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Novel imidazo[2,1-b][1,3,4]thiadiazole (ITD) hybrid compounds: Design, synthesis, efficient antibacterial activity and antioxidant effects

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ABSTRACT

In this study, novel imidazo[2,1-b][1,3,4]thiadiazole (ITD) compounds were synthesized and their antimicrobial and antioxidant capacity was examined. The C-2 position of the ITD structure was fixed with the 3,4-hydroxybenzene ring and the properties of the two series of compounds obtained by phenyl or 4-chlorophenyl in the C-6 position were compared. In the formation of these series, new properties were determined by the addition of different pharmacophore to the target product by binding of the groups known in the literature from the C-5 position to the structure. In the study, it was seen that the compounds **4a, 4b, 5a, 5b, 7f, 10, 12** and **13** had very high anti-tuberculosis activities at low concentrations, **3b** was found to exhibit moderate activity while other synthesis compounds exhibited moderate activity. In addition, it showed activity against gram positive and negative bacteria. In the determination of the antioxidant capacities of the newly synthesized compounds by FRAP and DPPH methods, the compounds showing activity were found to be **2, 3a, 3b, 6c, 9, 11** and **13**.

The structures of all synthesized compounds were solved by spectroscopic methods such as FT-IR, ^{1}H NMR, ^{13}C NMR and mass.

1. Introduction

Tuberculosis (TB) remains a very serious health problem today. The presence of this disease with the bacterium *Mycobacterium tuberculosis* by threatening one-third of the world's population has proven to be sick with this microorganism. It has become an important problem for the emergence of drug derivatives which contain new active groups that will resist the microorganism especially for the treatment of tuberculosis [1]. Therefore, there is a need for synthesis of hybrid compounds having a novel structure to destroy this effect. Some of the structures which have this effect and have proven effective in the literature are compounds having triazole compounds, thiadiazole compounds and imidazo[2,1-b][1,3,4] thiadiazole (ITD) skeleton.

Imidazo[2,1-b][1,3,4]thiadiazole (ITD) derivatives exhibit different biological activities such as antimicrobial [2], antifungal [3], anticancer [4,5], anticonvulsant [6], analgesic [7], antiinflammatory [8,9], anesthetic and diuretic [6], antitubercular [10–12] and are in the class of compounds that are open to potential development [13–16]. In this respect, it is important to create new derivatives. Research efforts over the last decade have mainly focused on the synthesis and evaluation of the ITD derivatives as antibacterial, antiTB and anticancer agents. In this context, it has been reported in the literature that the difference of groups or atoms in the 2- and 6- positions of the ITD-derived compounds to be synthesized had a significant effect on the variety and effect of their biological activities [6]. In addition, the presence of different groups in the ITD C-5 position has been reported in the literature that contributed to the various biological effects [17]. It has been shown that the groups bound in the C-5 position as mentioned below (Fig. 1) are effective against *Mycobacterium tuberculosis* [11,12,18,19].

In this study we report the design and synthesis of new kind of ITD derivatives bearing antimicrobial and anti-tubercular activity beside antioxidant activities (Fig. 2). Phenolic compounds are substances containing one or more hydroxyl groups, including functional derivatives linked to an aromatic ring [20–23]. Phenolic is one of the most active natural antioxidants, antioxidant effects of free radicals, binding with metals and chelates to form and inhibit lipoxygenase enzyme [20,24]. Therefore, in this study we choose 3,4-dihydroxybenzoic acid as a starting material of forming the ITD C-2 group and the aromatic hydroxy group to possess antioxidant effect.

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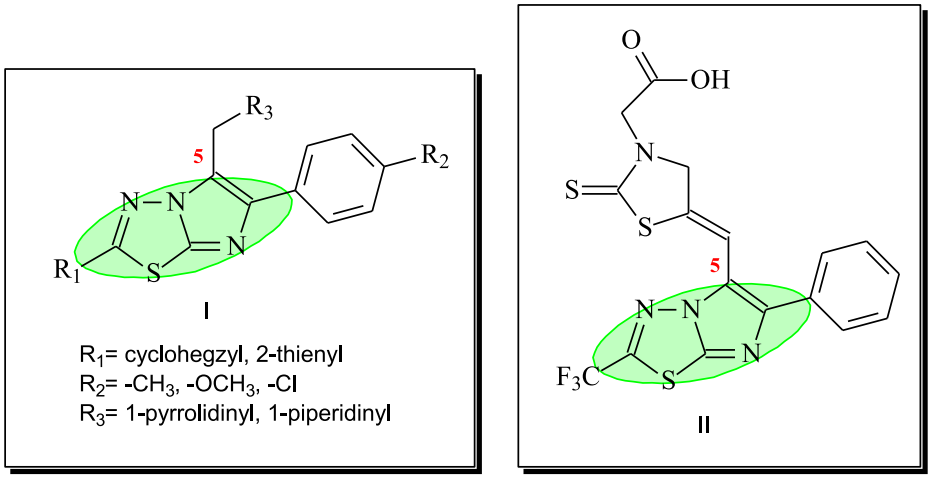


Fig. 1. Some ITD derivatives (I and II) that show anti-tubercular effect.

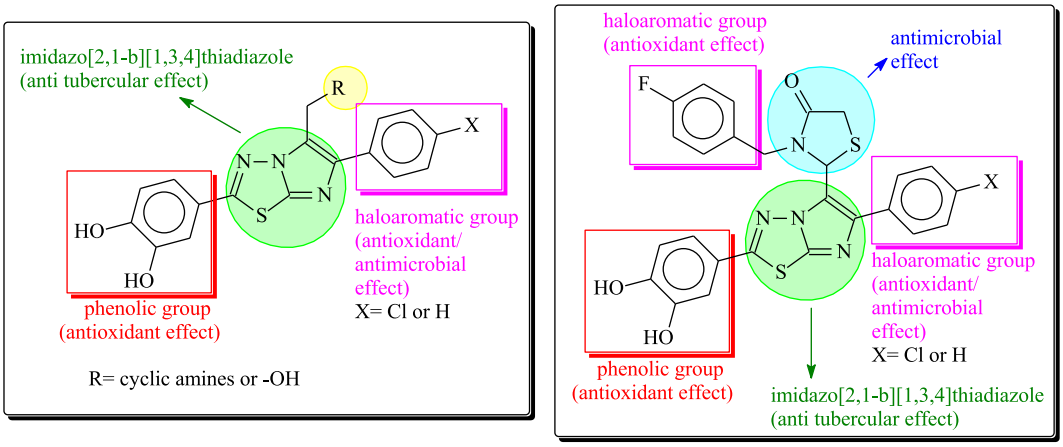


Fig. 2. Schematic representation of target products.

2. Chemistry

The ITD skeleton was occured in two reaction step. Condensation of 3,4-dihydroxybenzaldehyde with thiosemicarbazide under reflux in $POCl_3$ gave the mid-product of 2-amino-1,3,4-thiadiazole compound (**2**), then compound **2** was reacted with α-bromoarylketone by cycloaddition reaction to afford the ITD skeleton (**3a, 3b**) (Scheme 1) [25].

Mannich reaction of ITD (**2**) with Norfloxacin and Ciprofloxacin, which are still used as flouroquinolone drugs, and with different cyclic amines (morpholine, thiomorpholine, phenylpiperazine, 1-(2-pyrimidyl)piperazine, indole, piperidin-2,6-dione and piperidine in dimethylformamide was reacted with $InCl_3$ as a catalyst to yield the corresponding derivatives of compounds **4a, 4b, 5a** and **5b** (Scheme 1), **6a-6 g** and **7a-7 g** (Scheme 2) [25]. To afford the ITD C-5 formyl derivatives (**8** or **10**) of compound **3a** or **3b** Vilsmeier-Haack reaction were studied in DMF and $POCl_3$ [26]. Conventional reduction method was used for compounds **8** and **10** by $NaBH_4$ to afford the alcohol function group at C-5 position of ITD structure (**9** and **11**) [27]. A cyclisation reaction were occured with compounds **8** and **10** and 4-flourobenzylamine in thioglycolic acid to afford the corresponding 1,3-thiazolidin-4-one derivatives. In this reaction step a Schiff base intermediate were formed which has not been isolated. (**12, 13**) (Scheme 1) [28].

3. Results and discussion

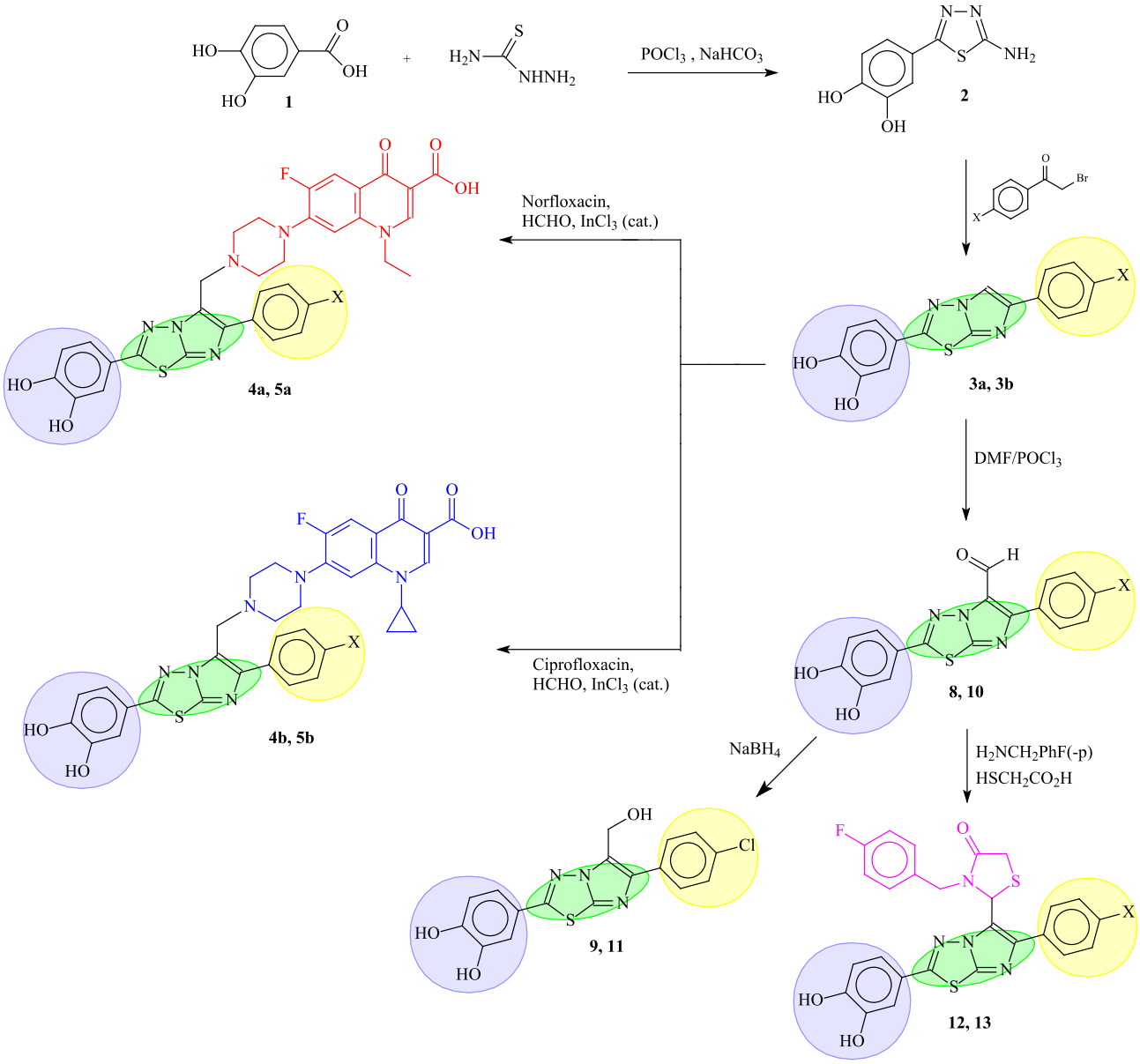
3.1. Antimicrobial activity

All the newly synthesized compounds were screened for their antimicrobial activity (Table 1). When the antimicrobial activity results of the new compounds synthesized were examined, *Mycobacterium smegmatis* was observed to be the most sensitive microorganism with the MIC value between < 0.24 and 0.49 μg/mL concentration according to the standard drug Streptomycin (4 g/mL) and it was observed that compound **4a, 4b, 5a, 5b, 10, 12** and **13** showed excellent anti-tuberculosis activity. Beside this, compound **3a, 3b, 6e, 7b** and **7e** showed moderate anti-tuberculosis activity.

The Mannich bases **4a, 4b, 5a**, **5b** and 1,3-thiazolidin-4-one derivatives **12, 13** as indicated in Table 1, were also found to be effective only on bacteria, ie prokaryotic cells, and showed high inhibition at low concentrations (< 0.24 μg/mL) compared with standard drug Ampicillin.

Compound **7f** including piperidin-2,6-dione and compound **10** an aldehyde at ITD C-5 position exhibited very excellent activity against all the test microorganisms shown in Table 1 and it is clear that compound **7f** and **10** is more effective than these standard drugs.

Compound **3a** an ITD derivative showed not good activity against gram negative microorganisms (*Ef., Sa.* and *Bc.*), whereas its chlorine



Scheme 1. Synthetic routes for target compounds **2–5** and **8–13**.

derivative (**3b**) showed very high activity (< 3.9 μg/mL) compared with standard drug Ampicillin (10–35 μg/mL). Also compound **6a** which is a derivative of ITD including a morpholine group at C-5 position showed no significant activity, whereas compound **7a**, which is the chlorine derivative, showed very good activity against *Sa.*, gram positive bacterial strains and *Sc.*, yeast fungi referenced with standard drugs mentioned at Table 1.

3.2. Antioxidant effect

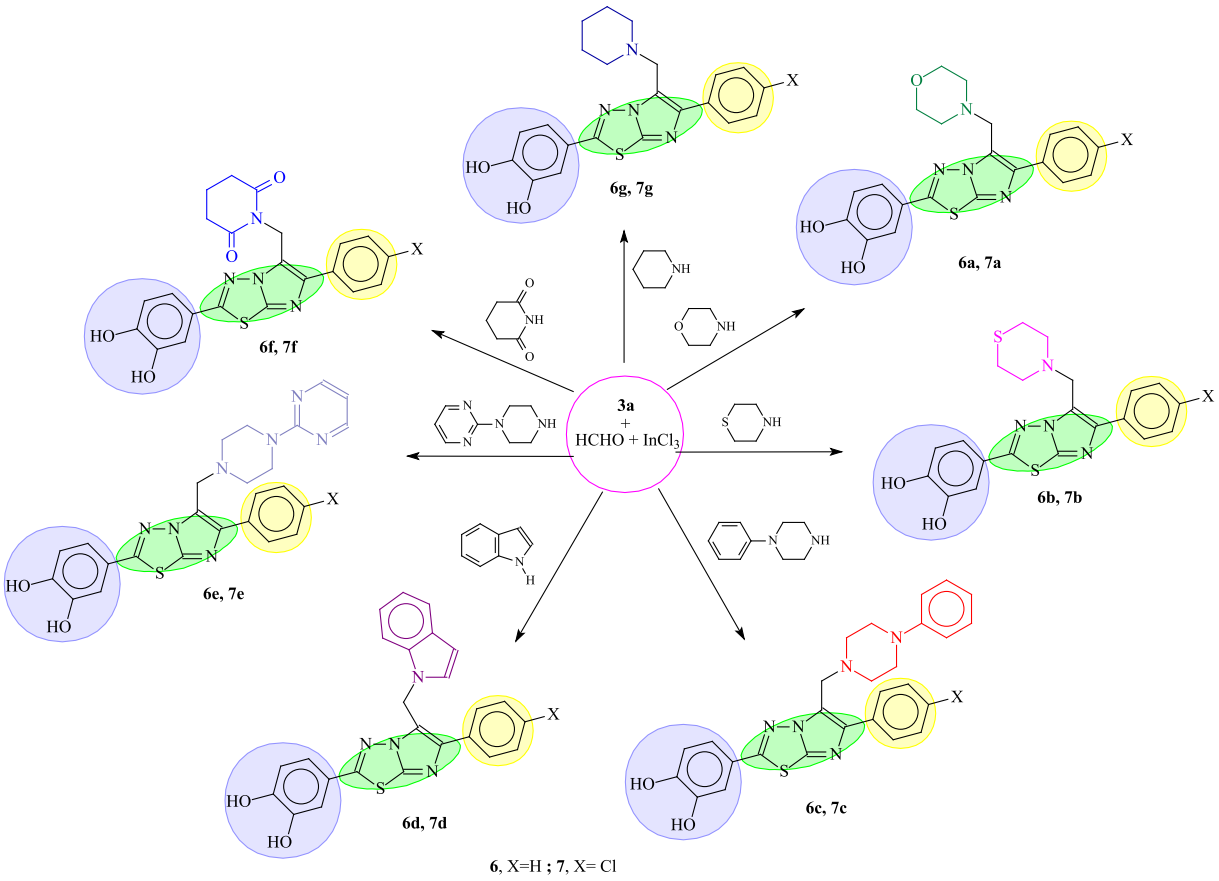
The IC_{50} values of the concentrations corresponding to the maximum absorbance of the DPPH˙ radical was calculated by mixing the DPPH˙ (2,2-diphenyl-1-picrylhydrazyl) radical with the sample solutions (purple violet color of the DPPH˙ radical). Assessments were made against the Quercetin standard.

When the antioxidant capacity of DPPH method is examined among all synthesized compounds, compounds **2, 3a, 3b, 4a, 6c, 6e, 7a, 7b, 7f, 9, 11** and **13** show a higher effect than Quercetin, which is the reference standard substance, and thus has also a more reducing properties (Table 2).

The FRAP method is based on the reduction of Fe(III) ion in Fe(III) complex in the presence of an antioxidant. Fe(III) ions form the (Fe(III)-TPTZ-2,4,6-tris(2-pyridyl)-S-triazine) complex with the ligand called TPTZ. Evaluation was made in terms of μmolTE/g according to trolox equivalent antioxidant capacity. In this connection, compounds **2, 3a** and **3b** exhibit a far more effective antioxidant capacity than trolox, which is used as standard. On the other hand, compounds which showed an antioxidant property similar to or higher than the trolox standard were compounds **4a, 4b, 5a, 6c, 6d, 6f, 7g, 9, 10, 11** and **13**.

4. Conclusion

In summary we report here some new kind of ITD compounds bearing different pharmacophores at C-2, C-5 and C-6 position that possess excellent anti-tubercular, antimicrobial activity and antioxidant effect.



Scheme 2. Synthetic routes for target compounds **6** and **7**.

5. Experimental

5.1. General methods for chemistry

All the chemicals were purchased from Fluka Chemie AG Buchs (Switzerland) and used without further purification. Melting points of the synthesized compounds were determined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminium sheets. The mobile phase was ethyl acetate:diethyl ether (1:2), and detection was made using UV light. FT-IR spectra were recorded using a *Perkin Elmer* 1600 series FTIR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were registered in DMSO-d_6 on a *BRUKER AVENE II* 400 MHz NMR Spectrometer (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C). The chemical shifts are given in ppm relative to Me_4Si as an internal reference and *J* values are given in Hz. All the compounds gave C, H and N analysis within ± 0.4% of the theoretical values. The Mass spectra were obtained on Thermo Scientific TSQ Quantum Access MAX Triple Quadrupole Mass Spectrometer.

5.2. 4-(5-Amino-1,3,4-thiadiazol-2-yl)benzen-1,2-diol **(2)**

To a round bottom flask was added 3,4-dihydroxybenzoic acid (10 mmol), thiosemicarbazide (20 mmol) and $POCl_3$ solution (15 mL). The mixture was refluxed at 100 °C for 8 h (the reaction time was determined by TLC). The mixture was removed in an evaporator flask and the solvent was evaporated under reduced pressure. $NaHCO_3$ solution was added to the resulting crude product, the resulting solid was filtered off, dried and purified by crystallization from DMF: H_2O (1: 3) mixture. Yield 87%, m.p: 258–260 °C.

FT-IR (υ_{max}, cm^{-1}): 3046 (Aromatic CH), 1613 (C=C), 1561 (C=N), 1264 (C–O).

^{1}H NMR (DMSO-d_6, δ ppm): 6.87 (1H, d *J* = 8.0 Hz, arH), 7.08 (1H, d *J* = 8.0 Hz, arH), 7.23 (1H, s, arH), 7.70 (2H, s, 2OH), 9.14 (2H, brs, NH_2).

^{13}C NMR (DMSO-d_6, δ ppm): 113.65 (arCH), 116.60 (arCH), 119.43 (arCH), 120.42 (arC), 146.41 (arC), 149.29 (arC), 156.69 (thiadiazole C-2), 168.67 (thiadiazole C-5).

EI MS *m/z* (%): [$C_8H_7N_3SO_2$ = 209.03], 132.67 (19), 178.67 (18), 209.83 ([M]$^{+}$, 100).

5.3. General synthesis method of compounds 3a and 3b

2-Bromoacetophenone (for compound **3a**) or 2-bromo-4-chloroacetophenone (for compound **3b**) (10 mmol) was separately added in a round bottom flask to compound **2** (10 mmol) in ethanol. The mixture was reflux for 12 h. The reaction solvent was evaporated and neutralized with K_2CO_3 solution. A solid appeared and filtered off. The crude product was purified by crystallization from DMSO:H_2O mixture (1:3).

5.3.1. 4-(6-Phenylimidazo[2,1-b][1,3,4]thiadiazol-2-yl)benzen-1,2-diol **(3a)**

Yield 88%, m.p: 153–155 °C.

FT IR (υ_{max}, cm^{-1}): 3422 ve 3309 (2OH), 3045 (Aromatic CH), 1625 (C=C), 1502 (C=N), 1296 (C–O).

^{1}H NMR (DMSO-d_6, δ ppm): 6.80 (1H, s, arH), 6.99 (1H, s, arH), 7.20 (4H, s, arH), 7.36 (2H, brs, arH), 7.88 and 8.07 (2H, brs, 2OH), 8.69 (1H, s, CH).

^{13}C NMR (DMSO-d_6, δ ppm): 113.65 (arCH + ITD CH), 116.42 (arCH), 118.93 (arCH), 122.60 (arC), 125.06 (arCH), 127.71 (arCH), 128.93 (arCH), 129.14 (2arCH), 134.36 (arC), 146.10 (ITD C-6), 147.82 (arC–OH), 150.00 (arC–OH), 157.32 (ITD C-2), 167.99 (ITD C-8).

EI MS *m/z* (%): [$C_{16}H_{11}N_3SO_2$ = 309.34], 309.78 ([M]$^{+}$, 44), 327.84 ([M + H_2O]$^{+}$, 100).

Table 1
Antimicrobial Activities of newly synthesized compounds **2–13**.

Comp. No	Microorganisms and Inhibition Concentrations (MIC) (μg/mL)									
	Gram-Negattive			Gram-Positive			No Gram	Yeast Fungi		
	Ec.	*Yp.*	*Pa.*	*Ef.*	*Sa.*	*Bc.*	*Ms.*	*Ca.*	*Ct.*	*Sc.*
2	–	–	500	–	500	125	62.5	125	125	125
3a	–	–	500	–	250	125	31.25	62.5	62.5	15.6
3b	–	–	500	< 3.9	< 3.9	< 3.9	15.6	125	125	62.5
4a	< 0.24	< 0.24	< 0.24	< 0.24	< 0.24	< 0.24	< 0.24	–	–	–
4b	< 0.24	< 0.24	< 0.24	< 0.24	< 0.24	< 0.24	< 0.24	500	–	–
5a	< 0.24	< 0.24	< 0.24	< 0.24	< 0.24	< 0.24	< 0.24	–	500	–
5b	< 0.24	< 0.24	< 0.24	< 0.24	< 0.24	< 0.24	< 0.24	250	250	250
6a	–	–	–	–	500	–	62.5	500	–	–
6b	–	–	–	–	500	–	62.5	500	–	–
6c	–	–	–	–	–	–	62.5	500	–	–
6d	–	–	–	–	250	–	125	250	500	500
6e	–	–	–	–	250	–	31.25	125	125	125
6f	–	–	–	500	500	500	125	125	125	125
6g	–	–	–	–	500	–	62.5	250	125	125
7a	–	–	–	31.25	7.8	31.25	62.5	15.6	31.25	0.98
7b	–	–	–	–	500	500	31.25	250	500	250
7c	–	–	–	–	500	250	62.5	62.5	625	< 0.24
7d	–	–	–	125	125	250	62.5	62.5	62.5	< 0.24
7e	–	–	–	125	16	500	31.25	62.5	125	0.49
7f	< 0.24	15.6	31.25	< 0.24	< 0.24	< 0.24	< 0.24	3.9	3.9	0.49
7g	–	–	–	–	250	125	62.5	250	250	250
8	–	–	–	–	250	–	62.5	20	16	< 0.24
9	–	–	–	–	–	–	62.5	500	500	62.25
10	3.9	62.5	250	< 0.24	< 0.24	< 0.24	0.49	3.9	3.9	< 0.24
11	–	–	–	–	250	–	62.5	62.5	500	31.25
12	< 0.24	< 0.24	< 0.24	< 0.24	< 0.24	< 0.24	< 0.24	–	–	–
13	< 0.24	< 0.24	< 0.24	< 0.24	< 0.24	< 0.24	< 0.24	–	–	–
Amp.	10	18	> 128	35	10	15				
Strep							4			
Flu.								< 8	< 8	< 8

Ec: *Escherichia coli* ATCC 25922, **Yp:** *Yersinia pseudotuberculosis* ATCC 911, **Pa:** *Pseudomonas aeruginosa* ATCC 27853, **Sa:** *Staphylococcus aureus* ATCC 25923, **Ef:** *Enterococcus faecalis* ATCC 29212**, Bc:** *Bacillus cereus* 702 Roma, **Ms:** *Mycobacterium smegmatis* ATCC607, **Ca:** *Candida albicans* ATCC 60193, **Ct:** *Candita tropicalis* ATCC 13803, **Sc:** *Saccharomyces cerevisiae* RSKK 251, **Amp.:** Ampicillin 10 μg/mL, **Strep.:** Streptomycin10 μg/mL, **Flu.:** Fluconazole 5 μg/mL, **(–):** no activity.

Table 2
Antioxidant activities of compounds by DPPH and FRAP method.

Comp. No	DPPH IC_{50} (μg/mL)	FRAP μmol TE/mL
2	7.05 ± 0.40	3692.94 ± 72.40
3a	1.14 ± 0.52	6683.33 ± 439.02
3b	3.37 ± 0.22	3890.38 ± 161.54
4a	8.66 ± 0.05	1068.58 ± 29.87
4b	23.17 ± 0.80	896.79 ± 51.22
5a	13.81 ± 0.22	976.28 ± 411.92
5b	44.26 ± 1.16	800.64 ± 128.85
6a	23.41 ± 0.11	1378.84 ± 106.59
6b	16.54 ± 0.95	699.35 ± 17.34
6c	8.30 ± 0.47	954.48 ± 28.35
6d	20.30 ± 0.11	1001.92 ± 46.79
6e	8.84 ± 0.54	2505.76 ± 137.66
6f	14.26 ± 0.05	905.76 ± 119.66
6g	11.15 ± 0.07	1800.64 ± 45.89
7a	8.56 ± 0.27	1439.10 ± 71.47
7b	7.41 ± 0.08	1531.41 ± 91.77
7c	16.61 ± 0.93	805.76 ± 97.07
7d	26.05 ± 0.96	1230.12 ± 27.82
7e	27.01 ± 1.42	406.53 ± 32.75
7f	7.49 ± 0.27	1469.23 ± 144.27
7g	17.08 ± 0.09	1009.61 ± 50.29
8	18.01 ± 0.38	1912.17 ± 25.02
9	10.05 ± 0.18	1000.25 ± 49.81
10	18.34 ± 0.01	955.76 ± 60.32
11	9.82 ± 0.18	912.17 ± 34.90
12	22.42 ± 0.12	1268.72 ± 99.60
13	10.11 ± 0.18	998.12 ± 57.55
Quercetin	10.25 ± 1.45	–

*5.3.2. 4-[6-(4-Chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl] benzen-1,2-diol **(3b)***

Yield 94%, m.p: 139–141 °C.

FT IR (υ_{max}, cm^{-1}): 3415 ve 3309 (2OH), 3045 (Aromatic CH), 2982 (Aliphatic CH), 1602 (C=C), 1504 (C=N), 1295 (C−O).

^{1}H NMR (DMSO-d_6, δ ppm): 6.78 (1H, d J = 8.0 Hz, arH), 6.99 (2H, d J = 8.0 Hz, arH), 7.20 (2H, brs, arH), 7.43 (2H, brs, arH), 7.90 and 8.07 (2H, brs, 2OH), 8.72 (1H, s, CH).

^{13}C NMR (DMSO-d_6, δ ppm): 113.64 (arCH + ITD CH), 116.42 (arCH), 118.96 (arCH), 122.47 (arC), 126.72 (arCH), 129.18 (arCH), 130.83 (2arCH), 132.83 (arC), 134.44 (arC), 146.12 (ITD C-6), 147.91 (arC−OH), 157.28 (arC−OH), 168.04 (ITD C-2 and ITD C-8).

EI MS m/z (%): [$C_{16}H_{10}N_3SO_2Cl$ = 343.79], 343.80 ([M]$^+$, 50), 361.65 ([M + H_2O]$^+$, 100), 363.75 ([M + 2 + H_2O]$^+$, 40).

*5.4. General synthesis method of compounds **4a, 4b, 5a** and **5b***

To a solution of corresponding compound **3a** (for compound **4a** and **5a**) or **3b** (for compound **4b** and **5b**) (10 mmol) in dimethylformamide containing indium (III) chloride (catalytic amount) and Norfloxain or Ciprofloxacin (10 mmol) was added and the mixture was stirred at room temperature in the presence of formaldehyde (37%, 50 mmol) for 24 h. The solid precipitated was collected by filtration and re-crystallized from dimethylsulfoxside:water (1:3) to give the desired compound.

*5.4.1. 7-(4-{[2-(3,4-Dihydroxyphenil)-6-phenylimidazo [2,1-b][1,3,4] thiadiazol-5-yl]methyl}piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinolon-3-carboxylic acid **(4a)***

Yield 74%, m.p: 193–194 °C.

FT IR (υ_{max}, cm^{-1}): 3056 (Aromatic CH), 2918 (Aliphatic CH), 1716 and 1667 (2C=O), 1625 (C=C), 1255 (C−O).

^{1}H NMR (DMSO-d_6, δ ppm): 1.40 (3H, t J = 8.0 Hz, CH_3), 2.73 (2H, s, CH_2), 2.89 (2H, s, CH_2), 3.36 (4H, brs, $2CH_2$), 4.18 (2H, s, CH_2), 4.59 (2H, brs, CH_2), 6.78–7.25 (7H, m, arH), 7.90–7.95 (3H, m, 3arH), 8.96 (1H, brs, quinolone CH), 9.34–9.43 (2H, m, 2OH, D_2O exchange), 15.38 (1H, s, OH, D_2O exchange).

^{13}C NMR (DMSO-d_6, δ ppm): 14.79 (CH_3), 43.23 (CH_2), 47.06 (CH_2), 49.70 ($2CH_2$), 52.25 ($2CH_2$), 106.37 (arCH), 107.53 (C), 111.48 (arCH), 113.67 (arCH), 116.39 (arCH), 118.85 (2arCH), 119.64 (ITD C-5), 119.71 (arC), 122.28 (arC), 122.88 (arC), 127.42 (arCH), 128.97 (arCH), 129.11 (2arCH), 137.63 (arC), 145.87 (C−F), 146.07 (ITD C-6), 147.76 (arC), 148.88 (quinolone CH), 152.11 (arC−OH), 154.58 (arC−OH), 157.60 (C−F), 166.46 (ITD C-2 and ITD C-8), 168.34 (C=O), 176.61 (C=O).

EI MS *m/z* (%): [$C_{33}H_{29}N_6SO_5F$ = 640.69], 541.02 (83), 640.77 ($[M]^+$, 29), 662.96 ($[M-1+Na]^+$, 41), 748.99 (100).

*5.4.2. 7-(4-{[2-(3,4-Dihydroxyphenyl)-6-(4-chlorophenyl)imidazo[2,1-b] [1,3,4] thiadiazol-5-yl]methyl}piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinolon-3-carboxylic acid **(5a)***

Yield 64%, m.p:121–122 °C.

FT IR (υ_{max}, cm^{-1}): 3060 (Aromatic CH), 2928 (Aliphatic CH), 1707 and 1662 (2C=O), 1626 (C=C), 1589 (C=N), 1256 (C−O).

^{1}H NMR (DMSO-d_6, δ ppm): 1.42 (3H, t J = 8.0 Hz, CH_3), 2.74 (2H, s, CH_2), 2.90 (2H, s, CH_2), 3.40 (6H, brs, $3CH_2$), 4.58 (2H, brs, CH_2), 7.19–7.62 (7H, m, arH), 7.88–8.04 (4H, m, 2arH + 2OH), 8.95 (1H, s, quinolone CH), 15.34 (1H, brs, OH).

^{13}C NMR (DMSO-d_6, δ ppm): 14.81 (CH_3), 47.11 (CH_2), 49.54 (CH_2), 49.84 ($2CH_2$), 52.28 ($2CH_2$), 102.18 (arCH), 107.56 (C), 111.71 (arCH), 113.66 (arCH), 116.71 (arCH), 119.83 (arC), 120.83 (ITD C-5), 126.68 (2arCH), 129.16 (arCH), 131.64 (2arCH), 132.69 (arC), 133.26 (arC), 133.77 (arC), 137.60 (2arC), 146.56 (ITD C-6), 148.85 (quinolone CH), 150.06 (C−F), 152.10 (arC−OH), 154.58 (arC−OH), 162.26 (C-F), 166.57 (ITD C-2 and ITD C-8), 168.57 (C=O), 176.58 (C=O).

EI MS *m/z* (%): [$C_{33}H_{28}N_6SO_5FCl$ = 675.13], 540.67 (100), 674.94 ($[M]^+$, 58), 696.64 ($[M-1+Na]^+$, 52).

*5.4.3. 1-Cyclopropyl-7-(4-{[2-(3,4-dihydroxyphenyl)-6-phenylimidazo [2,1-b][1,3,4]thiadi azol-5-yl]methyl}piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinolon-3-carboxylic acid **(4b)***

Yield 76%, m.p: 213–215 °C.

FT IR (υ_{max}, cm^{-1}): 3065 (Aromatic CH), 2957 (Aliphatic CH), 1707 and 1659 (2C=O), 1626 (C=C), 1593 (C=N), 1258 (C−O).

^{1}H NMR (DMSO-d_6, δ ppm): 1.18–1.32 (4H, m, $2CH_2$), 2.73 (2H, s, CH_2), 2.89 (2H, s, CH_2), 3.35 (4H, s, $2CH_2$ + H_2O), 3.82 (2H, brs, CH_2), 4.18 (1H, brs, cyclopropyl-CH), 6.77–8.28 (10H, m, arH), 8.67 (1H, s, quinolone CH), 9.34–9.43 (2H, m, 2OH), 15.25 (1H, brs, OH).

^{13}C NMR (DMSO-d_6, δ ppm): 8.02 ($2CH_2$), 36.25 (CH), 49.71 (CH_2), 52.24 ($4CH_2$), 106.87 (2arCH), 107.24 (C), 111.25 (arCH), 113.71 (arCH), 116.41 (2arCH), 118.86 (arCH), 119.08 (ITD C-5), 122.69 (arC), 127.45 (arCH), 128.96 (2arCH), 134.17 (2arC) 139.60 (arC), 145.49 (ITD C-6), 145.89 (arC), 146.07 (C-F), 148.32 (quinolone CH), 152.25 (arC−OH), 154.71 (arC−OH), 166.36 (ITD C-2 and ITD C-8), 168.35 (C=O), 176,78 (C=O).

EI MS *m/z* (%): [$C_{34}H_{29}N_6SO_5F$ = 652.69], 652.54 ($[M]^+$, 39), 675.22 ($[M+Na]^+$, 19), 687.12 ($[M+Cl]^+$, 67).

*5.4.4. 1-Cyclopropyl-7-(4-{[2-(3,4-dihydroxyphenyl)-6-(4-chlorophenyl) imidazo[2,1-b][1,3,4]thiadiazol-5-yl]methyl}piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinolon-3-carboxylic acid **(5b)***

Yield 68%, m.p: 201–203 °C.

FT IR (υ_{max}, cm^{-1}): 3072 (Aromatic CH), 2937 (Aliphatic CH), 1717 and 1665 (2C=O), 1626 (C=C), 1591 (C=N), 1258 (C−O).

^{1}H NMR (DMSO-d_6, δ ppm): 1.18–1.31 (4H, m, $2CH_2$), 2.73 (2H, s, CH_2), 2.89 (2H, s, CH_2), 3.35 (4H, brs, $2CH_2$), 3.82 (2H, brs, CH_2), 4.18 (1H, s, cyclopropyl-CH), 6.77–8.28 (9H, m, arH), 8.67–8.71 (1H, s, quinolone CH), 9.34–9.43 (2H, m, 2OH, D_2O exchange), 15.25 (1H, brs, OH, D_2O exchange).

^{13}C NMR (DMSO-d_6, δ ppm): 8.03 ($2CH_2$), 31.24 (CH), 49.76 (CH_2), 63.57 ($4CH_2$), 102.19 (arCH), 106.93 (arCH), 107.21 (C), 116.73 (arCH), 119.10 (ITD C-5), 119.51 (arCH), 121.06 (arC), 126.70 (2arCH), 129.19 (arCH), 131.60 (2arCH), 131.66 (arC), 132.37 (arC), 132.53 (2arC), 139.62 (arC), 146.56 (ITD C-6), 148.42 (quinolone CH), 150.29 (C-F), 152.24 (arC−OH), 154.72 (arC−OH), 162.67 (ITD C-2 and ITD C-8), 166.38 (C=O), 176.81 (C=O).

EI MS *m/z* (%): [$C_{34}H_{28}N_6SO_5FCl$ = 687.14], 552.92 (100), 668.43 ($[M-F]^+$, 46), 686.84($[M-1]^+$, 56), 705.11 ($[M+H_2O]^+$, 36).

*5.5. General synthesis method of compounds **6a–6g** and **7a–7g***

To a solution of corresponding compound **3a** (for compounds **6a–6g**) or **3b** (for compounds **7a–7g**) (10 mmol) in dimethylformamide containing indium (III) chloride (catalytic amount) and suitable secondary amine (morpholine, thiomorpholine, phenylpiperazine, 1-(2-pyrimidyl)piperazine, indole, piperidine-2,6-dione and piperidine (10 mmol) was added and the mixture was stirred at room temperature in the presence of formaldehyde (37%, 50 mmol) for 24 h. The solid precipitated was collected by filtration and recrystallized from DMF:H_2O (1:3) to give the desired compound.

*5.5.1. 4-[5-(morpholin-4-ylmethyl)-6-phenylimidazo[2,1-b][1,3,4] thiadiazol-2-yl]benzen-1,2-diol **(6a)***

Yield 78%, m.p: 122–123 °C.

FT IR (υ_{max}, cm^{-1}): 3062 (Aromatic CH), 2927 (Aliphatic CH), 1655 (C=C), 1597 (C=N), 1287 (C−O).

^{1}H NMR (DMSO-d_6, δ ppm): 2.74 (2H, s, CH_2), 2.90 (2H, s, CH_2), 3.40 (6H, brs, $3CH_2$), 6.78–7.58 (8H, m, 7arH + OH, D_2O exchange), 7.96 (2H, s, arH + OH, D_2O exchange).

^{13}C NMR (DMSO-d_6, δ ppm): 52.69 (CH_2), 53.35 ($2CH_2$), 66.64 ($2CH_2$), 113.65 (arCH), 116.45 (arCH), 119.06 (2arCH), 121.15 (ITD C-5), 122.55 (arC), 125.07 (arCH), 127.42 (arCH), 128.96 (2arCH), 134.87 (arC), 146.13 (ITD C-6), 147.91 (arC−OH), 150.02 (arC−OH), 166.94 (ITD C-2 and ITD C-8).

EI MS *m/z* (%): [$C_{21}H_{20}N_4SO_3$ = 408.47], 371.76 (100), 408.93 ($[M]^+$, 64), 449.88 ($[M+2+K]^+$, 56).

*5.5.2. 4-[6-Phenyl-5-(thiomorpholin-4-ylmethyl)imidazo[2,1-b][1,3,4] thiadiazol-2-yl] benzen-1,2-diol **(6b)***

Yield 75%, m.p: 223–224 °C.

FT IR (υ_{max}, cm^{-1}): 3061 (Aromatic CH), 2922 (Aliphatic CH), 1657 (C=C), 1597 (C=N), 1293 (C−O).

^{1}H NMR (DMSO-d_6, δ ppm): 2.74 (2H, s, CH_2), 2.90 (2H, s, CH_2), 3.39 (6H, s, $3CH_2$ + H2O), 6.91–7.96 (10H, m, arH + 2OH).

^{13}C NMR (DMSO-d_6, δ ppm): 24.08 ($2CH_2$), 45.25 (CH_2), 54.31 ($2CH_2$), 113.76 (arCH), 116.25 (arCH), 119.64 (2arCH), 120.75 (ITD C-5), 121.18 (arC), 127.57 (2arCH), 129.02 (2arCH), 135.06 (ITD C-6), 135.35 (arC), 146.59 (arC−OH), 150.13 (arC−OH), 161.57 (ITD C-2 and ITD C-8)

EI MS *m/z* (%): [$C_{21}H_{20}N_4S_2O_2$ = 424.54], 424.77($[M]^+$, 73), 442.55 ($[M+H_2O]$, 14), 457.81 (100).

*5.5.3. 4-{6-Phenyl-5-[(4-phenylpiperazin-1-yl)methyl]imidazo[2,1-b] [1,3,4]ttiadiazol-2-yl}benzen 1,2-diol **(6c)***

Yield 68%, m.p: 115–116 °C.

FT IR (υ_{max}, cm^{-1}): 3060 (Aromatic CH), 2920 (Aliphatic CH), 1598 (C=N), 1228 (C−O).

^{1}H NMR (DMSO-d_6, δ ppm): 2.74 (2H, s, CH_2), 2.90 (2H, s, CH_2),

3.15 (2H, s, CH_2), 3.44 (2H, s, CH_2), 3.93 (2H, s, CH_2), 6.62–8.00 (13H, m, arH + 2OH), 8.35–8.43 (2H, m, arH).

EI MS *m/z* (%): [$C_{27}H_{25}N_5SO_2$ = 483.58], 385.77 (42), 371.98 (46), 443.88 (35), 472.16 (25), 485.81 ($[M+2]^+$, 100), 500.93 ($([M-1+H_2O]^+$, 24).

5.5.4. 4-[5-(1H-indol-1-yl)-6-phenylimidazo[2,1-b][1,3,4]thiadiazol-2-yl]benzen-1,2-diol ***(6d)***

Yield 57%, m.p: 86–88 °C.

FT IR (υ_{max}, cm^{-1}): 3055 (Aromatic CH), 2926 (Aliphatic CH), 1652 (C═C), 1597 (C═N), 1290 (C—O).

^{1}H NMR (DMSO-d_6, δ ppm): 5.44 (2H, s, CH_2), 6.85–7.98 (16H, m, 14arH + 2OH).

^{13}C NMR (DMSO-d_6, δ ppm): 48.14 (CH_2), 110.60 (CH), 111.76 (arCH), 114.67 (arC), 118.47 (arCH), 119.11 (arCH), 121.19 (arCH), 121.54 (arCH), 122.77 (ITD C-5), 123.21 (arCH), 125.09 (2arCH), 126.37 (arCH), 127.66 (arCH), 128.80 (arC), 129.13 (2arCH), 129.43 (CH), 134.39 (arC), 136.39 (ITD C-6), 136.86 (arC), 146.60 (arC—OH), 150.05 (arC—OH), 162.08 (ITD C-2 ve ITD C-8).

EI MS *m/z* (%): [$C_{25}H_{18}N_4SO_2$, 438.50], 327.77 (55), 427.90 838), 439.03 ($[M+1]^+$, 14), 456.74 ($[M+H_2O]^+$, 100).

5.5.5. 4-{6-Phenyl-5-[(4-pyrimidin-2-ylpiperazin-1yl)methyl]imidazo[2,1-b][1,3,4]thiadi azol-2-yl}benzen-1,2-diol ***(6e)***

Yield 78%, m.p: 179–180 °C.

FT IR (υ_{max}, cm^{-1}): 3023 (Aromatic CH), 2830 (Aliphatic CH), 1655 (C═C), 1598 (C═N), 1293 (C—O).

^{1}H NMR (DMSO-d_6, δ ppm): 2.73 (2H, s,CH_2), 2.89 (2H, s,CH_2), 3.32–3.36 (6H, m, 3CH_2), 6.77–7.49 (11H, m, arH + OH), 7.96 (2H, brs, arH + OH).

EI MS *m/z* (%): [$C_{25}H_{23}N_7SO_2$ = 485.56], 371.69 (100), 383.94 (40), 483.97 ($[M-2]^+$, 16), 507.84 ($[M-1+Na]^+$, 12).

5.5.6. 1-{[2-(3,4-Dihydroxyphenyl)-6-phenylimidazo[2,1-b][1,3,4] thiadiazol-5-yl]methyl} pyperidin-2,6-dione ***(6f)***

Yield 79%, m.p: 230–231 °C.

FT IR (υ_{max}, cm^{-1}): 3075 (Aromatic CH), 2984 (Aliphatic CH), 1698 (C═O), 1650 (C═C), 1595 (C═N), 1298 (C—O).

^{1}H NMR (DMSO-d_6, δ ppm): 1.09–1.11 (2H, m, CH2), 2.73 (2H, s,CH_2), 2.89 (2H, s, CH_2), 4.91 (2H, s,CH_2), 6.89–8.06 (10H, m, arH + 2OH).

EI MS *m/z* (%): [$C_{22}H_{18}N_4SO_4$ = 434.46], 327.65 (100), 427.83 (88), 436.30 ($[M+2]^+$, 10), 459.96 ($[M+2+Na]^+$, 41).

5.5.7. 4-[6-Penyl-5-(pyperidin-1-ylmethyl)imidazo[2,1-b][1,3,4] thiadiazol-2-yl]benzen-1,2-diol ***(6g)***

Yield 69%, m.p: 223–224 °C.

FT IR (υ_{max}, cm^{-1}): 3032 (Aromatic CH), 2936 (Aliphatic CH), 1596 (C═N), 1286 (C—O).

^{1}H NMR (DMSO-d_6, δ ppm): 1.13–1.17 (4H, m, 2CH_2), 1.29–1.32 (2H, m, CH_2), 2.74 (2H, s, CH_2), 2.90 (2H, s, CH_2), 3.38 (2H, s, CH_2), 6.91–8.05 (10H, m, arH + 2OH).

^{13}C NMR (DMSO-d_6, δ ppm): 23.67 (CH_2), 26.06 (2CH_2), 55.82 (CH_2), 58.49 (2CH_2), 113.69 (arCH), 116.75 (arCH), 119.47 (2arCH), 121.15 (ITD C-5 and arC), 125.08 (arCH), 129.12 (arCH), 130.06 (2arCH), 134.38 (arC), 145.55 (ITD C-6), 146.59 (arC—OH), 150.04 (arC—OH), 162.06 (ITD C-2 and ITD C-8).

EI MS *m/z* (%): [$C_{22}H_{22}N_4SO_2$ = 406.56], 342.97 (100), 374.98 (27).

5.5.8. 4-[6-(4-Chlorophenyl)-5-(morpholin-4-ylmethyl)imidazo[2,1-b] [1,3,4]thiadiazol-2-yl]benzen-1,2-diol ***(7a)***

Yield 83%, m.p: 109–110 °C.

FT IR (υ_{max}, cm^{-1}): 3061 (Aromatic CH), 2978 (Aliphatic CH), 1656 (C═C), 1587 (C═N), 1286 (C—O).

^{1}H NMR (DMSO-d_6, δ ppm): 2.73 (2H, s,CH_2), 2.89 (2H, s, CH_2), 3.41 (6H, s, 3CH_2), 6.90–8.03 (9H, m, 7arH + 2OH).

^{13}C NMR (DMSO-d_6, δ ppm): 53.27 (CH_2), 63.58 (2CH_2), 66.60 (2CH_2), 113.70 (arCH), 116.74 (arCH), 121.07 (ITD C-5), 126.72 (2arCH), 129.18 (arCH), 131.66 (2arCH), 133.29 (arC), 139.04 (arC), 144.32 (ITD C-6), 144.79 (arC—Cl), 146.58 (arC—OH), 150.09 (arC—OH), 162.31 (ITD C-2 and ITD C-8).

EI MS *m/z* (%): [$C_{21}H_{19}N_4SO_3Cl$ = 442.92], 313.93 (63), 343.82 (61), 380.93 (61), 405.78 (100), 442.81 ($[M]^+$, 58), 467.24 ($[M+2+Na]^+$, 32).

5.5.9. 4-[6-(4-Chlorophenyl)-5-(thiomorpholin-4-ylmetihy)imidazo[2,1-b] [1,3,4] thiadi azol-2-yl]benzen-1,2-diol ***(7b)***

Yield 87%, m.p:117–118 °C.

FT IR (υ_{max}, cm^{-1}): 3025 (Aromatic CH), 2926 (Aliphatic CH), 1588 (C═N), 1285 (C—O).

^{1}H NMR (DMSO-d_6, δ ppm): 2.73 (2H, s,CH_2), 2.89 (2H, s,CH_2), 3.37 (6H, s, 3CH_2), 6.90–8.06 (9H, m, arH + 2OH).

^{13}C NMR (DMSO-d_6, δ ppm): 27.53 (2CH_2), 54.42 (CH_2), 63.58 (2CH_2), 116.75 (arCH), 119.52 (arCH), 121.08 (ITD C-5), 126.73 (2arCH), 129.17 (arCH), 131.65 (2arCH), 133.30 (arC), 139.03 (ITD C-6), 144.33 (arC), 144.79 (arC—Cl), 146.58 (arC—OH), 150.09 (arC—OH), 162.30 (ITD C-2 and ITD C-8).

EI MS *m/z* (%): [$C_{21}H_{19}N_4S_2O_2Cl$ = 458.98], 412.85 (100), 414.67 (47), 444.98 (70), 446.66 (30), 458.91 ($[M]^+$, 10), 476.41 ($[M+H_2O]^+$, 10).

5.5.10. 4-{6-(4-Chlorophenyl)-5-[(4-phenylpiperazin-1-yl)methyl]imidazo [2,1-b][1,3,4] thiadiazol-2-yl}benzen-1,2-diol ***(7c)***

Yeld 75%, m.p: 100–101 °C.

FT IR (υ_{max}, cm^{-1}): 3039 (Aromatic CH), 2929 (Aliphatic CH), 1598 (C═N), 1230 (C—O).

^{1}H NMR (DMSO-d_6, δ ppm): 2.74 (2H, s, CH_2), 2.90 (2H, s, CH_2), 3.41 (6H, s, 3CH_2), 6.77–8.07 (14H, m, arH + 2OH).

^{13}C NMR (DMSO-d_6, δ ppm): 46.07 (2CH_2), 48.55 (CH_2), 52.49 (2CH_2), 116.05 (2arCH), 116.51 (arCH), 120.01 (arCH), 120.48 (arCH), 121.06 (ITD C-5), 126.72 (2arCH), 129.19 (arCH), 129.36 (2arCH), 131.67 (2arCH), 133.30 (arC), 139.03 (arC), 144.31 (arC—Cl), 146.58 (ITD C-6), 150.09 (arC—OH), 151.21 (arC—OH), 162.31 (ITD C-2 and ITD C-8).

EI MS *m/z* (%): [$C_{27}H_{24}N_5SO_2Cl$ = 518.03], 530.96 (100), 533.13 ($[M-2+H_2O]^+$, 38), 536.84 ($[M+H_2O]^+$, 22).

5.5.11. 4-[6-(4-Chlorophenyl)-5-(1H-indol-1-ylmethyl)imidazo[2,1-b] [1,3,4]thiadiazol-2-yl]benzen-1,2-diol ***(7d)***

Yield 60%, m.p: 256–258 °C.

FT IR (υ_{max}, cm^{-1}): 3056 (Aromatic CH), 2975 (Aliphatic CH), 1590 (C═N), 1277 (C—O).

^{1}H NMR (DMSO-d_6, δ ppm): 3.44 (2H, s, CH_2), 6.75–8.07 (15H, m, arH + 2OH).

EI MS *m/z* (%): [$C_{25}H_{17}N_4SO_2Cl$ = 472.95], 331.90 (79), 413.21 (36), 490.85 ($[M+H_2O]^+$, 100).

5.5.12. 4-{6-(4-Chlorophenyl)-5-[(4-pyrimidin-2-ylpiperazin-1-yl)methyl] imidazo[2,1-b] [1,3,4]thiadiazol-2-yl}benzen-1,2-diol ***(7e)***

Yield 83%, m.p: 116–118 °C.

FT IR (υ_{max}, cm^{-1}): 3027 (Aromatic CH), 2935 (Aliphatic CH), 1584 (C═N), 1259 (C—O).

^{1}H NMR (DMSO-d_6, δ ppm): 2.74 (2H, s,CH_2), 2.90 (2H, s, CH_2), 3.38 (6H, brs, 3CH_2), 6.63–8.36 (12H, m, arH + 2OH).

^{13}C NMR (DMSO-d_6, δ ppm): 52.38 (2CH_2), 54.27 (CH_2), 57.19 (2CH_2), 113.69 (arCH), 116.74 (arCH), 119.54 (arCH), 121.07 (ITD C-5), 126.75 (2arCH), 129.17 (arCH), 131.66 (2arCH), 132.04 (arC), 133.30 (arC), 139.02 (ITD C-6 and arC—Cl), 146.57 (arC—OH), 150.07 (arC—OH), 158.57 (2arCH), 161.57 (ITD C-2), 162.30 (ITD C-8).

EI MS *m/z* (%): [$C_{27}H_{24}N_5SO_2Cl$ = 520.00], 534.88 (100), 519.90 ($[M-1]^+$, 10), 537.05 ($[M-1+H_2O]^+$, 42).

*5.5.13. 1-{[6-(4-Chlorophenyl)-2-(3,4-dihydroxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl]methyl}pyperidin-2,6-dione **(7f)***

Yield 72%, m.p: 82–83 °C.

FT IR (υ_{max}, cm^{-1}): 3063 (Aromatic CH), 2953 (Aliphatic CH), 1686 (C=O), 1589 (C=N), 1209 (C−O).

^{1}H NMR (DMSO-d_6, δ ppm): 1.13–1.17 (2H, m, CH_2), 2.74 (2H, s, CH_2), 2.90 (2H, s, CH_2), 5.21 (2H, s, CH_2), 6.90–8.08 (9H, m, arH + 2OH).

EI MS *m/z* (%): [$C_{22}H_{17}N_4SO_4Cl$ = 468.91], 313.86 (75), 343.68 (64), 361.81 (100), 363.84 (46), 412.92 (51), 430.35 (51), 467.10 ($[M-1]^+$, 12), 487.12 ($[M+1+H_2O]^+$, 16).

*5.5.14. 4-[6-(4-Chlorophenyl)-5-(pyperidin-1-ylmethyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]benzen-1,2-diol **(7g)***

Yield 76%, m.p: 118–119 °C.

FT IR (υ_{max}, cm^{-1}): 3042 (Aromatic CH), 2935 (Aliphatic CH), 1587 (C=N), 1285 (C−O).

^{1}H NMR (DMSO-d_6, δ ppm): 1.13–1.17 (4H, m, $2CH_2$), 1.29–1.32 (2H, m, CH_2), 2.74 (2H, s, CH_2), 2.90 (2H, s, CH_2), 3.41 (2H, brs, CH_2), 6.82–8.06 (9H, m, arH + 2OH).

^{13}C NMR (DMSO-d_6, δ ppm): 23.65 (CH_2), 26.01 ($2CH_2$), 55.75 (CH_2), 58.49 ($2CH_2$), 116.76 (arCH), 119.47 (arCH), 121.06 (ITD C-5), 126.73 (2arCH), 129.16 (arCH), 131.65 (2arCH), 133.60 (2arC), 139.07 (ITD C-6), 144.33 (arC−Cl), 146.63 (arC−OH), 150.16 (arC−OH), 162.32 (ITD C-2 ve ITD C-8).

EI MS *m/z* (%): [$C_{22}H_{21}N_4SO_2Cl$ = 440.95], 376.87 (100), 408.93 (43).

*5.6. General synthesis method of compounds **8** and **10***

$POCl_3$ (2 mL) in *N,N*-dimethylformamide (2 mL) was added dropwise under nitrogen gas with a dropping funnel into a round-bottomed flask and stirred for 15 min. Compound **3a** or **3b** dissolved in DMF (10 mmol) was added and reflux for 9 h at 60 °C (the reaction time was determined by TLC). The reaction mixture was cooled to room temperature and poured into ice water. The resulting crude product was decanted and dried and treated with diethyl ether to provide solidity of the oily product. The crude product was purified by crystallization from a mixture of DMF:H_2O (1:3).

*5.6.1. 2-(3,4-Dihydroxyphenyl)-6-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-carbaldehyde **(8)***

Yield 80%, m.p: 68–70 °C.

FT IR (υ_{max}, cm^{-1}): 3062 (Aromatic CH), 1731 (C=O), 1648 (C=C), 1597 (C=N), 1259 (C−O).

^{1}H NMR (DMSO-d_6, δ ppm): 6.89–8.29 (10H, m, arH + 2OH), 10.02 (1H, s, aldehyde-H).

^{13}C NMR (DMSO-d_6, δ ppm): 116.74 (arCH), 119.47 (arCH), 121.08 (ITD C-5), 125.05 (2arCH), 127.84 (2arCH), 129.16 (2arCH), 132.88 (arC), 134.30 (arC), 145.42 (ITD C-6), 146.57 (arC−OH), 150.03 (arC−OH), 162.10 (ITD C-2 and ITD C-8), 162.80 (C=O).

EI MS *m/z* (%): [$C_{17}H_{11}N_3SO_3$ = 337.35], 353.20 ($[M-2+H_2O]^+$, 15), 382.88 (100).

*5.6.2. 6-(4-Chlorophenyl)-2-(3,4-dihydroxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-5- carbaldehyde **(10)***

Yield 89%, m.p: 82–83 °C.

FT IR (υ_{max}, cm^{-1}): 3002 (Aromatic CH), 1686 (C=O), 1589 (C=N), 1209 (C−O).

^{1}H NMR (DMSO-d_6, δ ppm): 6.91–8.06 (9H, m, arH + 2OH), 9.89 (1H, s, aldehyde-H).

^{13}C NMR (DMSO-d_6, δ ppm): 116.76 (arCH), 119.55 (arCH), 120.87 (ITD C-5), 126.74 (2arCH), 129.21 (arCH), 130.71 (2arCH), 132.18 (2arC), 139.26 (arC−Cl), 143.78 (ITD C-6), 146.60 (arC−OH), 150.19 (arC−OH), 162.58 (ITD C-2 and ITD C-8), 179.19 (C=O).

EI MS *m/z* (%): [$C_{17}H_{10}N_3SO_3Cl$ = 371.79], 285.99 (33), 345.84 ($[M-3-Na]^+$, 12), 352.91 ($[M-1-H_2O]^+$, 21), 361.80 (100), 391.20 ($[M-3+Na]^+$, 10).

*5.7. General synthesis method of compounds **9** and **11***

Compound **8** or **10** (10 mmol) was stirred with $NaBH_4$ (30 mmol) in 15 mL ethanol at room temperature for 24 h (the reaction time was determined by TLC). The solvent was evaporated and a solid appeared by adding water. The solid was filtered off and purified by crystallization in a DMF:H_2O (1: 3) mixture to afford the corresponding compounds **9** or **11**.

*5.7.1. 4-[5-(Hydroxymethyl)-6-phenylimidazo[2,1-b][1,3,4]thiadiazol-2-yl]benzen-1,2-diol **(9)***

Yield 76%, m.p: 179–180 °C.

FT IR (υ_{max}, cm^{-1}): 3061 (Aromatic CH), 2933 (Aliphatic CH), 1603 (C=C), 1257 (C−O).

^{1}H NMR (DMSO-d_6, δ ppm): 3.38 (2H, s, H_2O + CH_2), 6.58 (1H, s, OH, D_2O exchange), 6.72–7.53 (7H, m, arH), 7.90 (1H, brs, arH), arOH was not observed.

^{13}C NMR (DMSO-d_6, δ ppm): 53.82 (CH_2), 116.70 (arCH), 119.64 (arCH), 120.04 (arC), 126.70 (2arCH), 128.50 (arCH), 128.88 (arCH), 129.10 (2arCH), 132.02 (ITD C-5), 133.33 (arC), 143.00 (ITD C-6), 150.07 (arC−OH), 152.54 (arC−OH), 155.96 (ITD C-2), 163.30 (ITD C-8).

EI MS *m/z* (%): [$C_{17}H_{12}N_3SO_3Cl$ = 339.36], 309.79 (27), 329.88 (73), 338.84 ($[M-1]^+$, 10), 381.06 (30).

*5.7.2. 4-[6-(4-Chlorophenyl)-5-(hydroxymethyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]ben-zen-1,2-diol **(11)***

Yield 81%, m.p: 117–118 °C.

FT IR (υ_{max}, cm^{-1}): 3059 (Aromatic CH), 2972 (Aliphatic CH), 1659 (C=C), 1598 (C=N), 1263 (C−O).

^{1}H NMR (DMSO-d_6, δ ppm): 3.39 (2H, brs, H_2O + CH_2), 6.25–6.69 (9H, m, arH + OH, D_2O exchangei), 7.89 (1H, brs, arH).

^{13}C NMR (DMSO-d_6, δ ppm): 54.01 (CH_2), 116.74 (arCH), 119.63 (arCH), 120.04 (arC), 126.70 (2arCH), 128.55 (arCH), 129.15 (2arCH), 131.94 (ITD C-5), 133.91 (arC), 141.11 (arC−Cl), 144.14 (ITD C-6), 150.07 (arC−OH), 152.54 (arC−OH), 155.98 (ITD C-2), 163.98 (ITD C-8).

EI MS *m/z* (%): [$C_{17}H_{12}N_3SO_3Cl$ = 373.81], 353.89 ($[M + 3\text{-}Na]^+$, 100), 355.78 ($[M-H_2O]^+$, 68), 363.62 (59).

*5.8. General synthesis method of compounds **12** and **13***

Compound **8** or **10**, which is a carbaldehyde derivative of ITD, (10 mmol) was heated with 4-fluorobenzyl amine (10 mmol) with thioglycolic acid (catalytic amount) on the oil bath for 3 h. The reaction mixture was then further heated by the addition of thioglycolic acid (30 mmol) for a further 3 h (The elution time was determined by TLC). The resulting crude product was then neutralized with $NaHCO_3$ solution. The resulting oily product was dried under vacuum and the corresponding 1,3-thiazolidin-4-one derivatives were synthesized.

*5.8.1. 2-[2-(3,4-Dihydroxyphenyl)-6-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-yl]-3-(4-fluorobenzyl)-1,3-thiazolidin-4-one **(12)***

Yield 52%.

FT IR (υ_{max}, cm^{-1}): 3075 (Aromatic CH), 1718 (C=O), 1252 (C−O).

^{1}H NMR (DMSO-d_6, δ ppm): 3.37 (2H, s, CH_2), 4.25 (2H, brs, CH_2), 6.77–8.18 (15H, m, 13arH + 2OH).

^{13}C NMR (DMSO-d_6, δ ppm): 34.55 (CH_2), 48.20 (CH_2), 65.84 (CH), 113.69 (2arCH), 116.70 (arCH), 119.56 (arCH), 120.85 (ITD C-5), 125.56 (arC), 126.76 (2arCH), 129.24 (arCH), 130.00 (arCH), 130.71 (2arCH), 131.61 (2arCH), 132.89 (arC), 133.48 (arC), 140.15 (ITD C-6), 146.55 (arC−OH), 150.20 (arC−OH), 159.54 and 162.56 (arC−F, d

J = 302.0 Hz), 166.93 (ITD C-2 and ITD C-8), 179.84 (C).

5.8.2. 2-[2-(3,4-Dihydroxyphenyl)-6-(4-chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl]-3-(4-fluorobenzyl)-1,3-thiazolidin-4-one **(13)**

Yield 65%.

FT IR (υ_{max}, cm^{-1}): 3074 (Aromatic CH), 1722 (C═O), 1278 (C−O).

^{1}H NMR (DMSO-d_6, δ ppm): 3.68 (2H, s, CH_2), 4.37 (2H, brs, CH_2), 6.71 (1H, d J = 8.0 Hz, arH), 6.93–8.27 (13H, m, arH + 2OH).

^{13}C NMR (DMSO-d_6, δ ppm): 34.80 (CH_2), 48.24 (CH_2), 66.51 (CH), 113.76 (2arCH), 116.80 (arCH), 119.53 (arCH), 120.90 (ITD C-5), 125.21 (arC), 126.78 (2arCH), 129.35 (arCH), 130.11 (2arCH), 130.75 (2arCH), 132.85 (arC), 133.34 (arC−Cl), 134.45 (arC), 141.08 (ITD C-6), 146.56 (arC−OH), 150.23 (arC−OH), 159.17 and 162.60 (arC−F, d J = 343.0 Hz), 167.76 (ITD C-2 and ITD C-8), 178.54 (C).

5.9. Antimicrobial activity/sensitivity studies

Antimicrobial activity or susceptibility tests were performed to determine the in-vitro activity of the antimicrobial agent against a particular bacterial species. Two techniques are used to measure susceptibility testing of microorganisms, including “diffusion” and “dilution”. Disc diffusion technique is a frequently used technique and the sensitivity of the antibiotic absorbed into paper discs (the solution to be measured by antibacterial activity) is based on the diffusion of the organism to the medium in which the organism is inoculated. After the impregnated paper discs are placed on solid media in which the microorganism is inoculated, the discs dissolve and diffuse into the agar, while the inoculated microorganism begins to multiply. There is no growth in the vicinity of the disc where the inhibitor concentrations of the drug are achieved after a certain incubation period. The more sensitive the microorganism is to the drug, the larger the zone of inhibition around the disc. The diameter of the zone of inhibition is measured in mm and evaluations are made according to standard zone tables and the susceptibility status of the microorganism to the antimicrobial agents used is determined.

Minimal Inhibition Test measurements are tested by applying the microdilution technique to determine the dose value of the effectiveness of the substances determined to be effective in the agar well. The quantity of the material is diluted with serial dilutions to the lowest doses and the same amount of microorganism is added to each diluent. With this test, the efficacy dose of the lowest amount of substance is determined.

5.9.1. Minimal inhibition concentration (MIC) method

In order to determine the minimum amount of substance that shows antimicrobial activity, in other words, to determine the quantitative effect value, the micro-dilution liquid method is applied in the liquid medium and the minimal inhibition concentration (MIC) is determined as microgram/milliliter (μg/mL) [29]. For the determination of antimicrobial activity, liquid media were used for determining the antifungal activity of Mueller-Hinton liquid (MHB, pH.7.3) (Difco, Detroit, MI) and yeast extract liquid medium (YEG, pH 7.0) (Difco, Detroit, MI). ELISA plates were used for micro-dilution tests and serial dilutions were made with 0.1 mL of dissolved chemicals. McFarland 0.5 turbidity (1×10^8 cfu/mL) from overnight cultures of inoculated microorganisms was prepared for reconstitution and diluted 1:10 and 0.005 mL of microorganism (final assay concentration 5×10^4 cfu/well) was added to each well. Plates were incubated at 35 °C for 16–24 h under aerobic conditions. The MIC value was completely inhibited by the growth of the microorganism in the micro-dilution wells and was determined as the lowest antimicrobial concentration that could be determined by the naked eye. Ampicillin (10 μg), fluconazole for yeast (5 μg) and standard solvent control were used as standard control drugs.

5.10. Determination of antioxidant activity

Two different methods were used for the determination of antioxidant activity. They are briefly DPPH and FRAP.

5.10.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) - radical scavenging activity

The cleaning efficacy of different chemicals was determined using free radical DPPH (2,2-diphenyl-1-picrylhydrazase) as indicated by Blois [30]. A 100 mL chemical solution was mixed with 1 mL of a newly prepared methanolic DPPH solution. The reaction mixture was incubated for 30 min at room temperature in the dark and then measured at 520 nm. The concentration of the sample that removes 50% of the total amount of DPPH radical by plotting the concentrations against the absorbance is considered to be IC_{50} (mg/mL).

5.10.2. FRAP - ferric reducing ability of plasma

FRAP was measured by some changes in the method described by Benzie and Strain [31]. 2.9 mL new containing 100 mL of each sample, 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ (2,4,6-tryptiryl-s-triazine) and 20 mmol/L $FeCl{\cdot}6H_2O$ prepared FRAP reagent in 10:1:1 (v:v:v) ratios. The mixture was incubated at 37 °C for 30 min and measured at 593 nm. Values are expressed in mmol Trolox/g.

Declaration of Competing Interest

The authors declare that there is no conflict of interests regarding the publication of the paper.

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Appendix A. Supplementary material

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