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Design, Synthesis, SAR and Molecular Modeling Studies of Novel Imidazo[2,1-*b*][1,3,4]Thiadiazole Derivatives as Highly Potent Antimicrobial Agents

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Abstract: In this study, a novel series of phenyl substituted imidazo[2,1-*b*][1,3,4]thiadiazole derivatives were synthesized, characterized and explored for antibacterial activity against Gram-negative *Escherichia coli,* Gram-positive *Staphylococcus aureus* and *Bacillus subtilis* and antifungal activity against *Candida albicans*.

Most of the synthesized compounds exhibited remarkable antimicrobial activities, some of which being ten times more potent than positive controls. The most promising compound showed excellent activity with MIC value of 0.03 μg/ml against both *S. aureus* and *B. subtilis* (MIC values of positive compound Chloramphenicol are 0.4 μg/ml and 0.85 μg/ml, respectively).

Furthermore, structure-activity relationship was also investigated with the help of computational tools. Some physicochemical and ADME properties of the compounds were calculated too. The combination of electronic structure calculations performed at PM6 level and molecular docking simulations using Glide extra-precision mode showed that the hydrophobic nature of keto aryl ring with no electron withdrawing substituents at para position enhances activity while electron-donating substituents at the second aryl ring is detrimental to activity.

Keywords: Imidazo[2,1-*b*][1,3,4]thiadiazole · Molecular Docking · Antimicrobial Activity · SAR · Electronic Structure Calculations.

1 Introduction

There have been significant increases in the synthesis of compounds having antimicrobial activities in recent years.[1] However, the use of such compounds has been very limited due to drug resistance, high toxicity, various side effects and the deficiencies in biological activities. Medicinal chemists, therefore, put great efforts to synthesize new compounds having antimicrobial activities with minimum side effects, which can be used as a precursor or lead compound in the drug discovery.

Heterocyclic compounds are one of the most preferred compounds in antimicrobial studies and many involve the imidazole, 1,3,4-thiadiazole and their derivatives. Hence, synthesis and characterization of such bioactive compounds containing imidazole and 1,3,4-thiadiazole are being studied intensely.[2–6] Furthermore, the synthesis of compounds including these bioactive groups with various biological activities is currently being studied.[7–9] These heterocyclic systems where the imidazole and 1,3,4-thiadiazole rings are fused with a bridgehead nitrogen atom are known as imidazo[2,1-*b*][1,3,4]thiadiazoles.[10–12]

Owing to the fact that imidazo[2,1-*b*][1,3,4]thiadiazoles and their derivatives exhibit many biological activities such as antimicrobial,[13,14] antifungal,[15] antibacterial,[16–18] anti-inflammatory,[19–21] antituberculosis,[22–25] analgesic,[26] diuretic[27] activities, the synthesis of these compounds and their derivatives increases gradually.[28,29]

Besides being used as an anthelmintic drug, the fact that these compounds are bio-isostere of Tetramisole and its enantiomers Levamisole and Dexamisole[30,31] that are used as regulating and strengthening elements for immune system, has caused the anti-cancer activity studies to focus on imidazo[2,1-*b*][1,3,4]thiadiazole derivatives.[32–38] It was reported that, apart from their biological activities, imidazo [2,1-*b*][1,3,4]thiadiazole and their heterocyclic derivatives are also preferred in different industrial fields such as dye production.[39] Therefore, imidazo[2,1-*b*][1,3,4]thiadiazole and their derivatives have become important heterocyclic

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compounds commonly studied in the pharmaceutical chemistry and industry.

It is known that the most frequently used method in synthesizing the imidazo[2,1-*b*][1,3,4]thiadiazole derivatives is based on the reactions of 2-amino-1,3,4-thiadiazole derivatives with 2-bromo acetophenone derivatives.[40,41]

The process of novel drug design also includes computational applications. Molecular docking studies of drug candidates are one of the important steps to enlighten ligand (candidate molecule)-receptor (target) interactions. A relationship between a ligand's activity and its interaction with the target can provide useful information in future SAR studies.[42]

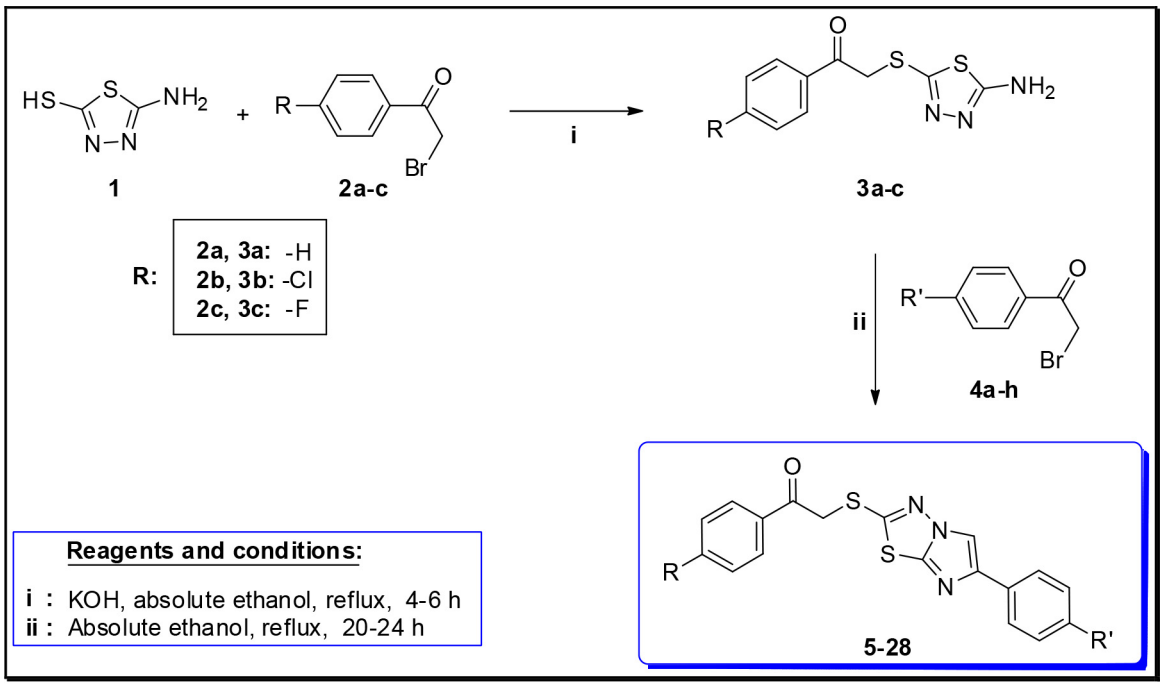
In the light of these aforementioned literature, the main purpose of this study is to synthesize imidazo[2,1-*b*][1,3,4] thiadiazole derivatives, to characterize those structures using different spectroscopic methods, and to investigate the antimicrobial activities of all compounds. The synthetic method employed to synthesize these compounds is given in Scheme 1. Computational tools such as electronic structure calculations and molecular docking studies were also used to explain SAR of those compounds and investigate their activities based on the binding interactions with the receptor and their calculated molecular properties.

2 Material and Methods

2.1 Computational

2.1.1 Ligand Preparation

The 3D models of the compounds **5**–**28** and Chloramphenicol were prepared using the LigPrep[43] tool of the Schrödinger software with OPLS3 force field[44] Ionization



Comp.	R	R'	Comp.	R	R'	Comp.	R	R'
5	–H	–H	**13**	–Cl	–H	**21**	–F	–H
6	–H	–Br	**14**	–Cl	–Br	**22**	–F	–Br
7	–H	–Cl	**15**	–Cl	–Cl	**23**	–F	–Cl
8	–H	–F	**16**	–Cl	–F	**24**	–F	–F
9	–H	$–OCH_3$	**17**	–Cl	$–OCH_3$	**25**	–F	$–OCH_3$
10	–H	–CN	**18**	–Cl	–CN	**26**	–F	–CN
11	–H	–Ph	**19**	–Cl	–Ph	**27**	–F	–Ph
12	–H	$–NO_2$	**20**	–Cl	$–NO_2$	**28**	–F	$–NO_2$

Scheme 1. Synthetic route for the synthesis of substituted imidazo[2,1-*b*][1,3,4]thiadiazole derivatives (**5**–**28**).

states at pH 7.0 ± 2.0 as well as tautomers were generated retaining up to 32 possible stereoisomers per ligand.

2.1.2 Protein Preparation

A high-resolution three-dimensional crystal structure of enoyl-ACP reductase (FabI) in complexed with nicotinamide cofactor (NAD+) and Triclosan (PDB id: 1C14) was downloaded from the protein data bank. Schrödinger's multi-step Protein Preparation Wizard was used for the preparation of the receptor site that was used as the target for molecular docking. Charges and bond orders were assigned, hydrogens were added to the heavy atoms and chain B of the protein and all waters were deleted.

Then, the hydrogen bonds were assigned and optimized at neutral pH. Energy minimization was carried out using default constraint of 0.3 Å RMSD and OPLS3 force field.

2.1.3 Molecular Docking

The prepared Fab-NAD+ binary complex model was used as the receptor model for docking simulations, which were performed using the Glide XP (extra precision) module of Schrödinger Suite.[45–47] Receptor grid has to be calculated to represent the binding pocket such that the ligand poses bind within the active site during docking. Receptor Grid was generated keeping the default parameters. A cubic box of specific dimensions centered on the centroid of the native ligand was generated for receptor.

2.1.4 Calculation of Physicochemical and ADME Properties

Highest molecular orbital (HOMO) energy values were calculated using Wavefunction's Spartan '16 parallel suite (Spartan 16, Wavefunction Inc., Irvine CA). In calculations of such descriptors, semi empirical PM6 method[48] was reported to be of similar quality as DFT-based models, which is much more expensive.[49] Thus, this level was selected and the docked pose of each compound at neutral form was used as equilibrium geometry. QikProp module of Schrodinger was used to calculate molecular weight, percent human oral absorption, polar surface area (PSA) and logarithm of octanol-water partition coefficient (QPlogPo/w).[50]

2.2 Crystallographic Analysis

X-ray diffraction data of compound **10** was collected with a Bruker AXS APEX CCD[51] diffractometer using MoKα beam. The crystal was kept at 293(2) K during data collection. Using Olex2,[52] the structure was solved with the ShelXT[53] structure solution program using Direct Methods and refined with the ShelXL[54] refinement package using Least Squares minimization.

2.3 Chemistry

The ^{1}H NMR and ^{13}C NMR spectra of the compounds were measured in DMSO-d_6 using an Agilent NMR V NMRS spectrometer at 400 MHz and 100 MHz, respectively. Chemical shift values are given in ppm (δ) relative to tetramethylsilane (TMS) as internal standard. The IR spectra were recorded in a Bruker Optics Alpha FT-IR in ATR. The mass spectra were measured with a Thermo TSQ Quantum Access Max LC-MS/MS spectrometer. Elemental analyses were performed on a LECO 932 CHNS (Leco-932, St. Joseph, MI, USA) instrument and the results were within ±0.4% of the theoretical values. Melting points were recorded on a Thermo Scientific IA9000 series apparatus and were uncorrected. X-Ray analysis was performed on a Bruker D8 QUEST instrument. All the chemicals, reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification, unless mentioned otherwise.

2.3.1 General Procedure for the Synthesis of 2-((5-Amino-1,3,4-thiadiazol-2-yl)thio)-1-(4-(un) substitutedphenyl)ethanone (3 a–c)

Compounds **3 a–c** were synthesized according to a method given in the literature.[55]

In a round-bottomed flask, 5-amino-1,3,4-thiadiazole-2-thiol **(1)** (0.08 mol) and KOH (0.08 mol) were dissolved in absolute ethanol. The solution was stirred for about 30 minutes at room temperature. 2-Bromoacetophenone derivatives (0.08 mol) **(2 a–c)** was dissolved in absolute ethanol (25 mL) and added dropwise to this solution at room temperature with the assistance of a dropping funnel. The mixture was refluxed and stirred for 4–6 h. The progress of reaction was monitored by TLC at appropriate time intervals. After completion of the reaction, the mixture was filtered and the solid matter was obtained. It was washed with deionized water, ethanol and diethyl ether, respectively. The solid was recrystallized from the appropriate solvent. The physical properties and spectral data derived from the obtained products are listed below.

2-((5-Amino-1,3,4-thiadiazol-2-yl)thio)-1-phenylethanone (3 a)

Light gray solid, yield: 16.29 g (81%); mp: 176–177 °C (from DMF-EtOH, 1:4); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.79 (s, 2H, –CH_2), Phenyl-H [7.53 (t, J = 16.0 Hz, 2H), 7.66 (t, J = 16.0 Hz, 1H), 7.99 (d, J = 8.0 Hz, 2H)], 7.28 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.02 (–CH_2), Phenyl-C [128.94 (CH), 129.26 (CH), 134.19 (C), 135.61 (C)], Thiadiazole-C [149.68 (C), 170.27 (C)], 193.86 (C=O); IR (ATR, cm^{-1}): 3259, 3108 (–NH_2), 3067 (Ar–CH), 2940 (Aliph. CH),

1693 (C=O), 1589 (C=N); MS: *m/z* 252.85 (M+1, 100); *Anal.* Calcd. for $C_{10}H_9N_3OS_2$: C 47.79, H 3.61, N 16.72, found: C 47.71, H 3.59, N 16.77.

2-((5-Amino-1,3,4-thiadiazol-2-yl)thio)-1-(4-chlorophenyl)ethanone (3b)

White solid, yield: 18.75 g (82%); mp: 184–186 °C (from DMF-EtOH, 1:4); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.78 (s, 2H, –CH_2), Phenyl-H [7.61 (d, *J*=8.0 Hz, 2H), 8.00 (d, *J*=8.0 Hz, 2H)], 7.32 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 41.92 (–CH_2), Phenyl-C [129.38 (CH), 130.88 (CH), 134.32 (C), 139.10 (C)], Thiadiazole-C [149.51 (C), 170.32 (C)], 192.99 (C=O); IR (ATR, cm^{-1}): 3263, 3116 (–NH_2), 3059 (Ar–CH), 2936 (Aliph. CH), 1674 (C=O), 1588 (C=N); MS: *m/z* 287.81 (M+2, 100); *Anal.* Calcd. for $C_{10}H_8ClN_3OS_2$: C 42.03, H 2.82, N 14.70, found: C 42.00, H 2.77, N 14.68.

2-((5-Amino-1,3,4-thiadiazol-2-yl)thio)-1-(4-fluorophenyl)ethanone (3c)

Light gray solid, yield: 18.09 g (84%); mp: 162–163 °C (from EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.76 (s, 2H, –CH_2), Phenyl-H [7.36 (d, *J*=16.0 Hz, 2H), 8.08 (d, *J*=16.0 Hz, 2H)], 7.27 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 41.92 (–CH_2), Phenyl-C [116.41 (CH), 132.08 (CH), 164.48 (C), 166.99 (C)], Thiadiazole-C [149.53 (C), 170.30 (C)], 192.54 (C=O); IR (ATR, cm^{-1}): 3251, 3074 (–NH_2), 3031 (Ar–CH), 2946 (Aliph. CH), 1671 (C=O), 1598 (C=N); MS: *m/z* 270.22 (M+1, 100); *Anal.* Calcd. for $C_{10}H_8FN_3OS_2$: C 44.60, H 2.99, N 15.60, found: C 44.58, H 3.01, N 15.55.

2.3.2 General Procedure for the Synthesis of Imidazo[2,1-b][1,3,4]thiadiazole Derivatives (5-28)

In a two-necked flask, 2-amino-1,3,4-thiadiazole derivatives **(3a–c)** (4 mmol) were dissolved in absolute ethanol (30 mL). 2-Bromoacetophenone derivatives **(4a–h)** (4 mmol) were dissolved in absolute ethanol (20 mL) and then added dropwise to this solution at room temperature with the assistance of a dropping funnel. Then, the mixture was refluxed and stirred for 20–24 h. The progress of reaction was monitored by TLC at appropriate time intervals. The excess of solvent was removed under reduced pressure and neutralized by aqueous sodium carbonate (Na_2CO_3) solution. The solution was filtered and washed with deionized water. The solid matter was recrystallized from acetone. The synthesized compounds were dried with P_2O_5 in a vacuum oven. The physical properties and spectral data derived from the obtained products are listed below.

1-Phenyl-2-((6-phenylimidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)ethanone (5)

White solid, yield 0.93 g (66%); mp: 158–160 °C (from Acetone); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.17 (s, 2H, –CH_2), Phenyl-H [7.57 (t, *J*=16.0 Hz, 2H), 7.70 (t, *J*=16.0 Hz, 1H), 8.05 (d, *J*=8.0 Hz, 2H], Imidazole-Phenyl-H [7.25 (t, *J*=16.0 Hz, 1H), 7.38 (t, *J*=16.0 Hz, 2H), 7.81 (d, *J*=8.0 Hz, 2H)], Imidazole-H [8.61 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.46 (–CH_2), Phenyl-C [127.78 (CH), 128.74 (CH), 129.80 (CH), 134.06 (C)], Imidazole-Phenyl-C [125.04 (CH), 129.04 (CH), 129.38 (CH), 133.72 (C)], Imidazole-C [110.99 (CH), 145.17 (C)], Thiadiazole-C [135.41 (C), 160.23 (C)], 193.05 (C=O); IR (ATR, cm^{-1}): 3131 (Ar–CH), 2951 (Aliph. CH), 1686 (C=O), 1595 (C=N); MS: *m/z* 351.76 (M$^+$, 100); *Anal.* Calcd. for $C_{18}H_{13}N_3OS_2$: C 61.52, H 3.73, N 11.96, found: C 61.45, H 3.77, N 11.89.

2-((6-(4-Bromophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)-1-phenylethanone (6)

Light gray crystals, yield 0.95 g (55%); mp: 185–186 °C (from Acetone); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.17 (s, 2H, –CH_2), Phenyl-H [7.57 (d, *J*=4.0 Hz, 2H), 7.69 (t, *J*=8.0 Hz, 1H), 8.05 (d, *J*=8.0 Hz, 2H], Imidazole-Phenyl-H [7.57 (d, *J*=8.0 Hz, 2H), 7.77 (d, *J*=8.0 Hz, 2H)], Imidazole-H [8.65 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.46 (–CH_2), Phenyl-C [127.00 (CH), 128.98 (CH), 134.50 (C), 144.09 (C)], Imidazole-Phenyl-C [120.61 (C), 129.38 (CH), 132.04 (C), 133.47 (CH)], Imidazole-C [111.43 (CH), 145.29 (C)], Thiadiazole-C [135.43 (C), 160.56 (C)], 193.05 (C=O); IR (ATR, cm^{-1}): 3126 (Ar–CH), 2945 (Aliph. CH), 1679 (C=O), 1582 (C=N); MS: *m/z* 430.56 (M$^+$, 100); *Anal.* Calcd. for $C_{18}H_{12}BrN_3OS_2$: C 50.24, H 2.81, N 9.76, found: C 50.21, H 2.87, N, 9.69.

2-((6-(4-Chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)-1-phenylethanone (7)

White solid, yield 0.96 g (62%); mp: 180–181 °C (from Acetone); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.17 (s, 2H, –CH_2), Phenyl-H [7.57 (t, *J*=12.0 Hz, 2H), 7.70 (t, *J*=12.0 Hz, 1H), 8.05 (d, *J*=8.0 Hz, 2H], Imidazole-Phenyl-H [7.43 (d, *J*=8.0 Hz, 2H), 7.83 (d, *J*=8.0 Hz, 2H)], Imidazole-H [8.64 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.46 (–CH_2), Phenyl-C [126.68 (CH), 128.90 (CH), 133.11 (C), 144.06 (C)], Imidazole-Phenyl-C [128.98 (CH), 129.32 (CH), 132.08 (C), 134.50 (C)], Imidazole-C [111.40 (CH), 145.27 (C)], Thiadiazole-C [135.42 (C), 160.53 (C)], 193.04 (C=O); IR (ATR, cm^{-1}): 3121 (Ar–CH), 2971 (Aliph. CH), 1679 (C=O), 1596 (C=N); MS: *m/z* 385.74 (M$^+$, 100); *Anal.* Calcd. for $C_{18}H_{12}ClN_3OS_2$: C 56.02, H 3.13, N 10.89, found: C 55.96, H 3.17, N 10.81.

2-((6-(4-Fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)-1-phenylethanone (8)

White solid, yield 0.99 g (67%); mp: 164–165 °C (from Acetone); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.16 (s, 2H, –CH_2), Phenyl-H [7.57 (bs, 2H), 7.70 (bs, 1H), 8.04 (bs, 2H], Imidazole-Phenyl-H [7.21 (t, *J*=12.0 Hz, 2H), 7.84 (bs, 2H)], Imidazole-H [8.58 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.46 (–CH_2), Phenyl-C [128.89 (CH), 129.38 (CH), 134.49 (CH), 144.36 (C)], Imidazole-Phenyl-C [116.09 (CH), 126.92 (C), 130.74 (CH), 163.16 (C)], Imidazole-C [110.83 (CH), 145.10 (C)], Thiadiazole-C [135.42 (C), 160.21 (C)], 193.05 (C=O); IR (ATR, cm^{-1}): 3067 (Ar–CH), 2941 (Aliph. CH), 1676 (C=O), 1597 (C=N); MS: *m/z* 369.57 (M$^+$, 100); *Anal.* Calcd. for $C_{18}H_{12}FN_3OS_2$: C 58.52, H 3.27, N 11.37, found: C 58.48, H 3.21, N 11.35.

2-((6-(4-Methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)-1-phenylethanone (9)

Light white crystals, yield 1.13 g (74%); mp: 138–139 °C (from Acetone); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.76

(s, 3H, –OCH_3), 5.17 (s, 2H, –CH_2), Phenyl-H [7.57 (t, J = 16.0 Hz, 2H), 7.70 (t, J = 16.0 Hz, 1H), 8.04 (d, J = 8.0 Hz, 2H], Imidazole-Phenyl-H [6.96 (d, J = 8.0 Hz, 2H), 7.73 (d, J = 8.0 Hz, 2H)], Imidazole-H [8.51 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.50 (–CH_2), 55.63 (–OCH_3), Phenyl-C [125.56 (CH), 128.96 (CH), 134.46 (C), 141.47 (C)], Imidazole-Phenyl-C [114.54 (CH), 128.00 (C), 129.36 (CH), 159.41 (C)], Imidazole-C [109.91 (CH), 144.34 (C)], Thiadiazole-C [135.53 (C), 161.09 (C)], 193.33 (C=O); IR (ATR, cm^{-1}): 3067 (Ar–CH), 2997 (Aliph. CH), 1667 (C=O), 1595 (C=N); MS: *m/z* 381.55 (M^+, 100); *Anal.* Calcd. for $C_{19}H_{15}N_3O_2S_2$: C 59.82, H 3.96, N 11.02, found: C 59.85, H 3.91, N 10.98.

4-(2-((2-Oxo-2-phenylethyl)thio)imidazo[2,1-b][1,3,4] thiadiazol-6-yl)benzonitrile (10)

Light white crystals, yield 1.01 g (67%); mp: 210–211 °C (from Acetone); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.19 (s, 2H, –CH_2), Phenyl-H [7.58 (t, J = 12.0 Hz, 2H), 7.70 (t, J = 12.0 Hz, 1H), 8.05 (d, J = 8.0 Hz, 2H], Imidazole-Phenyl-H [7.83 (d, J = 12.0 Hz, 2H), 7.99 (d, J = 12.0 Hz, 2H)], Imidazole-H [8.82 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.42 (–CH_2), Phenyl-C [128.98 (CH), 129.40 (CH), 134.51 (CH), 143.36 (C)], Imidazole-Phenyl-C [109.73 (C), 125.48 (CH), 133.21 (CH), 138.72 (C)], Imidazole-C [111.07 (CH), 145.88 (C)], 119.48 (C≡N), Thiadiazole-C [135.42 (C), 161.48 (C)], 192.98 (C=O); IR (ATR, cm^{-1}): 3067 (Ar–CH), 2957 (Aliph. CH), 2219 (C≡N), 1677 (C=O), 1595 (C=N); MS: *m/z* 376.57 (M^+, 100); *Anal.* Calcd. for $C_{19}H_{12}N_4OS_2$: C 60.62, H 3.21, N 14.88, found: C 60.55, H 3.16, N 14.81.

2-((6-([1, 1′-Biphenyl]-4-yl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)-1-phenylethanone (11)

Gray solid, yield 1.16 g (68%); mp: 185–187 °C (from DMF-EtOH, 1:3); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.17 (s, 2H, –CH_2), Phenyl-H [7.58 (t, J = 12.0 Hz, 2H), 7.71 (t, J = 12.0 Hz, 1H), 8.05 (d, J = 8.0 Hz, 2H], Imidazole-Phenyl-H [7.34 (t, J = 16.0 Hz, 1H), 7.45 (t, J = 16.0 Hz, 2H), 7.69 (d, J = 8.0 Hz, 4H), 7.91 (d, J = 8.0 Hz, 2H)], Imidazole-H [8.66 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.49 (–CH_2), Phenyl-C [127.86 (CH), 128.99 (CH), 133.34 (CH), 144.93 (C)], Imidazole-Phenyl-C [125.59 (CH), 126.89 (CH), 127.35 (CH), 129.39 (CH), 134.50 (CH), 139.33 (C), 140.16 (CH)], Imidazole-C [111.17 (CH), 145.19 (C)], Thiadiazole-C [135.44 (C), 160.21 (C)], 193.06 (C=O); IR (ATR, cm^{-1}): 3033 (Ar–CH), 2933 (Aliph. CH), 1675 (C=O), 1597 (C=N); MS: *m/z* 427.81 (M^+, 100); *Anal.* Calcd. for $C_{24}H_{17}N_3OS_2$: C 67.42, H 4.01, N 9.83, found: C 67.36, H 3.97, N 9.89.

2-((6-(4-Nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)-1-phenylethanone (12)

Yellow solid, yield 1.22 g (77%); mp: 231–233 °C (from DMF-EtOH, 1:1); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.07 (s, 2H, –CH_2), Phenyl-H [7.52 (t, J = 16.0 Hz, 2H), 7.65 (t, J = 16.0 Hz, 1H), 7.99 (d, J = 8.0 Hz, 2H], Imidazole-Phenyl-H [8.23 (d, J = 8.0 Hz, 2H), 8.40 (d, J = 8.0 Hz, 2H)], Imidazole-H [8.88 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.25 (–CH_2), 4Cl-Phenyl-C [129.31 (CH), 130.38 (CH), 139.40 (C), 143.50 (C)], Imidazole-Phenyl-C [113.25 (C), 124.58 (CH), 133.70 (CH), 135.63 (C)], Imidazole-C [109.12 (CH), 145.77 (C)], Thiadiazole-C [134.11 (C), 166.53 (C)], 191.75 (C=O); IR (ATR, cm^{-1}): 3105 (Ar–CH), 2972 (Aliph. CH), 1673 (C=O), 1597 (C=N), 1522, 1338 (–NO_2); MS: *m/z* 396.56 (M^+, 100); *Anal.* Calcd. for $C_{18}H_{12}N_4O_3S_2$: C 54.53, H 3.05, N 14.13, found: C 54.50, H 3.00, N 14.05.

1-(4-Chlorophenyl)-2-((6-phenylimidazo[2,1-b][1,3,4] thiadiazol-2-yl)thio)ethanone (13)

White solid, yield 1.13 g (73%); mp: 154–156 °C (from Acetone); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.15 (s, 2H, –CH_2), 4Cl-Phenyl-H [7.65 (d, J = 8.0 Hz, 2H), 8.06 (d, J = 8.0 Hz, 2H)], Imidazole-Phenyl-H [7.25 (t, J = 12.0 Hz, 1H), 7.37 (t, J = 12.0 Hz, 2H), 7.81 (d, J = 8.0 Hz, 2H)], Imidazole-H [8.59 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.36 (–CH_2), 4Cl-Phenyl-C [129.10 (CH), 129.51 (CH), 139.42 (C), 145.06 (C)], Imidazole-Phenyl-C [125.04 (CH), 127.76 (CH), 129.41 (CH), 130.90 (C)], Imidazole-C [110.99 (CH), 145.27 (C)], Thiadiazole-C [134.14 (C), 160.01 (C)], 192.25 (C=O); IR (ATR, cm^{-1}): 3039 (Ar–CH), 2932 (Aliph. CH), 1674 (C=O), 1599 (C=N); MS: *m/z* 385.78 (M^+, 100); *Anal.* Calcd. for $C_{18}H_{12}ClN_3OS_2$: C 56.02, H 3.13, N 10.89, found: C 55.96, H 3.04, N 10.93.

2-((6-(4-Bromophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)-1-(4-chlorophenyl)ethanone (14)

White solid, yield 1.19 g (64%); mp: 215–217 °C (from DMF-EtOH, 1:4); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.15 (s, 2H, –CH_2), 4Cl-Phenyl-H [7.65 (d, J = 8.0 Hz, 2H), 8.06 (d, J = 8.0 Hz, 2H)], Imidazole-Phenyl-H [7.57 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 8.0 Hz, 2H)], Imidazole-H [8.65 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.34 (–CH_2), 4Cl-Phenyl-C [129.51 (CH), 130.91 (CH), 139.42 (C), 144.40 (C)], Imidazole-Phenyl-C [120.62 (C), 127.00 (CH), 132.05 (C), 133.58 (CH)], Imidazole-C [111.44 (CH), 145.31 (C)], Thiadiazole-C [134.16 (C), 160.11 (C)], 192.24 (C=O); R (ATR, cm^{-1}): 3124 (Ar–CH), 2976 (Aliph. CH), 1678 (C=O), 1590 (C=N); MS: *m/z* 465.20 (M + 1, 100); *Anal.* Calcd. for $C_{18}H_{11}BrClN_3OS_2$: C 46.51, H 2.39, N 9.04, found: C 46.56, H 2.30, N 9.01.

1-(4-Chlorophenyl)-2-((6-(4-chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)ethanone (15)

White solid, yield 1.14 g (68%); mp: 216–218 °C (from DMF-EtOH, 1:4); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.15 (s, 2H, –CH_2), 4Cl-Phenyl-H [7.65 (d, J = 8.0 Hz, 2H), 8.06 (d, J = 8.0 Hz, 2H)], Imidazole-Phenyl-H [7.44 (d, J = 8.0 Hz, 2H), 7.83 (d, J = 8.0 Hz, 2H)], Imidazole-H [8.64 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.34 (–CH_2), 4Cl-Phenyl-C [129.51 (CH), 130.91 (CH), 139.42 (C), 144.08 (C)], Imidazole-Phenyl-C [126.70 (CH), 129.14 (CH), 132.09 (C), 133.10 (C)], Imidazole-C [111.41 (CH), 145.29 (C)], Thiadiazole-C [134.16 (C), 160.39 (C)], 192.25 (C=O); IR (ATR, cm^{-1}): 3120 (Ar–CH), 2947 (Aliph. CH), 1678 (C=O), 1591 (C=N); MS: *m/z* 420.69 (M^+, 100); *Anal.* Calcd. for $C_{18}H_{11}Cl_2N_3OS_2$: C 51.43, H 2.64, N 10.00, found: C 51.38, H 2.69, N 10.09.

1-(4-Chlorophenyl)-2-((6-(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)ethanone (16)

Light gray solid, yield 1.06 g (66%); mp: 188–190 °C (from Acetone); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.14 (s, 2H, –CH_2), 4Cl-Phenyl-H [7.65 (d, J = 8.0 Hz, 2H), 8.05 (d,

J = 8.0 Hz, 2H)], Imidazole-Phenyl-H [7.21 (t, J = 12.0 Hz, 2H), 7.85 (t, J = 12.0 Hz, 2H)], Imidazole-H [8.57 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.34 (–CH_2), 4Cl-Phenyl-C [129.50 (CH), 130.89 (CH), 139.43 (C), 144.38 (C)], Imidazole-Phenyl-C [116.09 (CH), 127.01 (CH), 130.89 (C), 160.74 (C)], Imidazole-C [110.84 (CH), 145.11 (C)], Thiadiazole-C [134.13 (C), 160.05 (C)], 192.24 (C=O); IR (ATR, cm^{-1}): 3116 (Ar–CH), 2946 (Aliph. CH), 1673 (C=O), 1591 (C=N); MS: *m/z* 403.66 (M^+, 100); *Anal.* Calcd. for $C_{18}H_{11}ClFN_3OS_2$: C 53.53, H 2.75, N 10.40, found: C 53.48, H 2.67, N 10.31.

1-(4-Chlorophenyl)-2-((6-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)ethanone (17)

Gray solid, yield 1.25 g (75%); mp: 184–185 °C (from Acetone); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.75 (s, 3H, –OCH_3), 5.13 (s, 2H, –CH_2), 4Cl-Phenyl-H [7.65 (d, J = 8.0 Hz, 2H), 8.06 (d, J = 8.0 Hz, 2H)], Imidazole-Phenyl-H [6.95 (d, J = 12.0 Hz, 2H), 7.74 (d, J = 12.0 Hz, 2H)], Imidazole-H [8.47 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.37 (–CH_2), 55.58 (–OCH_3), 4Cl-Phenyl-C [129.51 (CH), 130.90 (CH), 139.40 (C), 144.79 (C)], Imidazole-Phenyl-C [114.54 (CH), 126.39 (C), 126.80 (CH), 159.14 (C)], Imidazole-C [109.87 (CH), 145.37 (C)], Thiadiazole-C [134.16 (C), 160.02 (C)], 192.31 (C=O); IR (ATR, cm^{-1}): 3067 (Ar–CH), 2931 (Aliph. CH), 1673 (C=O), 1587 (C=N); MS: *m/z* 415.74 (M^+, 100); *Anal.* Calcd. for $C_{19}H_{14}ClN_3O_2S_2$: C 54.87, H 3.39, N 10.10, found: C 54.81, H 3.30, N 10.02.

4-(2-((2-(4-Chlorophenyl)-2-oxoethyl)thio)imidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzonitrile (18)

White solid, yield 1.18 g (72%); mp: 235–236 °C (from DMF-EtOH, 1:4); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.16 (s, 2H, –CH_2), 4Cl-Phenyl-H [7.65 (d, J = 8.0 Hz, 2H), 8.06 (d, J = 8.0 Hz, 2H)], Imidazole-Phenyl-H [7.84 (d, J = 8.0 Hz, 2H), 7.99 (d, J = 8.0 Hz, 2H)], Imidazole-H [8.81 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.30 (–CH_2), 119.48 (C≡N), 4Cl-Phenyl-C [129.52 (CH), 130.90 (CH), 139.43 (C), 143.37 (C)], Imidazole-Phenyl-C [113.06 (C), 125.48 (CH), 133.21 (CH), 138.70 (C)], Imidazole-C [109.74 (CH), 145.90 (C)], Thiadiazole-C [134.14 (C), 161.34 (C)], 192.18 (C=O); IR (ATR, cm^{-1}): 3122 (Ar–CH), 2944 (Aliph. CH), 2220 (C≡N), 1677 (C=O), 1589 (C=N); MS: *m/z* 411.76 (M + 1, 100); *Anal.* Calcd. for $C_{19}H_{11}ClN_4OS_2$: C 55.54, H 2.70, N 13.64, found: C 55.50, H 2.62, N 13.54.

2-((6-([1,1′-Biphenyl]-4-yl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)-1-(4-chlorophenyl)ethanone (19)

Gray solid, yield 1.31 g (71%); mp: 215–217 °C (from from DMF-EtOH, 1:3); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.16 (s, 2H, –CH_2), 4Cl-Phenyl-H [7.65 (d, J = 8.0 Hz, 2H), 8.07 (d, J = 8.0 Hz, 2H)], Imidazole-Phenyl-H [7.34 (t, J = 12.0 Hz, 1H), 7.45 (t, J = 12.0 Hz, 2H), 7.69 (d, J = 8.0 Hz, 4H), 7.91 (d, J = 8.0 Hz, 2H)], Imidazole-H [8.66 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.37 (–CH_2), 4Cl-Phenyl-C [129.52 (CH), 130.92 (CH), 139.42 (C), 144.94 (C)], Imidazole-Phenyl-C [125.58 (CH), 126.90 (CH), 127.36 (CH), 127.87 (CH), 129.40 (CH), 133.31 (C), 139.35 (C), 140.15 (C)], Imidazole-C [111.18 (CH), 145.22 (C)], Thiadiazole-C [134.16 (C), 160.06 (C)], 192.28 (C=O); IR (ATR, cm^{-1}): 3040 (Ar–CH), 2932 (Aliph. CH), 1673 (C=O), 1588 (C=N); MS: *m/z* 461.63 (M^+, 100); *Anal.* Calcd. for $C_{24}H_{16}ClN_3OS_2$: C 62.40, H 3.49, N 9.10, found: C 62.32, H 3.53, N 9.06.

1-(4-Chlorophenyl)-2-((6-(4-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)ethanone (20)

Yellow solid, yield 1.26 g (73%); mp: 238–240 °C (from DMF-EtOH, 1:1); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.17 (s, 2H, –CH_2), 4Cl-Phenyl-H [7.66 (d, J = 8.0 Hz, 2H), 8.07 (d, J = 8.0 Hz, 2H)], Imidazole-Phenyl-H [8.07 (d, J = 8.0 Hz, 2H)), 8.25 (d, J = 8.0 Hz, 2H)], Imidazole-H [8.88 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.30 (–CH_2), 4Cl-Phenyl-C [129.51 (CH), 130.91 (CH), 139.44 (C), 143.38 (C)], Imidazole-Phenyl-C [113.07 (C), 125.42 (CH), 133.27 (CH), 138.72 (C)], Imidazole-C [109.72 (CH), 145.89 (C)], Thiadiazole-C [134.12 (C), 161.33 (C)], 192.17 (C=O); IR (ATR, cm^{-1}): 3048 (Ar–CH), 2923 (Aliph. CH), 1673 (C=O), 1587 (C=N), 1506, 1343 (-NO_2); MS: *m/z* 430.76 (M^+, 100); *Anal.* Calcd. for $C_{18}H_{11}ClN_4O_3S_2$: C 50.17, H 2.57, N 13.00, found: C 50.12, H 2.59, N 13.08.

1-(4-Fluorophenyl)-2-((6-phenylimidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)ethanone (21)

Light yellowish crystals, yield 0.96 g (65%); mp: 183–184 °C (from Acetone); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.15 (s, 2H, –CH_2), 4F-Phenyl-H [7.41 (t, J = 12.0 Hz, 2H), 8.14 (t, J = 12.0 Hz, 2H)], Imidazole-Phenyl-H [7.25 (t, J = 12.0 Hz, 1H), 7.37 (t, J = 12.0 Hz, 2H), 7.82 (d, J = 8.0 Hz, 2H)], Imidazole-H [8.59 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.34 (–CH_2), 4F-Phenyl-C [116.37 (CH), 132.22 (CH), 164.66 (CH), 167.17 (C)], Imidazole-Phenyl-C [125.04 (CH), 127.75 (CH), 129.10 (CH), 132.14 (C)], Imidazole-C [110.99 (CH), 145.27 (C)], Thiadiazole-C [145.06 (C), 160.05 (C)], 191.75 (C=O); IR (ATR, cm^{-1}): 3072 (Ar–CH), 2951 (Aliph. CH), 1686 (C=O), 1593 (C=N); MS: *m/z* 369.82 (M^+, 100); *Anal.* Calcd. for $C_{18}H_{12}FN_3OS_2$: C 58.52, H 3.27, N 11.37, found: C 58.49, H 3.36, N 11.30.

2-((6-(4-Bromophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)-1-(4-fluorophenyl)ethanone (22)

Gray solid, yield 1.27 g (71%); mp: 198–199 °C (from Acetone); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.15 (s, 2H, –CH_2), 4F-Phenyl-H [7.41 (t, J = 12.0 Hz, 2H), 8.14 (t, J = 12.0 Hz, 2H)], Imidazole-Phenyl-H [7.57 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 8.0 Hz, 2H)], Imidazole-H [8.65 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.31 (–CH_2), 4F-Phenyl-C [116.37 (CH), 132.16 (CH), 162.25 (CH), 164.37 (C)], Imidazole-Phenyl-C [120.61 (C), 127.01 (CH), 132.05 (C), 133.46 (CH)], Imidazole-C [111.43 (CH), 145.31 (C)], Thiadiazole-C [144.10 (C), 160.49 (C)], 191.74 (C=O); IR (ATR, cm^{-1}): 3128 (Ar–CH), 2954 (Aliph. CH), 1679 (C=O), 1596 (C=N); MS: *m/z* 449.65 (M + 1, 100); *Anal.* Calcd. for $C_{18}H_{11}BrFN_3OS_2$: C 48.22, H 2.47, N 9.37, found: C 48.15, H 2.51, N 9.32.

2-((6-(4-Chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)-1-(4-fluorophenyl)ethanone (23)

Gray solid, yield 0.95 g (59%); mp: 197–199 °C (from Acetone); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.15 (s, 2H, –CH_2), 4F-Phenyl-H [7.41 (t, J = 12.0 Hz, 2H), 8.14 (t, J = 12.0 Hz, 2H)], Imidazole-Phenyl-H [7.43 (d, J = 8.0 Hz, 2H), 7.83 (d, J = 8.0 Hz, 2H)], Imidazole-H [8.64 (s, 1H)]; ^{13}C NMR

(100 MHz, DMSO-d_6, δ ppm): 42.32 (–CH_2), 4F-Phenyl-C [116.36 (CH), 132.23 (CH), 164.66 (CH), 167.18 (C)], Imidazole-Phenyl-C [126.69 (CH), 129.14 (CH), 132.06 (C), 133.10 (C)], Imidazole-C [111.40 (CH), 145.28 (C)], Thiadiazole-C [144.07 (C), 160.45 (C)], 191.74 (C=O); IR (ATR, cm^{-1}): 3124 (Ar–CH), 2945 (Aliph. CH), 1680 (C=O), 1596 (C=N); MS: *m/z* 403.73 (M^+, 100); *Anal.* Calcd. for $C_{18}H_{11}ClFN_3OS_2$: C 53.53, H 2.75, N 10.40, found: C 53.60, H 2.69, N 10.31.

1-(4-Fluorophenyl)-2-((6-(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)ethanone (24)

White solid, yield 1.07 g (69%); mp: 183–184 °C (from Acetone); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.17 (s, 2H, –CH_2), 4F-Phenyl-H [7.41 (t, *J* = 12.0 Hz, 2H), 8.14 (t, *J* = 12.0 Hz, 2H)], Imidazole-Phenyl-H [7.22 (t, *J* = 12.0 Hz, 2H), 7.85 (t, *J* = 12.0 Hz, 2H)], Imidazole-H [8.58 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.33 (–CH_2), 4F-Phenyl-C [116.36 (CH), 132.15 (CH), 164.66 (CH), 167.17 (C)], Imidazole-Phenyl-C [116.10 (CH), 127.00 (CH), 130.74 (C), 163.17 (C)], Imidazole-C [110.84 (CH), 145.11 (C)], Thiadiazole-C [144.38 (C), 160.75 (C)], 191.76 (C=O); IR (ATR, cm^{-1}): 3120 (Ar–CH), 2941 (Aliph. CH), 1677 (C=O), 1596 (C=N); MS: *m/z* 387.70 (M^+, 100); *Anal.* Calcd. for $C_{18}H_{11}F_2N_3OS_2$: C 55.80, H 2.86, N 10.85, found: C 55.71, H 2.78, N 10.80.

1-(4-Fluorophenyl)-2-((6-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)ethanone (25)

Yellowish solid, yield 1.10 g (69%); mp: 161–162 °C (from DMF-EtOH, 1:3); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.75 (s, 3H, –OCH_3), 5.13 (s, 2H, –CH_2), 4F-Phenyl-H [7.41 (t, *J* = 12.0 Hz, 2H), 8.14 (t, *J* = 12.0 Hz, 2H)], Imidazole-Phenyl-H [6.94 (d, *J* = 8.0 Hz, 2H), 7.74 (d, *J* = 8.0 Hz, 2H)], Imidazole-H [8.46 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.36 (–CH_2), 55.56 (–OCH_3), 4F-Phenyl-C [116.35 (CH), 132.15 (CH), 164.66 (CH), 167.16 (C)], Imidazole-Phenyl-C [114.53 (CH), 126.39 (C), 126.82 (CH), 159.14 (C)], Imidazole-C [109.86 (CH), 145.37 (C)], Thiadiazole-C [144.78 (C), 159.40 (C)], 191.80 (C=O); IR (ATR, cm^{-1}): 3148 (Ar–CH), 2944 (Aliph. CH), 1671 (C=O), 1593 (C=N); MS: *m/z* 399.74 (M^+, 100); *Anal.* Calcd. for $C_{19}H_{14}FN_3O_2S_2$: C 57.13, H 3.53, N 10.52, found: C 57.06, H 3.60, N 10.48.

4-(2-((2-(4-Fluorophenyl)-2-oxoethyl)thio)imidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzonitrile (26)

Light gray solid, yield 0.99 g (63%); mp: 211–212 °C (from Acetone); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.17 (s, 2H, –CH_2), 4F-Phenyl-H [7.41 (t, *J* = 12.0 Hz, 2H), 8.14 (t, *J* = 12.0 Hz, 2H)], Imidazole-Phenyl-H [7.83 (d, *J* = 8.0 Hz, 2H), 7.99 (d, *J* = 8.0 Hz, 2H)], Imidazole-H [8.81 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.30 (–CH_2), 119.48 (C≡N), 4F-Phenyl-C [116.37 (CH), 132.15 (CH), 164.67 (CH), 167.13 (C)], Imidazole-Phenyl-C [113.06 (C), 125.47 (CH), 133.20 (CH), 138.71 (C)], Imidazole-C [109.73 (CH), 145.88 (C)], Thiadiazole-C [143.37 (C), 161.38 (C)], 191.67 (C=O); IR (ATR, cm^{-1}): 3129 (Ar–CH), 2944 (Aliph. CH), 2226 (C≡N), 1674 (C=O), 1594 (C=N); MS: *m/z* 394.84 (M^+, 100); *Anal.* Calcd. for $C_{19}H_{11}FN_4OS_2$: C 57.85, H 2.81, N 14.20, found: C 57.79, H 2.75, N 14.23.

2-((6-([1, 1′-Biphenyl]-4-yl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)-1-(4-fluorophenyl)ethanone (27)

Gray solid, yield 1.28 g (72%); mp: 185–186 °C (from Acetone); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.16 (s, 2H, –CH_2), 4F-Phenyl-H [7.41 (t, *J* = 12.0 Hz, 2H), 8.15 (t, *J* = 12.0 Hz, 2H)], Imidazole-Phenyl-H [7.35 (t, *J* = 12.0 Hz, 1H), 7.45 (t, *J* = 12.0 Hz, 2H), 7.69 (d, *J* = 8.0 Hz, 4H), 7.91 (d, *J* = 8.0 Hz, 2H)], Imidazole-H [8.66 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.36 (–CH_2), 4F-Phenyl-C [116.36 (CH), 132.17 (CH), 164.66 (CH), 167.15 (C)], Imidazole-Phenyl-C [125.58 (CH), 126.90 (CH), 127.36 (CH), 127.87 (CH), 129.40 (CH), 133.33 (C), 139.34 (C), 140.15 (C)], Imidazole-C [111.17 (CH), 145.21 (C)], Thiadiazole-C [144.94 (C), 160.14 (C)], 191.78 (C=O); IR (ATR, cm^{-1}): 3137 (Ar–CH), 2932 (Aliph. CH), 1674 (C=O), 1596 (C=N); MS: *m/z* 445.73 (M^+, 100); *Anal.* Calcd. for $C_{24}H_{16}FN_3OS_2$: C 64.70, H 3.62, N 9.43, found: C 64.59, H 3.58, N 9.50.

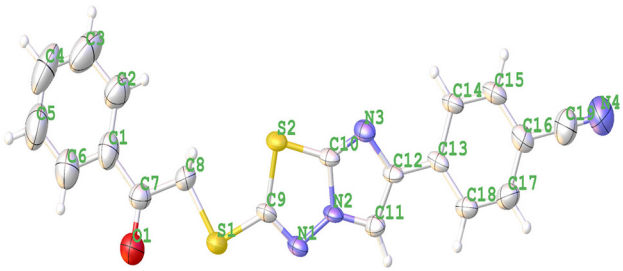


Figure 1. The crystal structure of compound **10**.

1-(4-Fluorophenyl)-2-((6-(4-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)ethanone (28)

Yellow solid, yield 1.19 g (72%); mp: 229–230 °C (from DMF-EtOH, 1:1); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.13 (s, 2H, –CH_2), 4F-Phenyl-H [7.41 (t, *J* = 12.0 Hz, 2H), 8.14 (t, *J* = 12.0 Hz, 2H)], Imidazole-Phenyl-H [8.07 (d, *J* = 12.0 Hz, 2H), 8.24 (d, *J* = 12.0 Hz, 2H))], Imidazole-H [8.87 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.36 (–CH_2), 4F-Phenyl-C [116.57 (CH), 131.28 (CH), 163.89 (CH), 164.14 (C)], Imidazole-Phenyl-C [125.73 (CH), 126.94 (CH), 139.11 (C), 141.27 (C)], Imidazole-C [110.61 (CH), 145.37 (C)], Thiadiazole-C [144.48 (C), 161.47 (C)], 195.74 (C=O); IR (ATR, cm^{-1}): 3115 (Ar–CH), 2986 (Aliph. CH), 1679 (C=O), 1596 (C=N), 1505, 1336 (–NO_2); MS: *m/z* 432.85 ($M + H_2O$, 100); *Anal.* Calcd. for $C_{18}H_{11}FN_4O_3S_2$: C 52.17, H 2.68, N 13.52, found: C 52.22, H 32.62, N 13.48.

2.4 Determination of *in vitro* Antimicrobial Activity and Minimal Inhibition Concentration (MIC)

2.4.1 The Screening of Antimicrobial Activity

Antimicrobial activities of all compounds were screened by agar well method. The test was performed on Mueller

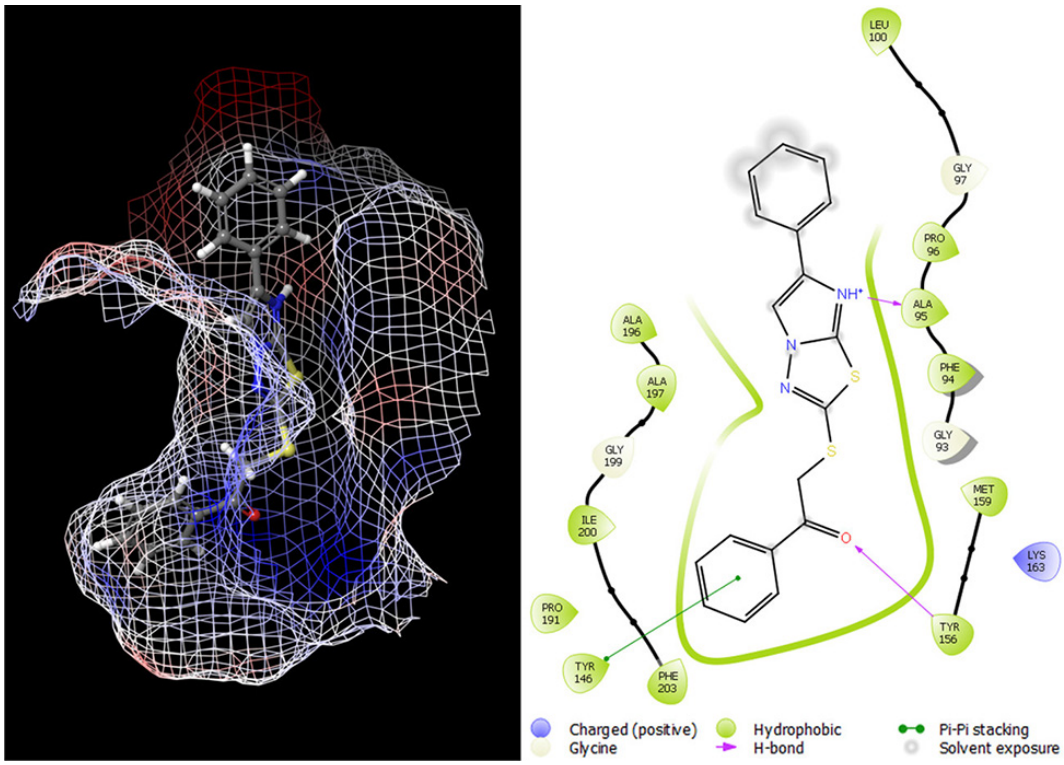


Figure 2. Docked pose of compound **5** in the active site of the enoyl-ACP reductase mapped with electrostatic potential (left) and 2D ligand interaction diagram (right).

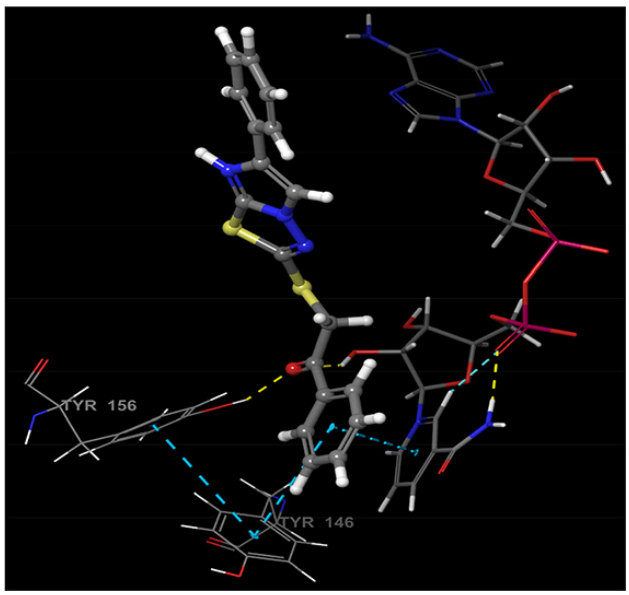


Figure 3. Binding interaction of compound **5** with the amino acid residues of the enoyl-ACP reductase and NAD+.

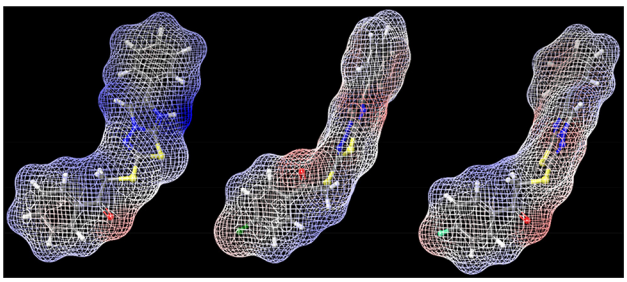


Figure 4. Electrostatic potential map surfaces of the compounds **5** (left), **13** (middle) and **21** (right).

Hinton Agar (MHA).[56] Agar surface were digged on wells. Fresh cultures of *E. coli* ATCC 8739, *S. aureus* ATCC 29213, *B. subtilis* ATCC 6633 and *C. albicans* ATCC 10231 were prepared in Mueller Hinton Broth. Fresh bacterial culture was adjusted to 0.5 McFarland [about 10^8 cfu (colony forming unit)/ml] in 0,85 % (w/v) sterile phosphate buffer saline (PBS) by using nephelometer (Crystal Spec™, Becton Dickenson, USA). 100 µl of each 0.5 McFarland bacterial suspension were streaked on MHA via sterile swab. Then, 20 mg of all compounds were dissolved in 1 ml Dimethyl Sulfoxide (DMSO) separately. The wells on agar plate were filled with 100 µl of these compounds in DMSO. A Petri dish was used for 4 compounds. Antimicrobial screening test were performed duplicate for each sample. DMSO was used as negative control, Chloramphenicol disk (30 µg) (HIMEDIA) for bacteria and Nystatin (10000 u/ml) for fungi were used as positive controls. Inoculated Petri dishes were incubated

at 37 °C for 24 h. After 24 h incubation, clear zones around the wells were measured and recorded as mm.

2.4.2 Minimum Inhibitory Concentration (MIC)

According to antimicrobial activity screening test results, compounds that have the most activity value against all microorganisms were selected. The MIC values of selected compounds were screened by 96 well plates in Mueller Hinton Broth (MHB) using the twofold serial dilution technique.[57,58] Dilutions of all compounds were prepared from 256 µg/ml to 0.03 µg/ml with 14 serial dilutions in 96 well plates with MHB. All fresh bacteria culture in Mueller Hinton broth was centrifuged at 4500 g for 10 min and bacteria pellets were adjusted to 0.5 McFarland using sterile PBS (pH 7.4). 100 µl from each bacterial suspension were inoculated in plate wells, which included diluted compounds. All plates were incubated on 37 °C for 24 h. Their absorbance (OD_{600}) was evaluated by micro plaque reader (BMG LABTECH, FLUOstar Omega, USA). Chloramphenicol was used as positive control. Fresh bacteria culture in MHB was used as negative control. The results which have half of negative control's absorbance value were recorded as MIC value (µg/ml). All assays for every dilution of MIC were carried out duplicate.

3 Results and Discussion

3.1 Chemistry

In this study, as the first step for the target compounds, 2-amino-1,3,4-thiadiazole derivatives **(3 a–c)** were synthesized by reacting 5-amino-1,3,4-thiadiazole-2-thiol **(1)** with α-bromoacetophenone derivatives **(2 a–c)** via dehydrobromination mechanism, in the presence of ethanolic potassium hydroxide in high yields (84-81 %). These compounds were obtained in analogy to the literature[55] but in higher yields. The synthesized compounds **3 a–c** were then converted to the target compounds **5–28** by reacting with α-bromoacetophenone derivatives **(4 a–h)** in absolute alcohol with moderate to good (55–77 %) yields. The synthetic method employed to synthesize these compounds is given in Scheme 1.

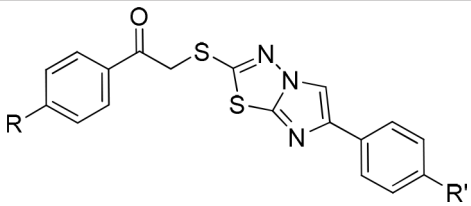
The structures of the synthesized compounds were elucidated based on physical constants, spectral data (FTIR, ^{1}H NMR, ^{13}C NMR, X-ray (compound **10**) and MASS) and elemental analysis. ^{1}H NMR and ^{13}C NMR spectra of all synthesized compounds and X-ray data of compound **10** are provided in the Supplementary Material Section. The crystal structure of compound **10** is shown in Figure 1.

In general, the characteristic singlet for $-CH_2$ methylene proton was observed in ^{1}H NMR spectra of compounds **3 a–c** around δ 4.76-4.79 ppm, which confirmed the reaction between 5-amino-1,3,4-thiadiazole-2-thiol **(1)** and α-bromoketones **(2 a–c)**. In ^{13}C NMR spectra, the resonance values related to C-2 and C-5 carbons were in consistent with similar compounds found in the literature.[59–61]

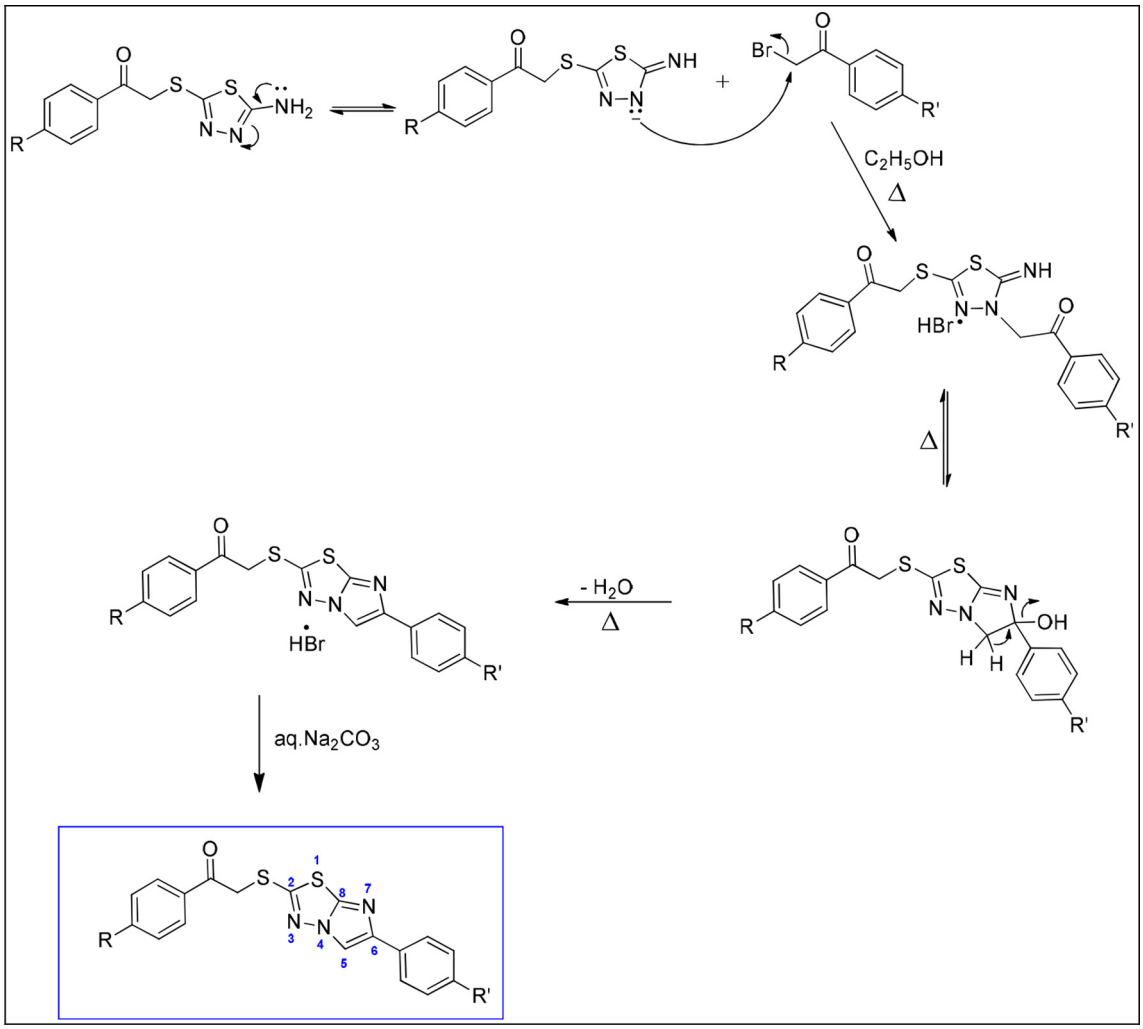
The most significant evidence of formation of the target compounds **(5-28)** in the IR spectra is the disappearance of $-NH_2$ symmetric and asymmetric absorption bands, which were observed in the 3263–3074 cm^{-1} range, in 2-amino-1,3,4-thiadiazole derivatives **(3 a–c)**. Furthermore, $-NH_2$ proton signals of the starting compounds observed in the δ 7.27–7.32 ppm range **(3 a–c)** were disappeared in the ^{1}H NMR spectra and instead, a singlet peak corresponding to one proton of the C5-H was observed in the δ 8.46–8.88 ppm. This clearly proves the formation of compounds **5–28**. These values are also similar to those given in relevant studies in the literature.[13,35] The formation mechanism[34] of target compounds is illustrated in Scheme 2.

Other ^{1}H NMR and ^{13}C NMR spectral data of the compounds are given in detail in the experimental section. The physical and structural properties of the target compounds **(5-28)** are given in Table 1. In addition, mass spectra of all synthesized compounds were also found as

Table 1. Physical and structural properties of target compounds **(5–28)**.



Compound	R	R′	Yield (%)	m.p (°C)
5	–H	–H	66	158–160
6	–H	–Br	55	185–186
7	–H	–Cl	62	180–181
8	–H	–F	67	164–165
9	–H	$-OCH_3$	74	138–139
10	–H	–CN	67	210–211
11	–H	–Ph	68	185–187
12	–H	$-NO_2$	77	231–233
13	–Cl	–H	73	154–156
14	–Cl	–Br	64	215–217
15	–Cl	–Cl	68	216–218
16	–Cl	–F	66	188–190
17	–Cl	$-OCH_3$	75	184–185
18	–Cl	–CN	72	235–236
19	–Cl	–Ph	71	215–217
20	–Cl	$-NO_2$	73	238–240
21	–F	–H	65	183-184
22	–F	–Br	71	198–199
23	–F	–Cl	59	197–199
24	–F	–F	69	183–184
25	–F	$-OCH_3$	69	161–162
26	–F	–CN	63	211–212
27	–F	–Ph	72	185–186
28	–F	$-NO_2$	72	229–230



Scheme 2. Mechanism for the synthesis of target compounds **(5–28)**.

expected and the molecular ion peaks supported the structures.

3.2 Computational Studies

Computational (*in silico*) methods are without doubt rapidly advancing and proved to be essential tools that are widely used for the design of novel enzyme inhibitors.

As an initial step, we have calculated some molecular descriptors commonly used in absorption, distribution, metabolism and excretion (ADME) analysis (Table 2).

Accordingly, molecular weight (MW), percent human oral absorption, predicted octanol/water partition coefficient (QPlogPo/w), polar surface area (PSA) and number of violations of Lipinski's rule of five[62] were estimated. Highest molecular orbital (HOMO) energies were also calculated to evaluate the effect of electronic structure on the activity.

The results show that most of the active compounds obey Lipinski's rule of five, which is an indication of the drug-likeness of a molecule, and PSA values are within the range that Veber et al. suggested.[63] According to the Table 2, electron-donating substituents are clearly one of the factors that diminishes activity. As an example, the least active compound **9** that has a powerful electron-donating group (–OCH_3) has highest HOMO value.

In the current study, molecular docking simulations were also employed to gain insight into the major binding motifs of the ligands. One of the main aim of the molecular docking is to map the ligand/target interactions to assist the rational drug design. The commonly used docking software available at the market are able to accurately and reliably predict the binding pose of the ligands in many cases, however, the scoring functions have a lower success rate in discriminating between active and inactive compounds.[15,64,65] Docking scores of the compounds **5–28** together with that of the reference compound, Chloram-

Table 2. Docking scores and selected molecular properties of compounds **5**–**28** and Chloramphenicol (reference drug).

Compound	Dscore (kcal/mol)	MW[a] (g/mol)	%Human Oral Absorption[b]	EHOMO[c] (eV)	PSA[d] Å^2	QPlogPo/w[e]	Rule of Five
5	−8.01	351.44	100.00	−8.98	55.94	4.52	+
28	−7.74	414.43	90.38	−9.55	102.04	3.86	+
10	−7.73	376.45	93.82	−9.38	82.59	3.69	+
27	−7.51	445.53	100.00	−8.79	55.89	6.26	−
21	−7.46	369.43	100.00	−9.04	57.31	4.60	+
8	−7.43	369.43	100.00	−9.25	56.84	4.78	+
6	−7.36	430.34	100.00	−9.16	58.09	4.94	+
12	−7.34	396.44	89.79	−9.58	102.60	3.88	+
9	−7.33	381.47	100.00	−8.64	65.78	4.46	+
15	−7.28	420.33	100.00	−9.44	59.68	5.46	−
11	−6.84	427.54	100.00	−8.77	56.04	6.15	−
24	−6.78	387.42	100.00	−9.23	58.84	4.87	+
7	−6.37	385.89	100.00	−9.05	57.84	4.87	+
26	−6.20	394.44	92.82	−9.38	83.76	3.67	+
25	−6.16	399.46	100.00	−8.70	66.55	4.57	+
22	−6.08	448.33	100.00	−9.19	58.19	5.10	−
Chloramphenicol	−6.05	323.13	65.67	−10.76	121.13	1.14	+
23	−6.04	403.88	100.00	−9.17	58.29	5.02	−
13	−5.93	385.89	100.00	−9.04	57.85	4.78	+
17	−5.90	415.91	100.00	−8.69	66.48	4.83	+
18	−5.88	410.90	93.30	−9.44	84.16	3.92	+
20	−5.84	430.88	91.64	−9.51	102.29	4.10	+
16	−5.72	403.88	100.00	−9.04	53.26	5.33	−
14	−5.57	464.78	100.00	−9.21	58.10	5.36	−
19	−3.46	461.98	100.00	−8.69	52.51	6.63	−

[a] Molecular weight (recommended value <500). [b] Percentage of human oral absorption ($<25\%$ is weak and $>80\%$ is strong). [c] Calculated at Semi Empirical PM6 method with Spartan '16 parallel suite. [d] Polar surface area (recommended value ≤ 140 Å^2).[62] [e] Logarithm of the partition coefficient of the compound between n-octanol and water (recommended value <5)

phenicol are given in Table 2. Glide XP docking performed well in ranking the active compound **5**, which has the highest inhibition zone and lowest MIC value. Another active compound **28** ranked second at the top.

Molecular modeling of the binding of the imidazo[2,1-*b*][1,3,4]thiadiazole derivatives on enoyl-acyl carrier protein reductase, the molecular target site of several antibacterial agents like triclosan or substituted imidazoles,[66–68] indicated that these compounds fit within the binding pocket occupied by triclosan. The binding pocket of the target receptor can be divided into two main regions: the binding-site entrance, which is lined with both hydrophobic and hydrophilic groups, and a deep cavity, which is mainly hydrophobic. The active compound **5,** docked into the active site mapped with electrostatic potential, and 2D ligand interaction diagram are depicted in Figure 2. Red color reveals lowest potential (electron-rich) while blue color indicates highest potential (electron poor) regions. Favorable interactions between compound **5** and the target can be observed in the Figure 2. Negative potential of carbonyl oxygen interacts with the high potential region of the receptor (blue region), and hydrogens attached to the rings are oriented towards the negative potential regions of the receptor.

The aromatic ring next to the carbonyl of compound **5** is nested into hydrophobic pocket located at the bottom of the deep cavity. It stacks parallel to the nicotinamide cofactor (NAD+) and almost perpendicular to the TYR146 making π-π stacking interaction with NAD+ and a T shaped π-π interaction with TYR146 (Figure 3). The carbonyl acts as a hydrogen bond acceptor and it makes a hydrogen bond with the Tyrosine 156 and NAD+.

We believe that those overall favorable interactions account for the high activity of compound **5**.

3.3 *In vitro* Antimicrobial Activity and SAR (Structure-activity Relationship) Studies

The *in vitro* antimicrobial activity of the synthesized compounds (**5**-**28**) against Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, Gram-negative bacterium *Escherichia coli* and a fungal species *Candida albicans* was tested, and the inhibition zones are given in Table 3. The compounds **5**, **6**, **7**, **8**, **15**, **16**, **17**, **20**, **27** and **28** showed promising activities against all bacteria species (*S. aureus, B. subtilis, E. coli*) when the inhibition zone diameters are compared to that of the reference drug Chloramphenicol

Table 3. Antimicrobial activity test results, inhibition zones of target compounds.

Compound	Inhibition zones diameter (mm) *S. aureus* ATCC 29213	*B. subtilis* ATCC 6633	*E. coli* ATCC 8739	*C. albicans** ATCC 10231
5	40	36	35	8
6	38	34	35	7
7	34	34	32.5	7
8	40	32	33	9
9	24	22	31	7
10	30	26	27	10
11	30	24	24	11
12	40	34	29	10
13	30	26	26	11
14	38	26	31	6
15	40	34	30.8	6
16	40	30	36	12
17	36	34	31	12
18	30	28	27	11
19	28	22	24	11
20	38	32	30	12
21	24	24	23	11
22	36	30	30	11
23	36	30	36	11
24	30	30	24	14
25	20	20	30	12
26	26	27	30	13
27	38	30	30	11
28	40	32	30	14
DMSO (Negative control)	–	–	–	–
Chloramphenicol	30	25	27	–
Nystatin	–	–	–	30

* *C. albicans* has high resistance to all compounds, according to zone diameter of positive control nystatin.

(30 µg – C[30]). However, according to the comparison between the inhibition zone diameters of the positive control Nystatin and the synthesized compounds, no remarkable antifungal activity was observed against *C. albicans*, (Table 3).

Minimal inhibition concentration (MIC) values of the compounds **5**, **6**, **7**, **8**, **15**, **16**, **17**, **20**, **27** and **28** were determined and are given in Table 4. All compounds showed noteworthy inhibition zone diameters against all bacteria species.

The MIC values tabulated in Table 4 shows that almost all of the selected compounds were found to be more potent than the reference drug Chloramphenicol against *S. aureus* and *B. subtilis*. Gram-negative bacterium *Escherichia coli* seems to be more resistant to the synthesized compounds. Gram-negative bacteria have an additional outer membrane (lipopolysaccharide) which initially protects the bacteria. This behavior was observed in different studies found in the literature.[69–72] Hence, the presence of this outer membrane as a permeation barrier could be the key reason for the diminished potency in *Escherichia coli*.

A direct correlation of the substituents' effect on the activities of the synthesized compounds could not be seen at a first glimpse. At this point, we referred to the computational calculations. A small change in a compound might have a dramatic effect on the activity. Hence, in discussing SAR, it would be more meaningful to compare and focus on the compounds on which only one parameter has been changed. All the synthesized compounds adopted same orientations as compound **5** when docked into the active site of the target: the aryl keto group is buried deeply inside the hydrophobic pocket while the other aryl moiety on which different R′ substituents were applied, interacts with the binding site entrance. Hence, one would think that increasing the hydrophobic character of the R should increase the activity. If we compare the compounds **5**, **13**, and **21** (R′: –H for all), although hydrophobicity increases

Table 4. Minimum Inhibitory Concentration (MIC) of selected compounds.

Compound	R	R′	Minimum Inhibitory Concentration in µg/ml (MIC) Gr positive coccus *S. aureus*	Gr positive rod (spore forming bacteria) *B. subtilis*	Gr negative rod *E. coli*
5	–H	–H	0.03	0.03	0.5
6	–H	–Br	0.03	0.03	1
7	–H	–Cl	0.06	0.06	1
8	–H	–F	0.03	0.03	1
9	–H	$-OCH_3$	64	64	>256
10	–H	–CN	2	1	32
15	–Cl	–Cl	0.03	0.03	1
16	–Cl	–F	0.03	0.03	1
17	–Cl	$-OCH_3$	0.25	0.25	4
20	–Cl	$-NO_2$	0.25	0.25	2
27	–F	–Ph	0.12	0.03	1
28	–F	$-NO_2$	0.03	0.03	2
Chloramphenicol			0.4	0.85	0.4

(Table 2) by replacing -H with –Cl or –F, respectively, activity decreases. As can be seen from Figure 3, there is a T shaped π-π interaction of TYR146 with the positive quadrupole of phenyl ring, proximal to the R. Inductive effect of the -F and -Cl increases the electron density in this area that weakens π-π interaction. This can clearly be seen in Figure 4 (Lower left part of each molecule). Compound **5** has higher potential (electron poor, more positive) where it interacts with the negative quadrupole of TYR146, than compounds **13** and **21**.

The effect of R′ on activity was also investigated. The compounds with less hindered groups on R′ were found to have potent activities against *S. aureus* and *B. subtilis* (**5**, **6**, **7** and **8; R:** –H for all). On the other hand, electron-donating groups were observed to be detrimental to the activity. For instance, keeping R as –H but applying a methoxy group on R′ (compound **9**) decreases activity by more than 1000-fold (Table 4). As stated before, this compound has highest HOMO energy value in consequence of this electron donating feature (Table 2). This behavior can also be clearly observed for the compounds **17** and **16** where R is –Cl for both. If the methoxy substituent on R′ of **17** is being replaced with a strong e-withdrawing group -F as in **16**, a ten-fold increase in activity is observed (Table 4).

Interestingly, the most active compound (**5**) against all bacteria species has no substituents as R or R′. Leaving hydrogen on both positions, removes a possible inductive electron withdrawing effect on R that in turn weakens π-π interaction with the amino acid residue of the target and also avoids a possible steric clash of R′ with the target.

4 Conclusion

In the current study, imidazo[2,1-*b*][1,3,4]thiadiazole derivatives were synthesized using simple reaction methodologies and evaluated for anti-bacterial activities against Gram-negative (*Escherichia coli)*, Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis) bacteria* and antifungal activities against *Candida albicans*. Their structures were elucidated by spectroscopic methods. Although none of the compounds showed a remarkable antifungal activity, most of the compounds were found to have potent anti-bacterial activities, some were with MIC values as low as 0.03 μg/ml, ten times more potent than positive control.

According to the electronic structure calculations, almost all active compounds obey the drug likeness properties. Molecular docking studies further supported inhibitory activities of the compounds and helped understanding the various interactions between the ligands and enzyme active sites.

While hydrophobicity was found to be an important factor in the aryl keto part of the compounds, electron withdrawing substituents on this region weakens a T shaped π-π interaction with the amino acid residue of the target that results in a decrease in the activity. On the other hand, electron donating substituents at the second aryl ring was observed to be detrimental to the activity. The results obtained from the experimental and computational studies in the present work provide insights in designing potential antibacterial drug candidates.

Conflict of Interest

None declared.

Acknowledgement

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