20; H, 3.86; Cl, 6.07; N, 7.29; O, 11.10; S, 5.48.

(Z)-5-(5-(1-(4-ethoxyphenyl)-4,5-diphenyl-1H-imidazol-2-yl)-2-hydroxy-3-methoxybenzylidene)thiazolidine-2,4-dione (7c) Pale yellow solid; yield: 85%; m.p.: 161.88 °C; FT-IR (KBr) ν cm^{-1}: 3407 (N–H); 3056 (aromatic C–H stretching); 2959, 2933 (aliphatic C–H); 1738, 1702 (C = O); 1605 (C = N stretch); 1511, 1480 (C = C stretch); 1249, 1074 (C–O stretch); 1119 (C-N stretch); 838, 775, 697 (aromatic C-H out of plane bend). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.53 (s, 1H), 10.36 (s, 1H), 7.93 (s, 1H), 7.49–7.48 (d, J = 7.4 Hz, 2H), 7.34–7.23 (m, 11H), 7.07 (s, 1H), 6.88–6.87 (d, J = 8.7 Hz, 2H), 3.96–3.95 (d, J = 8.7 Hz, 2H), 3.73 (s, 3H), 1.27–1.24 (t, J = 8.7 Hz,3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 167.86, 167.24, 158.79, 147.53, 144.84, 135.99, 131.55, 131.14, 129.89, 129.78, 128.98, 128.57, 128.47, 126.93, 125.77, 123.37, 120.37, 115.01, 114.30, 63.38, 55.95, and 14.37. ^{13}C NMR-DEPT (135): CH (131.13, 129.91, 129.79, 128.59, 128.49, 126.84, 125.67, 114.95, 114.77); CH_2 (-62.13) and CH_3 (55.90, 14.40). HRMS-ESI (*mlz*) [M^+] Calcd. For $C_{34}H_{27}N_3O_5S$ 589.1743, found 589.1702. Exact mass [M^+] (*m/z*) = 346, 340(100), 279, 189,181, 171, 163, 158, 96. Elemental analysis Calcd. For $C_{34}H_{27}N_3O_5S$ (%): C, 69.26; H, 4.62; N, 7.13; O, 13.57; S, 5.44 Found: C, 69.30; H, 4.55; N, 7.16; O, 13.48; S, 5.51.

(Z)-5-(5-(4,5-diphenyl-1-(p-tolyl)-1H-imidazol-2-yl)-2-hydroxy-3-methoxybenzylidene)thiazolidine-2,4-dione (7d) Dark yellow solid; yield: 83%; m.p.: 252.69 °C; FT-IR (KBr) ν cm^{-1}: 3398 (N–H); 3036 (aromatic C–H stretching); 2930 (aliphatic C–H); 1738, 1701 (C = O); 1600 (C = N stretch); 1511, 1478

(C = C stretch); 1269, 1076 (C–O stretch); 1158 (C–N stretch); 774, 697 (aromatic C-H out of plane bend). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.52 (s, 1H), 10.25 (s, 1H), 7.93 (s, 1H), 7.50–7.48 (d, J = 7.8 Hz, 2H), 7.33–7.30 (m, 5H), 7.26–7.23 (m, 5H), 7.20–7.16 (t, J = 8.1 Hz, 3H), 7.05 (s, 1H), 3.71 (s, 3H), 2.51 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 167.96, 167.26, 147.50, 144.95, 138.84, 131.31, 131.16, 130.00, 128.79, 128.53, 128.38, 128.35, 127.20, 126.93, 125.88, 123.26, 120.05, 114.14, 55.87, and 20.56. ^{13}C NMR-DEPT (135): CH (131.14, 130.01, 129.75, 128.78, 128.54, 128.36, 127.15, 126.67, 125.79) and CH_3 (55.79, 20.56). HRMS-ESI (*mlz*) [M^+] Calcd. For $C_{33}H_{25}N_3O_4S$ 559.1597, found 559.1634. Exact mass [M^+] (*m*/*z*) = 346, 340(100), 279, 189,181, 171, 165, 158,145, 116, 91. Elemental analysis Calcd. For $C_{33}H_{25}N_3O_4S$ (%): C, 70.82; H, 4.50; N, 7.51; O, 11.44; S, 5.73 Found: C, 70.79; H, 4.53; N, 7.47; O, 11.46; S, 5.75.

(Z)-5-(2-hydroxy-3-methoxy-5-(1-(3-nitrophenyl)-4,5-diphenyl-1H-imidazol-2-yl) benzylidene)thiazolidine-2,4-dione (7e) Pale yellow solid; yield: 80%; m.p.: 240.77 °C; FT-IR (KBr) ν cm^{-1}: 3417(N–H); 3059 (aromatic C–H stretching); 2959, 2933 (aliphatic C–H); 1738, 1702 (C = O); 1641, 1604 (C = N stretch); 1535, 1350 (NO_2 symmetric stretch); 1488, 1425 (C = C stretch); 1270, 1075 (C-O stretch); 1158 (C–N stretch); 860, 775, 697 (aromatic C-H out of plane bend). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.55 (s, 1H), 10.26 (s, 1H), 8.42–8.40 (d, J = 9.7 Hz, 1H), 8.24–8.22 (d, J = 9.3 Hz, 1H), 7.91 (s, 1H), 7.71–7.67 (t, J = 8.7 Hz, 1H), 7.54–7.52 (d, J = 7.6 Hz, 2H), 7.41–7.33 (m, 10H), 7.01 (s, 1H), 3.74 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 167.48, 167.15, 156.39, 154.52, 154.25, 148.37, 147.78, 147.10, 147.10, 145.10, 136.97,135.40, 131.26, 131.21, 130.90, 129.48, 128.89, 128.86, 128.68, 127.05, 125.36, 123.92, 122.48, 121.44, 120.93, 120.19, 56.14. ^{13}C NMR-DEPT (135): CH (135.41, 131.24, 131.19, 130.92, 129.57, 128.88, 128.74, 128.70, 127.05, 125.19, 124.52, 123.95, 120.92, 116.96, 114.56) and CH_3 (56.10). HRMS-ESI (*mlz*) [M^+] Calcd. For $C_{32}H_{22}N_4O_6S$ 590.1265, found 590.1331. Exact mass [M^+] (*m*/*z*) = 346, 340(100), 279, 189,181, 171, 165, 158, 91. Elemental analysis Calcd. For $C_{32}H_{22}N_4O_6S$ (%): C, 65.08; H, 3.75; N, 9.49; O, 16.25; S, 5.43 Found: C, 65.11; H, 3.70; N, 9.53; O, 16.17; S, 5.49.

(Z)-5-(5-(4,5-diphenyl-1H-imidazol-2-yl)-2-hydroxy-3-methoxybenzylidene)thiazolidine-2,4-dione (7f) Shiny yellow solid; yield: 75%; m.p.: 200.13 °C; FT-IR (KBr) ν cm^{-1}: 3393 (N–H); 3059 (aromatic C–H stretching); 2959, 2930 (aliphatic C–H); 1732, 1689 (C = O); 1650, 1603 (C = N stretch); 1483, 1446 (C = C stretch); 1261, 1075 (C–O stretch); 1164 (C–N stretch); 768, 697 (aromatic C–H out of plane bend). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 11.74 (s, 1H), 9.81 (s, 1H), 9.55 (s, 1H), 9.41 (s, 1H), 7.24–7.21 (d, J = 6.2 Hz, 1H), 6.95–6.91 (d, J = 8.2 Hz, 1H), 6.71–6.69 (d, J = 8.2 Hz, 4H), 6.60–6.57 (d, J = 7.5 Hz, 4H), 6.53–6.50 (d, J = 7.6 Hz, 2H), 3.12–3.13 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 168.24, 167.39, 158.80, 148.07, 147.34, 144.65, 135.13, 128.59, 127.93, 126.61, 123.50, 120.53, 117.21, 125.54, 123.64, 120.93, 120.23, 113.88, 110.96, and 56.26.

^{13}C NMR-DEPT (135): CH (128.60, 127.94, 127.70, 126.61) and CH_3 (56.26). HRMS-ESI (*mlz*) [M^+] Calcd. For $C_{26}H_{19}N_3O_4S$ 469.1095, found 469.1101. Exact mass [M^+] (*m/z*): 318, 268(100), 235, 218, 194, 178, 162, 151, 126, 93. Elemental analysis Calcd. For $C_{26}H_{19}N_3O_4S$ (%): C, 66.51; H, 4.08; N, 8.95; O, 13.63; S, 6.83 Found: C, 66.46; H, 4.12; N, 8.92; O, 13.71; S, 6.79.

Biolog

Antimicrobial activities by Inhibition zone diameter assay

Antibacterial activity of the synthesized compounds **7a–7b** was determined, using a slightly modified cup plate method. Mueller Hinton agar was used for the growth of bacterial strains such as gram-positive (*B. anthracis* & *S. aureus*) and gram-negative (*E. coli* & *P. aeruginosa*). All of the test compounds were dissolved in DMSO at a Conc. of 5 mg/ml. Each plate was inoculated with 20 µl of microbial suspension. Thirty microliters of the test compounds was added to each cup. The plates containing bacteria were incubated at 37 °C for 24 h, the positive antimicrobial activity was read based on the growth inhibition zone, and compared with Chloramphenicol was used as a standard and pioglitazone as a drug, as shown in Table 2 and Fig. 2**.**

Antioxidant activity by DPPH assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical. MeOH solution of DPPH is used to evaluate the antioxidant activity of several synthesized compounds. Antioxidant on interaction with DPPH both transfers electron or hydrogen atom to DPPH, thus neutralizing its free radical character. The degree of the scavenging activity displays change in the color from purple to yellow. The change in absorbance produced at 517 nm has been used as a measure of its antioxidant activity. DPPH radical scavenging activity in percent (I %) was calculated as follows:

$$\mathrm{I}\,\% \;=\; \left[(\mathrm{Ab}-\mathrm{As})\,/\mathrm{Ab}\right] \;\times\; 100$$

where Ab is the absorbance of blank reaction (DPPH + MeOH) and as the absorbance of the test sample compounds.

The suitable amount of DPPH was dissolved in MeOH to give a Conc. of 6.25×10^{-5} M. The sample solutions at Conc. of 2000, 1000, 500, 250, and 125 µg/mL in MeOH were prepared and ascorbic acid as a standard. To 0.5 mL of each sample, the solution was added 3.5 mL of fresh DPPH solution and was shaken vigorously. Samples were kept in darkness for 30 min at 37 °C, and then their absorbance was measured at 517 nm. The investigation of antioxidant activity revealed that all the newly synthesized compounds showed potent to moderate radical scavenging activity when compared with ascorbic acid as a standard; the results are shown in Figs. 3, 4 and Table3.

Cytotoxic activity by MTT assay

Cell lines

The human breast cancer cells (MCF-7) were obtained from the Pasteur Institute of Iran (Tehran, Iran). The cells were maintained at 37 °C in a humidified atmosphere (90%) containing 5% CO2 and then cultured in DMEM (Dulbecco's Modified Eagle's Medium) with 10% (v/v) FBS (fetal bovine serum), 100 units/mL penicillin, and 100 µg/mL streptomycin. The cells were seeded overnight and then incubated with different concentrations of compounds **7a–7f** and cisplatin.

Cell viability assay

Cell viability following exposure to synthetic compounds was estimated by using the MTT (3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide) reduction assay[42]. The (MCF-7) cells were seeded in 96-well plates and at 10,000 cells per well containing 200 µL medium. After 24-h incubation at 37 °C, different concentrations of test compounds were added to the wells. The samples including **7a–7f** and cisplatin were tested at 25, 50, 75, 100, 125, 125, 150, 150, 175, and 200 µg/mL concentrations. The samples were dissolved in dimethyl sulfoxide (DMSO) and further diluted with cell culture medium. The final concentration of DMSO was adjusted to 1% of the total volume of the medium in all treatment, including the blank. A control medium without DMSO was also incubated. After the treatment, 5 mg/mL of MTT solution was added and incubated for 3 h at 37 °C in a dark place. The absorbance of formazan creation was measured at a wavelength of 570 nm with an enzyme-linked immunosorbent assay (ELISA) reader (Molecular Devices, Sunnyvale, CA, USA). The cell viability by MTT assay was calculated as a percentage of the control value (untreated cells) (Fig. 5).

Conclusions

In this project, we were able to provide an easy and efficient method with relatively high-efficiency and high-purity products for the synthesis of compounds **7a**–**7f**, which includes two rings of thiazolidine-2,4-dione and imidazole. Also, the obtained products **7e, 7d**, and **7f** had high antioxidant properties, among which the synthesized product **7e** had stronger antioxidant properties due to the presence of the NO_2 group in the DPPH method. This presents the potential electron donation capacity of products besides their hydrogen atom transfer capacity. Among the synthesized products, compound **7c** had a lower IC50 than ascorbic acid. The IC50 value of product **7c** may be due to the presence of a *p*-OEt functional group in the structure of this compound. Compounds **7a**–**7f** and pioglitazone were also microbial evaluated and showed low to moderate antimicrobial activity against gram-positive and gram-negative bacteria. Compound **7c**, among other compounds, showed very good inhibition of zone diameter assay compared to gram-positive (*B. anthracis*) bacteria, and only product **7d** showed inhibition of zone diameter assay against

gram-negative (*E. coli*) bacteria, and the drug pioglitazone only shows inhibitory activity against gram-negative (*P. aeruginosa*) bacteria. The cytotoxic effects of the compounds against breast cancer cells (MCF-7) have been studied using the modified MTT method. The presence of chlorine and nitrogen dioxide groups in the structure of the compound increased the growth inhibition of cancer cells, and compound **7b** had the best results.

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