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CATALYST FREE SYNTHESIS OF *N*-(SUSBTITUTED-BUTYL)-6-(5-(4-SUBSTIUTED-PHENYL)FURAN/THIOPHENE-2-YL)-2-PHENYLIMIDAZO[2,1-*B*][1,3,4]THIADIAZOL-5-AMINE AND THEIR BIOLOGICAL AND DOCKING STUDIES

Ramasamudra Nagabhushana Sathish Kumar[a], Ramesh S. Bhat[b], Yariyuru B. Basavaraju[a*]

[a]*Department of Chemistry, Manasagangotri, University of Mysore, Mysuru, Karnataka, India-570006.*
[b]*Department of Chemistry, NMAM Institute of Technology, NITTE (Deemed to be University), Nitte -574110, India*
**Corresponding author: basavaraju_yb@yahoo.co.in*

ABSTRACT:
A concise and effective one-pot synthesis of thiadiazoles has been carried out using the three-component Groebke-Blackburn-Bienaymé (GBB) reaction, which uses isonitriles, aromatic aldehydes, and amines. The reactions took place in ultimate green conditions, devoid of catalysts and solvents, under microwave irradiation. Additionally, the protocol's "greenness" was assessed following green metrics, and the procedure demonstrated exceptional performance in terms of the specified criteria, including carbon efficiency, reaction mass efficiency, E-factor, atom economy, and process mass intensity. This ecologically friendly GBB process makes it simple to synthesize scaffolds with important pharmacological properties. Further, synthesized compounds were screened for antioxidant and anti-inflammatory activities. Molecular studies enlightened the interactions of the target with the synthesized compounds.

KEYWORDS Groebke-Blackburn-Bienaymé reaction, Microwave conditions, catalyst-free, biological activity

1. INTRODUCTION

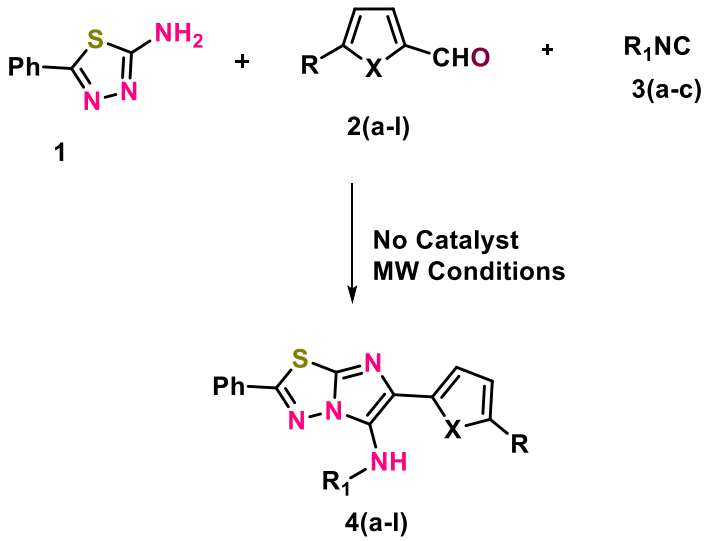
In the last few decades, fused heterocyclic structures have opened up a lot of possibilities for the domains of therapeutics and drug creation. [i], [ii] One such scaffold that is commonly visible in a variety of pharmacophoric actions, including antidiabetic,[iii] antitubercular,[iv] anticancer,[v] anti-Alzheimer's,[vi] DNA binding,[vii] anti-inflammatory,[viii] larvicidal,[ix] antiviral,[x] and antibacterial [xi] properties is the bicyclic core of thiadiazoles.
From a synthetic perspective, there are currently very few ways to synthesize thiadiazoles. The primary technique used in the literature to synthesize this core involved substituting 1,3,4-thiadiazol-2-amines and reacting them with various reagents, such as functionalized α-

halo ketones,[xii] α-haloacetic acid,[xiii] chloroacetyl chloride,[xiv] acetophenones,[xv] and *N*,*N*-dimethylformamide dimethyl acetal. These traditional methods have several drawbacks, such as the use of hazardous reagents, organic solvents, lengthy reaction times, laborious work-up procedures, and moderate yields. Furthermore, these approaches are not appropriate for a diversity-oriented thiadiazole scaffold synthesis. Microwave-Assisted Organic Synthesis (MAOS) has been more well-known over the last ten years, especially in the development of solvent- and catalyst-free sustainable versions of laborious processes (CFR and SFR). Furthermore, and this is a common outcome, these methods prevent time-consuming column chromatography by reducing impurities, sharply shortening reaction times, and forming relatively pure products. As a result, it offers a strong environmentally friendly substitute for traditional synthesis. In this situation, a viable method for synthesizing *N*-(substituted-butyl)-6-(5-(4 substituted-phenyl)furan/thiophene-2-yl)-2-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-amine using microwave-assisted multi-component Groebke-Blackburn-Bienaymé (GBB). By doing this, we anticipated reaping some of the advantages related to MAOS, as mentioned.

2 RESULTS AND DISCUSSION

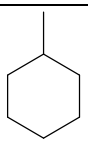
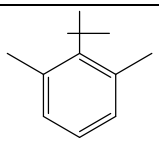
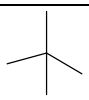
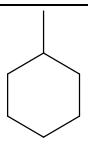
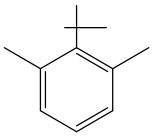
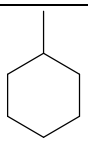
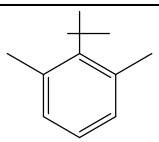
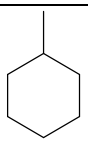
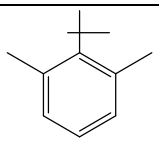
2.1 Chemistry

The synthetic strategy for the target amines is represented in **Scheme 1** and the possible mechanism is in **Scheme 2**. The structures of the synthesized amines are symbolized in **Fig. 1**. In an effort to create a catalyst-and solvent-free method for the synthesis of *N*-(thiadiazol-5-amine)[2,1-*b*][1,3,4]thiadiazol-6-(5-(4-substituted-phenyl)furan/thiophene-2-yl)-2 phenylimidazo[2,1-*b*]. Utilizing 5-phenyl-1,3,4-thiadiazol-2-amines (**1a**) (0.25 mmol), aldehyde (**2a**) (0.27 mmol), and *N*-tert-butyl isonitrile (**3a**) (0.30 mmol) as model substrates under microwave irradiation, we began our investigation with the optimization of the three component GBB reaction (**Table 1**).



Scheme 1: Synthesis of *N*-(substituted-butyl)-6-(5-(4-substituted-phenyl)furan/thiophene-2-yl)-2-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-amine. Initial conditions for the reaction included 30 minutes at a moderate 40 °C in a sealed vial without the presence of a catalyst or solvent (**Table 1**, entry 1).

Table 1. Characterization data of synthesized compounds

Comp. No.	**R**	**R_1**	**%Yield**	**X**	**Temperature (℃)**	**Time (Min)**
4a	4-Cl C_6H_4		90	O	40	10
4b	4-CH_3 C_6H_4		95	O	80	10
4c	3-Cl-C_6H_4		92	O	100	10
4d	4-Br-C_6H_4		85	O	120	10
4e	4-NO_2-C_6H_4		80	O	140	10
4f	4-OH-C_6H_4		75	O	160	5
4g	4-Cl C_6H_4		70	S	120	2
4h	4-CH_3 C_6H_4		65	S	120	10
4i	3-Cl-C_6H_4		60	S	40	10
4j	4-Br-C_6H_4		72	S	80	10
4k	4-NO_2-C_6H_4		70	S	100	10
4l	4-OH-C_6H_4		80	S	120	10

The beginning components were entirely unused, and the reaction never happened. However, increasing the temperature to 80 °C resulted in moderate yields of the intended product, with some unreacted starting material remaining. The reaction was then conducted at different substrates.

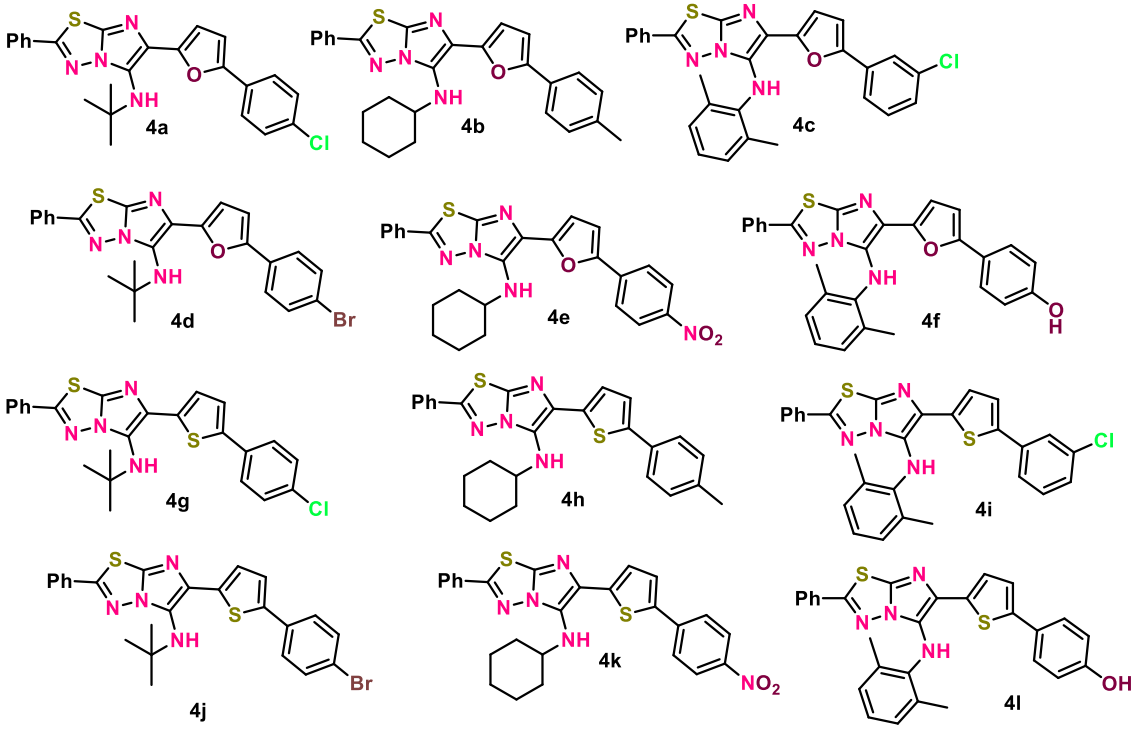


Figure 1. Structure of the newly prepared derivatives

Following confirmation of the reaction's viability, a variety of amines, aldehydes, and isocyanides were used to assess the breadth and resilience of this one-pot, three-component domino thiadiazoles synthesis (**Fig. 2.** and **Table 2**). We tried an amine reaction to examine how heteroatoms affected amine functionality and reaction efficiency, however, the process only produced starting material and failed to produce the desired result.

The amines' and aldehydes' electronic properties affected the effectiveness of the process. Under the right reaction conditions, it was discovered that a range of amines produced from benzoic acid were safe to use and had phenyl ring groups that were both electron-donating and electron-withdrawing. Amines with electron-donating groups produced high product yields. Conversely, yields were marginally reduced for groups that withdrew electrons from the amine functionality.

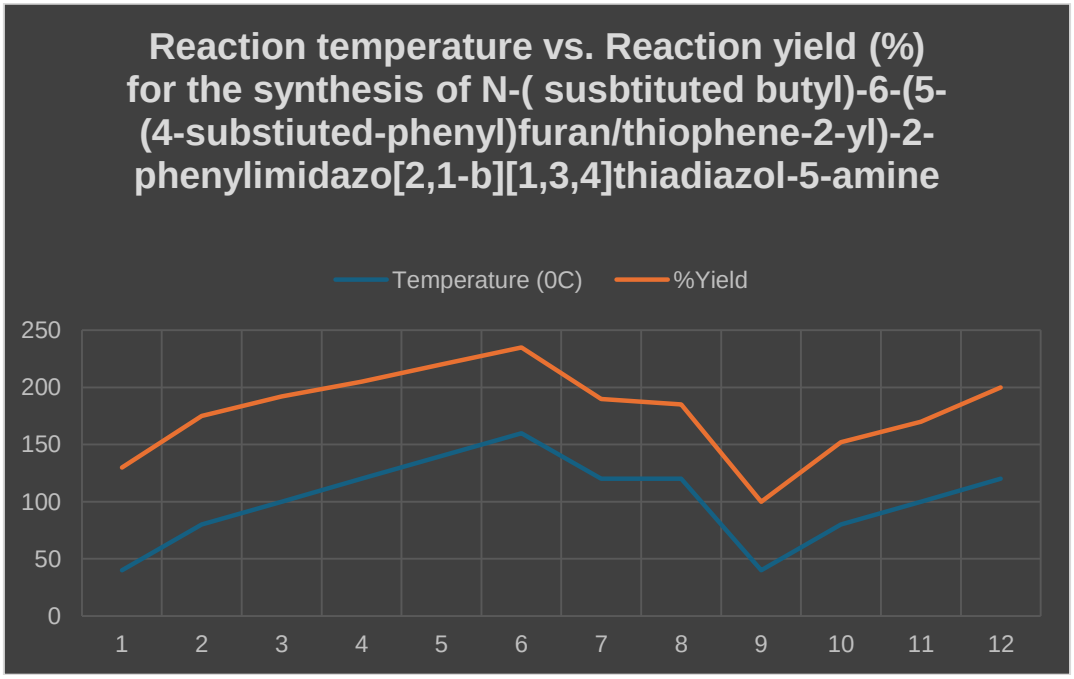
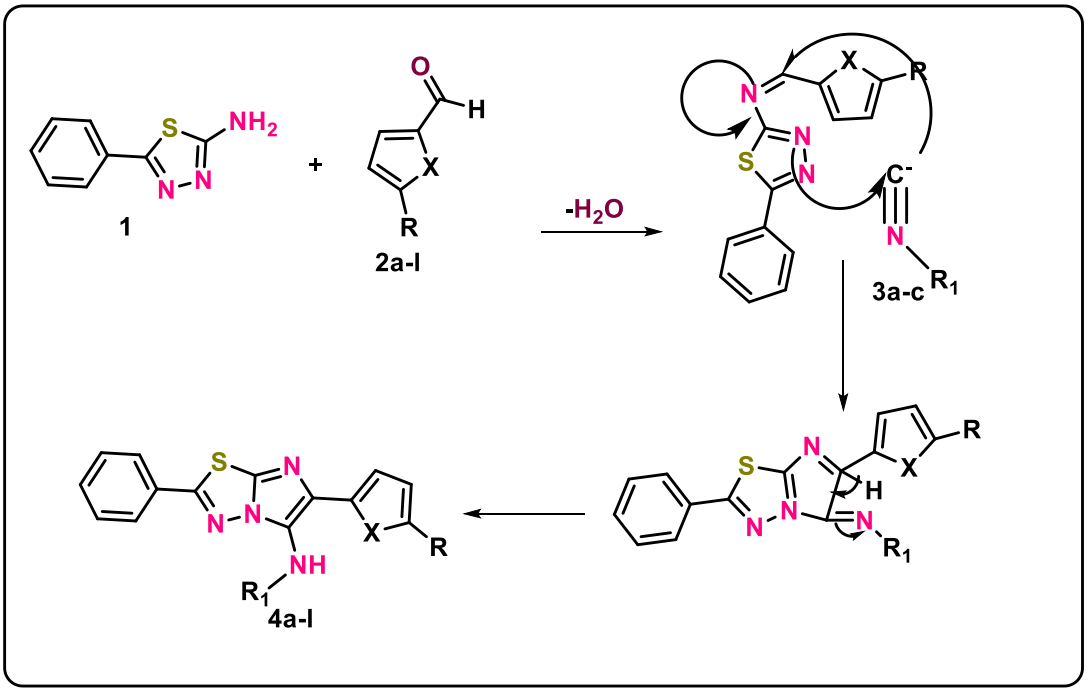


Fig. 2: Reaction temperature *vs.* Reaction yield (%) for the synthesis of *N*-(substituted-butyl)-6-(5-(4-substituted-phenyl)furan/thiophene-2-yl)-2-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-amine **4(a-l)**

Both electron-releasing as well as electron-withdrawing aromatic aldehydes were well-accommodated. It's important to note that, as predicted, aromatic aldehydes with an electron-withdrawing group produced good product yields. On the other hand, electron-donating substituents in aromatic aldehydes are also widely recognized. As such, the current methodology functions effectively in both deactivated and activated systems. It is important to note that deactivating systems with elements like nitro and fluoro also produced good yields without the need for additional catalysts or promoters, and in this case, they outperformed it.

In a similar way, the reaction has appeared to be very resistant to a variety of isocyanides, including aromatic (2,6-dimethylphenyl), aliphatic (*N*-tert-butyl), and alicyclic (cyclohexyl) isocyanides. Based on the findings, it appeared that the reaction between *N*-tert-butyl and 2,6-dimethylphenyl isocyanides was effective and smooth, leading to the crystallization of the pure products. Though in great yields, column chromatography was necessary for the isolation of the pure products in processes involving cyclohexyl isocyanide **4f**, **4i**, **4k**, and **4l**.



Scheme 2: Tentative reaction mechanism for the synthesis of *N*-(substituted butyl)-6-(5-(4-substituted-phenyl)furan/thiophene-2-yl)-2-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-amine

2.2 Biology

2.2.1. Antioxidant activity

Table 3 presents the results of the DPPH radical inhibition assay. The data shows that compound **4f** demonstrated a higher efficacy in inhibiting radical damage, with an inhibition rate of **11.28%**. This performance surpasses that of the standard antioxidant, ascorbic acid, which exhibited an inhibition rate of **20.25%**. This suggests that compound **4f** is more effective than DPPH radicals compared to ascorbic acid in this assay.

Table 3. Antioxidant activity measured by the synthesized amines' DPPH radical inhibition test

Compound	**Antioxidant activity of the synthesized compounds 4(a-l)**
	% of DPPH inhibition (IC_{50})
4a	19.25
4b	20.15
4c	33.58
4d	47.89
4e	62.18
4f	11.28
4g	78.95
4h	15.97
4i	63.78
4j	56.12
4k	88.52
4l	44.21
Ascorbic acid	20.25

2.2.2. Anti-inflammatory efficacy *in vitro* (using the denaturation of bovine serum albumin technique)

The percentage inhibition of the generated compounds was determined by utilizing a range of concentrations, specifically 20-100 µg/mL. Using the inhibition of protein denaturation (bovine albumin), diclofenac sodium was thought to be a reference drug to evaluate the *in vitro* anti-inflammatory efficacy of all the produced amine derivatives. **Table 4** provides an overview of the results obtained from determining the IC_{50} values. It was clear from the results that a number of synthesized substances had higher activity. The IC_{50} values of the synthesized compounds varied from 21.78 to 85.27 µg/mL because of the structural variations in the substrate. Not only were the replacements changed, but the compound's potency was also changed.

Table 4. Anti-inflammatory activity in terms of protein denaturation assay of the synthesized hydrazones

Compound	**IC_{50} value of the synthesized hydrazones**
4a	25.78
4b	23.12
4c	30.18
4d	44.87
4e	58.23
4f	21.78
4g	69.79
4h	85.27
4i	40.12
4j	74.59
4k	69.58
4l	74.57
Diclofenac sodium	39.23

2.2.3. *In vitro* antimicrobial activity:

Because of the substrate's structural changes, the minimal inhibitory concentration of the produced compounds displayed the greatest to lowest values. **Table 5** lists the antibacterial activity's minimal inhibitory concentration values.

Table 5. MIC values (µg/mL) of antibacterial evaluation of the titled compounds

Compound	***S. aureus***	***B. subtilis***	***E. coli***	***P. aeruginosa***
4a	2.44	3.22	2.02	4.12
4b	1.22	2.12	2.88	3.22
4c	3.24	4.16	6.46	5.22
4d	6.88	7.64	8.42	7.84
4e	7.64	5.66	6.12	6.44
4f	2.16	2.14	2.78	2.32
4g	4.48	3.44	**1.52**	1.30
4h	5.24	4.82	5.68	4.86
4i	8.88	8.86	8.96	8.94

4j	**1.20**	**1.82**	**1.52**	**1.42**
4k	7.68	6.42	6.44	7.22
4l	5.64	6.42	7.88	6.32
Tetracycline	0.68	0.66	1.66	1.66
Streptomycin	1.00	1.00	1.66	1.00

2.3. Molecular docking studies

To determine the potential binding mode of active compounds against the target protein, *E. coli* 24kDa domain in complex with clorobiocin, docking studies were meticulously conducted. The molecular docking analysis revealed promising binding energies for all the compounds, which spanned from -7.2 to -8.9 kcal/mol, as illustrated in **Table 6**. Notably, compound **4c** demonstrated a single hydrogen bonding interaction at the enzyme's active site, as depicted in **Figure 3**. This hydrogen bonding interaction originated from the oxygen atom of the furan ring group with the HIS23 amino acid residue. Furthermore, compound **4c** exhibited multiple interactions, including a carbon-hydrogen bond with the protein *via* the GLY19 residue, pi-cation interactions with GLU171 and HIS23, a pi-sigma interaction with THR20, a pi-sulfur interaction with HIS23, and a pi-pi T-shaped interaction with the PHE168 amino acid residue.

Table 6. The binding affinity of the synthesized compounds.

Code	Binding Affinity (kcal/mol)
4a	-7.5
4b	-8.1
4c	-8.9
4d	-7.4
4e	-7.9
4f	-8.3
4g	-7.2
4h	-7.8
4i	-7.8
4j	-7.2
4k	-7.8
4l	-7.7

In a similar way, compound **4f** displayed two hydrogen bonding interactions at the enzyme's active site, as shown in **Figure 4**. The first interaction was established between the oxygen atom of the furan ring group and the HIS23 amino acid residue, while the second interaction involved the secondary amine group and the GLU171 amino acid residue. Additionally, compound **4f** also formed a carbon-hydrogen bond with the HIS24 residue, engaged in pi-cation interactions with GLU171 and HIS23, exhibited a pi-sulfur interaction with HIS23, and a pi-pi T-shaped interaction with the PHE168 amino acid residue. The hydrophobic interactions around the molecule **4c** and **4f** at the active site of E. *coli* are depicted in **Figure 5**.

These interactions highlight the potential binding modes and affinities of the compounds, suggesting their capability to effectively engage with the target protein. The detailed insights into hydrogen bonding and other non-covalent interactions provide a comprehensive understanding of the binding mechanisms, paving the way for further optimization and development of these compounds as potential therapeutic agents.

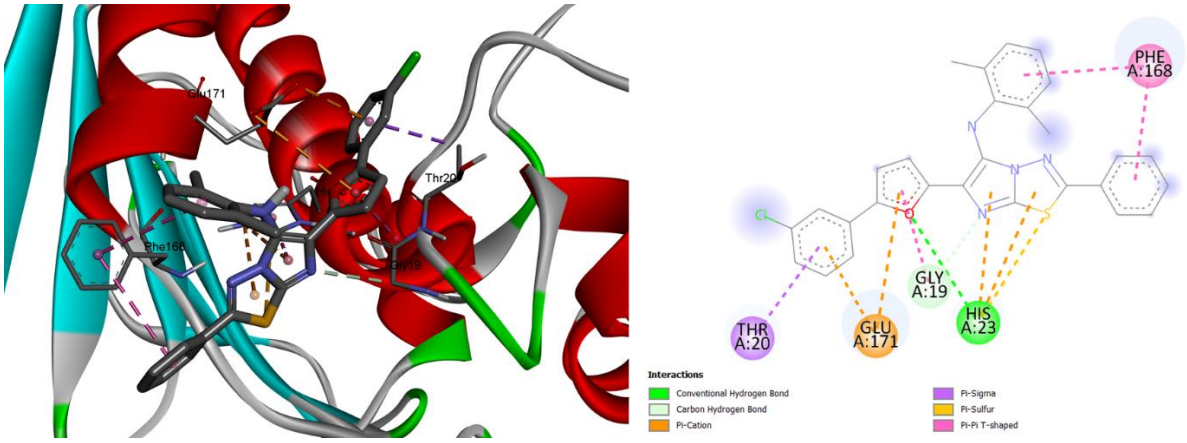


Figure 3. The crystal structure of *E. coli* 24kDa domain in complex with clorobiocin (PDB:1KZN) with active compound **4c.**

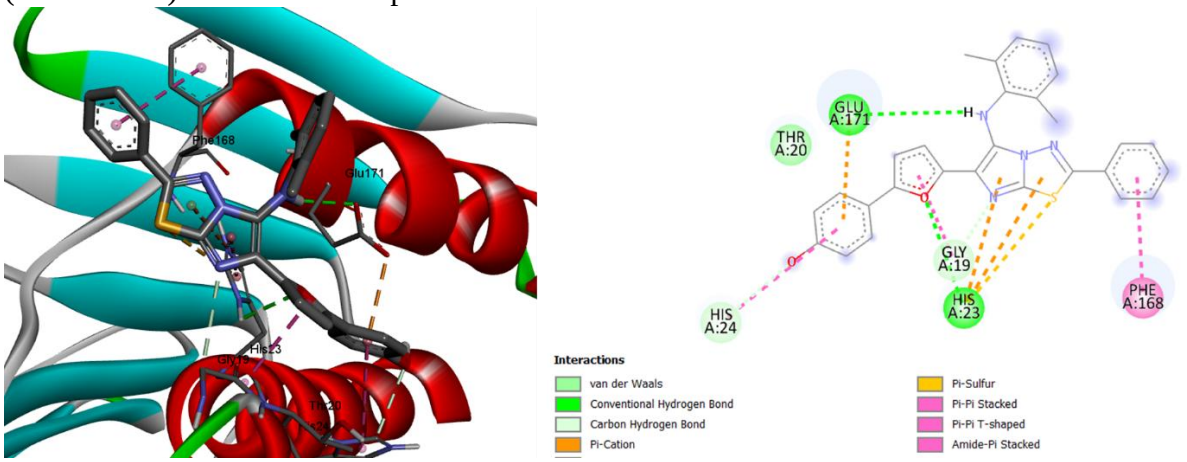


Figure 4. The crystal structure of *E. coli* 24kDa domain in complex with clorobiocin (PDB:1KZN) with active compound **4f.**

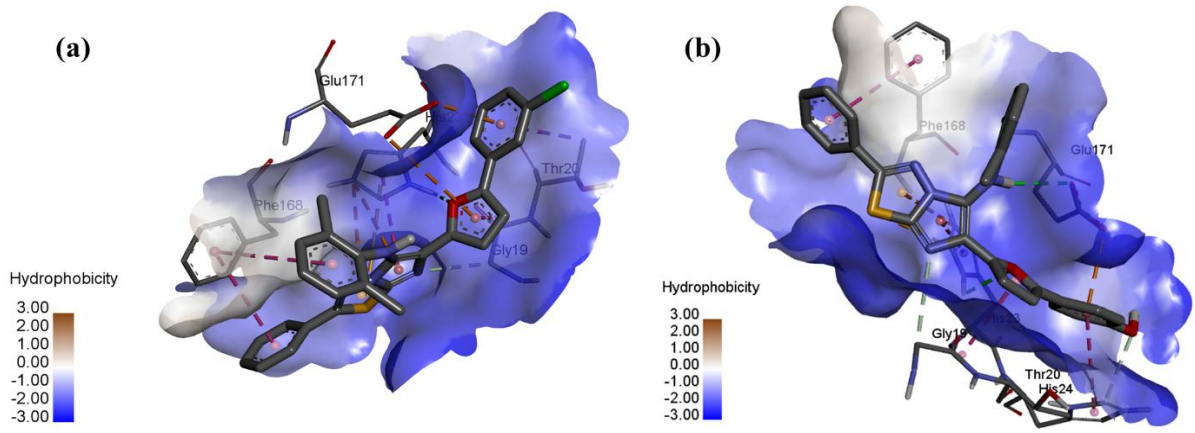


Figure 5. Hydrophobic interactions around the molecule (a) **4c** and (b) **4f** at the active site of E. *coli* 24kDa domain in complex with clorobiocin (PDB:1KZN)

3 EXPERIMENTAL

3.1 Chemistry

3.1.1 General

The recently synthesized amine compounds' melting points were measured using an open capillary approach, and they are uncorrected. On a Shimadzu FTIR spectrophotometer, the IR

spectra in the KBr pellet were recorded. Using DMSO-*d*6 as the solvent and TMS as the internal standard, the ^{1}H NMR spectra were captured using a JEOL 400 MHz NMR spectrometer. Proton signals were denoted as s = singlet, d = doublet, t = triplet, and m = multiplet. All chemical shift values are expressed in eight scales downfield from TMS. A Micro mass Q-Tof Micro LC mass spectrometer was used to record the mass spectra. The Vario-El Elementar-III model analyzer was used to do elemental analysis. Using silica gel plates (MERCK) and petroleum ether:ethyl acetate (1:1) as the mobile phase, TLC was used to confirm the purity of the compounds.

3.1.2. General Procedure for Synthesis of *N*-(substituted-butyl)-6-(5-(4-substituted-phenyl)furan/thiophene-2-yl)-2-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-amine (**4a-l)**

In a G-10 glass vial with a Teflon septum sealed, 5-aryl-1,3,4-thiadiazol-2-amines **1** (0.25 mmol), aromatic aldehyde **2(a-l)** (0.27 mmol), and isocyanide **3(a-c)** (0.3 mmol) were added. The vial was exposed to microwave irradiation with an initial ramp duration of one minute at 60 °C following a pre-stirring period of one or two minutes at room temperature. After that, the temperature was raised to 120 °C and held for five minutes. EtOH was used to recrystallize the products. Using ethyl acetate/hexane silica gel column chromatography, some of the viscous products were purified. All compounds were thoroughly characterized using mass spectrum data, NMR, and IR data.

Spectral data of synthesized compounds

***N*-(Tert-butyl)-6-(5-(4-chlorophenyl)furan-2-yl)-2-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-amine (4a)**

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (dd, J = 7.0, 3.6, 1.8 Hz, 2H), 7.73-7.63 (m, 4H), 7.50-7.44 (m, 2H), 7.21-7.16 (m, 1H), 6.60 (t, J = 2.7 Hz, 1H), 6.39 (t, J = 2.7 Hz, 1H), 5.07-5.04 (m, 1H), 1.35-1.20 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.7, 175.6, 159.2, 155.2, 155.2, 149.4, 133.7, 129.1, 128.7, 128.4, 127.8, 127.4, 126.7, 112.6, 107.9, 80.5, 56.60, 28.9. Mass (m/z): 448.28 (M^+). IR (KBr): 3522 (NH), 3057 (CH aromatic), 2222 (CN), 1690 (CO), 1645 (C=C), 1637 (C=N).

***N*-Cyclohexyl-2-phenyl-6-(5-(*p*-tolyl)furan-2-yl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-amine (4b)**

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (dd, J = 7.9, 1.5 Hz, 2H), 7.53-7.45 (m, 4H), 7.24 -7.17 (m, 3H), 6.60 (d, J = 3.4 Hz, 1H), 6.36 (d, J = 3.4 Hz, 1H), 5.08 (d, J = 8.1 Hz, 1H), 2.70 (dd, J = 13.0, 6.5 Hz, 1H), 2.27 (s, 3H), 1.57 (m, 4H), 1.50-1.36 (m, 4H), 0.91-0.90 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 175.7, 159.2, 155.2, 149.4, 141.5, 129.1, 128.4, 127.8, 126.9, 126.7, 112.6, 107.9, 80.5, 56.6, 33.4, 31.6, 21.3, 20.0. Mass (m/z): 454.22; IR(KBr): 3538 (NH), 3041 (CH aromatic), 2225 (CN), 1698 (CO), 1643 (C=C), 1641 (C=N).

6-(5-(3-Chlorophenyl)furan-2-yl)-*N*-(2,6-dimethylphenyl)-2-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-amine (4c)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24-8.21 (m, 2H), 7.72 (t, J = 1.2 Hz, 1H), 7.54 (m, 1H), 7.49 (d, J = 6.8 Hz, 1H), 7.28 (t, J = 7.7 Hz, 1H), 7.22-7.18 (m, 1H), 6.96-6.89 (m, 2H), 6.86-6.84 (m, 2H), 6.61 (s, 2H), 6.38 (d, J = 3.4 Hz, 1H), 5.04 (s, 1H), 2.29 (s, 3H), 2.12 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.7, 159.2, 155.2, 149.4, 137.2, 134.8, 129.2, 129.0, 128.4, 128.1, 128.0, 127.8, 127.4, 126.7, 125.7, 125.2, 112.6, 107.9, 80.5, 56.6, 21.3, 20.1. Mass (m/z): 497.19 IR (KBr): 3428 (NH), 3046 (CH aromatic), 2258 (CN), 1686 (CO), 1632 (C=C), 1626 (C=N).

6-(5-(4-Bromophenyl)furan-2-yl)-*N*-(tert-butyl)-2-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-amine (4d)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23-8.18 (m, 2H), 7.71 (d, *J* = 9.1 Hz, 2H), 7.61 (d, *J* = 9.1 Hz, 2H), 7.48 (s, 2H), 7.19 (dd, *J* = 7.7, 4.6, 1.4 Hz, 1H), 6.56 (d, *J* = 3.4 Hz, 1H), 6.39 (d, *J* = 3.4 Hz, 1H), 5.07 (d, *J* = 8.1 Hz, 1H), 1.29 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.7, 159.2, 155.2, 149.4, 131.7, 129.1, 128.4, 127.8, 126.7, 126.6, 122.3, 112.6, 107.9, 80.5, 56.6, 28.9. Mass (m/z): 492.45 (M^+), 494.35 (M^+2); IR (KBr): 3512 (NH), 3014 (CH aromatic), 2258 (CN), 1685 (CO).

_N_-Cyclohexyl-6-(5-(4-nitrophenyl)furan-2-yl)-2-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-amine (4e)

^{1}H NMR (400 MHz, $CDCl_3$) 8.30-8.09 (m, 2H), 7.77-7.73 (m, 2H), 7.59-7.56 (m, 2H), 7.50-7.43 (m, 2H), 7.19 (m, 1H), 6.51 (d, *J* = 3.6 Hz, 1H), 6.39 (d, *J* = 3.6 Hz, 1H), 5.04 (d, *J* = 8.1 Hz, 1H), 2.70-2.59 (m, 1H), 1.61-1.45 (m, 8H), 1.40-1.28 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.7, 159.2, 155.2, 149.4, 139.5, 129.1, 128.4, 127.8, 126.7, 126.0, 117.7, 112.6, 107.9, 80.5, 56.6, 32.9, 25.6, 24.8. Mass (m/z): 485.26; IR (KBr): 3472 (NH), 3063 (CH aromatic), 2231 (CN), 1697 (CO).

4-(5-(5-((2,6-Dimethylphenyl)amino)-2-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-6-yl)furan-2-yl)phenol (4f)

^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.23-8.18 (m, 2H), 7.79-7.71 (m, 2H), 7.57-7.53 (m, 1H), 7.48 (s, 1H), 7.19-7.17 (m, 2H), 6.92 (s, 2H), 6.78 (s, 2H), 6.51 (s, 2H), 6.38 (d, *J* = 3.5 Hz, 1H), 5.01 (s, 1H), 2.12 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.7, 159.2, 157.4, 155.2, 149.4, 137.2, 129.3, 128.8, 128.4, 128.1, 127.6, 126.7, 115.7, 112.6, 107.9, 80.5, 56.6, 20.1, 17.7; Mass (m/z): 478.23; IR (KBr): 3425 (NH), 3017 (CH aromatic), 2232 (CN), 1684 (CO).

_N_-(Tert-butyl)-6-(5-(4-chlorophenyl)thiophen-2-yl)-2-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-amine (4g)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (dd, *J* = 7.9, 1.5 Hz, 2H), 7.61 (d, *J* = 9.1 Hz, 2H), 7.53-7.45 (m, 4H), 7.21-7.16 (m, 2H), 7.08 (d, *J* = 8.8 Hz, 1H), 5.12 (d, *J* = 8.1 Hz, 1H), 1.29 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.7, 159.2, 151.1, 141.0, 134.3, 133.7, 128.7, 128.4, 127.8, 127.6, 127.0, 126.7, 124.0, 80.5, 56.6, 28.9; Mass (m/z): 465.03 (M^+), 467.15 (M^+2); IR (KBr): 3512 (NH), 3037 (CH aromatic), 2243 (CN), 1672 (CO).

_N_-Cyclohexyl-2-phenyl-6-(5-(*p*-tolyl)thiophen-2-yl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-amine (4h)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (m, 2H), 7.54-7.47 (m, 2H), 7.46-7.38 (m, 3H), 7.20-7.15 (m, 2H), 6.94 (dd, *J* = 11.7, 3.5 Hz, 2H), 5.14 (d, *J* = 8.1 Hz, 1H), 3.95 (m, 1H), 2.16 (s, 3H), 1.84-1.74 (m, 4H), 1.57-1.44 (m, 4H), 1.43-1.31 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3, 149.1, 143.9, 139.8, 137.7, 133.9, 132.4, 128.6, 128.1, 126.7, 124.4, 117.0, 52.9, 34.1, 30.9, 28.9, 25.3, 24.7; Mass (m/z): 470.47 (M^+); IR (KBr): 3435 (NH), 3061 (CH aromatic), 1686 (CO).

6-(5-(3-Chlorophenyl)thiophen-2-yl)-_N_-(2,6-dimethylphenyl)-2-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-amine (4i)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12-8.03 (m, 1H), 7.76-7.70 (m, 2H), 7.60 (t, *J* = 1.5 Hz, 1H), 7.56-7.48 (m, 3H), 7.37-7.31 (m, 2H), 7.00-6.98 (m, 3H), 6.90 (d, *J* = 3.5 Hz, 1H), 6.82-6.77 (m, 1H), 5.08 (d, *J* = 8.1 Hz, 1H), 2.17 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3, 149.1, 146.5, 143.9, 138.1, 137.7, 135.1, 133.9, 133.1, 132.4, 130.5, 129.8, 129.4, 128.6, 128.1, 127.0, 126.7, 125.2, 124.4, 124.2, 112.8, 107.9, 81.3, 58.2, 21.9, 19.4; Mass (m/z): 512.24 (M^+); IR (KBr): 3318 (NH), 3014 (CH aromatic), 1678 (CO).

6-(5-(4-Bromophenyl)thiophen-2-yl)-*N*-(tert-butyl)-2-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-amine (4j)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04-8.00 (m, 2H), 7.55-7.48 (m, 3H), 7.45-7.41 (m, 2H), 7.39-7.31 (m, 3H), 7.27 (d, *J* = 8.8 Hz, 1H), 5.06 (d, *J* = 8.1 Hz, 1H), 1.37 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3, 149.1, 146.5, 143.9, 137.7, 133.9, 133.7, 132.4, 131.9, 128.6, 128.1, 126.7, 124.4, 124.2, 122.0, 80.9, 52.9, 28.9; Mass (m/z): 508.36 (M^+), 510.42 (M+2); IR (KBr): 3478 (NH), 3049 (CH aromatic), 1685 (CO).

***N*-Cyclohexyl-6-(5-(4-nitrophenyl)thiophen-2-yl)-2-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-amine (4k)**

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14-7.89 (m, 2H), 7.60-7.54 (m, 2H), 7.51-7.44 (m, 3H), 6.83-6.49 (m, 4H), 5.08 (d, *J* = 8.1 Hz, 1H), 3.91 (m, 1H), 2.01-1.70 (m, 4H), 1.59-1.46 (m, 4H), 1.37 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3, 149.1, 147.6, 146.5, 143.9, 137.7, 133.9, 133.7, 132.4, 128.6, 128.1, 126.7, 124.4, 124.2, 123.8, 112.8, 107.5, 81.2, 56.3, 33.3, 25.3, 24.7; Mass (m/z): 501.43 (M^+); IR (KBr): 3438 (NH), 3024 (CH aromatic), 1696 (CO).

4-(5-(5-((2,6-Dimethylphenyl)amino)-2-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-6-yl)thiophen-2-yl)phenol (4l)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04-7.99 (m, 2H), 7.49-7.44 (m, 6H), 7.36 (d, *J* = 8.8 Hz, 1H), 7.27-7.16 (m, 4H), 6.99-6.93 (m, 2H), 5.04 (s, 1H), 2.19 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3, 157.2, 149.1, 146.5, 146.2, 143.9, 137.7, 133.9, 133.7, 132.4, 129.0, 128.6, 128.1, 128.0, 126.7, 124.4, 124.2, 115.5, 56.3, 28.1; Mass (m/z): 494.08; IR (KBr): 3426 (NH), 3018 (CH aromatic), 1675 (CO).

3.2. Biological activities

3.2.1. Antioxidant activity in terms of DPPH radical inhibition assay

The Blois method was used to examine hydrazone analogs' ability to scavenge free radicals. At various doses (20-100 µg/mL), a recently prepared DPPH solution (0.004% w/v) was added to the sample solutions in methanol. The solution was allowed to sit at room temperature for thirty minutes in the dark. Next, using a spectrometer, the mixture's absorbance at 517 nm was measured. Ascorbic acid was the drug used as a reference. Methanol served as the blank, and the same volume of DPPH was used to create the control sample in the absence of any test samples. The lower absorbance value of the reaction mixture indicates that it has a higher free radical scavenging activity. Every test was administered three times in duplicate.[xvi] Using the following formula, the fraction of the DPPH free radical that was scavenged was calculated:

$$\%Inhibition = \frac{(A_{control} - A_{test})}{A_{control}} \times 100$$

where A_{test} is the absorbance of the test sample and $A_{control}$ is the absorbance of the control reaction.

3.2.2. *In vitro* anti-inflammatory activity (denaturation of bovine serum albumin method)

The anti-inflammatory activity of the target compounds was measured using the denaturation of bovine serum albumin technique, adhering to the protocols outlined in the literature.[xvii], [xviii] In the test sample, the pH of the combination-which contained the test chemical and a 1% aqueous solution of bovine albumin fraction-was increased to 7.4. Furthermore, test samples were incubated for 20 minutes at 37 °C before being heated to 51 °C for 20 minutes. Using a UV-visible spectrophotometer, the sample's turbidity was measured at 660 nm after it had cooled to room temperature. Diclofenac sodium, the typical medicine used in the

investigation, was administered in triplicate.

Based on the percentage of inhibition of albumin denaturation, the anti-inflammatory activity of the substances listed was computed as follows:

$$\% \text{ Inhibition} = \frac{[\text{control absorbance} - \text{sample absorbance}]}{\text{control absorbance}} \times 100$$

3.2.3. Antimicrobial activity by macro dilution broth method:

Using the NCCLS macro dilution broth method, the target compounds' susceptibility to antibiotics was assessed. The Clinical and Laboratory Standards Institute (CLSI) standard was followed in evaluating the target drugs' minimal inhibitory concentration (MIC).[xix] "The minimum inhibitory concentration (MIC) is the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in the tubes or microdilution wells as detected by the unaided eye," according to the definition provided in the Clinical and Laboratory Standards Institute publication.[xx] Thus, the established protocol was adhered to. The synthesized compounds underwent antimicrobial evaluation at varying concentrations (0.125-128 μg/mL) against strains of bacteria, including *Bacillus subtilis* (*B. subtilis*) (MTCC 441), *Escherichia coli* (*E. coli*) (MTCC 443), and *Pseudomonas aeruginosa* (*P. aeruginosa*) (MTCC 424). Tetracycline was employed as a standard medication for the assessment of its antibacterial properties. The test microorganisms were introduced to a sterile Mueller Hinton Broth (MHB) medium for bacteria, which contained sterile test tubes containing the target chemicals. As a control, the MHB tube with and without target compounds was employed. When the target compound-containing tube exhibited no discernible growth of the test microorganism, the minimum inhibitory concentration (MIC) was found.

3.3. Molecular docking study

3.3.1. Protein preparation

The protein database (www.rcsb.org) provided the crystal structures of the *E. coli* 24 kDa domain in interaction with clorobiocin (PDB:1KZN).[xxi] To obtain clean protein, the inhibitors, additional ligands, and water molecules were removed from the protein prior to protein production. Prior to docking, polar hydrogen atoms bearing Gasteiger-Huckel charges were incorporated into the protein.[xxii] There were 62, 66, and 38 points in the middle of the protein (PDB ID: 3LN1) grid box, and 37.738, -26.122, and -6.412 Å were the number of points in the x, y, and z dimensions, respectively.

3.3.2. Ligand preparation

The synthesised molecules' 2D orientations were ascertained using the Marvin Sketch tool, and a minimization technique was then employed to transform them into the most energy-efficient 3D forms. Additionally, the assertions made by Gasteiger Using AutoDock 4.2, rotatable bonds and nonpolar hydrogen atoms were built. Vina was used to dock each molecule.[xxiii] The docking outcome as shown by the Discovery Studio program.[xxiv]

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Conflicts of Interest

There are no conflicts of interest to declare.

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