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SYNTHESIS, ANTIMICROBIAL AND COMPUTATIONAL STUDIES OF NEW BRANCHED AZAPHENOTHIAZINONES DERIVATIVES

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Abstract. In a continued search for new medicinally active nonlinear phenothiazines, novel angular chloro-azaphenothiazinone derivatives have been synthesized *via* transition metal-catalyzed cross-coupling reactions. The structural elucidation of the synthesized compounds was established by a combined spectroscopic and elemental analysis. The synthesized compounds were tested for their antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Enterococus faecalis, Escherichia coli, Candida albican*, and *Aspergillus niger* isolates by the convectional agar-well dilution method and compounds **5c** and **8c** disclosed excellent *in vitro* activity against some of the tested microorganisms. *In silico,* the study showed that the synthesized compounds possessed promising physicochemical properties and fit well in the active site of a Biotin-Protein Ligase (BPL) forming essential hydrogen bonding and hydrophobic interactions.

Keywords: phenothiazine, Buchwald-Hartwig cross-coupling, antimicrobial, *in silico, in vitro*.

1. Introduction

Numerous applications of phenothiazine heterocycles and their derivatives as drugs have stimulated the continued study of this class of organo-sulfur compounds.[1-2] In medicine, phenothiazine derivatives possess a wide range of biological activities including antibacterial,[3] antifungal,[4] antipsychotic,[5] anti-inflammatory,[6] anti-parkinsonian,[7] anti-tubercular,[8] anticonvulsant,[9] and cardiovascular[10] activities. In addition to the main neuroleptic action of phenothiazine family, their antitumor activities are profusely reported.[11-14] The increasing reports of bacterial and fungal resistance against currently available antibiotics have necessitated the continued search for novel drug candidates amongst the phenothiazine and phenoxazines.[15-16] One of the usual methods of obtaining benzo[a]phenothiazine parent molecule is by anhydrous base-catalyzed coupling of 2,3-dichloro-1,4-naphthoquinone with aminothiophenol.[17-19] Recently, we reported the synthesis of novel derivatives of angular azaphenoxazinone and angular azaphenothiazinones with a promising antimicrobial activity.[20] They were prepared by a condensation reaction between 2-6-diamino-4-chloropyrimidine-5-thiol with 7-chloro-5,8-quinolinequinone in the presence of anhydrous sodium carbonate followed by the conversion into their derivatives *via* palladium(o)/piperazine ligand, utilizing Mizoroki–Heck cross coupling reaction.

In continuation of our search for novel potent phenothiazinones, we herein report the synthesis of angular aza chlorophenothiazinone *via* Buchwald-Hartwig protocol and the evaluation of their antimicrobial properties. Molecular docking has also been carried out to rationalize the *in vitro* results.

2. Experimental

2.1 General Information

Starting materials and reagents were of analytical grades and were purchased from Sigma-Aldrich chemical company. Melting points were determined with a Fischer-Johns melting point apparatus and were uncorrected. UV-visible and IR spectra were recorded on UV2500PC series using matched 1cm quartz cells and on a Shimadzu FTIR-8400s Fourier Transform Infrared (KBr pellets) respectively at National Research Institute Chemical Technology Zaria. Nuclear Magnetic Resonance (^{1}HNMR and ^{13}CNMR) spectra were determined using a Bruker AV-400 spectrometer and a JEOL-JNM LA-400 spectrometer at Natural Products Research Group, Strathclyde Institute of Pharmacy and Biomedical Science, University of Strathclyde, Glasglow, Scotland. All chemical shifts were recorded on the δ-scale (neat) and coupling constants

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(J) reported in hertz. The antimicrobial screening was done at the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

2.2. 10-amino-6,8-dichloro-5H-naphtho[2,1-b]pyrimido[4,5-e][1,4]thiazin-5-one, 3

2,6-Diamino-4-chloropyrimidine-5-thiol **1** (0.88 g, 5 mmol), sodium carbonate anhydrous (1.06 g, 10 mmol), benzene (40 mL) and dimethylformamide (DMF)(5 mL) were charged into 100 mL three-necked flask fitted with a short magnetic stirring bar and a reflux condenser. The mixture was stirred while heating on a water-bath at 70–75 ^{o}C for 45 min. Then 2,3–dichloro–1,4 naphthoquinone **2** (1.14 g, 5 mmol) was added and the mixture was stirred at the same temperature for 6 h. The colour of the reaction mixture changed from bright red, to reddish-brown and finally to an intense red product as the reaction progressed. At the end of 6 h reflux, the mixture was filtered to obtain 10-amino-6,8-dichloro-5*H*-naphtho[2,1-b]pyrimido[4,5-e][1,4]thiazin-5-one **3**. Reddish-brown powder, yield=1.35 g (85%), mp. 188–190 ^{o}C (dec.). UV-Vis (Methanol) λ_{max} (nm): 473 nm (3.086). IR (KBr) (cm^{-1}): 3100 cm^{-1} (C-H aromatic), 1682 (C=O), 1568 (C=C), 887. ^{1}HNMR (400 MHz, DMSO-d_6): δ=8.09-7.67 (4H, m, Ar-H), 6.50 (2H, s, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 178.4 (C = O), 168.8, 164.6, 162.9 (C=N), 146.1, 143.8, 138.5, 136.9, 135.3, 131.1, 124.7, 121.3.

2.3. The general procedure for Buchwald-Hartwig nickel−catalyzed synthesis of 10-amino-6,8-dichloro-5H-naphtho[2,1-b]pyrimido[4,5-e][1,4]thiazin-5-ones, 4a-e

Nickel complex $NiCl_2(PPh_3)_2$ (0.019 g, 0.03 mmol), triphenylphosphine, PPh_3 (0.016 g, 0.06 mmol) and K_3PO_4. $3H_2O$ (0.692 g, 2.6 mmol) were charged into a flask equipped with a magnetic stirring bar and a reflux condenser. After thorough flushing the flask with nitrogen gas, it was charged with tertiary butanol (2 mL) and water (1 mL) followed by the addition of a mixture of phenothiazinone **3** (1.0 mmol) and amides (1.4 mmol). The resulting mixture was stirred under reflux for 3 h at 110 ^{o}C. At the end of the reaction, the solvent was evaporated and the desired product was recrystallized from aqueous acetone to obtain derivatives **4a-e.**

2.3.1. 6-acetamido-10-amino-8-chloro-9,11-diaza-5*H*-benzo[a]phenothiazin-5-one, 4a

Brown solid, yield=0.32 g (76%), m.p. 140–142 ^{o}C (dec.). UV-Vis (Methanol) λ_{max} (nm): 331 (2.324), 662.5 (0.200). IR (KBr) (cm^{-1}): 3406 (N-H), 3201 (C-H aromatic), 1674 (C=O), 1591 (C=C), 709. ^{1}HNMR (400 MHz, DMSO-d_6): δ=7.72-8.07 (4H, m, Ar-H) 9.32 (1H, s, CONH); 6.70 (2H, s, NH_2), 1.76 (3H, s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): 178.4, 175.4 (C = O), 164.1, 162.5 (C=N), 146.2, 136.4, 135.3, 131.0, 131.3, 124.7, 126.4, 121.5. Elemental Analysis (%) calcd (Found) $C_{16}H_{10}N_5ClSO_2$: C=54.99(54.59), H=3.00 (2.60), N=19.24 (19.04), S=7.34 (7.04).

2.3.2. 6-(10-amino-8-chloro-5-oxo-5H-naphtho[2,1-b]pyrimido[4,5-e][1,4]thiazin-6-yl)benzamide, 4b

Brown solid, yield=0.30 g (78%), m.p. 100–102 ^{o}C. UV-Vis (Methanol) λ_{max} (nm): 328 (2.875) and 367 (5.00). IR (KBr) (cm^{-1}): 3369 (N-H), 3174 (C-H aromatic), 1665 (C=O), 1572 (C=C), 696, ^{1}HNMR (400 MHz, DMSO-d_6): δ=9.78 (1H, s, CONH), 7.62- 8.03 (8H, m, Ar-H), 6.75 (2H, s, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 178.8, 175.3 (C=O), 166.3, 162.9 (C=N), 146.3, 136.1, 134.5, 133.2, 132.1. 131.3, 131.2, 131.1, 128.9, 127.4, 126.4, 124.6. Elemental Analysis (%) calcd (Found) $C_{21}H_{12}N_5ClSO_2$: C=55.24 (54.84), H=2.55 (2.15), N=19.33 (19.30), S=7.37 (7.30).

2.3.3. 6-(10-amino-8-chloro-5-oxo-5H-naphtho[2,1-b]pyrimido[4,5-e][1,4]thiazin-6-yl)-4-nitrobenzamide, 4c

Brown solid, yield=0.37 g (71%), m.p. 115–117 ^{o}C. UV-Vis (Methanol) λ_{max} (nm): 367 (5.00). IR (KBr) (cm^{-1}): 3329 (NH), 3070 (C-H aromatic), 1681 (C=O), 1591 (C=N), 1336 (N=O), 709. ^{1}HNMR (400 MHz, DMSO-d_6): δ=9.29 (1H, s, CONH), 7.58-8.55 (8H, m, Ar-H), 6.71 (2H, s, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 176.3, 175.6 (C=O), 166.2, 164.5, 162.8 (C=N), 151.3, 146.4, 136.3, 134.5, 131.0, 131.2, 131.5, 126.4, 129.4, 124.0, 122.4, 109.7. Elemental Analysis (%) calcd (Found): $C_{21}H_{11}N_6ClSO_4$. C=50.06 (49.66), H=2.10 (1.70), N=20.43 (20.03), S=6.68 (6.28).

2.3.4. 6-(10-amino-8-chloro-5-oxo-5H-naphtho[2,1-b]pyrimido[4,5-e][1,4]thiazin-6-yl)-2-hydroxybenzamide, 4d

Dark brown solid, yield=0.35 g (78%), m.p. 105–107 ^{o}C. UV-Vis (Methanol) λ_{max} (nm): 367 (4.971). IR (KBr) (cm^{-1}): 3425 (OH), 3369 (NH), 3176 (C-H aromatic), 1678 (C=O), 1544 (C=C), 709. ^{1}HNMR (400 MHz, DMSO-d_6): δ=10.13 (1H, s, OH), 9.30 (1H, s, CONH), 6.70-8.09 (8H, m, Ar-H), 6.50 (2H, s, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 177.3, 175.5 (C=O), 164.5, 163.8 (C=N), 159.4, 146.2, 136.3, 134.5, 133.4, 131.5, 131.2,

131.1, 128.9, 126.4, 124.7, 122.6, 121.2, 119.7, 117.9, 109.5. Elemental Analysis (%) calcd (Found): $C_{21}H_{12}N_5ClSO_3$ C=58.14 (57.74), H=2.79 (2.39), N=16.14 (16.54), S=7.39 (7.09).

2.4. General procedure for copper(II)-catalyzed N-Arylation for the synthesis of derivatives 8a-c and 5a-c

The copper(II)-catalyzed *N*-Arylation reaction was carried out following the Yamamoto protocol.[21-23] A mixture of potassium aryltriolborate (0.366 g, 1.5 mmol), copper(II)acetate, $Cu(OAc)_2$, (0.018 g, 0.10 mmol), trimethylamine *N*-oxide, Me_3NO (0.083 g, 1.1 mmol), potassium phosphate K_3PO_4 (0.030 g, 0.14 mmol) and powdered molecular sieves, 4Å (0.300 g) in a toluene solvent (6.0 mL) was stirred for 5 min at room temperature. Phenothiazin-5-one **3** or **7** (1.0 mmol) was then added. The resulting mixture was stirred for 20 h at room temperature. The crude mixture was filtered and the filtrate was concentrated to obtain the pure products after recrystallization from aqueous ethanol.

2.4.1. 8-Chloro-10-(phenylamino)-1, 9, 11-triaza-5*H*-benzo[a]phenothiazin-5-one, 8a

Yield 0.25 g (64%), m.pt. 245–247 °C. (dec.). UV – Vis (Methanol) λ_{max} (nm): 267 (4.244) 325 (0.567) 338 (0.570) 437 (1.617). IR (KBr) (cm^{-1}) 3232 (NH), 1604 (C=O), 1585 (C=N), 749. ^{1}HNMR (400 MHz, DMSO-d_6): δ=9.33 (1H, s, NH), 8.81-7.02 (9H, m, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6): 180.2 (C=O), 172.3, 168.8, 165.6 (C=N), 154.9, 154.5, 146.2, 138.9, 133.9, 133.0, 129.5, 121.4, 117.5. Elemental Analysis (%) calcd (Found) C=58.09 (57.69), H=2.82 (2.42), N=17.83 (17.43), S=8.16 (7.76).

2.4.2. 8-Chloro-10-((3-chlorophenyl)amino)-1,9,11-triaza-5*H*-benzo[a]- phenothiazin-5-one, 8b

Yield=0.15 g (35%), mp 228–230 °C (dec.). UV – Vis (Methanol) λ_{max} (nm): 251 (2.005) 419 (0.137). IR (KBr) (cm^{-1}) 3325 (NH), 3124 (C–H aromatic), 1708 (C=O), 1583 (C=C), 761 (C–Cl). ^{1}HNMR (400 MHz, DMSO-d_6): δ=9.41 (1H, s, NH), 8.90-6.98 (8H, m, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6): 180.2 (C=O), 174.3, 169.8, 165.0 (C=N), 157.9, 156.5, 144.2, 135.9, 133.5, 132.0, 127.5, 125.4, 127.0. Elemental Analysis (%) calcd (Found) C=53.52 (53.12), H=2.11 (2.50), N=16.43 (16.03), S=7.51 (7.11).

2.4.3. 10-((4-bromo-phenyl)amino)-8-chloro-1,9,11-triaza-5*H*-benzo- [a]phenothiazin-5-one, 8c

Yield=0.12 g (25%), mp 178–180 °C. UV – Vis (DMSO) λ_{max} (nm): 320 (0.245) 326 (0.244) 337 (0.237) 430 (0.412**).** IR (KBr) (cm^{-1}) 3338 (N – H), 3156 (C–H, aromatic), 1681 (C=O) 1587 (C=C), 634. ^{1}HNMR (400 MHz, DMSO-d_6): δ=9.45 (1H, s, NH), 8.93-7.70 (3H, m, Ar–H), 7.38 (2H, d, J=7.15 Hz), 6.69-7.02 (3H, m, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6): 181.2 (C=O), 172.4, 168.0, 164.1 (C=N), 154.7, 154.1, 145.2, 137.7, 135.0, 133.9, 133.0, 126.5, 118.5, 117.5, 116.5. Elemental Analysis (%) calcd (Found) C=48.46 (48.06), H=1.91 (2.30), N=14.88 (14.48), S=6.80 (6.40).

2.4.4. 8-chloro-10-(phenylamino)-5H-naphtho [2,1-b]pyrimido[4,5-e][1,4]thiazin-5-one, 5a

Yield=0.26 g (64%), m.p 230–232 °C. (dec.). UV – Vis (DMSO) λmax (nm): 269 (5.00) 463 (0.45) 755 (0.05). IR (KBr) (cm^{-1}) 3414 (NH), 3178 (C–H aromatic), 1658 (C=O), 1567 (C=N), 655. ^{1}HNMR (400 MHz, DMSO-d_6): δ=9.43 (1H, s, NH), 8.03-7.66 (5H, m, Ar-H), 7.73-7.02 (5H, m, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6): 182.0 (C=O), 175.8, 168.9, 164.3 (C=N), 138.9, 136.7, 135.5, 134.7, 131.6, 131.3, 129.2, 126.4, 124.7, 122.4, 117.8, 117.4. Elemental Analysis (%) calcd (Found) C=56.47 (56.87), H=2.35 (2.70), N=13.18 (12.75), S=7.53 (7.13).

2.4.5. 8-chloro-10-((3-chlorophenyl)amino)-5H-naphtho[2,1-b]pyrimido[4,5-e][1,4]thiazin-5-one, 5b

Yield=0.10 g (40%), m.p. 189–191 °C (dec.). UV – Vis (Methanol) λmax (nm): 260 (0.34) 307 (0.16) 312 (0.15) 326 (0.15) 424 (0.13). IR (KBr) (cm^{-1}) 3371 (N–H), 3145 (C–H aromatic), 1683 (C=O), 1589 (C=C), 727. ^{1}HNMR (400 MHz, DMSO-d_6): δ=9.42 (1H, s, NH), 8.00-7.67 (5H, m, Ar-H), 7.51-6.98 (4H, m, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6): 182.3 (C=O), 175.4, 169.1, 164.5 (C=N), 146.2, 143.7, 136.2, 135.1, 134.5, 131.1, 130.9, 126.5, 124.6, 122.3, 117.5, 116.7, 115.7. Elemental Analysis (%) calcd (Found) C=52.25 (52.65), H=1.97 (2.35), N=12.19 (12.09), S=6.97 (6.56).

2.4.6. 10-((4-bromophenyl)amino)-8-chloro-5H-naphtho[2,1-b]pyrimido[4,5-e][1,4] thiazin-5-one, 5c

Yield=0.20 g (25%), mp 178–180 °C. (dec.). UV – Vis (Methanol) $^{\lambda}$max (nm): 267 (4.73) , 396 (0.33). IR (KBr) (cm^{-1}) 3230 (NH), 3123(C–H aromatic), 1601 (C = O), 1583 (C=C), 761. ^{1}HNMR (400 MHz, DMSO-d_6): δ=9.35 (1H, s, NH), 8.03-7.68 (4H, m, Ar–H), 7.98 (1H, s, Ar–H), 7.39 (2H, d, J=7.15), 7.03 (2H, d, J=7.25). ^{13}C NMR (100 MHz, DMSO-d_6): 182.2 (C=O), 175.6, 168.5, 164.6 (C=N), 146.1, 137.6, 136.3, 135.8, 134.4, 132.6, 131.1, 118.5, 117.5, 116.4. Elemental Analysis (%) calcd (Found) C=56.47 (56.87), H=2.35 (2.70), N=13.18 (12.75), S=7.53 (7.13).

2.5. Antimicrobial evaluation of the synthesized compounds

The antimicrobial evaluation was carried out using the agar-well dilution method.[24-25] Fresh and pure clinical isolates of *Bacillus subtillis, staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Candida albicans* and *Aspergilus niger* obtained from the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, were used for the tests. Stock solutions of the respective synthesized compounds were prepared by initially dissolving 0.04 g in 2 mL of dimethyl sulphoxide, DMSO, to obtain stock solutions of concentration 20 mg/mL each. From the stock solution, concentration of 10, 5, 2.5, 1.25, 0.125 and 0.3125 mg/mL were prepared by a serial dilution. Inoculation of the prepared agar plates with the organism was done using a wire loop to transfer a strand of the organism into the plate followed by a cross-streaking with the same wire loop to achieve uniform spread on the plate. The bores (8 mm in diameter) were aseptically made in the plates using a sterilized cork borer. A synthesized compound of known concentration was introduced into the well using a sterilized syringe. The plates were incubated at 37 ^{o} C for bacteria and 25 ^{o}C for fungi for 24 h. At the expiration of the time the plates were examined for inhibition zones and the observed zones were measured and recorded in millimeters.

2.5.1. Determination of Minimum Inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using the agar-well dilution method. A set of six capped small test tubes was used for each synthesized sample against each organism. Nutrient agar solution was prepared according to the level of turbidity of the solution in the test tubes. The test tube containing the solution with the lowest concentration of the sample that produced a clear solution was taken and recorded as the MIC of the synthesized sample. The screening effect of the synthesized compounds was compared with the standard drugs, ciprofloxacin and ketoconazole.

2.6. ADMET screening of the synthesized compounds

The online open access molinspiration (www.molinspiration.com) was used to calculate the physiochemical properties used to evaluate the drug-likeness of the synthesized compounds. The molecular descriptors calculated include molecular weight, partition coefficient, hydrogen bond acceptor, hydrogen bond donor, topological polar surface area, number of rotatable bonds and volume.

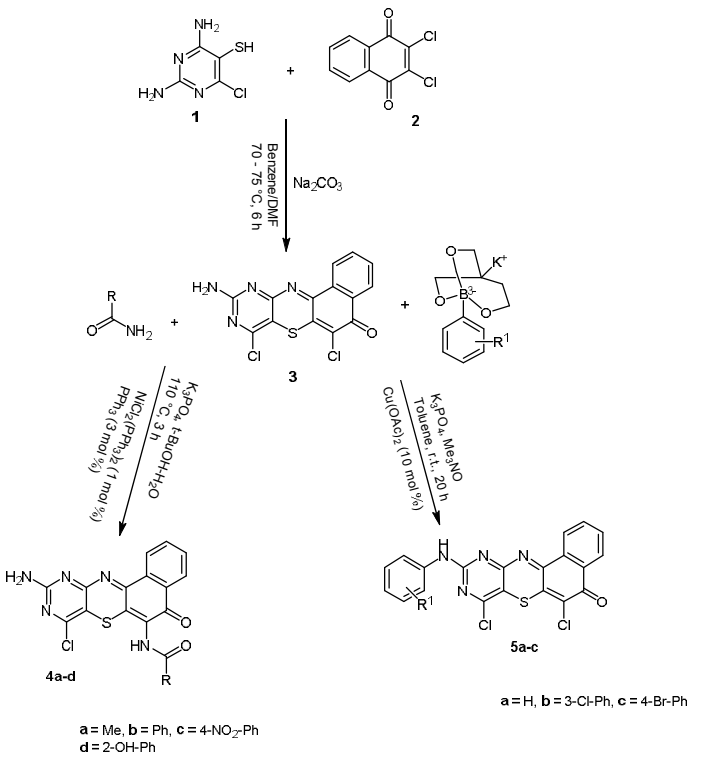
2.6.1. Molecular Docking study

Auto Dock permits the understanding of the molecular interactions between a ligand and a receptor in terms of free binding energy and bonding interactions. Molecular docking was carried out to determine the binding energy and bonding interactions of the synthesized compounds against a Biotin-Protein Ligase (BPL) (PDB: 3V7R). The structure of the target protein was retrieved from the protein data bank and prepared using BIOVIA Discovery Studio 2017 R2 version 17.2.0.16349. The preparations included deleting multiple chains, the water of crystallization, energy minimization and the binding site. The grid box size of the binding site was determined by checking the binding site attributes. The structures of the standards were downloaded from DrugBank. The synthesized compounds were drawn using ChemDraw Ultra 12.0 and were converted to their 3D form using Discovery Studio. The synthesized compounds were docked into the active site of the target protein using Autodock/Autodock vina.[26] During the docking, both the protein and the ligands were regarded as rigid ones. The docking results were analyzed using BIOVIA Discovery Studio. The binding modes with significant binding scores (lowest energy) were selected for further analysis.

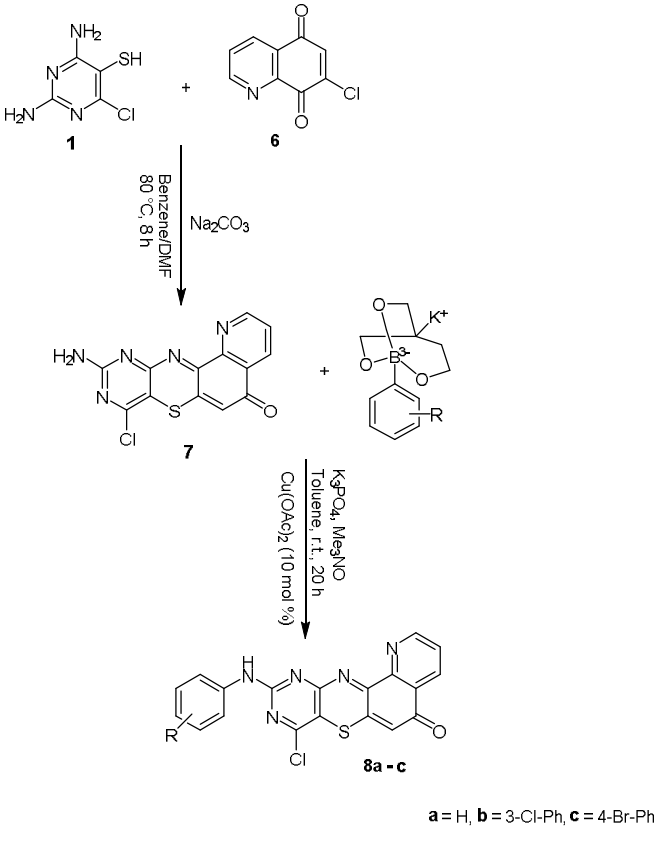
3. Results and Discussion

3.1. Chemistry

The base-mediated condensation of 2,4-diamino-6-chloropyrimidine-5-thiol **1** and 2,3-dichloro-1,4-naphthoquinone **2** or 7-chloroquinoline-5,8-dione **6** produced the functional intermediates **3** and **7**. The reaction of compound **3** with a variety of amides or aryltriborates (obtained from boronic acids as previously reported.[21-23]) *via* nickel or copper-catalyzed Buchwald-Hartwig protocol furnished the 10–amino–8–chloro–9,11–diaza–5*H*–benzo[a]phenothiazin–5–ones **4a-e** or the 8-chloro-5H-naphtho[2,1-b]pyrimido[4,5-e][1,4]thiazin-5-ones **5a-c**, (Scheme 1). Compound **7** was converted to the 8-Chloro-1,9,11-triaza-5*H*-benzo[a]phenothiazin-5-ones **8a-c** by the reaction with aryltriborates, (Scheme 2). The structures of the synthesized compounds were supported by analytical and spectral data.



Scheme 1. Synthetic route to derivatives 4a-e and 5a-c



Scheme 2. Synthetic route to derivatives 8a-c

3.2. Biological activity

3.2.1. Antimicrobial activity

The synthesized compounds were tested for their antimicrobial potentials against *B. subtilis*, *S. aureus*, *Enterococcus faecalis, E. coli, C. albicans*, and *A. niger*. Some of the synthesized compounds showed a good antimicrobial potential against some of the tested microorganisms as shown in Table 1. Compounds **5c** and **8c** showed to be 3-fold more potent than the ciprofloxacin standard against *B. subtilis* with the MIC of 0.251 mg/ml. Compounds **5c** and **8c** were also highly active against *S. aureus* and *E. coli*. Only compound **8c** had a significant antifungal activity among the tested compounds. It was observed that derivatives amongst **5a-c** and **8a-c** with a bromo-substituent exhibited an enhanced antimicrobial activity.

3.2.2. *In silico* ADMET analysis

In silico techniques are essential in drug discovery as it helps medicinal chemists to evaluate the physicochemical properties of potential drug candidates. The synthesized compounds were studied for prediction of the Lipinski's rule and other properties that influence the drug absorption. Lipinski's rule is a vital assessment of the drug-likeness of small molecules. Compounds that fail in more than one parameter are adjudged to have oral bioavailability difficulty.[27] The ADMET results are presented in Table 2. The figures in Table 2 indicate that almost all the predicted ADMET properties of the compound are within the recommended values, suggesting a good oral bioavailability.[28] However, compounds **5a-c** failed in one of Lipinski's parameters but passed the rule overall.

Table 1. Minimum inhibitory concentration (MIC) (mg/ml) of the synthesized compounds

Compound	*B. subtilis*	*S. aureus*	*Enterococus faecalis*	*E. coli*	*C. albicans*	*A. niger*
4a	1.000	1.150	1.585	+	1.585	+
4b	0.787	1.096	0.645	+	1.259	+
4c	1.000	1.202	1.514	+	1.995	+
4d	1.148	0.794	1.096	+	+	+
5a	0.501	+	+	+	1.585	+
5b	1.014	+	+	+	+	+
5c	0.251	0.501	+	0.420	+	1.975
8a	0.501	+	+	1.585	+	+
8b	+	+	+	+	+	+
8c	0.251	0.501	+	0.661	0.794	+
Ciprofloxacin	0.794	0.501	0.437	0.501	+	+
Ketoconazole					0.631	0.575

Key: +Slightly sensitive

Table 2. *In-silico* ADMET screening of the synthesized compounds

Compound	MW	acceptHB	donorHB	Log P	Rtb	Lipinski's Violation	tPSA [Å^2]	Volume
Recommended value	≤500	≤10	≤5	≤5	≤10	≤2	≤140	--
4a	371.81	7	3	2.14	1	0	110.87	286.02
4b	433.88	7	4	3.88	2	0	124.86	339.75
4c	478.88	10	4	3.82	3	0	170.69	363.08
4d	449.88	8	5	3.83	2	0	145.09	347.77
5a	390.86	5	1	5.90	2	1	76.77	310.59
5b	425.30	5	1	6.55	2	1	67.77	324.13
5c	469.75	5	1	6.71	2	1	67.77	342.36
8a	391.84	6	1	4.16	2	0	80.67	306.44
8b	426.29	6	1	4.81	2	0	80.67	319.97
8c	470.74	6	1	4.97	2	0	80.67	324.32

Abbreviations: MW- Molecular weight of the molecule, **donorHB -** approximated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution, **acceptHB-** approximated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution, **Rule Of Five-** Number of violations of Lipinski's rule of five, **Log P-** partition coefficient**, Rtb -** approximated number of rotatable bonds, **tPSA** – topological polar surface area**.**

3.2.3 Molecular docking analysis

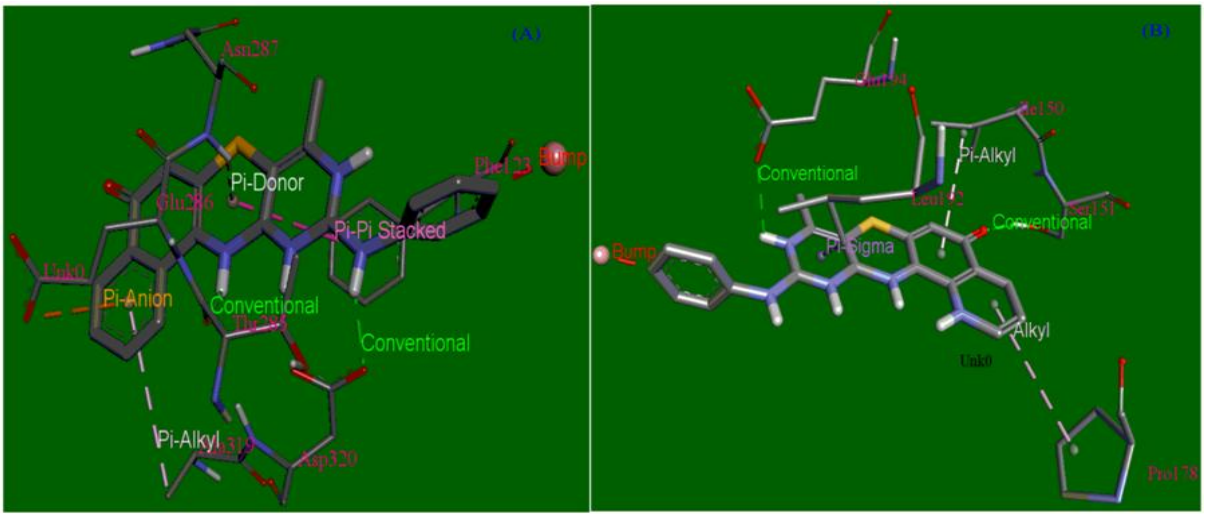
Biotin-protein ligase (BPL) is credited with the post-translational attachment of biotin onto biotin-dependent enzymes that perform the essential role of catalyzing key reactions in metabolic pathways. BPL plays a key role in the activation of acetyl CoA carboxylase (ACC) and pyruvate carboxylase (PC) that are closely engaged in the biosynthesis and anapluresis of fatty acids, hence, they have been identified as a potential antibacterial drug target. Studies have also shown that BPL plays essential roles in bacterial pathogens such as *S. aureus*, *E. coli*, *Mycobacterium tuberculosis* and *Streptococcus pneumonia*.[29-31] The synthesized compounds were docked in the active pocket of BPL, and the results were compared with ciprofloxacin and ketoconazole standards, Table 3. The synthesized compounds possessed higher binding affinity (lower binding energy) against the BPL compared to the ciprofloxacin standard. Due to the promising *in vitro* antibacterial potency of compounds **5c** and **8c**, their docking assay with the target protein and interactions have been depicted (Fig.). Compounds **5c** and **8c** fit well in the active pocket of the BPL, demonstrating significant hydrogen bonding and other hydrophobic interactions. Compound **8c** established hydrogen bonds with Glu 194 and Ser 151 residues and also formed hydrophobic interactions with Leu 192, Pro 178 and Ile 150 amino acid residues. Compound **5c** on the other hand formed hydrogen bonds with Thr 285 and Asp 320, and also established hydrophobic interactions in the active binding site with Glu 286, Asn 287, Phe 123, Ala 319 residues. The results hinted that the BPL protein might be one of the targets which explained why compounds **5c** and **8c** exerted potential potencies against *B. subtilis, S. aureus* and *E. coli*. Though other synthesized compounds revealed lower *in vitro* antimicrobial potency, they also showed significant interactions with the target protein.

Table 3. Binding energy, H-Bonds, H-Bond length, H-Bond with, hydrophobic and electrostatic interactions of compounds with the receptor, 3V7R

Compound	Affinity (Kcal/mol)	H-bonds	H-bond length (Å)	H-bond with	Hydrophobic/ electrostatic interactions	Type
1	2	3	4	5	6	7
4a	-8.80	4	2.60 2.71 2.56 2.27	Met A: 195 Arg A: 122 Phe A: 123 Asn A: 197	Glu A: 194 Ile A: 150	Pi-anion Pi-alkyl
4b	-8.20	4	2.67 2.63 2.61 2.83	Asp A: 322 Ile A: 321 Ser A: 223 Arg A: 227	Arg A: 127 Arg A: 227 Trp A: 127 Ile A: 224 Arg A: 125	Pi-cation Pi-cation Pi-Pi stacked Pi-alkyl Pi-alkyl
4c	-8.5	5	2.64 2.57 2.68 2.02 2.81	Asp A: 322 Ile A: 321 Ser A: 223 His A: 126 Arg A: 227	Arg A: 125 Arg A: 125 Arg A: 227 Trp A: 127 Ile A: 224 Arg A: 125	C-hydrogen Pi-cation Pi-cation Pi-Pi stacked Pi-alkyl Pi-alkyl
4d	-8.5	4	2.67 2.26 2.82 2.60	Asp A: 65 gly A: 132 Thr A: 214 Leu A: 45	Lys A: 131 Ile A: 66 Ile A: 66 Lys A: 131	Pi-cation Pi-sigma Pi-alkyl Pi-alkyl
5a	-8.5	3	2.74 2.48 2.83	Asp A: 65 Gly A: 132 Thr A: 214	Lys A: 131 Ile A: 66 Lys A: 131	Pi-cation Pi-sigma Pi-alkyl
5b	-8.8	3	2.73 2.44 2.82	Asp A: 65 Gly A: 132 Thr A: 214	Lys A: 131 Ile A: 66 Lys A: 131	Pi-cation Pi-sigma Pi-alkyl
5c	-8.60	2	2.29 2.07	Thr A: 285 Asp A: 320	Glu A: 286 Asn A: 287 Phe A: 123 Ala A: 319	Pi-anion Pi-donor Pi-Pi stacked Pi-alkyl

Continuation of the **Table 3**

1	2	3	4	5	6	7
8a	-8.2	2	2.06 2.62	His A: 126 Arg A: 227	Ser A: 128 ile A: 224 Trp A: 127 Ile A: 224 Lys A: 187 Arg A: 125 Trp A: 127	C-hydrogen Pi-sigma Pi-Pi stacked Alkyl Pi-alkyl Pi-alkyl Pi-alkyl
8b	-8.5		2.27 2.41	His A: 126 Arg A: 227	Ser A: 128 Ile A: 224 Trp A: 127 Ile A: 224 Arg A: 125 LYS A: 187 ARG A: 125 TRP A: 127	C-hydrogen Pi-sigma Pi-Pi stacked Alkyl Alkyl Pi-alkyl Pi-alkyl Pi-alkyl
8c	-8.40	2	2.97 2.10	Glu A: 194 Ser A: 151	Leu A: 192 Pro A: 178 Ile A: 150	Pi-sigma Alkyl Pi-alkyl
Ciprofloxacin	-7.1	3	2.28 2.27 2.20	Asn A: 185 Asp A: 322 Lys A: 187	Phe A: 323 Asn A: 185 Phe A: 323 Phe A: 323 Arg A: 227	C-hydrogen Halogen Pi-sigma Pi-Pi-T stacked Pi-alkyl
Ketoconazole	-8.4	2	2.88 2.21	Lys A: 99 Phe A: 123	Thr A: 193 Lys A: 99 Phe A: 123 Leu A: 192 Leu A: 96 Arg A: 120 Arg A: 122 Leu A: 192 Arg A: 120	C-hydrogen Pi-cation Pi-Pi-T stacked Alkyl Alkyl Alkyl Pi-alkyl Pi-alkyl Pi-alkyl



3D interactions of (A) 5c, (B) 8c with the target protein, 3V7R

4. Conclusions

Novel derivatives of non-linear azaphenothiazinones were synthesized using the Buchwald-Hartwig amination protocol and were evaluated for their antimicrobial activity against microbial strains of tropical interest. Compounds **5c** and **8c** showed the excellent antimicrobial activity against *B. subtilis, S. aureus, E. coli*, and *C. albican*. All the synthesized compounds showed the strong binding affinity and demonstrated significant interactions with the Biotin-protein ligase. Compounds **5c** and **8c** are indicated as potential antimicrobial candidates and are selected for further study.

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СИНТЕЗ, АНТИМІКРОБНІ Й ОБЧИСЛЮВАЛЬНІ ДОСЛІДЖЕННЯ НОВИХ ПОХІДНИХ РОЗГАЛУЖЕНИХ АЗАФЕНОТІАЗИНОНІВ

Анотація. *У процесі постійного пошуку нових медикаментозно активних нелінійних фенотіазинів синтезовано нові кутові похідні хлороазафенотіазинону за допомогою реакцій перехресного приєднання, каталізованих перехідними металами. Структурну будову синтезованих сполук встановлено за допомогою комбінованого спектроскопічного й елементного аналізу. Синтезовані сполуки були протестовані на антимікробну активність щодо ізолятів Bacillus subtilis, Staphylococcus aureus, Enterococus faecalis, Escherichia coli, Candida albican та Aspergillus niger методом конвекційного розведення в агаровому середовищі, і сполуки 5c та 8c виявили відмінну активність in vitro проти деяких з досліджуваних мікроорганізмів. Дослідження in silico показало, що синтезовані сполуки мають перспективні фізико-хімічні властивості та добре вписуються в активний центр біотин-протеїнової лігази (BPL), утворюючи необхідні водневі зв'язки та гідрофобні взаємодії.*

Ключові слова: *фенотіазин, реакція кроскопуляції Бухвальда-Гартвіга, антимікробна дія, in silico, in vitro.*