Azhar Int J Pharm Med Sci 2023; Vol 3 (1): 44-57
(Research Article)

Open Acces

Print ISSN: 2735-4598
Online ISSN: 2735-4601

Synthesis, antimicrobial evaluation, and molecular modelling studies of Niclosamide derivatives as biotin carboxylase inhibitors

Magda MF. Ismail [1*], Tamer Nasr [2, 3], Abeer Saeed [3], and Yousry A. Ammar [4]

[1] Department of Pharmaceutical Chemistry, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt
[2] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Helwan University, Helwan, Cairo, Egypt
[3] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, MTI University, Cairo, Egypt
[4] Department of Chemistry, Faculty of Science, Al-Azhar University, Cairo, Egypt

***** Correspondence: m.elalfy101@gmail.com

Article history: Received 2022-03-23 **Revised 2022-05-23** **Accepted 2022-05-27**

Abstract: New Niclosamide Schiff's bases and Niclosamide esters were synthesized and evaluated for their antimicrobial activity against Gram-positive and Gram-negative bacterial strains in addition to fungal strains. Moreover, the antimicrobial activity of the target compounds against the resistant bacterial strains MRSA10 and MRSA12 was evaluated. Minimum inhibition concentration (MIC) values of the target compounds were determined to evaluate their antimicrobial activities. Niclosamide esters **4**, **5** and **6** displayed remarkable activities against MRSA12 (MIC ≤ 4.03 µM) and moderate activities against C. albicans (MIC 7.8-31.25 µM). Strong antibacterial activities were elicited by Niclosamide esters 4 and 5 against B. subtilis (MIC 1.95 µM, 3.90 µM; respectively). Docking studies demonstrated the ability of the target compounds to bind with the active site of the microbial biotin carboxylase. Furthermore, physicochemical properties and ADME calculations indicated that the target compounds are available by oral route, with no blood–brain barrier (BBB) permeation. This study demonstrated that Niclosamide esters are potent antimicrobial agents against Gram-positive and resistant MRSA12 bacterial strains and were safe towards the normal human cell line (WI-38).

Keywords: Niclosamide esters; Niclosamide Shiff's bases; Biotin carboxylase; Antimicrobial; Docking; Cytotoxicity.

1. INTRODUCTION

Microbial drug resistance has become a threatening challenge in the last few decades[1]. Among these resistant microbes are the Gram-positive Methicillin Resistant *S. aureus*[2] (*MRSA*) and the Gram-negative *A. baumannii*, *P. aeruginosa* and *K. pneumonia* bacterial strains[3]. In order to mitigate this problem, new antibiotics directed against new target molecules are urgently needed. Since fatty acids are only used for membrane biogenesis in bacteria, the enzymes of the fatty acid biosynthetic pathway are potential targets for the development of novel antibacterial agents[4,5]. Biotin carboxylase acts as an important catalyst in the synthesis of malonyl-CoA which is an important precursor for the synthesis of fatty acids[6]. These are essential for formation of the bacterial cell wall. With the advent of drug resistant bacterial strains, the urgent need for drugs with potential for inhibiting drug resistant bacteria was felt[7,8]. Drug repurposing is a very imperative and practical approach to identify antimicrobial activities in several approved medicines. Repurposing clinically approved drugs with well-known pharmacological properties recommends a curtailed hazard and price advantages when related to *de-novo* advance

Cite this article: Ismail M., Naser T., Saeed A., Ammar Y. Synthesis, antimicrobial evaluation, and molecular modelling studies of Niclosamide derivatives as biotin carboxylase inhibitors. Azhar International Journal of Pharmaceutical and Medical Sciences, 2023; 3(1):44-57. doi: 10.21608/AIJPMS.2022.129287.1120

DOI : 10.21608/AIJPMS.2022.129287.1120

trials of new antimicrobial agents[9]. Niclosamide was approved by FDA as remedy for tape worm infection in humans[10]. Recently, it has been utilized as anticancer[11], antibacterial[12], antiviral[13], and it was seen that it improved diabetic symptoms in mice[14]. Niclosamide is considered as an alternative antibacterial therapy, its MICs values distant below that of Vancomycin, which is usually used in infections because of clinical resistant isolates of MRSA[15–19] Niclosamide demonstrated strong activity against the Gram-positive bacteria such as *E. faecium* and *S. aureus* (MIC: 0.25 µg/mL and 0.125 µg/mL; respectively). Whereas it displayed weak antibacterial activity against Gram-negative bacterial strains. Astonishingly, it has showed high antibacterial activity against *H. pylori* (0.25 µg/mL) with immunomodulatory activity by letting down IL-8 secretion in abdominal cancer cell line subsequent *H. pylori* infection[20]. Niclosamide is a safe drug because very little is absorbed from the gastrointestinal tract, and it is well tolerated in humans[21]. Its little systemic exposure when given orally may hinder its usage as treatment for systemic diseases. Therefore, the reported ester derivative of Niclosamide, DK-520, can be metabolized *in vivo* and greatly increase the plasma concentration of Niclosamide and its exposure duration[22]. Nonetheless, Niclosamide does not have perfect pharmacokinetic profile on account of its poor water solubility and little oral bioavailability[23, 24]. The absence of any announced resistance to Niclosamide, organized with its high efficiency against Gram-positive bacteria, makes it a gifted replacement to conservative antibiotics. Its main mode of action is through the inhibition of DNA gyrase. Molecular modeling studies showed that both Niclosamide Schiff's bases and Niclosamide esters can inhibit the bacterial biotin carboxylase enzyme. Niclosamide esters have advantage over Niclosamide Schiff's bases as after they metabolized *in-vivo*, they regenerate Niclosamide again in patients' plasma as reported[25] and consequently inhibit microbial DNA gyrase enzyme. So, Niclosamide esters has a dual antimicrobial mode of action. Based on the aforementioned information and docking studies we designed some new Niclosamide esters and Schiff's bases as new powerful antimicrobial agents specially against resistant strains (**Figure 1**).

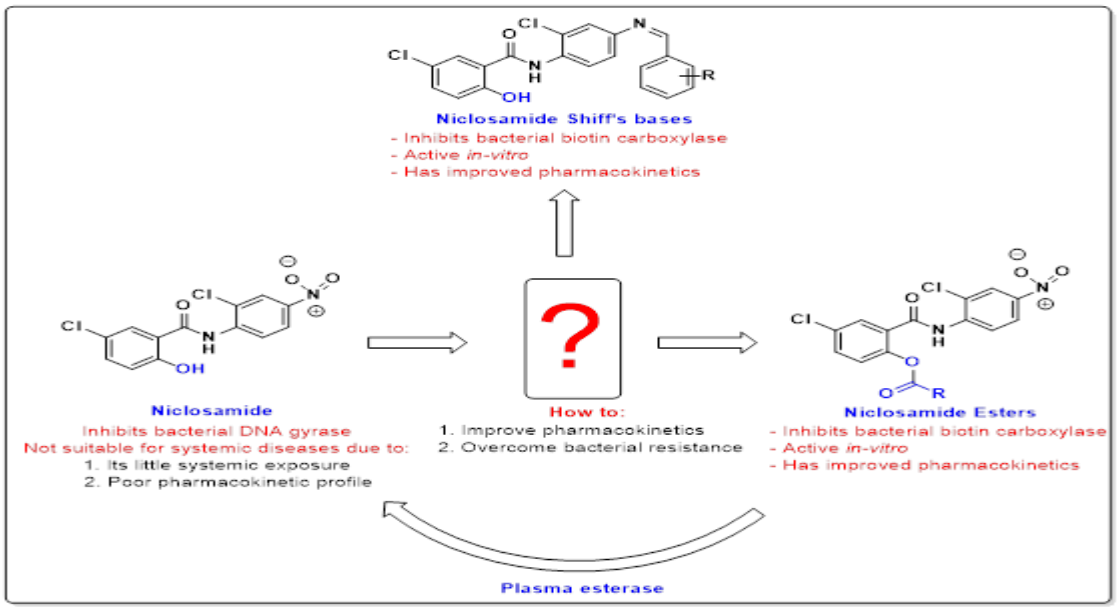


Figure 1. Rationale of the work.

2. METHODS

2.1. Chemistry

Without more refinements, chemical substances and reagents were attained from Aldrich Chemicals, and solvent from Fisher. Open capillaries were used to record the melting points (MPs) of all the newly developed compounds on a digital Gallen Kamp MFB-595 equipment. IR spectra were computed on a Shimadzu 440 spectrophotometer using the KBr disc

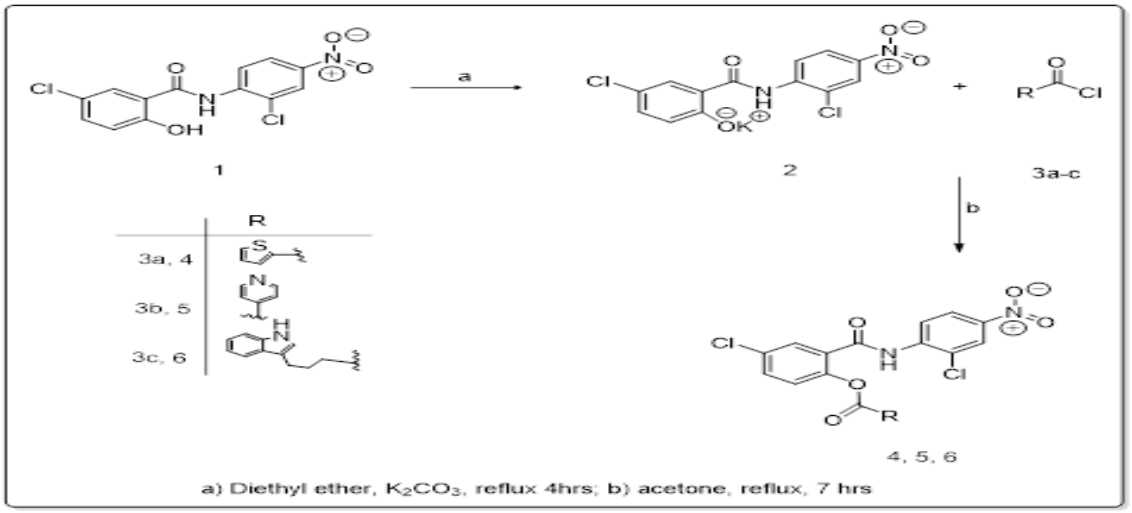
methodology in the 400–4000 cm^{-1} range. Chemical shifts were computed in ppm relative to tetra methyl silane (TMS) as an internal standard (= 0 ppm) in NMR spectra (^{1}H / ^{13}C), which were obtained on a JOEL spectrometer 500 / 125 MHz using $CDCl_3$ and DMSO-d6 as solvents. Elemental studies were done at Cairo University's Micro Analytical Unit in Cairo. Antimicrobial evaluation experiments were done at Al-Azhar University's Regional Center for Biotechnology. Thin layer chromatography (TLC) was done using silica gel-precoated aluminum sheets (Type 60, F 254, Merck, Darmstadt, Germany).

2.1.1. Synthesis of 4-chloro-2-((2-chloro-4-nitrophenyl) carbamoyl) phenyl aryl ester (4-6)

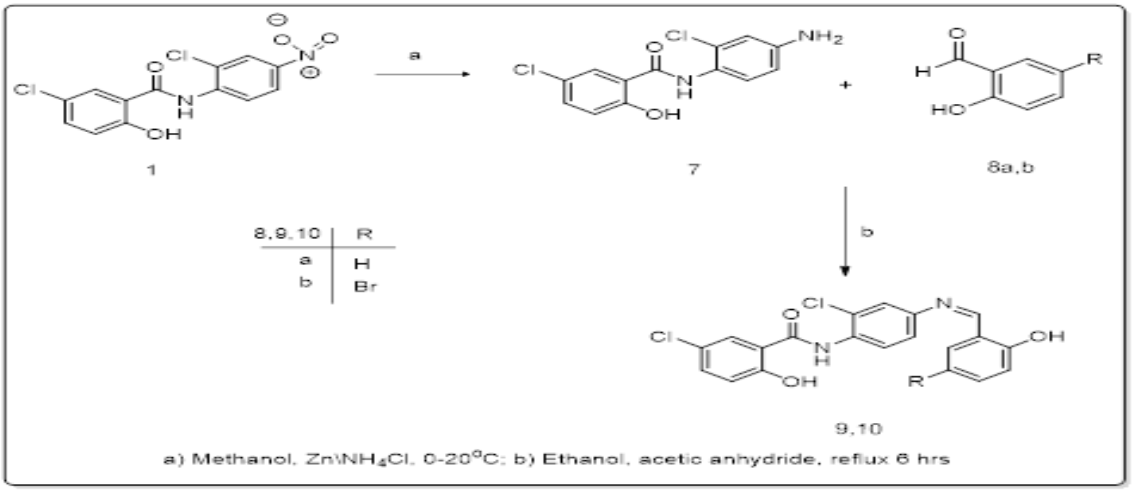
A solution of Niclosamide **1** in diethyl ether was refluxed with K_2CO_3 to afford Niclosamide potassium salt **2** and without separation was refluxed with solution of thiophene-2-carbonyl chloride **3a**, isonicotinoyl chloride **3b** or 4-(1*H*-indol-3-yl)butanoyl chloride **3c** in acetone for 7 hours[26] to yield the target prodrugs **4-6**; respectively **(Scheme 1).**

2.1.2. Synthesis of N-(4-Amino-2-chlorophenyl)-5-chloro-2-hydroxybenzamide (7)

A suspension of zinc dust (0.46 g, 7.08 mmol) in methanol was added slowly to a methanolic mixture of Niclosamide (2 g, 5 mmol) and acetic acid (12.5 mL)[27, 28]. After 5 minutes a precipitate was seen in the reaction mixture. Then CH_3OH (5 mL) was added every 30 minutes for 2 hours to assist stirring. After 3 hours, to remove the zinc, the reaction mixture was filtered over a Celite plug and concentrated to get a brown solid powder **(Scheme 2).**



Scheme 1. Synthesis of Niclosamide esters 4-6.



Scheme 2. Synthesis of the target Schiff's bases 9 and 10.

2.1.3. Synthesis of 5-chloro-N-(2-chloro-4-((5-substituted-2-hydroxybenzylidene)amino)phenyl) -2-hydroxybenzamide (9 and 10)

To a mixture of compound (7) (0.3 g, 1 mmol) in ethanolic solution,2-hydroxybenzaldehyde or 5-bromo salicylaldehyde (8 a, b) (1 mmol) was added, catalyzed with some drops of acetic acid. The produced mixture was heated for 4-5 hours under reflux

(The reaction mixture was monitored by TLC), allow to cool and resulting product was filtered and crystallized from ethanol to achieve the aim.

2.2. Microbiological evaluation

2.2.1. In vitro antimicrobial screening

2.2.1.1. Agar-diffusion method [29]

The target compounds **4, 5, 6, 9** and **10** were tested by disc-diffusion method for antimicrobial activity at concentration of 1 mg/mL against six strains of bacteria and fungi: two Gram-positive bacteria *(S. aureus ATCC 25923 and B. subtilis RCMB 015 (1) NRRL B-543),* two Gram-negative bacteria (*E. coli ATCC 25922 and P. vulgaris RCMB 004 (1) ATCC 13315) and* two fungal strains (*C. albican*s ATCC 10231 and *A. fumigatus RCMB 002008)* by using Gentamycin and Ketoconazole as standards for antibacterial and antifungal activities respectively.

2.2.1.2. Minimum inhibition concentration (MICs) method [30]

The MIC (µg/mL) of our new Niclosamide analogs were tested against standard bacterial strains of *B. subtilis, S. aureus, E. coli, P. vulgaris* and standard fungal strains of *C. albican*s and *A. fumigatus*. Moreover, investigation of our compounds against resistant strains of *S. aureus*, *MRSA10* and *MRSA12* was also performed using Amoxicillin and Nystatin as antibacterial and antifungal reference standards respectively.

2.3. *In silico* and molecular docking simulation

2.3.1. Molecular docking simulation

From the Protein Data Bank (PDB) biotin carboxylase (PDB code: 2V58)[31] was selected as the enzyme for docking simulation in binding pocket. After removing the ligand and solvent molecules, hydrogen atoms were added to each protein atom, energy minimization and partial charges calculation (using Force Field Amber12: EHT). Docking was carried out by MOE 2014 software binding pocket. As a scoring function, alpha PMI was used to estimate score. The database resulted in a kcal/mol score between the conformers of the ligands and the enzyme binding sites. Self-docking was used to confirm the reliability of docking results, the synthesized compounds were then docked into the active site of the protein. The top-ranked pose had an RMSD of less than 1.5 Å from the experimental crystal structure. This finding indicated that the MOE docking method can be used to accurately predict the docking pose of the chemicals investigated to an enzyme. Values of less than 1.5 or 2 Å were said to indicate a successful and reliable docking process.

3. RESULTS

3.1. Chemistry

4-Chloro-2-((2-chloro-4 nitrophenyl) carbamoyl) phenyl thiophene-2- carboxylate (4)

Light green powder; yield: (83%); melting point: 145-147°C. **IR**: unit υ/cm^{-1}: 3244 (NH), 3097, 3082 (aromatic CH), 1747 (C=O of ester), 1681 (C=O of amide). **^{1}H NMR** (500 MHz, in DMSO-d_6) unit δ/ppm: 9.91 (s, 1H, NH; D_2O exchangeable), 8.75 (d, J = 9.3 Hz, 1H, aromatic-H), 8.32 (s, 1H, aromatic-H), 8.19 (dd, J = 9.2, 2.3 Hz, 1H, aromatic-H), 7.91 (d, J = 2.3 Hz, 1H, aromatic-H), 7.86 (d, J = 2.5 Hz, 1H, aromatic-H), 7.69 (s, 1H, aromatic-H), 7.45 (dd, J = 8.7, 2.5 Hz, 1H), 7.15 (t, J = 4.6 Hz, 1H), 7.08 (d, J = 8.7 Hz, 1H, aromatic-H). **^{13}C NMR** (125 MHz, in DMSO-d_6) unit δ/ppm: 119.6,119.9, 120.10, 120.50, 121.50, 123.0, 124.50, 125.50, 126.30, 127.54, 128.50, 131.10, 133.50, 142.0, 134.50, 143.50, 155.50 (C=O), 163.50 (C=O). **Anal. Calc**. for **$C_{18}H_{10}Cl_2N_2O_5S$** (435.97): C, 49.44; H, 2.31; Cl, 16.22; N, 6.41; O, 18.30; S, 7.33 **Found**: C, 49.40; H, 2.35; Cl, 16.25; N, 6.37; O, 18.33; S, 7.28.

4-Chloro-2-((2-chloro-4-nitrophenyl) carbamoyl) phenyl isonicotinate (5)

Light brown powder; yield: (86%); melting point: 155-157°C. **IR**: unit υ/cm^{-1}: 3244 (NH), 3097, 3082 (aromatic CH), 1762 (C=O of ester), 1685 (C=O of amide). **^{1}H NMR** (500 MHz, in DMSO-d_6) unit δ/ppm: 9.61 (s, 1H, NH; D_2O exchangeable), 8.74 (d, J = 9.2 Hz, 1H, aromatic-H), 8.45 (d, J = 9.2 Hz, 2H, aromatic-H), 8.32 (s, 1H, aromatic-H) , 8.20 (d, J = 8.8 Hz, 1H, aromatic-H), 7.94 (d, J = 5.9, 2H, aromatic-H), 7.87 (s, 1H, aromatic-H), 7.45 (dd, J = 8.6, 2.0 Hz, 1H, aromatic-H), 7.08 (d, J = 8.6 Hz, 1H, aromatic-H).**^{13}C NMR** (125 MHz, in DMSO-d_6) unit δ/ppm: 119.51, 120.0, 121.23, 122.10, 123.20,

124.20, 125.30, 126.10, 129.8, 131.0, 131.70, 134.50, 135.0, 135.10, 141.80, 142.10, 144.50, 157.30 (C=O), 163.30 (C=O). **Anal. Calc**. for **$C_{19}H_{11}Cl_2N_3O_5$** (432.01): C, 52.80; H, 2.57; Cl, 16.41; N, 9.72; O, 18.51 **Found**: C, 52.84; H, 2.52; Cl, 16.38; N, 9.78; O, 18.54.

4-Chloro-2-((2-chloro-4-nitrophenyl) carbamoyl) phenyl 4-(1*H*-indol-3-yl) butanoate (6)

Light brown powder; yield: (86%); melting point: 250-252ºC. **IR**: unit υ/cm^{-1}: 3244 (NH), 3097, 3082 (aromatic CH), 2904 (aliphatic CH), 1747 (C=O of ester), 1682 (C=O of amide). **^{1}H NMR** (500 MHz, in DMSO-d_6) unit δ/ppm: 11.35 (s, 1H, NH -indole; D_2O exchangeable), 10.15 (s, 1H, NH; D_2O exchangeable), 9.29 (d, J = 9.5 Hz, 1H, aromatic-H), 8.90-8.80 (m, J = 7.5 Hz, 2H, aromatic-H), 8.65 (d, J = 10 Hz, 1H aromatic-H), 8.47 (d, J = 7.9 Hz, 1H, aromatic-H) , 8.40 (s, 1H, aromatic-H), 8.35-8.23 (m, J = 8.4 Hz, 2H 7,8-indole), 7.90 (s, 1H, 2-indole), 7.50 (d, J = 9.5 Hz, 1H, 5-indole), 7.30 (d, J = 10 Hz, 1H, 6-indole), 2.30 (t, J = 3.5 Hz, 2H, aliphatic-H), 1.50 (t, J = 1.5 Hz, 2H, aliphatic-H), 1.30 (m, J = 0.5 Hz, 2H, aliphatic-H).**^{13}C NMR** (125 MHz, in DMSO-d_6) δ/ppm: 39.87, 58.97, 59.37, 110.90, 112.23, 118.70, 119.87, 121.25, 123.02, 124.34, 125.42, 127.48, 130.49, 134.39, 136.84, 141.87, 143.18, 146.79 (2C), 152.20, 155.79, 157.79, 159.15, 163.10 (C=O), 165.29 (C=O). **Anal. Calc**. for **$C_{25}H_{19}Cl_2N_3O_5$** (512.34): C, 52.80; H, 2.57; Cl, 16.41; N, 9.72; O, 18.51 **Found:** C, 52.76; H, 2.62; Cl, 16.48; N, 9.66; O, 18.55.

2.1.2.

N-(4-Amino-2-chlorophenyl)-5-chloro-2-hydroxybenzamide (7)

Light brown powder; yield: (93%); melting point: 220-222ºC. **IR**: υ/cm^{-1}: 3437, 3358 ((NH_2, OH), 3284 (NH of amide), 3040 (aromatic CH), 1680 (C=O). **Anal. Calc**. for **$C_{13}H_{10}Cl_2N_2O_2$** (297.14): C, 52.55; H, 3.39; Cl, 23.86; N, 9.43; O, 10.77 **Found:** C, 52.59; H, 3.44; Cl, 23.82; N, 9.47; O, 10.73.

5-Chloro-*N*-(2-chloro-4-((2-hydroxybenzylidene) amino)phenyl)-2-hydroxybenzamide (9)

Light green powder; yield: (86%); melting point: 270-272ºC. **IR**: unit υ/cm^{-1}: 3439 (2OH of phenol), 3285 (NH of amide), 3091 (aromatic CH), 1675 (C=O of amide), 1623 (C=N). **^{1}H NMR** (500 MHz, in DMSO-d_6) unit δ/ppm: 11.15 (s, 1H, OH; D_2O exchangeable), 10.99 (s, 1H, NH; D_2O exchangeable), 8.99 (s, 1H, methine-H), 8.46 (s, 1H, aromatic-H), 7.96 (d, J = 4.6 Hz, 1H, aromatic-H), 7.71 (d, J = 4.6 Hz, 1H, aromatic-H), 7.62 (t, J = 5.6 Hz, 1H, aromatic-H), 7.38 (d, J = 4.2 Hz, 1H, aromatic-H), 7.05 (s, 1H, aromatic-H), 6.98-6.95 (m, 2H, aromatic-H), 6.67 (d, J = 2.4 Hz, 1H, aromatic-H), 6.51 (dd, J = 9.8, 3.5 Hz, 1H, aromatic-H), 5.40 (s, 1H, br-OH; D_2O exchangeable). **^{13}C NMR** (125 MHz, in DMSO-d_6) unit δ/ppm: 117.35, 117.81, 119.60, 121.46, 122.68, 123.90, 126.61, 129.38, 129.93 130.27, 132.98, 133.16, 134.19, 137.08, 140.26, 145.14, 157.60 (C-OH) 160.48 (CH=N), 161.20 (C-OH), 164.94 (C=O). **Anal. Calc**. for **$C_{20}H_{14}Cl_2N_2O_3$** (401.24): C, 59.87; H, 3.52; Cl, 17.67; N, 6.98; O, 11.96 **Found:** C, 59.82; H, 3.57; Cl, 17.63; N, 6.95; O, 11.93.

***N*-(4-((5-Bromo-2-hydroxybenzylidene)amino)-2-chlorophenyl)-5-chloro-2-hydroxybenzamide (10)**

Light green powder; yield: (86%); melting point: 290-292ºC. **IR**: unit υ/cm^{-1}: 3410 (NH-amide), 3410, 3255, 3224 (2OH, NH), 3100 (aromatic CH), 1643 (C=O of amide), 1620 (C=N). **^{1}H NMR** (500 MHz, in DMSO-d_6) δ/ppm: 12.72 (s, 1H, OH; D_2O exchangeable), 11.03 (s, 1H, NH; D_2O exchangeable), 8.92 (s, 1H, methine-H), 8.49 (d, J = 7.2 Hz, 1H, aromatic-H), 7.95 (d, J = 9.3 Hz, 1H, aromatic-H), 7.81 (s, 1H, aromatic-H), 7.65 (d J = 8.8 Hz, 1H, aromatic-H), 7.51 (d, J = 5.5 Hz, 1H, aromatic-H), 7.44 (s, 1H, aromatic-H), 7.03 (d, J = 7.1 Hz, 1H, aromatic-H), 6.98 (d, J = 6.8 Hz, 1H, aromatic-H), 6.92 (s, 1H, , aromatic-H), 5.40 (s, 1H, br-OH; D_2O exchangeable). **^{13}C NMR** (125 MHz, in DMSO-d_6) unit δ/ppm: 110.56, 113.38, 119.60, 121.90, 122.59, 123.56, 126.10, 128.10, 130.28, 133.95, 134.40, 133.0, 134.50, 139.50, 145.10, 147.23, 157.0, 157.10, 159.70 (CH=N), 162.40 (C=O). **Anal. Calc**. for **$C_{20}H_{13}BrCl_2N_2O_3$** (480.14): C, 57.57; H, 3.38; Cl, 16.99; N, 6.71; O, 15.34 **Found:** C, 57.52; H, 3.43; Cl, 16.93; N, 6.66; O, 15.39.

3.2. Microbiological evaluation

3.2.1. Agar-diffusion method

Niclosamide esters **4**, **5** and **6** displayed better antimicrobial profile when compared to Niclosamide. On contrary, the Schiff's bases of Niclosamide **9** and **10** elicited weaker antimicrobial activities compared to the parent Niclosamide. Concerning Gram-positive bacteria, compounds **4** and **6** have the best activity against *S. aureus.* Whereas compounds **4** and **5** have the best effect against *B. subtilis*. (**Table 1**).

3.2.2. Minimum inhibition concentration (MICs)

The antibacterial activities of the Niclosamide esters **4, 5** and **6** toward *B. subtilis* were varied according to the type of the ester, it ranged between strong activity as elicited by compound **4** (MIC 1.95 μM) and **5** (MIC 3.90 μM) and mild activity as shown for the compound **6** (MIC 31.25 μM). Particularly, enhanced activity was appeared for the compound **6** (MIC 6.25 μM) toward standard strain of *S. aureus* compared to amoxicillin (MIC 2.0 μM) and mild antibacterial activity for compound **5** (MIC 31. 5 μM) against *P. vulgaris.* All compounds showed poor antibacterial results against standard strain of *E. coli*.

Antibacterial activity against resistant strains, MRSA12 and MRSA10

Niclosamide esters **4, 5** and **6** displayed remarkable activities against the resistant strain of *S. aureus, MRSA12* (MIC ≤ 4.03 μM) **(Table 2)**.

Table 1. The antimicrobial activity (inhibition zone in mm) of the new compounds.

Compound\ Microorganism	**4**	**5**	**6**	**9**	**10**	**Amoxicillin**	**Gentamycin**	**ketoconazole**	**Niclosamide**
S. aureus *ATCC 25923*	12	11	12	8	10	14	-	-	9
B. subtilis *NRRL B-543*	20	18	17	10	9	18	-	-	20
E. coli *ATCC 25922*	10	12	11	9	11	-	30	-	10
P.vulgaris *ATCC 13315*	13	14	12	NA	10	-	25	-	15
Candida albicans	14	13	15	10	11	-	-	20	13
A.fumigatus *(RCMB 002008)*	NA	NA	NA	9	NA	-	-	17	NA

Table 2. The minimum inhibition concentration (MIC) of the new compounds.

Sample code/ Tested microorganism	**4**	**5**	**6**	**9**	**10**	**Niclosamide**	**AMOX/ NYS**
S. aureus	500	500	6.25	500	500	ND	2.00
B. subtilis	1.95	3.90	31.25	250	500	ND	ND
MRSA10	≤ 4.03	125	8.06	125	> 500	≤ 4.03	> 500
MRSA12	≤ 4.03	≤ 4.03	≤ 4.03	125	125	≤ 4.03	> 500
E. coli	500	125	62.5	500	250	ND	8.00
P.vulgaris	250	31.5	500	NA	250	ND	ND
Candida albicans	15.6	7.80	31.25	250	250	ND	1.95
A.fumigatus	ND	ND	ND	500	ND	ND	1.95

3.2.3. The effect on normal cell line (WI-38)[32–34]

The safety profile of the Niclosamide esters **4**, **5** and **6** was *in-vitro* examined via determination of their cytotoxicity on human normal cell line (WI-38). The obtained results displayed high safety margin of them, (CC_{50}: 61.32, 85.11 and 210.19 µM; respectively) **(Fig. 2)**.

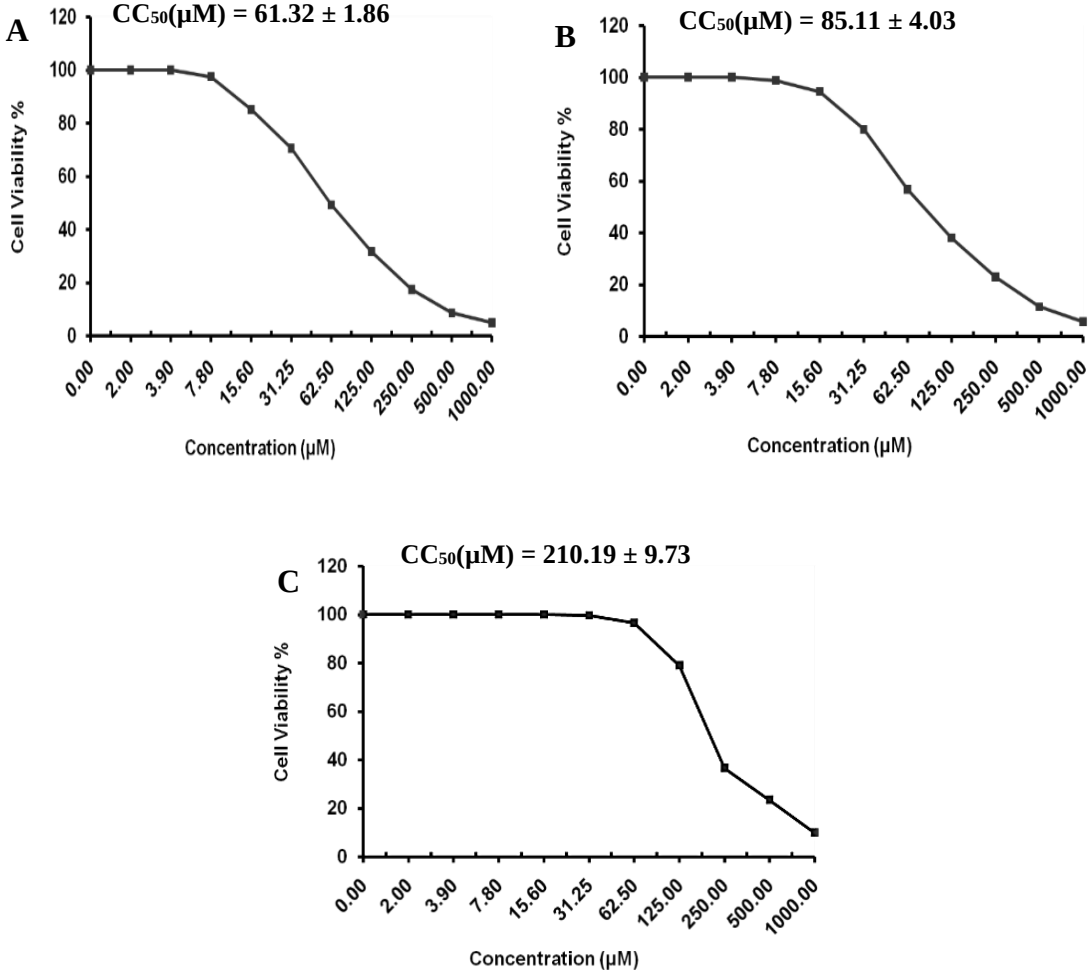


Figure 2. Cytotoxicity of Niclosamide esters against the human WI-38 cell line. (**A**) effect of compound 4 on WI-38 cell line, (**B**) effect of compound 5 on WI-38 cell line, (**C**) effect of compound 6 on WI-38 cell line.

3.3. Molecular modeling studies

MOE 2014 software was used to study the binding interaction of certain Niclosamide derivatives within the known biotin carboxylase enzyme's crystal structure (PDB ID: 2V58). Compounds **4**, **5** and **6** had lower docking score energies (-9.60, -9.75 and -11.50 Kcal/mol) compared to the reference ligand (-7.38 Kcal/mol) and the parent Niclosamide (-8.10 Kcal/mol). Furthermore, its binding mode to the protein was like that of the ligand; it used the pocket, displaying a network of hydrogen bonding interactions within the residue of hinge region. The docking results are tabulated in (**Table 3**) and depicted in (**Figures 3-6**).

Table 3. The docking scores and binding interaction of the target compounds, 4 -6 and 9, 10.

Compound	Docking score (Kcal/mol)	Interacting amino acids (Type of interaction)	Distance (A^0)
Ligand	-7.38	Lys 159 (2H-bond) Lys 202 (H-bond) Glu 201 (H-bond) Leu 204 (2H-bond)	2.01, 2.30 1.78 2.03 2.11, 3.41
Niclosamide	-8.1	Leu 204 (H-bond) Lys 159 (H-bond) His 209 (arene-H)	3.21 2.98 3.98
4	-9.6	Lys 159 (H-bond) Lys 238 (H-bond) Glu 201 (H-bond) Glu 288 (H-bond) Tyr 203 (H-bond) His 236 (arene-H)	3.42 3.0 3.82 3.36 2.96 4.13
5	-9.75	Lys 159 (H-bond) Lys 238 (H-bond) Glu 201 (H-bond) Tyr 203 (H-bond) Met 169 (H-bond) His 236 (arene-H)	3.42 3.02 3.77 2.90 3.30 4.37
6	-11.5	Lys 202 (H-bond) Tyr 203 (H-bond) His 209 (arene-H)	3.61 3.11 3.50
9	-8.4	Leu 204 (H-bond) Glu 288 (H-bond)	3.29 3.20
10	-8.55	Lys 159 (H-bond) Lys 238 (H-bond) His 236 (arene-H)	3.31 3.30 3.90

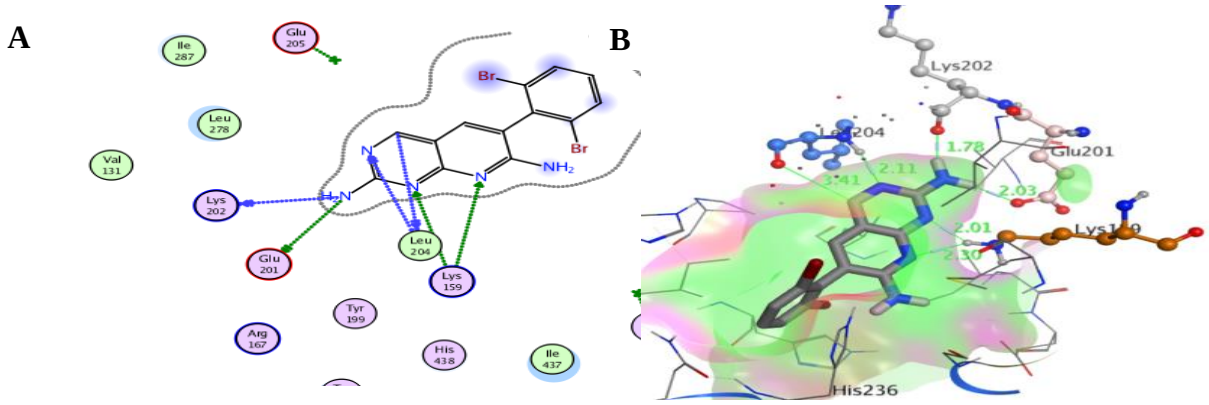


Figure 3. The proposed 2D (**A**) and 3D (**B**) binding interaction of co-crystalized ligand with 2V58.

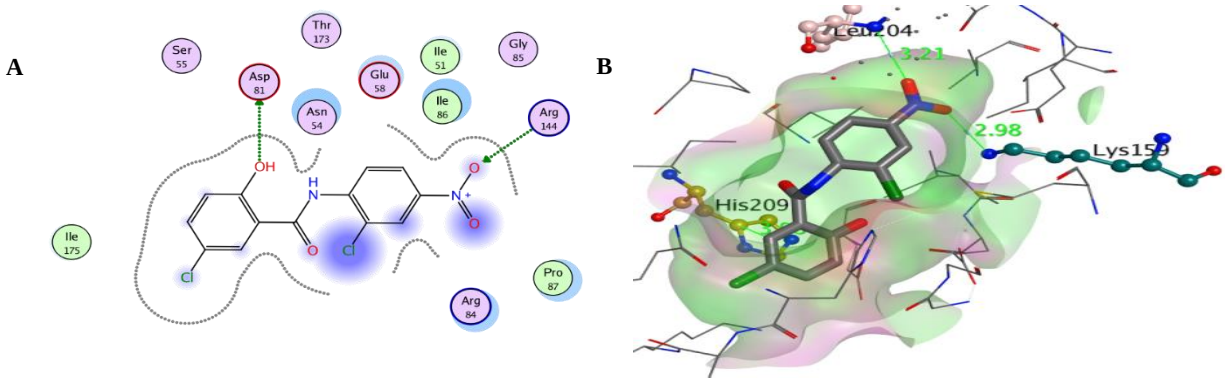


Figure 4. The proposed 2D (**A**) and 3D (**B**) binding interaction of co-crystalized Niclosamide with 2V58.

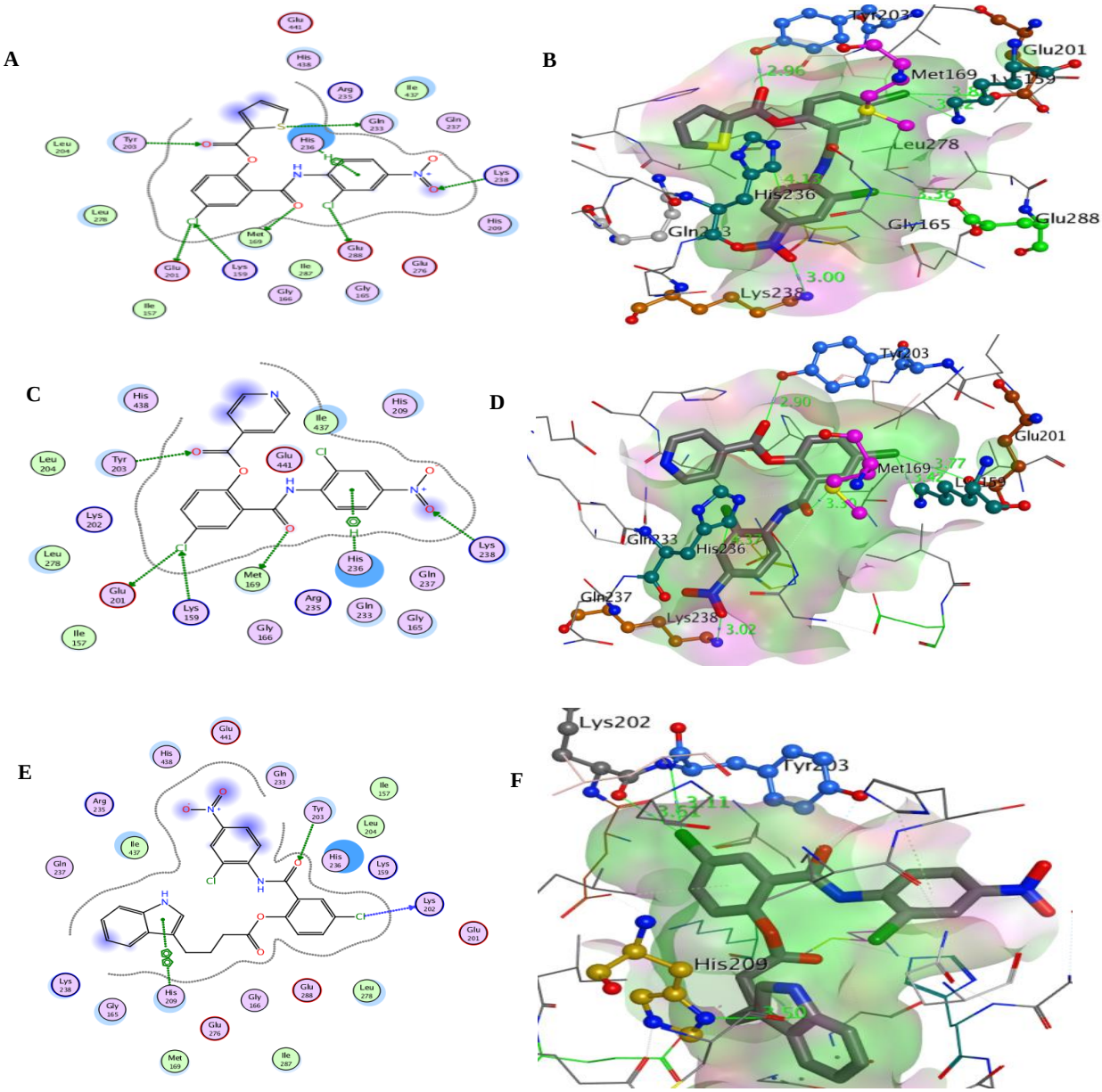


Figure 5. The proposed 2D (**A**) and 3D (**B**) binding interaction of co-crystalized compound **4** with 2V58, the proposed 2D (**C**) and 3D (**D**) binding interaction of co-crystalized compound **5** with 2V58 and the proposed 2D (**E**) and 3D (**F**) binding interaction of co-crystalized compound **6** with 2V58.

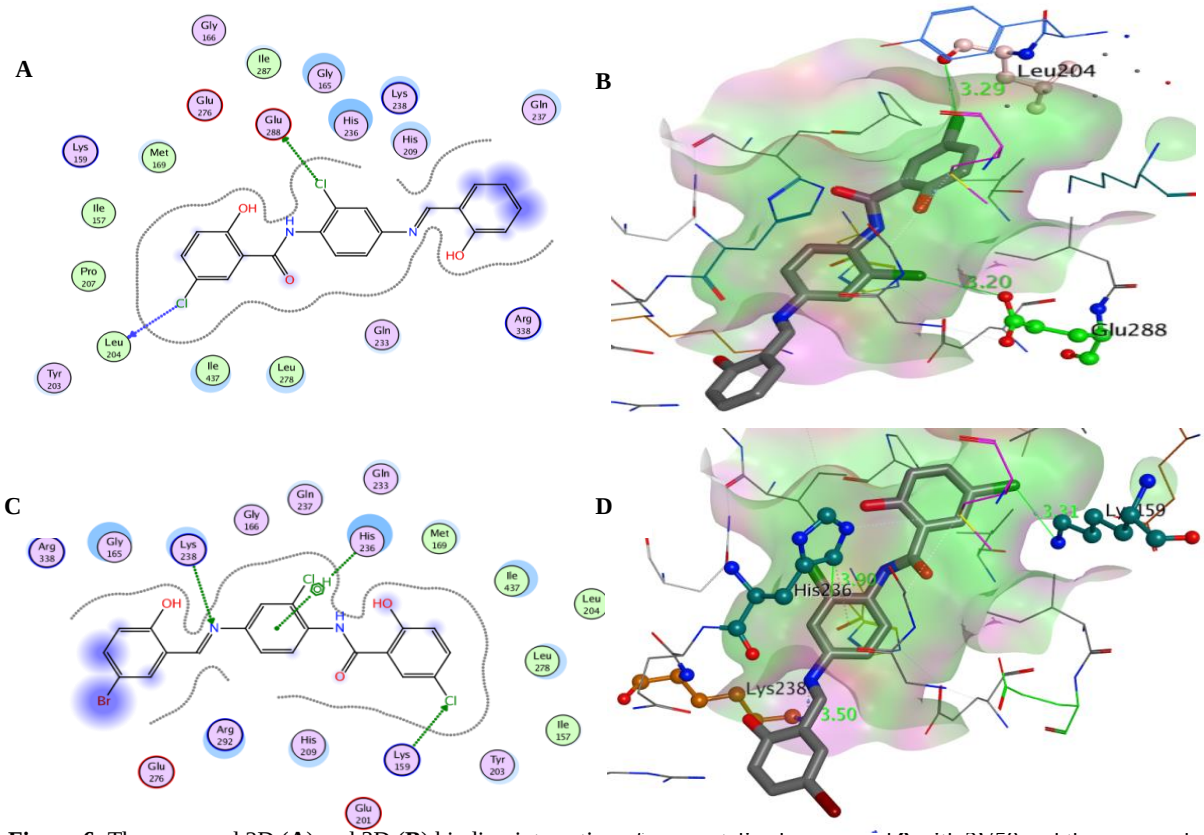


Figure 6. The proposed 2D (**A**) and 3D (**B**) binding interaction of co-crystalized compound **9** with 2V58 and the proposed 2D (**C**) and 3D (**D**) binding interaction of co-crystalized compound **10** with 2V58.

3.4. *In silico* study of physicochemical and pharmacokinetic properties.

In silico ADME data which refer to (absorption, distribution, biotransformation and elimination) provides the initial critical step into handling a drug by the human body. Because some drug candidates lack therapeutic action due to inadequate pharmacokinetic characteristics, evaluating the pharmacokinetic parameters of drug candidates at an primary stage of screening is an important stage in drug development that can lead to better hits[35] and lowering late-stage abrasion in drug development. Lipinski devised a set of parameters that take into account lipophilicity Log P (octanol–water partition), as well as the molecular weight (M Wt), the number of hydrogen bond acceptors (HBA), and the number of hydrogen bond donors (HBD). These concepts were well-considered as a basis for determining physicochemical characteristics with successful oral drug development and prospects. Lipinski's rule states: A drug candidate is regarded orally active if the HBD's number is < 5, HBA's number is < 10, and the MW is less than 500[36]. Moreover, Drug-likeness restraints are defined by Veber rule[37] as rotatable bond count ≤ 10; if a molecule is more flexible, it is less likely to be orally bioactive, whereas TPSA (polar surface area) can be used as an alternative aspect of the number of hydrogen bonding groups. In rats, a TPSA of less than 140 Å^2 would have a good oral bioavailability. To assess the pharmacokinetic parameters of our compounds **4**, **5**, **6**, **9** and **10**, we used swissADME[37] software It was clear that, the physicochemical properties of all the compounds **(Table 4)** have Lipinski zero or one violation so, they are orally bioavailable compounds**.** Moreover, all compounds displayed values of NROTB < 10 except compound 5 which equal 10, and values of TPSA range of 81.92 and 129.46 Å^2 (<140 Å^2), **(Table 4).** Furthermore, using percent ABS = 109 – (0.345 TPSA), it was discovered that the computed ABS percent of all compounds ranged from 64.33 percent to 80.73 percent, implying that these produced derivatives may have the requirements for cell membrane permeability and bioavailability. According to the SwissADME[38] website, the amount of medicine present in the plasma is referred to as bioavailability, and it is often regarded as the most critical factor influencing absorption. Surprisingly, all of the developed compounds had excellent bioavailability[39]**(Table 5).**

Table 4. *In silico* prediction of physicochemical properties of compounds **4**, **5**, **6**, **9** and **10.**

Compound No.	HBD	HBA	M.Wt.	MlogP	Lipiniski's violation	TPSA	No. of rotatable bonds
4	1	5	437.25	4.08	0	129.46	7
5	1	6	432.21	3.37	0	114.11	7
6	2	5	512.34	4.69	1	117.01	10
9	3	4	401.25	4.59	0	81.92	5
10	3	4	480.14	5.21	1	81.92	5

Table 5. *In silico* prediction of pharmacokinetic properties of compounds **4, 5, 6, 9** and **10.**

Compound No.	GIT absorption	BBB permeability	Bioavailability score	PAINS alert
4	Low	No	0.55	0
5	High	No	0.55	0
6	Low	No	0.55	0
9	High	No	0.55	0
10	High	No	0.55	0

4. DISCUSSION

The corresponding Niclosamive derivatives were obtained by refluxing compound **2** with formyl derivatives (3a-c). According to elemental analysis and spectrum data, the produced derivatives' structures were deduced. The IR spectral data of compound **4** showed disappearance of hydroxyl moiety absorption band and the existence of absorption peaks at υ 3244, 3097, 3082, 1747, 1681 cm^{-1} for NH, C=O of ester, C=N & of C=O amide moieties. Its 1HNMR data showed three signals at δ 8.09, 8.03 and 7.25 ppm for thiophen protons. 13C NMR analysis showed three signals at δ 134.2, 133.7, 128.3 for thiophen carbons. Additionally, the structure of compound (**9**) was assigned by elemental analysis and spectroscopic data. Its IR spectrum presented the existence of absorption peak at ν 3439 (2OH-phenolic), 3285, 3091, 1675, 1623 cm^{-1} for 2 OH, NH, CH. aromatic, C=N, C=O group; respectively. Its 1HNMR analysis demonstrated new exchangeable singlet (S) signal for phenolic protons at δ 7.01 ppm and new singlet signal at δ 8.92 ppm due to methine protons (H+) together with signals because of aromatic protons. Additionally, its 13C NMR data displayed signals at δ 159.70 ppm for CH=N, δ 162.40 ppm for C=O. as well as aromatic carbons in the range of δ 110.56-157.10 ppm.

Niclosamide esters **4**, **5** and **6** showed better antimicrobial profile when compared to Niclosamide. On the other hand, the Schiff's bases **9** and **10** have weaker antimicrobic activities compared to the parent Niclosamide. Concerning Gram-positive bacteria. The results showed that MIC value of compound **4** against B. subtilis was 1.95 µM while its CC50 against the human normal cell line (WI-38) was 61.32 µM. which means that it is effective against *B. subtilis* at dose thirty folds less than its cytotoxic concentration on the normal cell line. The MIC value of compound 6 against *S. aureus* was 6.25 µM while its CC50 against the human normal cell line (WI-38) was 210.19 µM. which means that it is effective against *B. subtilis* at dose thirty folds less than its cytotoxic concentration on the normal cell line Niclosamide esters **4**, **5** and **6** displayed remarkable activities against the resistant strain of *S. aureus* MRSA12 (MIC ≤ 4.03 µM). They

showed high docking score and better interactions with biotin carboxylase enzyme than compounds **9** and **10**.

Compound **5** has high GIT absorption, but compounds **4** and **6** have not. All Niclosamide esters don't have CNS side effects because they are unable permeate the blood brain barrier.

5. CONCLUSIONS

The existing study designates the synthesis of Niclosamide analogues as potential antimicrobial agents. Noticeably, the esters **4, 5** and **6** exhibited significant antimicrobial potential against tested bacteria and fungi than that for Schiff bases **9** and **10.** Niclosamide esters, **4** and **5** displayed strong antibacterial activity toward *B. subtilis.* Moreover, compounds **4, 5** and **6** displayed remarkable activities against the resistant strain of *Staphylococcus aureus (MRSA12)*, however they elicited moderate antifungal activities against *C. albicans*. The target compounds **4-6** also displayed safety profiles in cytotoxicity assay. They demonstrated binding patterns that were like those reported for most biotin carboxylase inhibitors in the docking studies. *In silico* study revealed that compound **5** had not only good antimicrobial activity but also acceptable pharmacokinetics and drug likeness properties. Thus, it could be well-thought-out as a promising starting point for additional modification and development in our future work.

Funding: This research received no external funding.

Acknowledgments: The authors would like to extend their sincere appreciation to all members of pharmaceutical chemistry department (Al-Azhar University (girls).

Conflicts of Interest: The authors declare no conflicts of interest.

Author Contribution: all authors reviewed the literature, drafted the manuscript, critically revised, and approved the final version before submission. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. Tamer N. Samir B. Tamer MI. et al. new acrylamide-sulfisoxazole conjugates as dihydropteroate synthase inhibitors. Bioorg. Med. Chem. Volume 28, Issue 9, 1 May (2020) 115444

2. Yasser MI. Ahmed MA. Tamer N. et al. Antibacterial and anti-quorum sensing activities of a substituted thiazole derivative against methicillin-resistant Staphylococcus aureus and other multidrug-resistant bacteria. Microb. Pathog. 149 (2020) 104500

3. Talan DA. Citron DM. Abrahamian FM. et al. Bacteriologic analysis of infected dog and cat bites. Emergency Medicine Animal Bite Infection Study Group. N Engl J Med. 1999; 340:85-92. Peleg AY, Hooper DC. Hospital-Acquired Infections Due to Gram-Negative Bacteria. N Engl J Med. (2010) 362(19).

4. Heath RJ. White SW. Rock CO. Lipid biosynthesis as a target for antibacterial agents. Vol. 40, Progress in Lipid Research. (2001).

5. Cronan JE Jr. Waldrop GL. Multi-subunit acetyl-CoA. Progress in Lipid Research 41 (2002) 407–435

6. Liang T. and H. James HJr. Acetyl-Coenzyme A Carboxylases: Versatile Targets for Drug Discovery. J. Cell. Biochem. (2006) 99:1476–1488

7. Mook RA, Wang J. Ren XR. Chen M. Spasojevic I, Barak LS, et al. Structure-activity studies of Wnt/β-catenin inhibition in the Niclosamide chemotype: Identification of derivatives with improved drug exposure. Bioorg. Med Chem. (2015) 23(17).

8. Garcia C. Burgain A. Chaillot J. et al. A phenotypic small-molecule screen identifies halogenated salicylanilides as inhibitors of fungal morphogenesis, biofilm formation and host cell invasion. Sci Rep. (2018) 8(1).

9. Corsello SM. Bittker JA. Liu Z. et al. The Drug Repurposing Hub: A next-generation drug library and information resource. (2017) Vol. 23, Nature Medicine.

10. Ye T. Xiong Y. Yan Y. et al. The anthelmintic drug niclosamide induces apoptosis, impairs metastasis and reduces immunosuppressive cells in breast cancer model. PLoS One. (2014) 9(1).

11. Rajamuthiah R. Fuchs BB. Conery AL. et al. Repurposing salicylanilide anthelmintic drugs to combat drug resistant

Staphylococcus aureus. PLoS One. (2015) 10(4).

12. Huang, C. Wang, Y. Li, X. et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet. (2020) 395, 497-506

13. Tao H. Zhang Y. Zeng X. et al. Niclosamide ethanolamine-induced mild mitochondrial uncoupling improves diabetic symptoms in mice. Nat Med. (2014) 20:1263–1269.

14. Pic E. Burgain A. Sellam A. Repurposing the anthelminthic salicylanilide oxyclozanide against susceptible and clinical resistant Candida albicans strains. Med Mycol. (2019) 57(3).

15. Gwisai T, Hollingsworth NR, Cowles S, Tharmalingam N, Mylonakis E, Fuchs BB, et al. Repurposing niclosamide as a versatile antimicrobial surface coating against device-associated, hospital-acquired bacterial infections. Biomed Mater. 2017;12(4).

16. Krátký M. Vinšová J. Novotná E. et al. Antibacterial activity of salicylanilide 4-(trifluoromethyl)benzoates. Molecules. (2013) 18(4).

17. Pauk K. Zadražilová I. Imramovský A. et al. new derivatives of salicylamides: Preparation and antimicrobial activity against various bacterial species. Bioorganic Med Chem. (2013) 21(21).

18. Tharmalingam N, Port J, Castillo D, Mylonakis E. Repurposing the anthelmintic drug niclosamide to combat Helicobacter pylori. Sci Rep. (2018) 8(1).

19. Weinbach EC. Garbus J. Mechanism of action of reagents that uncouple oxidative phosphorylation. Nature. (1969) 221(5185).

20. Yurui J. Chenglong Ch. Xijian H. et al. Niclosamide and its derivative DK-520 inhibit RANKL-induced osteoclastogenesis. FEBS Open Bio. (2020) 10(8).

21. Haijun Ch. Zhengduo Y. Chunyong D. et al. Discovery of O-alkylamino-tethered niclosamide derivatives as potent and orally bioavailable anticancer agents. ACS Med Chem Lett. (2013) 4(2).

22. ElKihel L. Loiseau PM. Bourass J. et al. Synthesis and orally macrofilaricidal evaluation of niclosamide lymphotropic prodrugs. Arzneimittel-Forschung/Drug Res. (1994) 44(11).

23. Navab M. Ruchala P. Waring AJ. et al. A novel method for oral delivery of apolipoprotein mimetic peptides synthesized from all L-amino acids. J Lipid Res. (2009) 50(8).

24. Mansbach, C. M., and R.F. Uptake and metabolism of circulating fatty acids by rat intestine. Am. J. Physiol. (1992) 263: G927–G933

25. Ben HT. Vandavasi JK. Shkoor M. et al. A Cross-Coupling Approach to Amide Bond Formation from Esters. ACS Catal. (2017) 7(3).

26. Tasqeeruddin S. Asiri YI. Shaheen S. NH4Cl/Zn powder: An efficient, chemoselective reducing catalyst for the microwave-assisted synthesis of 2,3-disubstituted quinolines via tandem Knoevenagel condensation. J Heterocycl Chem. (2021) 58(2).

27. Lejarazo GEF. Santos SE. Suarez TS. Reduction of Nitro Compounds, Through different Reaction Conditions (Combinatory Chemistry). J Chem Chem Eng. (2018) 12(2).

28. Bonev B. Hooper J. Parisot J. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. J Antimicrob Chemother. (2008) 61, 1295–1301

29. Pearson RD. Steigbigel RT. Davis HT. et al. Method for reliable determination of minimal lethal antibiotic concentrations. Antimicrob Agents Chemother. (1980) 18(5).

30. Miller JR. Dunham S. Mochalkin I. et al. A class of selective antibacterials derived from a protein kinase inhibitor pharmacophore. Proc Natl Acad Sci U S A. (2009) 106(6).

31. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods. (1983) 65(1–2).

32. Abd-El-Aziz AS. El-Ghezlani EG. Elaasser MM. et al. First Example of Cationic Cyclopentadienyliron Based Chromene Complexes and Polymers: Synthesis, Characterization, and Biological Applications. J Inorg Organomet Polym Mater. (2020) 30(1).

33. Gomha SM. Riyadh SM. Mahmmoud EA. Et al. Synthesis and anticancer activities of thiazoles, 1,3-thiazines, and thiazolidine using chitosan-grafted-poly(vinylpyridine) as basic catalyst. Heterocycles. (2015) 91(6).

34. Can NÖ. Osmaniye D. Levent S. et al. Design, synthesis and biological assessment of new thiazolylhydrazine derivatives as selective and reversible hMAO-A inhibitors. Eur J Med Chem. (2018) 144.

35. Rajanarendar E. Rama KS. Nagaraju D. et al. Environmentally benign synthesis, molecular properties prediction and anti-inflammatory activity of novel isoxazolo[5,4-d] isoxazol-3-yl-aryl-methanones via vinylogous Henry nitroaldol adducts as synthons. Bioorganic Med Chem Lett. (2015) 25(7).

36. Veber DF. Johnson SR. Cheng HY. Et al. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem. (2002) 45(12).

37. PreADMET | Prediction of ADME/Tox – Just another BMDRC Sites site, (n.d.). https://preadmet.bmdrc.kr/ (accessed June 15, 2020)

38. Daina A. Michielin O. Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. (2017) 7, 42717

39. Zhao YH. Abraham MH. Le J. et al. Rate-limited steps of human oral absorption and QSAR studies. Pharm Res. (2002) 19(10).