International Journal of Pharmaceutical Chemistry
ISSN: 2249-734X (Online)
CODEN: IJPCH3 (American Chemical Society)
Journal DOI: https://doi.org/10.7439/ijpc
Research Article

Molecular modeling and synthesis of *N*-benzoyl-*N'*-(3-triflouromethyl) phenylthiourea

Ariani Hutuba*

Airlangga University, Jl. Airlangga No.4 - 6, Airlangga, Gubeng, Kota SBY, Jawa Timur 60115, Indonesia

QR Code

***Correspondence Info:**
Ariani Hutuba,
Airlangga University, Jl. Airlangga
No.4 - 6, Airlangga, Gubeng, Kota SBY,
Jawa Timur 60115, Indonesia

***Article History:**
Received: 13/07/2018
Revised: 29/07/2018
Accepted: 29/07/2018
DOI: https://doi.org/10.7439/ijpc.v8i7.4913

Abstract

Objective: The purpose of this study was to explore interaction of *N*-Benzoyl-*N'*-3-(triflouromethyl) phenylthiourea with the active site of 2XCT to predict the antibacterial activity and synthesize the compound.

Materials and Methods: Molecular modeling which had been performed using Molegro Virtual Docker 5.5, explored interaction between *N*-benzoyl-*N'*-(3-triflouromethyl) phenylthiourea derivatives and DNA gyrase of *Staphylococcus aureus* (pdb. 2XCT) and determined total interaction energy of ligand-protein complex as Moldock Score. *N*-benzoyl-*N'*-(3-triflouromethyl) phenylthiourea derivatives was synthesis by Schotten-Baumann reaction modification.

Results: The result showed that *N*-benzoyl-*N'*-(3-triflouromethyl) phenylthiourea derivatives bound to 2XCT with the same pattern as ciprofloxacin where amino acid involved in ligand-protein interaction via hydrogen bonding is Arg 1033, and docking scores (Moldock) of all compounds are higher than internal ligand ciprofloxacin. The results of molecular modeling and docking indicates that Ser 1085, Val 1045, His 1079, His 1081, Arg 1033 is an important amino acid residues in the active site of DNA gyrase of *Staphylococcus aureus*.

Conclusion: We have accomplished the synthesis of *N*-benzoyl-*N'*-3-(triflouromethyl) phenylthiourea derivatives (1-4). Hence, it can be concluded that docking N-benzoyl-N'-3-(triflouromethyl) phenylthiourea derivatives interact with DNA gyrase of *Stapylococcus aureus* more efficiently, and these can be further developed to improve their antibacterial especially against *Staphylococcus aureus*.

Keywords: Molecular modeling, 3-(triflouromethyl) phenylthiourea, *Stahpylococcus aureus*.

1. Introduction

Staphylococcus aureus is a leading pathogen in surgical site, intensive care unit, and skin infections as well as health-care associated pneumonias. These infections are associated with an enormous burden of morbidity, mortality and increase of hospital length of stay and patient cost. The versatility of *S. Aureus* is reflected by the wide range of disease that it can cause. It's a leading cause of bacteraemia, infective endocarditis, osteomyelitis, pneumonia; indwelling medical device related infections, as well as skin and soft tissue infections (SSTIs) [1].

S. aureus pathogenicity is driven by the wealth of virulence factors and its ability to adapt to different environments. *S. Aureus* is impressively fast in acquiring antibiotic resistance and multidrug resistant strains are a serious threat to human health such as methicilin-resistant *Staphylococcus aureus* (MRSA) [2].

Thioureas, both symmetrical and unsymmetrical, have attracted much attention as antibacterial drug candidates. While unsubstituted 1,3-diphenylthiourea exerts no relevant antibacterial activity [3], its various structural modifications improve the biological effectiveness of a compound [4]. Literature survey reveals that incorporation of halogen atom(s) within the molecule is one of the most effective strategies to enhance its biopotency, bioavailability, and lipophilicity. Suresha *et al*., proved that fluoro-containing arylthiourea compounds show better activity as compared

to other analogues [5], however fluoro-methyl, methyl or metoxy substituent on the benzene ring also improve antibacterial potency. According to other authors findings [6-9], the antibacterial and efficiency depends on the presence of such electron-withdrawing substituent at C-3 and C-4 position of the phenyl ring. Bielenica *et al.*, proved thioureas containing 3-(trifluoromethyl) phenyl moiety exhibited potent to moderate antibacterial activity. Potent Gram-positive antibacterial activity of several analogs of thiourea and urea [10] derivatives is explained by an inhibition of the catalytic site of bacterial type II topoisomerases, in particular DNA gyrase and topoisomerase IV.

Computational Biology and bioinformatics have the potential not only of speeding up the drug discovery process thus reducing the costs, but also of changing the way drugs are designed. Rational drug design (RDD) helps to facilitate and speed up the drug designing process, which involves variety of methods to identify novel compounds [11]. One such method is the docking of the drug molecule with the receptor (target).

The main objective of this research is to explore interaction of *N*-Benzoyl-*N'*-3-(triflouromethyl) phenylthiourea with the active site of 2XCT to predict the antibacterial activity and synthesize the compound.

2. Material and Methodology

2.1 Receptor

The three-dimensional crystal structure of receptors taken from Protein Data Bank (PDB) (http://www.rcsb.org/) is as follows: *S. aureus* gyrase with ciprofloxacin and DNA (PDB ID: 2XCT). The PDB's were loaded in the Molegro virtual docker (MVD) with the removal of all water molecules. The standard Molegro algorithm was utilized for rendering the missing charges, protonation states, and assigning of polar hydrogen to the receptor.

2.2 Ligands

Structures of ligands were drawn using Chembiodraw Ultra 13.0 and energy minimization was done using MMFF94 force field on Chembio3D Ultra 13.0. Energy minimization is done to help the docking programme for identifying the bioactive conformer from the local minimal. One major advantage of MVD is that it helps in assigning the missing bond orders, charges, bonds, and hybridization states of the imported ligands. The 2D structures of *N*-benzoyl-*N'*-3-(triflouromethyl) phenylthiourea ligands are illustrated in (Table 1).

2.3 Validation and Analysis of Docked Receptor-Ligand Complex Structures

To ensure that ligands docked using the Molegro virtual docker represent valid score and accurate binding with receptor, the MVD scoring algorithm was to be validated first for the crystal structures (PDB: 2XCT). They served as control docking models as illustrated. The outcome of the docking showed that MVD determined the optimal orientation of the internal ligands.

2.4 Molecular Docking of ligands

We used MVD, which has been recently introduced and gained attention among medicinal chemists [12]. Bench mark results of MVD software provide very accurate predictions of ligand binding modes (87.0%) compared with other docking software such as Glide (81.8%), GOLD (78.2%), Surflex (75.3%), and FlexX2 (57.9%) . MVD is based on a differential evolution algorithm called MolDock; MolDock Score energy, E_{score}, is defined by (1), where E_{inter} is the ligand-receptor interaction energy and E_{intra} is the internal energy of the ligand. E_{inter} is calculated according to (2):

$$E_{Score} = E_{Inter} + E_{Intra} \quad (1)$$

$$E_{Inter} = \sum_{i=ligan} \sum_{j=protein} \left[E_{PLP}(r_{ij}) + 332.0 \frac{q_i q_j}{4r_{ij}^2} \right] \quad (2)$$

The E_{PLP} term is a "piecewise linear potential" [13, 14] that uses two different parameters, one for the estimate of the steric term (van der Waals) between atoms and another for the potential for hydrogen bonds; it describes the electrostatic interactions between charged atoms [12]. E_{intra} is calculated according to (3).

$$E_{Intra} = \sum_{i=ligan} \sum_{j=protein} [E_{PLP}(r_{ij})] + \sum_{flexible\ bond} A[1 - \cos(m\theta - \theta_\circ)] + E_{Clash} \quad (3)$$

The first term in (3) calculates all the energies involving pairs of atoms of the ligand, except those associated with two bonds. The second term represents the torsional energy, where his torsional angle of the bond. The average of the torsional energy bond contributions is used if several torsions can be determined.

The last term, *E*clash, assigns a penalty of 1,000 kcal/mol if the distance between two heavy atoms (more than two bonds apart) is smaller than 2.0˚ A, ignoring infeasible ligand conformations [12].

The molecular docking was performed for all the constituents with the predicted cavities of the receptor. The

MolDock score (GRID) function was used with a grid resolution (˚A) of 0.30 and a binding site radius of 15˚ A with respect to the origin of the respective cavities. The "MolDock SE" searching algorithm 10 runs using a maximum of 1500 iterations with a total population size of 50 was applied. The energy threshold used for the minimized final orientation is 100. The simplex evaluation with 300 maximum steps of neighbour distance factor 1 was completed.

3. Results and discussions

3.1 Chemistry

Studies were undertaken to synthesize the novel 3-(triflouromethyl) phenylthiourea ring bearing benzoyl chloride derivatives to investigate their antibacterial effects. N-benzoyl-N'-(3-triflouromethyl) phenylthiourea synthesis is achieved via Schotten-Baumann reaction modification by condensation of 3-(triflouromethyl) phenylthiourea with appropriate benzoyl chlorides (Figure 1, Table 1).

The NH2 in 3-(triflouromethyl) phenylthiourea is more nucleophilic than NH, which reacts preferentially with the more reactive carbonyl group, leading to the formation of N-benzoyl-N'-(3-triflouromethyl) phenylthiourea derivatives as major products specially if the reaction is carried out in the presence of a catalytic pyridine upon heating and without heating. In order to assure structural variability, electron-withdrawing (2, 3), and electron-donating groups (4) were inserted.

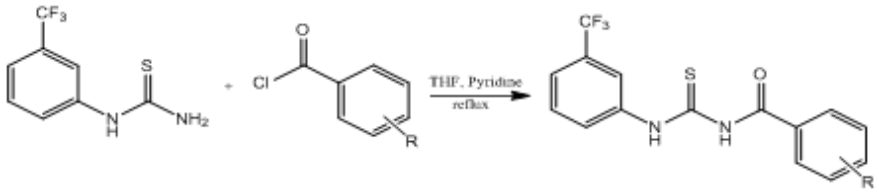
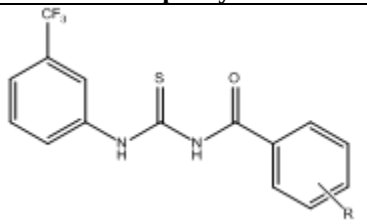
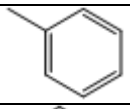
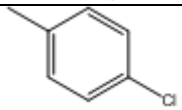
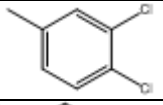
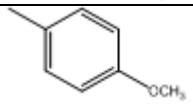


Figure 1: Synthetic procedure for *N*-Benzoyl-*N'*-3-(triflouromethyl) phenylthiourea derivatives

N-benzoyl-*N'*-(3-triflouromethyl) phenylthiourea were confirmed by FTIR and NMR. The compounds showed characteristic absorption bands 1666 cm^{-1} and 1678 cm^{-1} for carbonyl group at amide, the absence of NH stretching band around 3500-3100 cm^{-1}, and absorption bands 690 cm^{-1} for the C=S group. The ^{1}H NMR spectrum exhibited singlets at δ 12.76 – 7.55 ppm, which were assigned to the N-H protons. ^{13}C NMR revealed peaks at δ 178.8 ppm for C=S (thiourea), δ 165-167.2 ppm for (C=O amide) and quartets at 123.7–121 ppm with high coupling constants (272.8 – 270.0 Hz) for CF3 group. The second quartet at 131.5 – 127.6 ppm with low coupling constant (33.6 − 30.7 Hz) proved the presence of the carbon attached to CF3 (C3). Quartets of neighbouring carbons (C2 and C4) were also visible, both at 125.7 – 118.2 ppm (J ranged from 4.2 to 3.7 Hz). Signals at δ 165.0 – 178.8 ppm reflected the presence of the aniline carbon bound to the NH group.

Table 1: Structures, yields and properties of physical chemistry value of *N*-Benzoyl-*N'*-3-(triflouromethyl) phenylthiourea



Compound	R	Yield, %	CLogP
FT-1		72	3.9722
FT-2		74	4.75576
FT-3		71	5.37346
FT-4		45	4.09404

3.2 Molecular Modeling/Docking Studies

To obtained the newly synthesized of *N*-benzoyl-*N'*-(3-triflouromethyl) phenylthiourea derivatives against antibacterial agents were analyzed in detail by visually inspecting the docked complexes using MVD. The parameters measured in the process of docking is the score of energy involved, either a moldock score, rerank score, H bond, and RMSD (Root Mean square Deviation) [15].

To measure the affinity of ligand-receptors, we used moldock score. Docking results of *N*-benzoyl-*N'*-(3-triflouromethyl) phenylthiourea derivatives with DNA gyrase receptors (2XCT) have shown in Table 2.

Table 2: Docking score and predicted antibacterial activity

Compound	Moldock Score (kcal/mol)	Hydrogen bond*	Steric interaction*
Ciprofloxacin	-87.2432	2 (Arg 1033), 1 (Arg 1048)	His 1079, Arg 1033, Ala 1032, Val 1045
1	-110.039	1 (Arg 1033)	Ser 1085, Val 1045
2	-119.831	1 (Arg 1033)	Ser 1085, His 1079
3	-127.13	1 (Lys 1043)	Arg 1033, Val 1045
4	-127.423	2 (Arg 1033), 1 (Gly 1082), 1 (Ser 1085)	His 1081, His 1079, Ser 1085

N-benzoyl-*N'*-(3-triflouromethyl) phenylthiourea derivatives have a value of energy, moldock score, higher than internal ligand (ciprofloxacin). *N*-benzoyl-*N'*-(3-triflouromethyl) phenylthiourea derivatives, from low to high, is *N*-(4-metoxy) benzoyl-*N'*-(3-triflouromethyl) phenylthiourea > *N*-(3,4-dichloro)benzoyl-*N'*-(3-triflouromethyl) phenylthiourea > *N*-(4-chloro) benzoyl-*N'*-(3-triflouromethyl) phenylthiourea > *N*-benzoyl-*N'*- (3-triflouromethyl) phenylthiourea > *N*-benzoyl-*N'*-(3-triflouromethyl) phenylthiourea. *N*-(4-metoxy) benzoyl-*N'*-(3-triflouromethyl) phenylthiourea have binding affinity with receptors DNA gyrase of *Staphylococcus aureus* dengan receptor, which reflected the value of the Moldock score, the lowest compared with the third derivative of the other. Compounds with the lowest energy shows interactions ligand-receptors are the most stable, and can be predicted to have higher activity [16].

Interaction Ligand-receptor which includes: hydrogen bond and steric interactions (van der Waals). Hydrogen bonds involved in the processes of interaction of ligand (ciprofloxacin), and N-benzoyl-N'-(3-triflouromethyl) phenylthiourea derivatives, with receptors, types of amino acids are bound to Ligand, and the pharmacophore group, Shown in Figure 2.

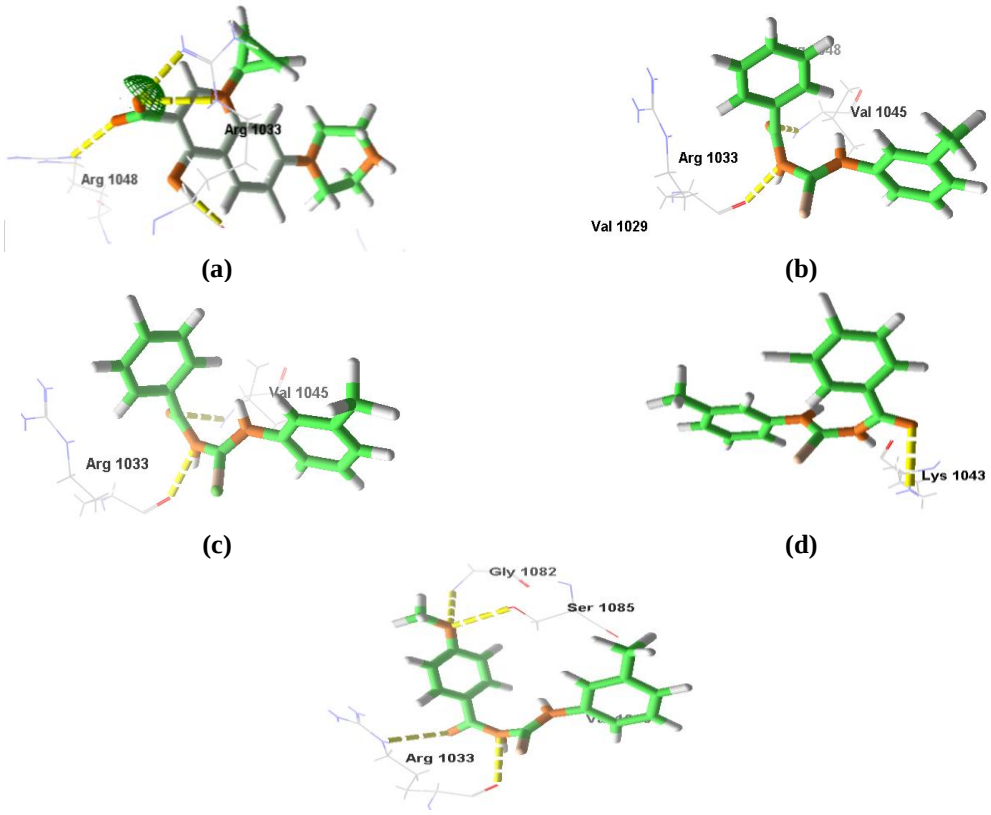


Figure 2: Docking 3D-conformation of Ciprofloxacin (a) and *N*-benzoyl-*N'*-(3-triflouromethyl) phenylthiourea derivatives 1-4 (b-e) have shown hydrogen bond (yellow dash line) with amino acids receptors 2XCT

The metabolites of ciprofloxacin and *N*-benzoyl-*N*'-(3-triflouromethyl) phenylthiourea derivatives (**1, 2, 4**) with receptors DNA gyrase of *Staphylococcus aureus* (2XCT) bound the same amino acids Arg 1033 through hydrogen bond, except as a compound (**3**) that form hydrogen bonds with amino acid Lys 1043. The compound (**4**) add three hydrogen bonds, because the presence of benzene and its substituent.

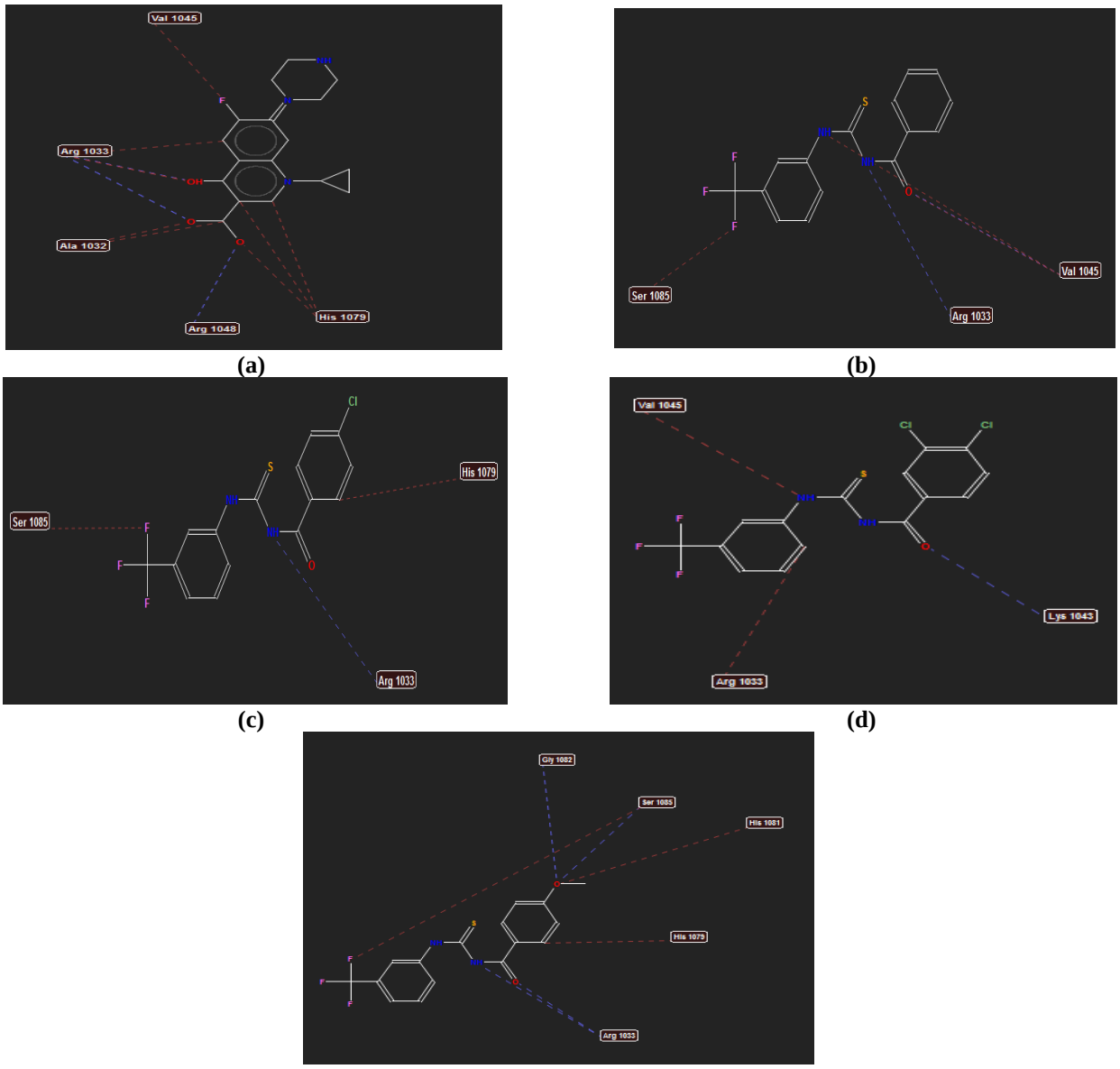


Figure 3: 2D-interaction between Ciprofloxacin (a), *N*-benzoyl-*N'*-(3-triflouromethyl) phenylthiourea derivatives (b-c), and receptors 2XCT

N-benzoyl-*N*'-(3-triflouromethyl) phenylthiourea derivatives was bound to similiar receptors with ciprofloxacin, and there is an additional interaction steric, between *N*-benzoyl-*N*'-(3-triflouromethyl) phenylthiourea derivatives with amino acids group Ser 1085, Val 1045, His 1079, His 1081, Arg 1033.

And it is also shown that the benzene ring and carbonyl group of *N*-benzoyl-*N*'-(3-triflouromethyl) phenylthiourea derivatives is a new pharmacophore group because it can bound with receptor through hydrogen bond and steric. The results of molecular modeling and docking indicates that Ser 1085, Val 1045, His 1079, His 1081, Arg 1033 is an important amino acid residues in the active site of DNA gyrase of *Staphylococcus aureus*.

3.3 Experimental section

FTIR spectra in KBr pellets were recorded on a One Perkin Elmer FTIR spectrophotometer. The 1H and 13C NMR spectra were recorded on a Jeol JNM-ECS 400 MHz, respectively; chemical shifts are expressed in parts per million (ppm) relative to TMS. The abbreviations used

to describe the peak patterns are: (b) broad, (s) singlet, (d) doublet, (t) triplet, (q) quartet, and (m) multiplet. Mass spectra (MS) were recorded in Schimadzu QP-5000 GC-Mass spectrometer.

3.3.1 General procedure for the synthesis of *N*-benzoyl-*N'*-3-(triflouromethyl) phenylthiourea: A solution of commercially available 3-(trifluoromethyl)phenylthiourea (0.0022 mol, 0.25 g) in tetrahydrofuran (10 mL) was treated with appropriate benzoyl chloride (0.005 mol) and the mixture was refluxed at room temperature for 2 h. Then solvent was removed on rotary evaporator. The solid separated was collected, washed with $NaHCO_3$ 10%, dried and recrystallized from aqueous ethanol. Analytical TLC was carried out on silica gel F254 (Merck) plates (0.25 mm thickness) using (chlorofom: methanol; 5:1) eluent.

***N*-benzoyl-*N'*-(3-triflouromethyl) phenylthiourea:** Yield (72%), white powder. FTIR (KBr): 3313 and 1598 (N-H secondary amides), 3118, 3095, 3030 (AR-H), 1666 (C=O amides), 1571 and 1481 (C=C aromatic), 690 (C=S thioamide) cm^{-1}; ^1H NMR (400 MHz, $CHCl_3$) δ ppm: 7.55 (m, 4H, Ar-H), 7.67 (m, 1H, *J*=9.6, Ar-H) 7.89 (dd, 2H, *J*= 7.2, Ar-H), 7.95 (m, 1H, Ar-H), 8.06 (s, 1H, Ar-H), 9.15 (s, -OC-NH-CS-), 12.76 (s, -SC-NH-Ar); ^{13}C NMR (400 MHz, $CHCl_3$) δ ppm: 178.80, 167.23, 138.20, 134.08, 131.45, 131.29, 129.54, 129.40, 127.65, 127.36, 123.52, 121.05.

N-(3, 4-dichloro) benzoyl-N'- (3-triflouromethyl) phenylthiourea: Yield (67%), white powder. FTIR (KBr): 3246 and 1602 (N-H secondary amides), 3053, 3026 (AR-H), 1678 (C=O amides), 1570 and 1463 (C=C aromatic), 690 (C=S thioamide) cm^{-1}; 1H NMR (400 MHz, $CHCl_3$) δ ppm: 7.55 (m**,** 2H, Ar-H), 7.63 (dd, 1H *J*= 8.4, Ar-H), 7.70 (dd, 1H, *J=8.8*, AR-H), 7.92 (m, 1H, Ar-H), 8.01 (t, 2H, *J*=6.8), 9.04 (s, -OC-NH-CS-), 12.55 (s, -SC-NH-Ar); ^{13}C NMR (400 MHz, $CHCl_3$) δ ppm: 178.31, 165, 137.99, 131.44, 131.17, 129.92, 129.62, 127.39, 126.41, 123.76, 121.07, 121.03.

4. Conclusion

We have accomplished the synthesis of *N*-benzoyl-*N'*-3-(triflouromethyl) phenylthiourea derivatives (1-4). The molecular modeling, docking *N*-benzoyl-*N'*-3-(triflouromethyl) phenylthiourea derivatives have Moldock higher than internal ligand (ciprofloxacin), and *N*-(4-metoxy) benzoyl-*N'*-3-(triflouromethyl) phenylthiourea as a compound with high moldock score. From molecular modeling results docking indicates that Ser 1085, Val 1045, His 1079, His 1081, Arg 1033 is an important amino acid residues in the active site of DNA gyrase of *Staphylococcus aureus*. Hence, it can be concluded that docking N-benzoyl-N'-3-(triflouromethyl) phenylthiourea derivatives interact with DNA gyrase of *Stapylococcus aureus* more efficiently, and these can be further developed to improve their antibacterial especially against *Staphylococcus aureus*.

Acknowledgments

The authors would like to acknowledge Nopic Factory for gum Arabic processing and Mr. El-Rashed Awad, central laboratory, at University of Khartoum, for technical support.

References

[1]. F. Bagnoli, R. Rappuoli, G. Grandi. 2016. *Staphylococcus aureus*: Microbiology, Pathology, Immunology, Therapy, Prophylaxis. Springer International Publishing: Switzerland

[2]. E.L. Palavecino, Y. Ji. 2014. Methicillin-Resistant *Staphylococcus aureus* (MRSA) protocols, 2t. Humana press: London

[3]. S. Cunha, F.C. Macedo Jr, G.A.N. Costa, M.T. Rodrigues Jr, R.B.V. Verde, L.C. De Souza Neta, I. Vencato, C. Lariucci, F.P. Sa, *Monatsh. Chem*. 138 (2007) 511-516.

[4]. A. Mishra, S. Batra, *Curr. Top. Med. Chem*. 13 (2013) 2011-2025.

[5]. G.P. Suresha, R. Suhas, W. Kapfo, D.C. Gowda, *Eur. J. Med. Chem*. 46 (2011) 2530-2540.

[6]. K.H. Chikhalia, M.J. Patel, *J. Enzyme Inhib. Med. Chem*. 24 (2009) 960-966.

[7]. A. Saeed, U. Shaheen, A. Hameed, S.Z.H. Naqvi, *J. Fluorine Chem.* 130 (2009) 1028-1034.

[8]. S. Saeed, N. Rashid, P.G. Jones, M. Ali, R. Hussain, *Eur. J. Med. Chem.* 45 (2010) 1323-1331.

[9]. H.M. Faidallah, S.A. Rostom, S.A. Basaif, M.S. Makki, K.A. Khan, Arch. Pharm. Res. 36 (2013) 1354-1368.

[10]. D.E. Ehmann, S.D. Lahiri, *Curr. Opin. Pharmacol.* 18 (2014) 76-83.

[11]. R. Thomsen and M. H. Christensen, "Mol Dock: a new technique for high-accuracy molecular docking," *Journal of Medicinal Chemistry*, 2006; 49 (11): 3315–3321.

[12]. D. K. Gehlhaar, G. Verkhivker, and P. A. Rejto, "Docking conformationally flexible small molecules into a protein binding site through evolutionary programming. Proceedings of the Fourth International Conference on Evolutionary Programming," *Lecture Notes in Computer Science*, 1999; 1447: 449–461.

[13]. J. Yang and C. Chen, "GEMDOCK: a generic evolutionary method for molecular docking," Proteins: Structure, *Function and Genetics*, 2004; 55 (2): 288-304.

[14]. CLCbio. 2012. Molegro virtual docker user manual, MVD 2012. 5.5 for windows, Linux, and Mac OS X, Molegro-A CLC bio company

[15]. Gundertofte, K. And Jorgensen R.S., 2000. Molecular Modelling and prediction of bioactivity. New York: Kluwer Academic Publ.