ORIGINAL ARTICLE

A C G publications

Org. Commun. 14:4 (2021) 323-333

organic communications

Novel naphthalene-1,5-diamine containing urea/thiourea derivatives – Promising antimicrobial agents

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(Received November 09, 2021; Revised December 08, 2021; Accepted December 16, 2021)

Abstract: A pioneering class of urea/thiourea derivatives of naphthalene-1,5-diamine was synthesized in excellent yields (89-96%) by one-pot procedure via treatments with phenyl isocyanates or phenyl isothiocyanates. All the constructed derivatives were evaluated for antimicrobial activity using in vitro and in silico methods. The obtained results showed that, all the titled compounds displayed the most significant antibacterial activity against gram-positive and gram-negative bacteria namely *B. substilis, B. sphaerius, S. aureus, P. aeruginosa, K. aerogenes, C. violaceum* and antifungal activity against *A.Niger, C. tropicum, R. oryzae, F. moniliforme and C. lunata* when compared with the standard drugs such as ciproflaxacin and clotrimazole. Among all, the compounds **2c, 2e** and **3d, 3e** displayed higher content of antimicrobial activity akin to the rest of the compounds due to the presence of fluoro substitution on aromatic ring. Furthermore, molecular docking studies provided support to the in vitro studies. Four of the synthesized compounds, 4-fluorophenyl, 3-trifluoromethylphenyl, 4-chlorophenyl exhibited significant binding modes and were the best target ligands as they fitted more stably into the DNA gyrase binding pocket. Henceforth, it is suggested that, the fabricated urea/thiourea derivatives of naphthalene-1,5-diamine would stand as the prosperous antimicrobial drug candidates for further studies.

Keywords: Naphthalene-1,5-diamine; antimicrobial activity; DNA Gyrases; urea/thiourea derivatives.
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1. Introduction

Urea and thiourea derivatives play a significant role in the uptake of nitrogen-containing analogues and besides they are the main constituent in the urine living thing. Urea is an internal product of amino acid and protein catabolism. They is produced from ammonia, which is a de-amination compound of amino acids. Every day nearly, 20-35 gm of urea is emitted from human urine. Urea is the earliest carbon-based compound was constructed from mineral substances by Friedrich Wohler.[1]In the

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The article was published by ACG Publications
http://www.acgpubs.org/journal/organic-communications © October-December 2021EISSN:1307-6175
DOI:http://doi.org/10.25135/acg.oc.115.2111.2257
Available online: December 24, 2021

literature, various researchers synthesized aromatic (Monophenyl, Diphenyl) and heterocyclic urea derivatives across the globe with potential biological applications.[2] Urea derivatives show good biological activities such as antimicrobial, anticancer[3] and act as anaplastic lymphoma kinase (ALK) inhibitors.[4] These derivatives also show a broad spectrum of biological activities such as antifungal, antiviral, anticonvulsant, analgesic and HDL elevating activities.[5-10]

The aryl and bis-aryl urea derivatives are one of the modest substances are used in clinics. Triclocarban is primarily used in cleaning as sterilizing solutions in primary health care centers, houses, face paint, dolls, cloths and plastics as it restricts the activity of ENR (enoyl-acyl carrier-protein redutase), an enzyme to develop the cell wall of the microorganisms and fungus.[11] Thiourea derivatives are essential Sulphur and nitrogen-containing mixtures these are evidenced useful constituents in drug examination in recent years.[12-17] Some urea derivatives have appreciated antibacterial. Antituberculosis, and anticonvulsant activities.[18,19] Majority of these derivatives consist of heterocyclic rings such as thiadiazoles, oxadiazoles, pyrazoles and triazoles. It is well-known that 1,2,4-triazole-derived N-bridged heterocycles finding applications in the area of cultivation medicine, and industry.

It was predicted that, these two energetic pharmacophores, associated together, would produce unique molecular patterns which are prospective to display promising pharmaceutical activities in animal models. In our ongoing studies are based on the various heterocyclic compounds which possess antimicrobial activity.[20-23]These results are encouraged us to construction of a novel sequence of mixtures and to conduct antimicrobial tests using in vitro and in silico methodologies. To the finest of our knowledge, this is the primary explanation to deliberate the preparation and bioactive properties of naphthalene-1,5-diamine urea/thiourea derivatives.

DNA gyrase is an important bacterial enzyme that catalyses the ATP-dependent -Ve super-coiling of double-stranded closed-circular DNA. Gyrase is a class of enzymes well-known as topoisomerases that are involved in the regulator of topological changes of DNA. The mechanism by which gyrase is able to effect the topological state of DNA grains is of typical attention from an enzymological perspective. Furthermore, considerable consideration has been focused on DNA gyrase as the intracellular target of a number of antibacterial agents and as an example for other DNA topoisomerases.

Therefore, in addition to synthesis and biological evaluation studies we performed docking studies of the synthesized compounds on DNA gyrase.

2. Experimental

2.1. General

Melting points were determined using a Cintex melting point apparatus and were uncorrected. Thin-layer chromatography (TLC) was performed by using Merck silica gel 60 F254 precoated plates (0.25 mm). Column chromatography was performed by using Silica gel (particle size 100-200 mesh). IR spectra (KBr) were recorded on a Perkin-Elmer BX series FTIR spectrometer. ^{1}H NMR spectra were recorded on a Bruker AMX 400 MHz spectrometer. ^{13}C NMR spectra were recorded at operating frequency of 100 MHz Chemical shift values are given in ppm (δ) With TMS as an internal standard. Mass spectra were determined on Agilent LC-1100 (LC-MS) series instrument. Elemental analyses were performed on a Carlo Erba 106 and Perkin Elmer model 240 analyzers. All the chemicals and reagents used in present investigation were purchased from Sigma-Aldrich.

2.2. Chemistry

2.2.1. Synthesis of Urea Derivatives of Naphthalene-1,5-diamine (22a-f & 23a-e)

Naphthalene-1,5-diamine (1.0 eq, 0.001 mol) and aryl-isocyanate (2.2 eq, 0.0025 mol) were dissolved in 10 mL of tetrahydrofuran (THF). N, N-dimethyl-piperazine (DMPi, 0.002 mol) was added at room temperature. The reaction mixture was heated to 50–55˚C and kept for stirring for 2.5-3 hrs. Reaction progress was monitored by TLC using ethyl acetate: heptane (3:2) as a mobile phase. After completion of reaction, the reaction mixture was concentrated to residual level under reduced pressure in a rota evaporator and the crude product was purified by using ethyl acetate: diethyl ether (1:4) to

obtain pure title compounds in 85-92% yield. All title compounds were synthesized by adopting the same procedure. The structures of newly synthesized urea/ thiourea derivatives **2a–f** and **3a-e** were confirmed by spectral (IR, ^{1}H, ^{13}C NMR & MS) and analytical data.

2.2.2. Spectral Data for Constructed Derivatives

*1,1'-(Naphthalene-1,5-diyl)bis(3-(3-(trifluoromethyl)phenyl)urea)(**2a**):* Yield: 90%, White solid, M.P.: 221-223°C; IR (KBr) ν_{max} (cm^{-1}): 3286, 1749, 1640, 1564, 1423, 1331, 1119, 904;^{1}H NMR (400 MHz, DMSO-d_6): δ 9.46 (s, 2H, NH), 8.92 (s, 2H, NH), 8.10 (s, 2H, Ar-H), 8.02-8.04 (d, 2H, *J*=8 Hz, Ar-H), 7.89-7.91 (d, 2H, *J*=8 Hz, Ar-H), 7.55-7.63 (m, 6H, Ar-H), 7.33-7.34 (m, 2H, Ar-H);^{13}C NMR (100 MHz, DMSO- d_6): δ 153.4 (C=O), 141.0, 134.9, 130.4, 127.1, 126.11(CF_3),125.8, 125.4, 122.8, 118.8, 118.58, 114.54; HR-MS: *m/z* 533.142 [M+H] $^{+}$. Anal. Calcd for $C_{26}H_{18}F_6N_4O_2$: C 58.65%, H 3.41%, N 10.52%. Found: C 58.78%, H 3.43%, N 10.57%.

*1,1'-(Naphthalene-1,5-diyl)bis(3-(3-chlorophenyl)urea) (**2b**)*: Yield: 87%, Off white powder, M.P.:175-179°C. IR (KBr) ν_{max} (cm^{-1}): 3252, 3120, 2957, 1725, 1638, 1558, 1426, 1327, 1129, 898;^{1}H NMR (400 MHz, DMSO-d_6): δ 9.38 (s, 2H, NH), 8.94 (s, 2H, NH), 8.08 (s, 2H, Ar-H), 7.99-8.01(d, 2H, *J*=8 Hz, Ar-H), 7.86-7.88 (d, 2H, *J*=8 Hz, Ar-H), 7.53-7.62 (m, 6H, Ar-H), 7.31-7.34(m, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO- d_6): δ153.5 (C=O), 141.4, 135.2, 130.4 (Ar-C-Cl), 127.4, 126.2, 125.8, 125.4, 121.8, 119, 118.6, 114; LC-MS: *m/z* 466.0[M+H]$^{+}$, 467.0[M+2]$^{+}$; Anal. Calcd for $C_{24}H_{18}Cl_2N_4O_2$: C 61.95%, H 3.90%, N 12.04%. Found: C 62.62%, H 3.92%, N 12.08%.

*1,1'-(Naphthalene-1,5-diyl)bis(3-(2,4-difluorophenyl)urea) (**2c**):* Yield: 91%, White crystalline powder, Melting range: 254-256°C. IR (KBr) ν_{max} (cm^{-1}): 3294, 3075, 2984, 1746, 1640, 1562, 1502, 1423, 1207, 1102, 963, 849; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.16 (s, 2H, NH), 9.06 (s, 2H, NH), 7.92-7.95 (d, 2H, *J*=12Hz) Ar-H), 7.53-7.64(m, 2H, Ar-H), 7.39-7.42 (m, 2H, Ar-H), 7.34-7.37 (m, 6H, Ar-H), 7.12-7.15(m 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO- d_6): δ163.9 (Ar-F), 160.5 (Ar-F-para),153.5 (C=O), 141.4, 134.2, 130.4, 127.5, 126.1, 125.5, 122.2,118.6, 114.5; LC-MS: *m/z* 469.0[M+H]$^{+}$. Anal. Calcd for $C_{24}H_{16}F_4N_4O_2$: C 61.54%, H 3.44%, N 11.96%. Found: C 61.65%, H 3.46%, N 11.99%.

*1,1'-(Naphthalene-1,5-diyl)bis(3-(4-fluorophenyl)urea) (**2d**):* Yield: 85%, White crystalline solid, M.P.:191-193°C. IR (KBr) ν_{max} (cm^{-1}): 3274, 3130, 2985, 1735, 1628, 1548, 1426, 1327, 1129, 898; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.38 (s, 2H, NH), 8.94 (s, 2H, NH), 8.02-8.04 (d, 2H, Ar-H), 7.94-7.98(d, 2H, *J*=16Hz, Ar-H), 7.75-7.79 (m, 2H, Ar-H), 7.53-7.60 (m, 6H, Ar-H), 7.21-7.24(d, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 162.5 (Ar-F), 153.7, 141.6, 135.5, 130.7, 128, 125.4, 121.8, 119, 105.3; LC-MS: *m/z* 433.2[M+H]$^{+}$. Anal. Calcd for $C_{24}H_{18}F_2N_4O$: C 66.66%, H 4.20%, N 12.96%. Found: C 66.62%, H 4.22%, N 12.99%.

*1,1'-(Naphthalene-1,5-diyl)bis(3-(4-chloro-3-(trifluoromethyl)phenyl)urea) (**2e**):* Yield: 92%, White crystalline powder, M.P.: 241-245°C. IR (KBr) ν_{max} (cm^{-1}): 3337, 3150, 3127, 3112, 2975, 1634, 1592, 1534, 1518, 1420, 843;^{1}H NMR (400 MHz, DMSO-d_6): δ 9.38 (s, 2H, NH), 8.94 (s, 2H, NH), 8.08 (s, 2H, Ar-H), 7.97-7.99 (d, 2H, *J*=8Hz, Ar-H), 7.73-7.75 (d, 2H, *J*=8Hz Ar-H), 7.52-7.59 (m, 6H, Ar-H), 7.25-7.28(m, 2H, Ar-H);^{13}C NMR (100 MHz, DMSO- d_6): δ 153.6(C=O), 145.3, 140.2, 134.9, 130.4(Ar-Cl), 127.1, 126.11,125.8, 125.4 (CF_3), 122.8, 118.58, 114.54; LC-MS: *m/z* 602.2[M+H]$^{+}$.Anal. Calcd for $C_{26}H_{16}Cl_2F_6N_4O_2$: C 51.93%, H 2.68%, N 9.32%. Found: C 52.04%, H 2.71%, N 9.35%.

*1,1'-(Naphthalene-1,5-diyl)bis(3-(4-bromophenyl)urea)(**2f**):* Yield: 85%, Pale brown color solid, M.P.: 224-225°C. IR (KBr) ν_{max} (cm^{-1}): 3254, 3110, 2977, 1735, 1627, 1548, 1416, 1337, 1139, 823; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.32 (s, 2H, NH), 8.96 (s, 2H, NH), 8.03-8.05 (d, 2H, *J*=8Hz, Ar-H), 7.96-7.99(m, 2H, Ar-H), 7.77-7.79 (d, 2H, *J*=8Hz, Ar-H), 7.54-7.61 (m, 6H, Ar-H), 7.22-7.25(d, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO- d_6): δ153.6(C=O), 141.5, 135.4, 130.9, 127.8, 126.5, 123.25(Ar-Br), 120.8, 117.5, 105.8; LC-MS: *m/z* 555.25 [M+H]$^{+}$, 556.3 [M+2]$^{+}$. Anal. Calcd for $C_{24}H_{18}Br_2N_4O_2$: C 52.01%, H 3.27%, N 10.11%. Found: C 52.14%, H 3.29%, N 10.13%.

*1,1'-(Naphthalene-1,5-diyl)bis(3-phenylthiourea) (**3a**):*Yield: 89%, Off white solid, M.P.:222-224ºC. IR (KBr) ν_{max} (cm^{-1}): 3336, 3164, 2958, 1597.6, 1531, 1416, 1223, 1156, 786.6; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.91 (brs, 2H, NH), 7.93 (brs, 2H, NH), 7.89-7.91 (d, 4H, *J*=8Hz, Ar-H); 7.77-7.79 (d, 4H, *J*=12Hz Ar-H), 7.45-7.51 (m, 6H, Ar-H); 7.26-7.29 (m, 2H, Ar-H);^{13}C NMR (100 MHz, DMSO-d_6): δ 181.5(C=S), 141.6, 141.2, 136.03, 131.39, 129.4, 126.16, 122.4;LC-MS: *m/z* 429.5[M+H]$^+$.Anal. Calcd for $C_{24}H_{20}N_4S_2$: C 67.26%, H 4.70%, N 13.07%; Found: C 67.38%, H 4.74%, N 13.14%.

*1,1'-(Naphthalene-1,5-diyl)bis(3-(4-nitrophenyl)thiourea) (**3b**):*Yield: 91%, Pale yellow color solid, M.P.: 234-236ºC. IR (KBr) ν_{max} (cm^{-1}): 3347, 3160, 2957.9, 1595.7, 1535, 1422, 1224, 1036, 786.6; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.88 (brs, 2H, NH), 7.91 (brs, 2H, NH), 7.16-7.18 (d, 4H, *J*=8Hz, Ar-H), 7.29-7.36 (m, 6H, Ar-H), 7.13-7.16 (m, 4H, Ar-H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 181.7(C=S), 145.9, 141.1, 136, 131.4, 129.7, 126.2, 122.4, 116.2, 109.7; LC-MS: *m/z* 519.2 [M+H]$^+$. Anal. Calcd for $C_{24}H_{18}N_6O_4S_2$: C 55.59%, H 3.50%, N 16.21%; Found: C 55.63%, H 3.55%, N 16.25%.

*1,1'-(Naphthalene-1,5-diyl)bis(3-(2,6-difluorophenyl)thiourea) (**3c**):*Yield: 90%, White crystal Solid, M.P.: 227-230ºC. IR (KBr) ν_{max} (cm^{-1}): 3294, 3075, 2977, 1746, 1640, 1562, 1502, 1423, 1324, 1102, 963, 786; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.26 (s, 2H, NH), 9.16(s, 2H, NH), 7.53-7.55 (d, 2H, *J*=8Hz, Ar-H), 7.53-7.64 (m, 4H, Ar-H), 7.32-7.42 (m, 2H, Ar-H), 7.12-7.17 (m, 4H, Ar-H);^{13}C NMR (100 MHz, DMSO- d_6): δ 180.9 (C=S), 169.4 (Ar-CF) 141.1, 134.9, 130.5, 127.5, 122.2, 118.8, 117.4, 114.5; LC-MS: *m/z* 501.48 [M+H]$^+$, 502.51 [M+2]$^+$. Anal. Calcd for $C_{24}H_{16}F_4N_4S_2$: C 57.59%, H 3.22%, N 11.19%.; Found: C 57.69%, H 3.24%, N 11.23%.

*1,1'-(Naphthalene-1,5-diyl)bis(3-(3,4-dichlorophenyl)thiourea) (**3d**):*Yield: 89%, Off white powder, M.P.: 247-248ºC. IR (KBr) ν_{max} (cm^{-1}): 3289, 3086, 2977, 1736, 1635, 1548, 1512, 1433, 1311, 1196, 963, 823, 795; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.73 (brs, 4H, NH), 7.85-7.98 (m, 4H, Ar-H), 7.50-7.61 (m, 8H, Ar-H); ^{13}C NMR (100 MHz, DMSO- d_6): δ181.4, 141.1, 140.3, 136.5, 131.2, 129.6, 126.2, 122.5, 116.3, 110.5, 109.6; LC-MS: *m/z* 567.1[M+H]$^+$. Anal. Calcd for $C_{24}H_{16}Cl_4N_4S_2$: C 50.90%, H 2.85%, N 9.89%: Found: C 50.98%, H 2.87%, N 9.99%.

*1,1'-(Naphthalene-1,5-diyl)bis(3-(m-tolyl)thiourea) (**3e**):*Yield: 92%, Off white solid, M.P.:135-137ºC. IR (KBr) ν_{max} (cm^{-1}): 3336, 3164, 2958, 1597, 1531, 1416, 1223, 1156, 1036, 786; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.46 (s, 2H, NH), 8.92 (s, 2H, NH), 8.10 (s, 2H, Ar-H), 8.02-8.04 (d, 2H, *J*=8Hz, Ar-H), 7.89-7.91 (d, 2H, *J*=8Hz, Ar-H), 7.55-7.63 (m, 6H, Ar-H), 7.33-7.34(quasi d, 2H, *J*=4Hz, Ar-H); 3.76 (s, 6H, Ar-H);^{13}C NMR (100 MHz, DMSO- d_6): δ 181.6(C=S) 141.1, 136, 131.4, 129.7, 126.2, 122.4, 116.2, 109.8, 55.55; LC-MS: *m/z* 457.3[M+H]$^+$. Anal. Calcd for $C_{26}H_{24}N_4S_2$: C 68.39%, H 5.30%, N 12.27%. Found: C 68.52%, H 5.34%, N 12.32%.

2.3. Biological Assay

2.3.1. Antibacterial Activity

The newly prepared compounds were studied in vitro for antibacterial activity against the growth of Gram-negative bacteria namely *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Chromobacterium violaceum* and Gram-positive bacteria namely *Bacillus subtilis*, *Bacillus sphaericus and Staphylococcus aureus* at 100 µg/mL concentration by using agar well diffusion method. Ciprofloxacin was used as the standard.[24] The obtained results are summarized in Table1.

2.3.2. Antifungal Activity

Antifungal activities of **2a-f** and **3a-e** were determined by using the agar cup bioassay method[25] with Clotrimazole as the standard. The compounds were tested for their antifungal activity against five test organisms namely *Aspergillus niger*, *Chrysosporium tropicum*, *Rhizopus oryzae*, *Fusarium*

moniliforme and Curvularia lunata using the agar cup bioassay method at 100 µg/mL concentration. The nutrient broth medium (HiMedia, 40g) was suspended in distilled water (1000 mL) and heated until it was dissolved completely. The medium and Petri dishes were autoclaved at a pressure of 15lb/inc for 20 min. The medium was poured into sterile Petri dishes under aseptic conditions in a laminar flow chamber. After media solidification, 0.5 mL culture of the test organism was inoculated by uniform spreading over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving plant extract in dimethyl sulfoxide (DMSO) at a concentration of 100 µg/mL. Agar inoculation cups were scooped out with a 6 mm sterile cork borer and the lids of the dishes were replaced. To each cup, 100 µg/mL of the test solution was added. Controls were maintained with DMSO and Clotrimazole (100 µg/mL). The treated and controls were kept at RT for 72-95 h. Inhibition zones were determined by measuring the diameter in millimeter. The obtain results are presented in Table 2.

2.3.3. Molecular Docking Analysis

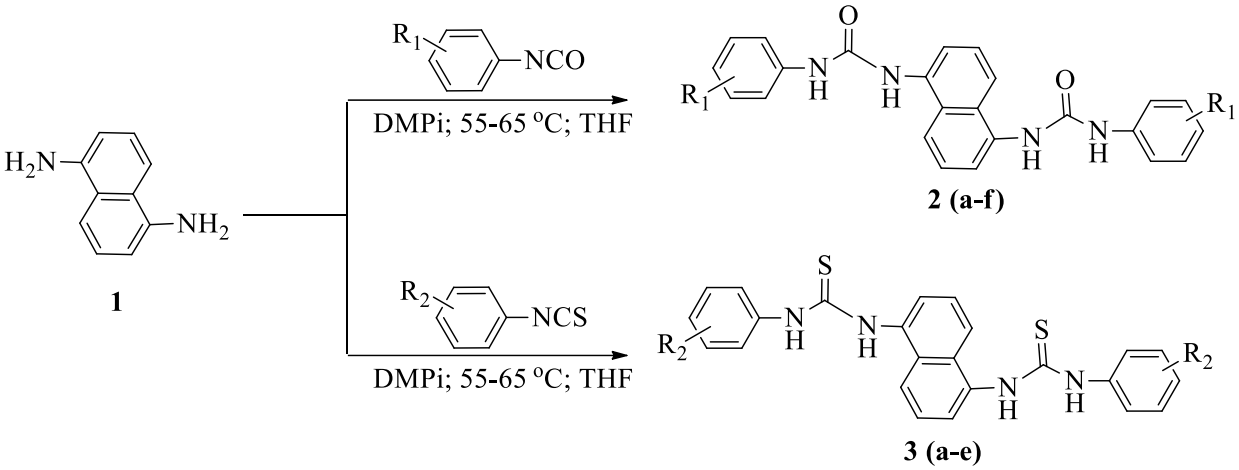
Streptomycin is an aminoglycoside antibiotic resulting from Streptomyces griseus having antibacterial activity. Streptomycin irreversibly binds to the 16S ribosomal RNA and S12 protein which are within 30S ribosomal subunit of bacteria. As a result, this agent inhibits with the assembly of initiation composite between mRNA and the bacterial ribosome, thereby preventing the beginning of protein construction. In addition, streptomycin gives misinterpretation of the mRNA pattern and causes translational frameshift, thereby resulting in premature termination followed by bacterial cell death.

Docking studies were voted for between DNA Gyrase A protein and the selected targets **2a-f & 3a-e** and the backing drug Streptomycin 1, using the docking segment executed in Pyrx2010.12. At first, the protein constructions were protonated with the addition of polar hydrogens, monitored by energy minimization with the MMFF94x force field, in order to get the constant conformer of the protein. Springy docking was carried, the inhibitor binding site was decreased and moderated, emphasized through the “Site Finder” module applied in the Pymol software. The network sizes were anticipated as ˚ X: 28.27, Y: 27.13, Z: 28.51 for Aromatase correspondingly. The docking was performed with factors i.e., placement: triangle matcher, recording 1: London dG, refinement: force field. All-out of 10 conformations of each target were permitted to be saved in a distinct database file in .mdb arrangement. After the docking method, the binding affinity and binding energy of the protein–ligand developments were calculated using Pymol viewer tool (www.pymol.org).

3.Results and Discussion

3.1. Chemistry

The title bis-urea derivatives **2a-f** and **3a-e** were fabricated by modest addition reaction of functionalized Naphthalene-1,5-diamine with aryl-isocyanates and aryl-isothio cyanates in presence of 1,4-dimethyl piperazine (DMPiz). The schematic representation is presented in Figure 1. The products were cleaned by column chromatography using Ethyl acetate: diethyl ether (3:2) to obtain pure title compounds in 85-92% yield. All the products were synthesized by adopting the same procedure. The structures of newly synthesized urea derivatives **2a-f** and **3a-e** were identified by spectral (IR, ^{1}H, ^{13}C NMR & MS) & analytical data.



R_1= 2a: 3-CF_3; 2b: 3-Cl; 2c: 2,4-F; 2d:4-F; 2e: 3-CF_3, 4-Cl; 2f: 4-Br
R_2= 3a: H; 3b: 4-NO_2; 3c: 2,6-F; 3d: 3,4-Cl; 3e: 3-CH_3

Figure 1. Schematic representation of synthesis of urea/thiourea derivatives ofnaphthalene-1, 5-diamine **(2a-f and 3a-e)**

IR spectral data were used to find out the functional groups existing in the constructed derivatives **2(a-f)/ 3(a-e)** and are presented in experimental data. The absorption bands in IR spectra were obtained in the region of 3286-3336 cm^{-1} presence of –NH group, the bands appeared in the range of 1749-1640 cm^{-1} for –C=O groups. Whereas the C=S group were observed in the region of 1597. The ^{1}H NMR spectroscopic data of compounds **2(a-f)/3(a-e)** are presented in experimental part and the obtained data was agreement with the proposed structures. In ^{1}H NMR spectra, two singlet signals were appeared in between 9.46-8.96 ppm confirming the -CONH protons, a singlet signal appeared in the range of 9.91ppm confirming the -CSNH protons, the chemical shifts in the region of 8.10-7.28 ppm were due to aromatic protons appeared as singlet, doublet and multiplet, respectively.

The ^{13}C NMR spectroscopic data of compounds **2(a-f)/3(a-e)** are presented in experimental part and the obtained data was agreement with the proposed structures. In ^{13}C NMR spectra, the peaks in the region of 159-109 ppm were assigned to carbons of aromatic ring. The signals in the region of 181 and 153 ppm corresponds to C=S and C=O respectively. The mass spectrometric data of compounds **2(a-f)/3(a-e)** are presented in the experimental part.

3.2. Biological Assay

3.2.1. Antibacterial Activity

The antibacterial activity results for compounds **2a-f** displayed decent activity. The compounds**2c** and **2e** containing fluoro and fluoro, chloro groups on the benzene ring exhibited high activity. The activity of the compound depends upon the position of the substituent at the phenyl group. The substituents particularly chloro, fluoro and trifluoromethyl groups attached to phenyl ring increases the activity extraordinarily. Compounds**2c** and **2e** shown significant activity. Among all these derivaties target **2c** revealed activity is equivalent to that of Ciproflaxacin. However, the degree of inhibition varied both with test compound as well as bacteria used in the present investigation. In conclusion, majority of compounds **2a-f** showed worthy activity by preventing growth of all the bacteria to a greater extent. These remarkable results may consider to the existence of urea is linked to aromatic ring.

Table 1. Antibacterial activity data of compounds **2a-f** and **3a-e** as MIC[a] (µg/mL)

	Gram-positive			Gram-negative		
Compound	***B.substilis***	***B.sphaerius***	***S.aureus***	***P.aeruginosa***	***K.aerogenes***	***C.violaceum***
2a	22	24	24	32	24	30
2b	21	24	21	28	25	26
2c	18	21	18	21	23	25
2d	24	25	30	35	26	28
2e	20	22	18	27	22	25
2f	24	25	30	29	26	28
3a	23	27	20	37	26	25
3b	21	25	24	31	24	22
3c	21	20	17	30	23	26
3d	23	24	25	27	24	24
3e	21	22	19	27	22	25
Ciproflaxacin	20	25	20	30	25	25

Note: [a]Negative control (DMSO) - No activity

3.2.2. Antifungal Activity

The antifungal activity results revealed that, compounds **2a-f** and **3a-e** are potential toxic towards all the five fungi and they are lethal even at 100 µg/mL concentration. In series **2**, targets **2c** and **2e** showed high antifungal activity owing to the presence of trifloromethyl, fluoro, chloro and bromo groups are present on the aromatic ring. In series **3**, targets **3d** and **3e** showed good antifungal activity owing to the fluoro, chloro, difluro and methyl groups are present on benzene ring. The obtained results compared with the standard drug clotrimazole revealed their promising activities. In conclusion, all the series of compounds **2a-f** and **3a-e** are moderately lethal towards the fungi under examination and they are lethal even at 100 µg/mL concentration in comparison with standard Clotrimazole at the same concentration.

Table 2. Antifungal activity screening data of compounds **2a-f** and **3a-e** as MIC[a,b] (µg/mL)

Compound	***A.niger***	***C. tropicum***	***R. oryzae***	***F. moniliformae***	***C. lunata***
2a	24	25	18	19	20
2b	23	28	14	17	23
2c	29	29	23	21	27
2d	27	24	21	18	25
2e	30	28	24	19	29
2f	28	26	21	19	20
3a	24	22	17	14	18
3b	23	25	14	17	22
3c	26	21	21	19	21
3d	24	25	22	19	25
3e	30	28	24	19	29
Clotrimazole	30	29	23	20	28

Note: [a]Negative control (DMSO)-no activity; [b]Concentration 100 µg/mL.

3.2.3. Molecular Docking Analysis

In order to get some information about the possible uses of synthesized compounds, docking study was carried out for targets (**2a-f** and **3a-e**) with particular pharmacological target such as DNA Gyrase A protein of *E. coli* which is appropriate target for anti-bacterial activity.

Table 3. Docking analysis of synthesized compounds (**2a-f** and **3a-e**) and streptomycin (standard) against *E. coli* DNA Gyrase A protein

Sl No	Compound	Binding Energy	Binding interaction	Bond Length (A^O)	Bond Angle (°)	Bond Type
1	Streptomycin	- 6.9	Asg 139 CG…HN	2.2	124.4	H- don
			Leu 135 CD…HN	2.7	125.7	H- don
			His 132 CB…OH	2.5	125.0	H- acc
			Asp 53 CG…OC	3.4	116.7	H- acc
			Asp 53 OC…OC	2.9	118.9	H- acc
			Asp 58 OD…OH	2.0	118.6	H- acc
			Asp 58 OD…HN	2.5	116.4	H- don
			His 132 ND…OC	2.8	126.2	H- acc
			His 132 ND…OC	2.7	120.0	H- acc
			His 132 OC…OH	2.5	119.8	H- acc
2	**2a**	- 7.9	Lys270 HZ…OC	1.9	110.3	H- acc
			Lys 270 HZ…OC	2.02	109.6	H- acc
			Asp 297 CA…HN	1.8	122.2	H- don
3	**2b**	- 7.9	Gly 110 OC…HN	1.9	121.7	H- don
			Gly 110 OC…HN	2.3	123.1	H- don
			Asn 108 CG…HO	2.3	121.5	H- don
			Phe 109 CA…OC	2.0	115.1	H- acc
			Gly 110 HN…OC	2.8	124.4	H- acc
4	**2c**	- 7.4	Thr 219 CA…HO	2.0	119.9	H- don
			Leu 264 HN…OC	2.2	114.7	H- acc
			Val 103 OC…HO	2.0	124.6	H- don
			Gly 105 HN…OC	2.2	124.7	H- acc
			Gly 105 OC…HN	2.2	124.2	H- don
5	**2d**	- 8.2	Gly 105 CA…HN	2.0	124.2	H- don
			Asp 104 CG…HN	2.4	118.7	H- don
6	**2e**	- 8.6	Glu 263 CD…NH	2.6	116.7	H- acc
			Gly 105 OC…NH	2.3	121.8	H- acc
			Gly 110 OC…HN	1.9	123.1	H- don
			Gly 110 OC…HN	2.4	121.7	H- don
7	**2f**	- 9.1	Gly 105 CA…HN	2.3	124.2	H- don
8	**3a**	- 8.8	Gly 110 OC…HN	2.0	123.1	H- don
			Gly 110 CA…HN	2.3	121.7	H- don
9	**3b**	- 8.6	Gly 110 OC…HN	2.3	121.7	H- don
			Met 301 HN…OC	2.1	122.2	H- acc
10	**3c**	- 8.1	Glu 263 CD…NH	2.6	116.7	H- acc
			Gly 110 OC…HN	2.4	121.3	H- don
11	**3d**	- 8.3	Arg 518 NE…OC	2.4	119.2	H- acc
			Asp 297 OC... HN	2.6	121.4	H- don
			Lys 270 NZ….OC	2.3	110.3	H- acc
12	**3e**	-8.8	Asp 297 CA….HN	2.0	122.4	H- don
			Lys 270 NZ…OC	2.3	110.3	H- acc

The crystal structure of DNA Gyrase A (PDB id: 3LPX) was recovered from the protein data bank (PDB) and the reference drug Streptomycin (**PC ID 19649)** from Pub Chem Drug bank. The docking results of DNA Gyrase A showed that, compounds **2a-f** and **3a-e**showed noteworthy binding modes compared to the control drug Streptomycin (-6.9). All the compounds have formed higher binding energies than the reference compound. The H-bonds, binding affinities and energy profiles of compounds **2a-f** and **3a-e**along with reference drugs, the active site amino acids of the enzyme are presented in Table 3. The binding modes of compounds **2f, 3a, 3b** and **3e** are suggested as the best target ligands as that they built-in more firmly into the DNA Gyrase binding pocket (Figure 3). Therefore, the current study exhibit that the synthesized compounds are promising next generation anti-microbial drugs, which can be efficiently used in the treatment of microbial and other related contagions.

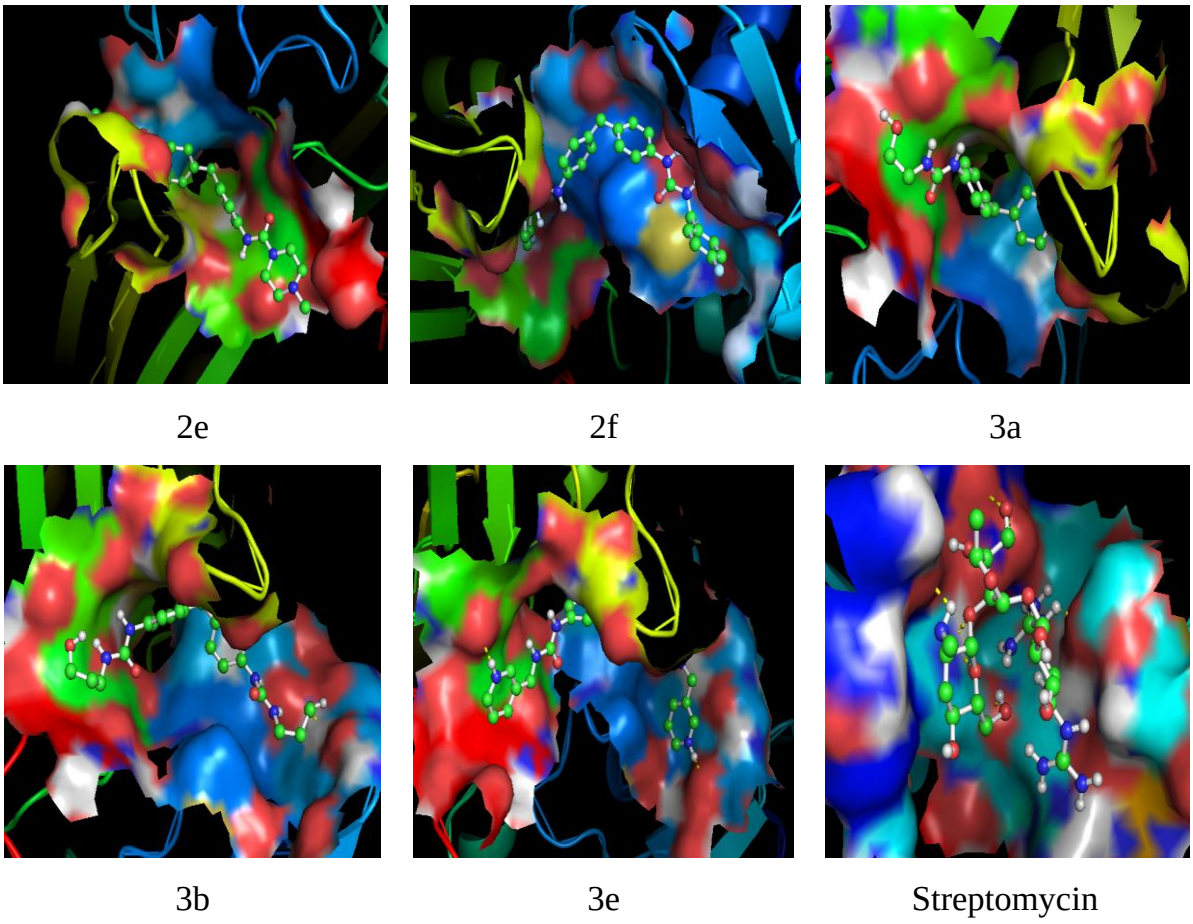


Figure 2. Docking poses of selected ligands with DNA Gyrase A Protein (3LPX)

4. Conclusion

A simple, efficient and practical method has been developed for the synthesis of novel urea and thiourea derivatives of naphthalene-1,5-diamine **2a-f** and **3a-e**. In series **2**, compounds **2c** and **2e**, in series **3**, compounds **3d** and **3e** showed high antifungal activity which may be due to the presence of fluoro, fluorochloro, trifloromethyl and methyl groups as substituents on the benzene ring. This method offers several advantages like mild reaction conditions, enhanced reaction rates, easy isolation of products and operational simplicity and compounds yields are high.

Acknowledgements

The authors are grateful to the management and principals of SV Colleges for their support and encouragement during the research.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/organic-communications

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Supporting Information

***Org. Commun.* 14:4 (2021) 323-333**

Novel naphthalene-1,5-diamine containing urea/thiourea derivatives – Promising antimicrobial agents

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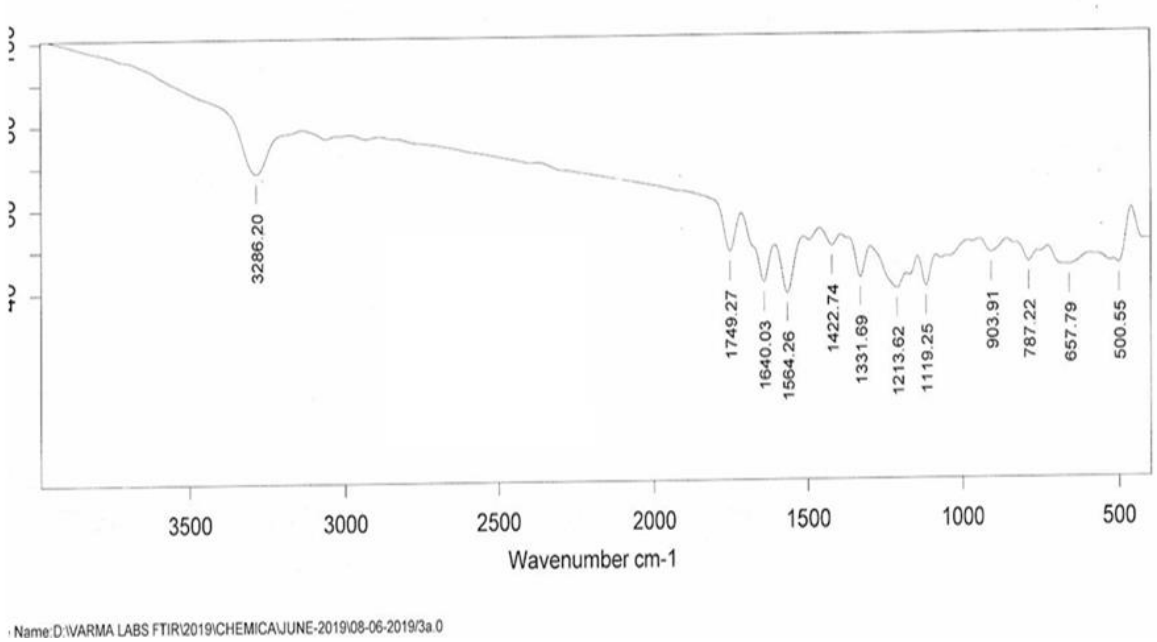

Figure S1 : IR spectrum of compound **2a**

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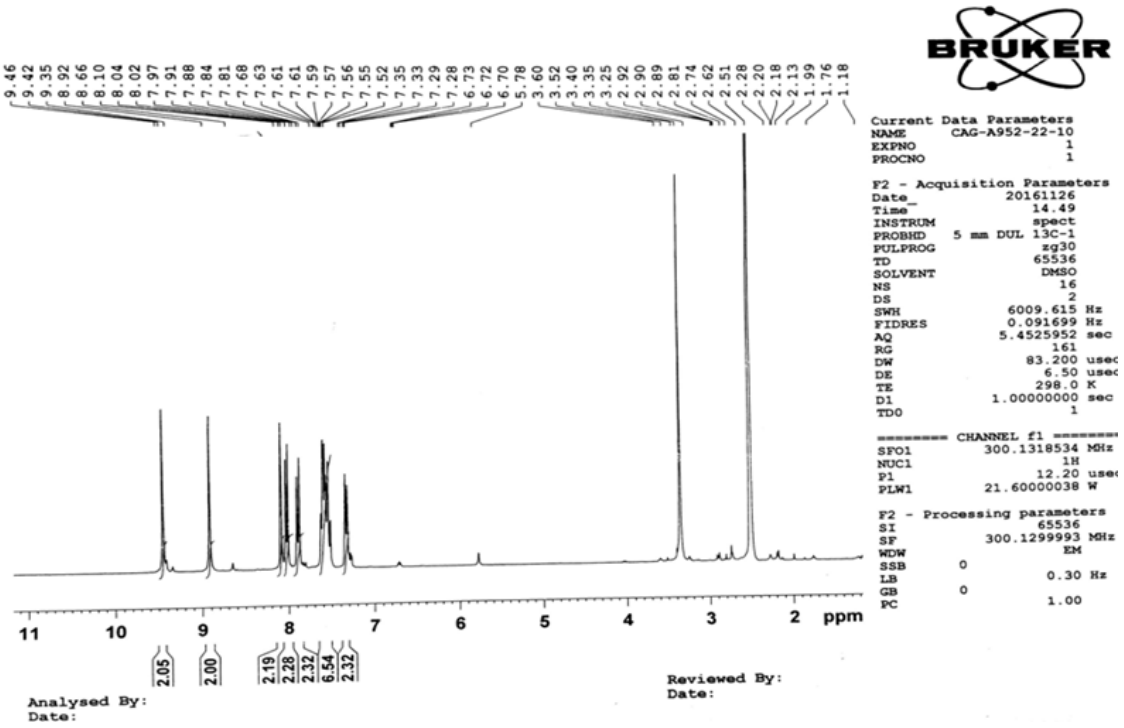

Figure S2 : ^{1}H NMR spectrum of compound **2a**

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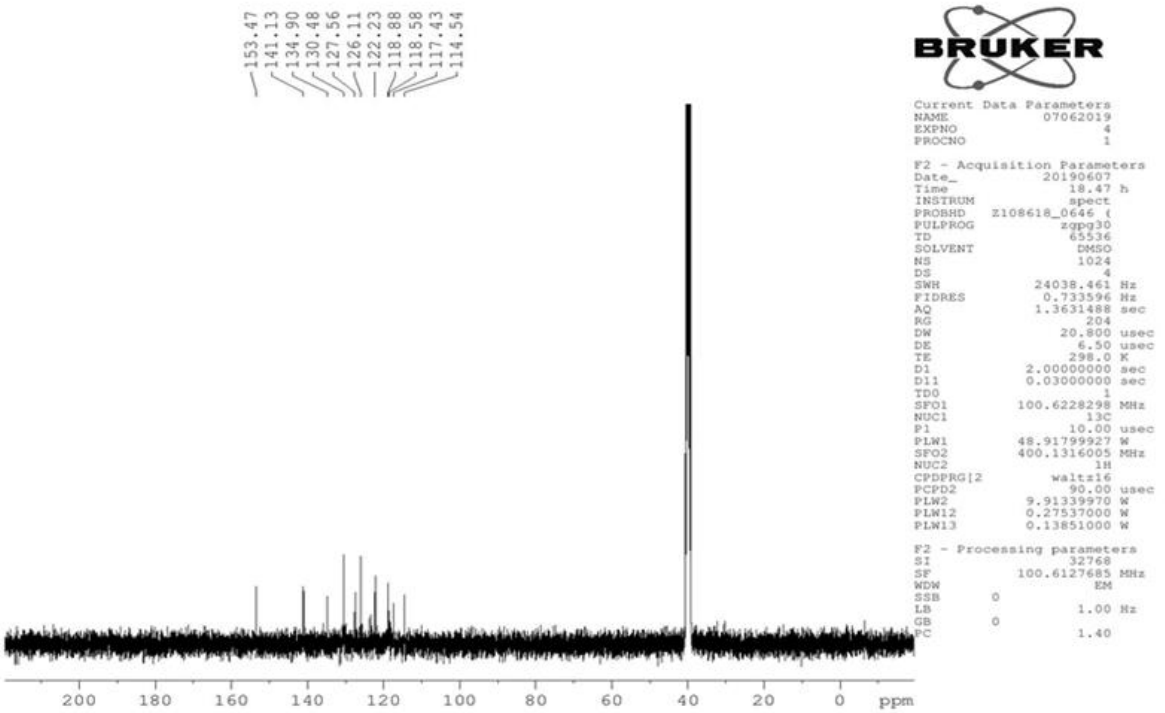

Figure S3 : ^{13}C NMR spectrum of compound **2a**

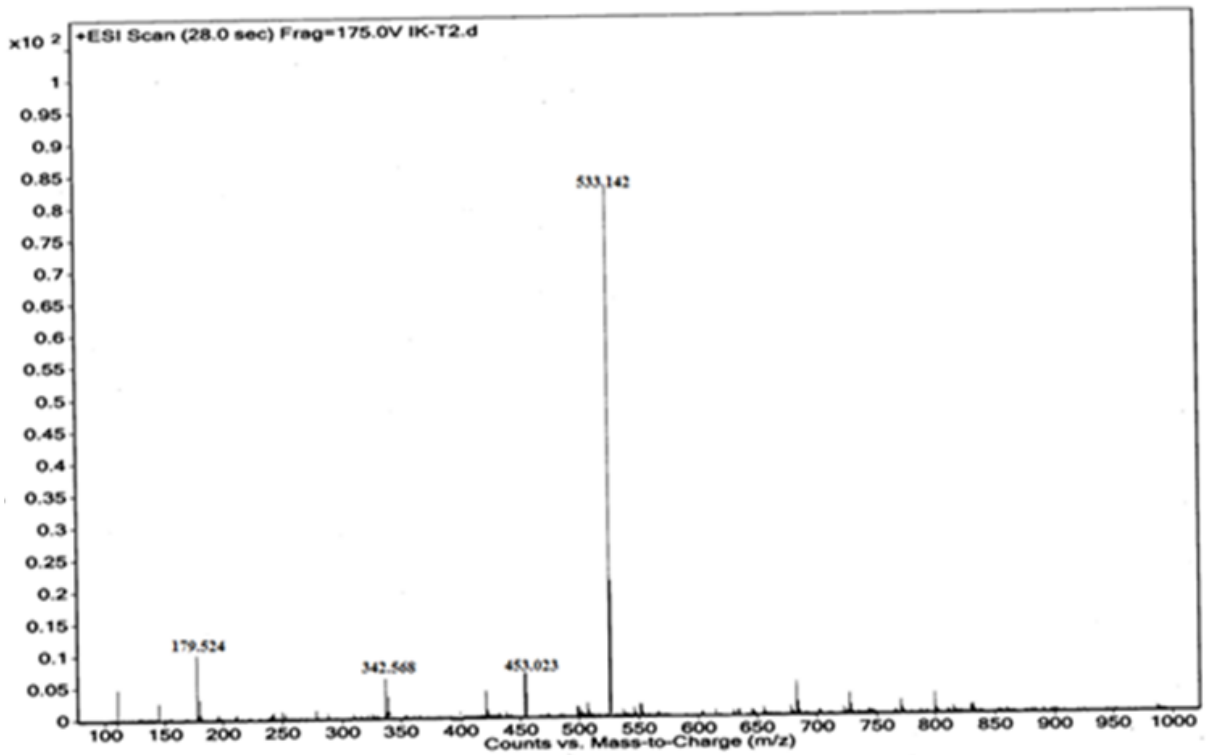

Figure S4 : Mass spectrum of compound **3j**

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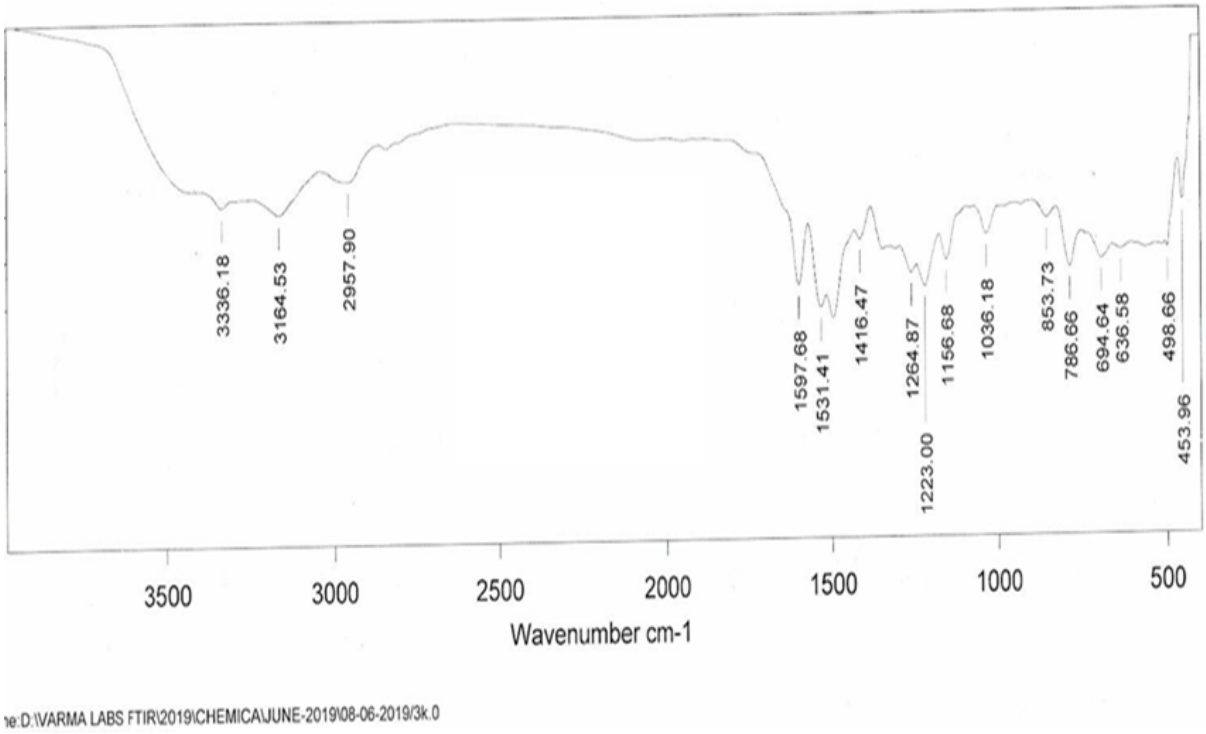

Figure S5 : IR spectrum of compound **3e**

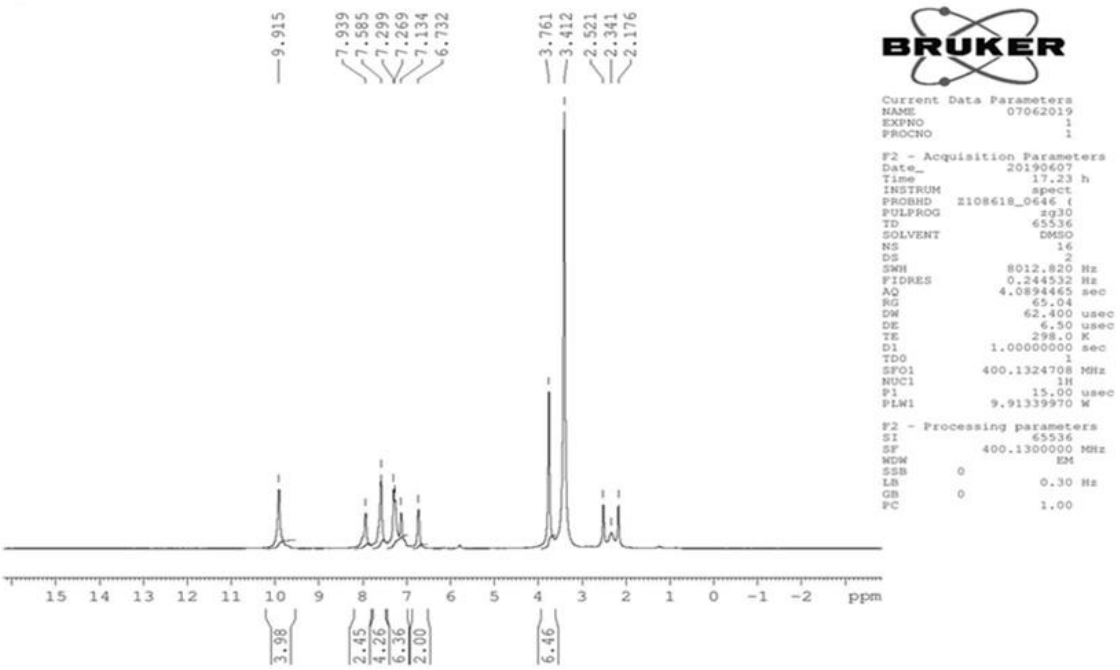

Figure S6 : ^{1}H NMR spectrum of compound **3e**

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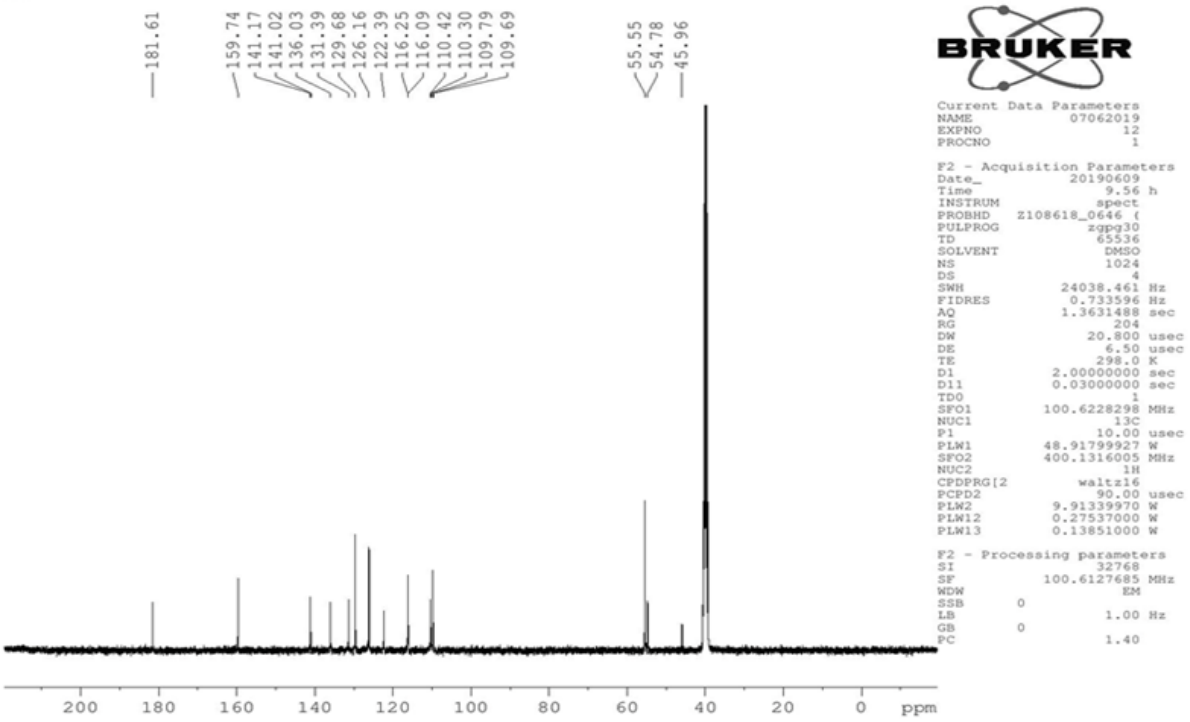

Figure S7 : ^{13}C NMR spectrum of compound **3e**

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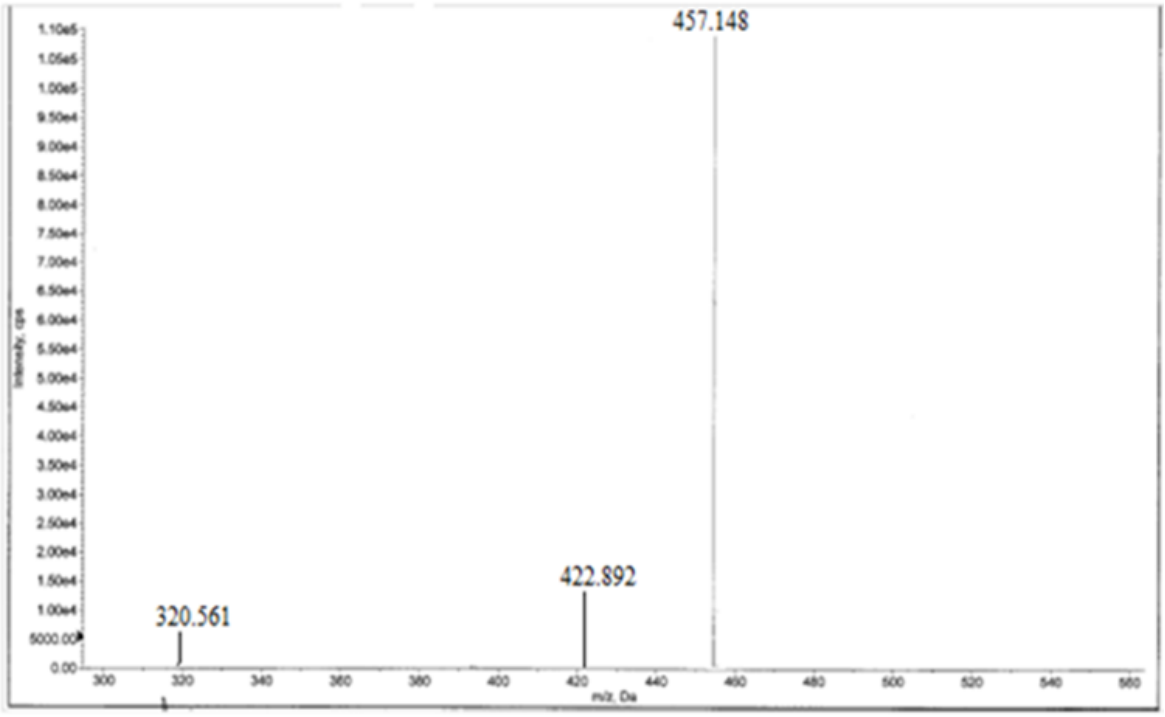

Figure S8 : Mass spectrum of compound **3e**

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