Journal of Research in Pharmacy

Research Article

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Design, synthesis and molecular docking studies of S-alkylated thiadiazole derivatives containing phthalimide moiety as antimicrobial agents

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Received: 29 November 2023 / Revised: 26 August 2024 / Accepted: 27 August 2024

ABSTRACT: Considering the incidence of infectious diseases and emergence of antibiotic-resistance bacterial and fungal strains, the discovery of new antimicrobial agents is essential. Thiadiazole as an useful pharmacophore is considered in drug discovery researches. Reported thiadiazole derivatives with antimicrobial properties indicate the importance of this scaffold as antimicrobial agent. Compounds bearing phthalimide moiety also have been identified as antimicrobial agents. The existing work was conducted to evaluate the antimicrobial property of new thiadiazole – phthalimide hybrid derivatives. Thiadiazole-phthalimide hybrid derivatives were prepared by the reaction of phthalic anhydride with amine group of various s-alkylated thiadiazole derivatives which followed by evaluation of antimicrobial properties using tube dilution technique against bacterial and fungal strains. The final molecules were docked in the active sites of *Escherichia coli* topoisomerase II DNA gyrase B to assay the possible interactions. Compound 3a was found to be active against *Staphylococcus aureus* , *Escherichia coli* and in particular *Candida albicans* with minimum inhibitory concentration values of 0.75, 0.18, 0.09 (mg/mL), respectively. None of the compounds had activity against *Pseudomonas aeruginosa* in studied concentrations. The highest docking score was -8.41kcal/mol for compound 3b which can confirm the experimental results.

KEYWORDS: Synthesis; Thiadiazole; Antimicrobial; Topoisomerase; Molecular Docking

1. INTRODUCTION

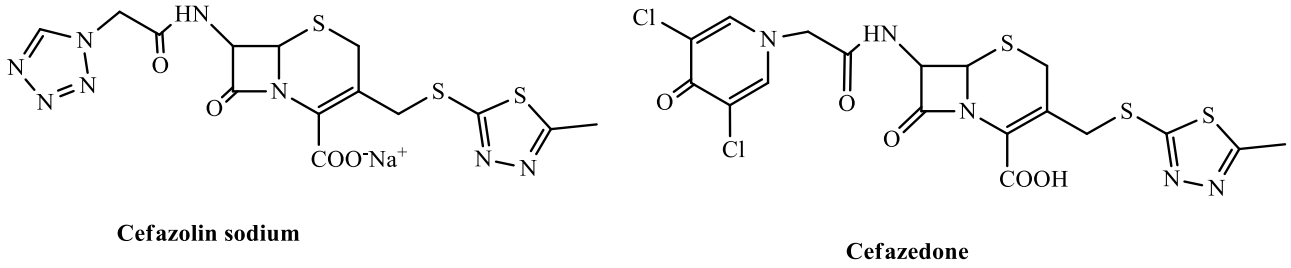
Despite the decline in mortality of microbial infections in the developed countries, these infections are still a challenge in the developing countries. Many opportunistic bacteria cause pernicious infections in people with defective immune systems. Today, antibiotics are obtained of many natural products, semi-synthetic and synthetic processes. Due to the wide side effects, and the increasing resistance of pathogenic microbes, there is a need to introduce new antimicrobial agents [1-5]. The compounds containing a heterocyclic ring especially azoles have always shown a special place in the treatment of microbial infections [2, 6-8]. Thiadiazole, (five-membered heterocycle) is considered as a pharmacophore in medicinal chemistry researches due to its wide range of biological properties [9]. Synthesized derivatives of thiadiazole have shown antimicrobial [10-15], antileishmania [16] and cytotoxic [17-20] effects in some reported studies. 2-Amino-thiadiazole derivatives have shown antimicrobial activity [2]. The presence of approved and commercial thiadiazole structure based -drugs with prominent antimicrobial properties [21] can confirm the antimicrobial properties of this pharmacophore (Figure1). In addition, phthalimide is an interested biological pharmacophore identified as antimicrobial, anti-inflammatory and anticancer agent. The structure of this cyclic imide is neutral and hydrophobic, which makes it easy to pass through biological membranes [22-24]. Many hybrid derivatives of thiadiazole including quinazolino-thiadiazole [25] and imidazo-thiadiazole [5], have shown significant antimicrobial effects. Derivatives containing thiadiazole-2-thioether have shown antifungal activity in previous reports [14]. In view of the high degree of bioactive thiadiazole and phthalimide derivatives in antimicrobial studies, the construction of a hybrid molecule based on 1, 3, 4-thiadiazole-thioether and

How to cite this article: Hassanzadeh F, Jafari E, Nicoobin M, Akbari V. Design, Synthesis, and Molecular Docking Studies of s-Alkylated Thiadiazole Derivatives Containing Phthalimide Moiety as Antimicrobial Agents. J Res Pharm. 2025; 29(4): 1573-1581.

ISSN: 2630-6344

https://doi.org/10.12991/ jrespharm.1734633

phthalimide was considered in this study to explore the additive effects towards their antimicrobial activities against fungal, and bacterial strains. The final compounds were docked into the binding sites of *Escherichia. coli* topoisomerase II DNA gyrase B and their binding energies were calculated. The purpose of choosing this enzyme (topoisomerase II DNA gyrase B) as target was the reported inhibitory mechanism of this enzyme by azole [26-29] or phthalimide [30] derivatives in previous studies.



Cefazolin sodium **Cefazedone**

Figure 1. Some approved drugs with thiadiazole scaffold

2. RESULTS and DISCUSSION

Nitrogen –based heterocyclic scaffolds are of absorbing compounds because of their various biological activities [6, 31, 32], especially antimicrobial properties [8]. Some of the reported phthalimide or thiadiazole based antimicrobial products have shown the importance of these scaffolds as antimicrobial agents [4, 5, 14]. In an experimental procedure, sulfhydryl group of thiadiazole was alkylated through the s-alkylation reactions using benzyl chloride or 2-bromo-1-phenylethanone derivatives in the presence of KOH (Figure 2).

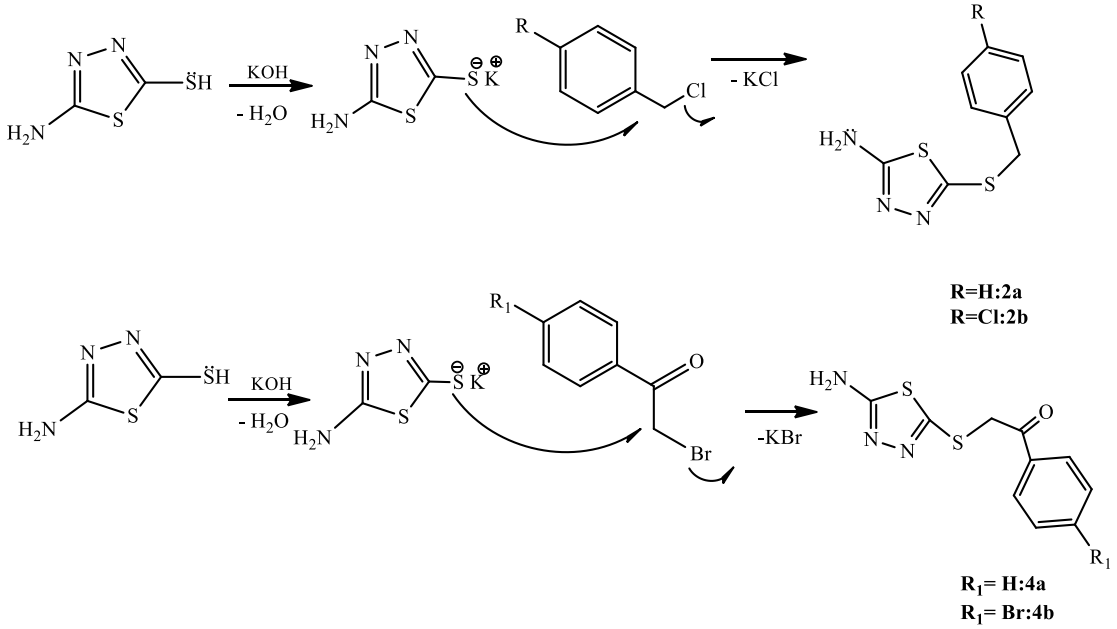
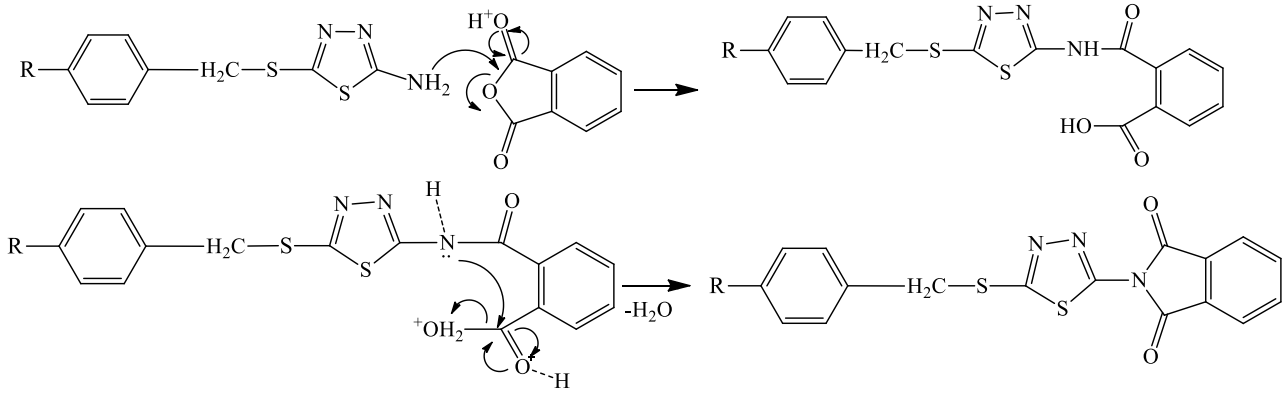


Figure 2. Mechanism for the synthesis of compounds **2a, 2b** and **4a, 4b.**

The reaction of obtained intermediates amine group with phthalic anhydride produced final products. Amine group of thiadiazole derivatives acts as a nucleophile and attacked to the carbonyl group of the phthalic anhydride molecule which resulted in the ring opening, subsequently the ione pair electron of nitrogen of the intermediate attacked to the carbonyl group of carboxylic acide which upon its dehydration produced final compounds (Figure 3). Final derivatives were confirmed based on spectral analysis. Methylene protons were observed as singlet in ^{1}H NMR spectra around δ 4.58 -4.99 ppm, which corroborated the reaction between 5-amino-1, 3, 4-thiadiazole-2-thiol (1) and benzyl chloride or 2-bromo-1-phenylethanone derivatives. The most remarkable confirmation in the IR spectra was the disappearance of-NH_2 absorption bands, which were observed in the 3263–3074 cm^{-1} range, in 2-amino-1,3,4-thiadiazole derivatives.



R=H, 3a
R=Cl, 3b

Figure 3. Proposed mechanism for the synthesis of final compounds.

2.1. Chemistry

2.1.1. 2-(5-Benzylthio)-1,3,4-thiadiazol-2-yl)-isoindole-1, 3-dione (3a)

Yield: 47 %; Pale yellow solid; M.p. 169-171°C; IR(ν_{max}) , 3052 (C-H, Ar), 1794 (C=O), 1734 (C=O) cm^{-1}; ^1H NMR: (400 MHz; $CDCl_3$): δ 8.06-8.03 ppm (2H, m, H-Phthalic), 7.90-7.88 ppm (2H, m, H-Phthalic), 7.47 ppm (2H, d, *J*=8Hz, H-Ar), 7.36-7.28 ppm (3H, m, H-Ar), 4.60 ppm (2H, s, CH_2); MS (m/z, %): 353 (M^+), for $C_{17}H_{11}N_3O_2S_2$, M.W. 353g/mol.

2.1.2. 2-[5-(4-Chloro-benzylthio) -1,3,4- thiadiazol-2-yl]-isoindole-1, 3-dion (3b)

Yield: 43%; Yellow solid; M.p. 161-164°C; IR(ν_{max}), 3128 (C-H, Ar), 2858 (C-H, aliphatic), 1787 (C=O), 1728 (C=O) cm^{-1}; ^{1}HNMR: (400 MHz; $CDCl_3$): δ 8.08-8.06 ppm (2H, m, H-Phthalic), 7.94-7.89 ppm (2H, m, H-Phthalic), 7.43 ppm (2H, d, *J*=8Hz, H-Ar), 7.31ppm (2H, d, *J*=8Hz, H-Ar), 4.58 ppm (2H, s, CH_2); MS (m/z, %): 387 (M^+), 389 (M+2) for $C_{17}H_{10}ClN_3O_2S_2$, M.W. 387g/mol.

2.1.3. 2-[5-(phenyl-ethanone thio)-1,3,4- thiadiazol-2-yl]-isoindole-1, 3-dione (5a)

Yield: 52%; Gray solid; M.p.180-183°C; IR(ν_{max}), 3060 (C-H, Ar), 2912 (C-H, aliphatic), 1792 (C=O), 1732 (C=O) cm^{-1}; ^{1}HNMR: (400 MHz; $CDCl_3$): δ 8.09-8.07 ppm (2H, m, H-Phthalic) , 8.05 ppm (2H, d, *J*=8HZ, H-Ar), 7.95-7.93 ppm (2H, m, H-Phthalic), 7.66 ppm (1H, t, *J*=8Hz, H-Ar), 7.54 ppm (2H, t, *J*=8Hz, H-Ar), 4.84 ppm (2H, s, CH_2), MS (m/z, %): 381 (M^+), for $C_{18}H_{11}N_3O_3S_2$, M.W. 381g/mol.

2.1.4. 2-[5-(4-Bromo-phenyl)ethanonethio)]-1,3,4- thiadiazol-2-yl}-isoindole-1, 3-dione (5b)

Yield: 65 %; Dark green solid; M.p. 170-173°C; IR(ν_{max}) 1792 (C=O), 1730 (C=O) cm^{-1}; ^1H NMR: (400 MHz; $CDCl_3$): δ 8.08-8.06 ppm (2H, m, H-Phthalic), 7.95 ppm (2H, d, *J*=8HZ, H-Ar), 7.93-7.90 ppm (2H, m, H-Phthalic), 7.68 ppm (2H, d, *J*=8Hz, H-Ar), 4.99 ppm (2H, s, CH_2), MS (m/z, %): 460 (M^+), 462 (M+2) for $C_{18}H_{10}BrN_3O_3S_2$, M.W. 460g/mol.

2.2. Biological evaluation

Final compounds were assayed for their *in vitro* antimicrobial properties and MIC values determined by tube dilution technique. The antimicrobial results are presented in Tables 1 and 2. Compounds (**3a, 3b**) bearing s-benzyle moiety were more effective than S-phenyl ethanone derivatives **(5a, 5b)** against studied strains. Replacing the substituent S-benzyl of the thiadiazole in compounds **3a, 3b** with s-phenyl ethanone (**5a, 5b**) lead to decrease in the antimicrobial activity to ›1 (mg/mL) (Table 1). In similar research performed by Rad et al, *S*-substituted 5-amino-2-mercapto-thiadiazoles revealed moderate activity against both bacterial strains, also moderate to good antifungal activity against *C. albicans* by agar diffusion technique [4]. In another study done byKaraburun et al S-phenyl ethanone derivatives of thiadiazole were synthesized. Compounds bearing 2, 4-dichlorophenyl or 2, 4-difluorophenyl possessed good activity against *C. albicans* [33]. *P. aeruginosa* seem to be more resistant. The characteristics and nature of substituents on either sulfhydryl group of thiadiazole and R have strong influence on activity of the compounds. Introduction of 4-chloro substitution on phenyl ring of S-benzyle moieties yielded the more selective activity against gram-negative bacteria

(compound **3b**). Reduced activity against Gram –positive and *C. albicans* was seen in mentioned compound. It is worth to mention that the nature of the substituents on sulfhydryl group of thiadiazole has a pronounced effect. MBC and MFC data indicated that tested compounds act as bactriostatic and fungistatic agents.

Table 1. MIC (mg/mL) of final compounds.

R_1	R	Final compounds	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*
-	**H**	**3a**	**0.75±0.25**	**0.18 ±0.06**	›1	**0.09 ±0.03**
-	**Cl**	**3b**	›1	**0.09 ±0.03**	›1	**0.18 ±0.06**
H	-	**5a**	›1	**0.75±0.25**	›1	›1
Br	-	**5b**	›1	›1	›1	›1
		Ciprofloxacin	0.25 (µg/mL)	0.1(µg/mL)	0.5 (µg/mL)	-
		Nystatine	-	-	-	2(µg/mL)

Table 2. MBC and MFC results (mg/mL) of final compounds.

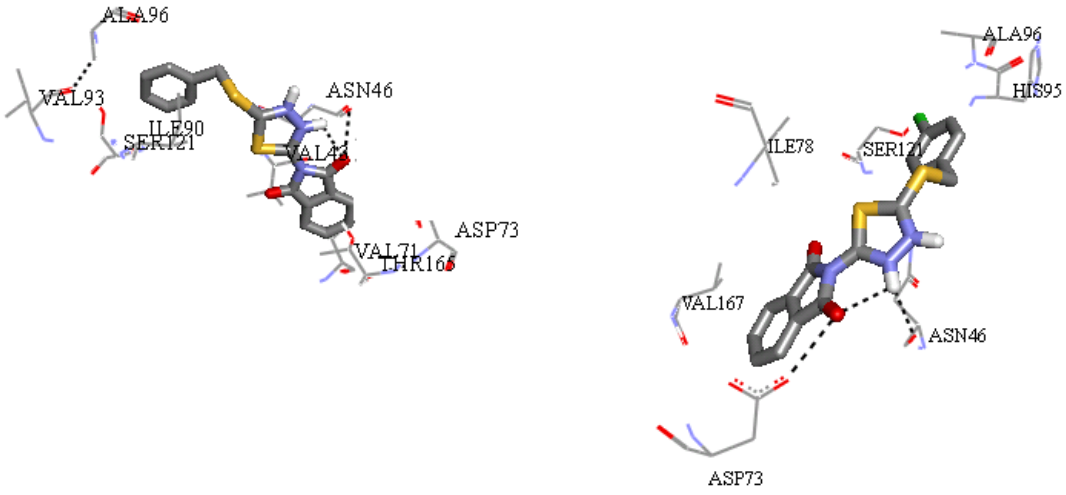
R_1	R	Final compounds	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*
-	**H**	**3a**	1	0.37 ±0.12	›1	0.37 ±0.12
-	**Cl**	**3b**	›1	0.25±0.03	›1	0.75 ±0.25
H	**-**	**5a**	›1	›1	›1	›1
Br	**-**	**5b**	›1	›1	›1	›1

2.3. Docking Results

Compounds **3a** and **3b** produced the highest docking scores indicating high binding affinity of the ligands toward topoisomerase II DNA gyrase for *E. coli*. Docking results were presented in Table 3 and Figure 4. Molecular docking results revealed that the carbonyl group of imide and NH of thiadiazole in compound **3a** interact mainly with Asn 46 residue. In the case of other effective compound, **3b**, Asn 46 and Asp 73 residues participated in hydrogen bonding interactions. No hydrogen bonds interaction for **5a,** and **5b** were predicted (Table 3, Figure 4). Chlorobiocin was docked to validate procedure which the residues Asp73, Asn46, and Arg136 were created hydrogen bond [34] as compounds **3a** and **3b** in this study showed a strong hydrogen bond with Asn 46 and Asp 73. Redocking result of Chlorobiocin in the active site of topoisomerase II DNA gyrase with acceptable RMSD: 0.55 Å is presented in Figure 5.

Table 3. Docking results of synthesized compounds **3a**, **3b** and **5a, 5b**

Tested Compounds	R	R_1	ΔG bind(Kcal/mol)	Hydrogen bond (Angstrom)	Ki (nM)
3a	H	-	-8.38	Asn 46 (2.34 A, 3.15 A)	723
3b	Cl	-	-8.41	Asn 46 (2.16 A), Asp 73 (3.1A)	689
5a	-	H	-8.27	-	866
5b	-	Br	-8.36	-	741



(A) (B)

Figure 4. Binding interaction of ligands with *E. coli* topoisomerase II DNA gyrase B domain. Binding pose of the highest scoring compound 3a (A) and 3b (B). Dotted black bond showing H-bond interaction with residue.

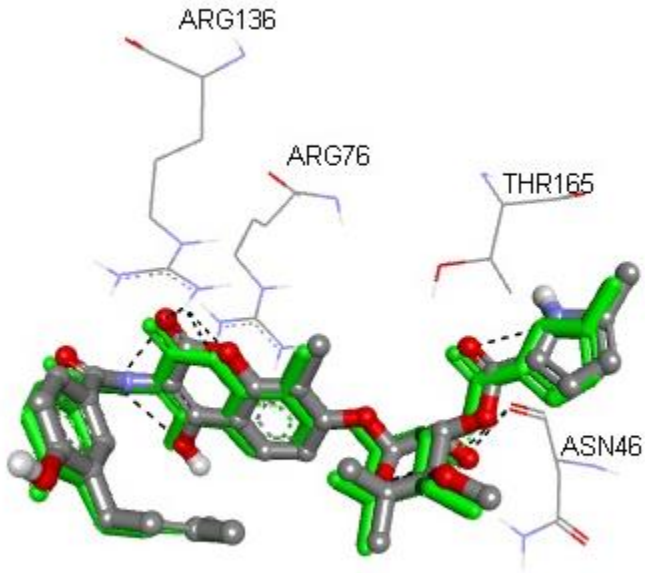


Figure 5. Redocking results of Chlorobiocin in the active site of topoisomerase II DNA gyrase with acceptable RMSD: 0.55Å

3. CONCLUSION

Some derivatives of phthalimide-thiadiazole as fused pharmacophore were synthesized as antimicrobial agents. Compounds 3a showed antimicrobial activity against *S. aureus*, *E. Coli* and *C. Albicans* with the MIC values (0.75, 0.18, 0.09 mg/mL), respectively. Compound 3b was found as active compounds with the MIC values of 0.09 and 0.18 mg/mL against *E. coli* and *C. albicans*, respectively. Both of them had more bacteriostatic than bactericidal activities. Studies indicated that the nature of the substituents on

sulfhydryl group of thiadiazole has a pronounced effect. Molecular docking revealed significant correlation with biological activity.The synthesized compounds could be considered as valuable templates for further modification to design more potent and selective antimicrobial agents.

4. MATERIALS AND METHODS

4.1. Material

Chemical reagents were obtained from Merck (Germany) and Samchun (Korea) Companies. Monitoring of reactions was carried out on Merck silica gel 60 F254plates (Germany). Melting points were determined in open capillary tubes using electrothermal IA9200 apparatus (England) and are uncorrected. Infrared (IR, KBr discs) was obtained with a WQF-510 Fourier-transform (FT)-IR spectrophotometer (China). Mass spectra were run on Agilent Technologies 5975C mass spectrometer (USA). Proton nuclear magnetic resonance spectrometer (1H NMR) were obtained by (Bruker, Germany, 400 MHz) spectrometer.

4.2. Method

4.2.1. Synthesis of (2a, 2b) and (4a, 4b)

5-Amino-2-mercapto-thiadiazole (10mmol, 1.3 g) (1) and KOH (10mmol, 0.5 g) were dissolved in ethanol (30 mL), followed by adding benzyl chloride or 2-bromo-1-phenylethanone derivatives (10mmol). Then the solution was stirred for 4h in room temperature [16]. After confirming the completion, the mixture was filtered and the ethanolic solution was evaporated to give compounds (2a, 2b) (yellow solids, 1.1 g, 49 %;) or (4a, 4b) (white solids, 2.7g, 80 %) (Figure 6).

4.2.2. Preparation of (3a) and (3b)

Compound (2a; 0.6 g, 3.5 mmol or 2b; 0.7 g, 3.5 mmol) was stirred in glacial acetic acid (30 mL) and heated up to 50 °C to complete dissolution. Then, phthalic anhydride (3.5 mmol, 0.4 g) was added and the solution was refluxed for 72 h [31]. The resulting precipitate was filtered and recrystallized from petroleum ether/chloroform (80/20) to yield the pale yellow products (3a, 0.5 g, 47%; 3b, 0.5 g, 43%)(Figure6).

4.2.3. Preparation of (5a) and (5b)

Compound (4a ;0.7 g, 3.5 mmol or 4b; 0.9g, 3.5 mmol), in glacial acetic acid (30 mL) was stirred and heated up to 50 °C to complete dissolution. After adding phthalic anhydride (3.5 mmol, 0.4 g), the solution was heated under reflux condition for 72h [31]. The resulted precipitate after cooling, was filtered and crystallized from petroleum ether/chloroform (80/20) to yield (5a: gray, 0.6 g, 52% and 5b: Dark green, 0.8 g, 65%) products (Figure 6).

4.3. In vitro Antimicrobial activity

Staphylococcus aureus (PTCC1337), *Pseudomonas aeruginosa* (PTCC1074), *Escherichia coli*(PTCC1338), and *Candida albicans* (PTCC5027) were purchased from the Persian Type Culture Collection. *S. aureus*, *P. aeruginosa*, and *E. coli* were grown on Nutrient agar (NA) plates and incubated at 37°C. *C. albicans* was grown on Sabouraud Dextrose Agar (SDA) plate and incubated at 25°C.

4.3.1. Microbial suspension preparation

The inocula of microorganisms were prepared from the cultures and the turbidity was determined and adjusted with spectrophotometer apparature at 580 nm. The concentration of inocula was approximately (1.5×10^8 CFU/mL) with transmittance 0.2 -0.3 and 0.5-0.6 for bacterial and fungal stains, respectively. The final concentrations of inocula were (1.5×10^4 CFU/mL) for bacteria and (1.5×10^5 CFU/mL) for fungi.

4.3.2. Preparation of synthesized compounds

Compounds (100 mg) were dissolved in DMSO (dimethyl sulfoxide) (1mL) to obtain concentration of (100 mg /mL) as stock solutions. It was diluted with Mueller-Hinton Broth (MHB) to make working solutions (1mg/mL) used to make serial dilution concentrations (0.5, 0.25, 0.125, 0.0625 (mg/mL).

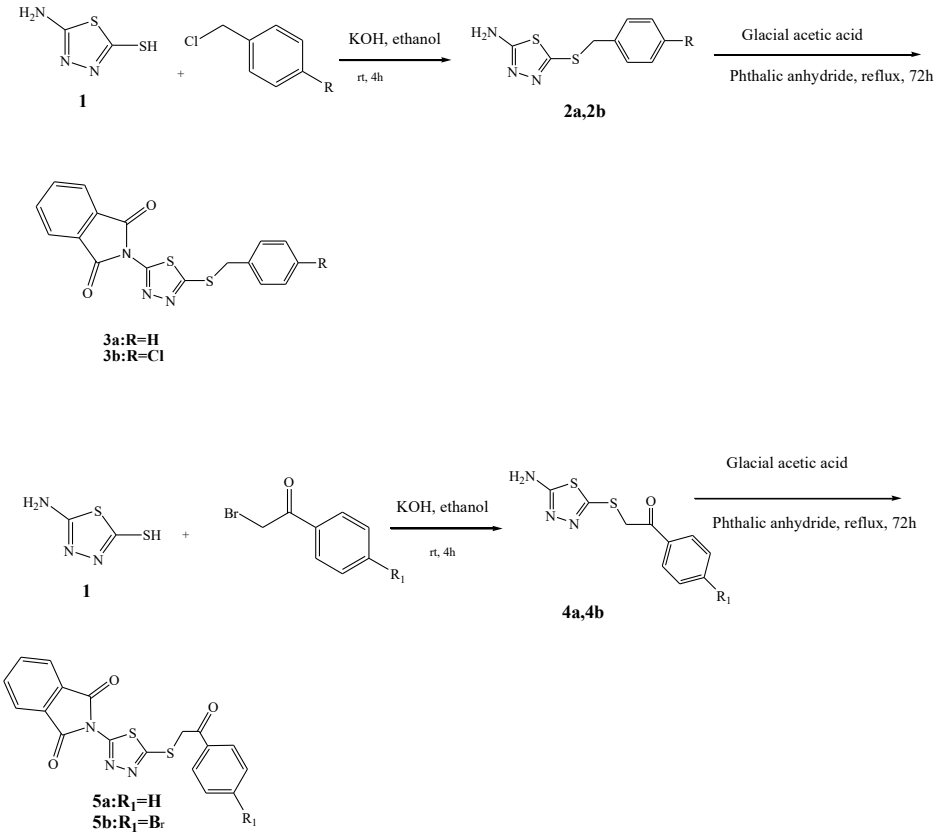


Figure 6. Synthetic route for the synthesis of thiadiazole -phthalimide hybrid derivatives.

4.3.3. Determination of Minimal Inhibitory Concentration (MIC) using tube dilution technique and Minimal Bactericidal Concentration and Minimal Fungicidal Concentration (MBC and MFC).

(MIC) was determined using tube dilution method**.** One milliliter of the prepared microbial suspension was added to each test tube with a certain dilution and incubates for 24 h at 37°C for the bacterial species and 48h at 25°C for fungal strain. The MIC was defined as the lowest concentration, which obstructed visible bacterial growth and showed the least turbidity. Positive control tub contained inoculated microbial suspension and Mueller-Hinton Broth without the synthesized compounds and tube contained medium supplemented with 1% DMSO inoculated with microbial suspension is considered as negative control. Following tube dilution technique, from each tube that shows no growth, contents (10 µL) were removed and spreaded onto (MHA) plates for bacteria and (SDA) for fungi and incubated nightly at 37°C and 25°C for bacteria and fungi, respectively. The lowest concentration of the compounds that restrained any residual colony formation was determined as the MBC and MFC [35] Tables 1 and 2.

4.4. Molecular Docking Study

Docking study was occurred by downloading PDB file: (1KZN) with resolution 2.3 Å for *E. coli* topoisomerase II DNA gyrase B from Protein Data Bank. The three-dimensional (3D) structures of synthesized compounds were built using HyperChem 7.0 software and pdb format of optimized ligands were used as inputs for AutoDock tools. PDBQT formats of ligands and protein were prepared with the procedure similar to the previous works using Autodock tools [36]. Gridbox dimensions were 60 × 60 × 60 with a 0.375Å grid points spacing. The center of the grid box was assigned (19.14, 30.33, and 34.79) according to the centroid of the co-crystal ligand. Study was conducted using the usual method and default parameters of molecular docking AutoDock 4.2 software. Ki Values were obtained of analyze section in the auto dock software (Table 3 and Figure 4).

Acknowledgements: This work was financially supported by the Vice-Chancellor of Research of Isfahan University of Medical Sciences, Isfahan. I.R.Iran through Grant No.399203
Author contributions: Concept – F.H., E.J., M.N,. V.A; Design – F.H., E.J.; Supervision – F.H., E.J.; V.A; Resources – E.J.; Materials – E.J; Data Collection and/or Processing – F.H., E.J., M.N.,V.A; Analysis and/or Interpretation – F.H., E.J., M.N., V.A; Literature Search – E.J; Writing – E.J., F.H.; Critical Reviews – E.J., F.H., M.N., V.A.
Conflict of interest statement: The authors declared no conflict of interest in the manuscript.

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