New symmetrical acyclic and alicyclic *bis*urea derivatives of 4,4'-methylene*bis*(phenyl isocyanate): Synthesis, characterization, bioactivity and antioxidant activity evaluation and molecular docking studies

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Abstract: A family of *bis*urea derivatives of 4,4′-methylene*bis*(phenyl isocyanate) have been synthesized with simple, effective and efficient procedure in high yields. The new compounds showed moderate bioactivity (at 32.0 µg/µL concentration) against selected bacterial pathogens, viz., *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa;* and two fungal species, *Candida albicans* and *Cryptococcus neoformans var. grubii.* Alternatively, their antioxidant activity was also evaluated by DPPH radical scavenging assay which revealed that the compounds, **10d, 10e, 10h** and **10m** exhibited moderate activity. However, the molecular docking studies of all the title compounds showed surprisingly higher binding energies with DNA gyrase A protein of *E. coli* when compared to the reference, streptomycin. Among the compounds **10e, 10f, 10g, 10k, 10l** and **10m** showed very good binding energies which implied that they could be promising next generation antimicrobials.

Keywords: 4,4′-Methylene*bis*(phenyl isocyanate); antimicrobial activity; antioxidant activity; molecular docking. ©2018 ACG Publication. All right reserved.

1. Introduction

The doom and gloom of antibiotic resistance dominates public perception of drugs for chemotherapy. The 2014 Report of WHO on global surveillance of antimicrobial resistance revealed

that "antibiotic resistance is no longer a prediction for the future; it is happening right now, across the world, and is putting at risk the ability to treat common infections in the community and hospitals".[1] Because of the marshaling of resistome (the accumulation of antibiotic-resistant genomes anchored in environmental bacterial populations and bacterial pathogens) genes into professional as well as opportunistic infectious agents there is always a necessity for the next generation of antimicrobial agents. The root of vancomycin resistance, for example, originate from the shipment of *vanHAX* genes from constitutionally resistant soil microbes such as *Lactobacilli* and *Leuconostoc* strains to *Enterococci* where they dwell on transposable elements.[2]

The morbific microbes especially "ESKAPE" pathogens, *Enterococcus faecium, Methicillin-Resistant Staphylococcus aureus* (MRSA), Vancomycin-Resistant *Enterococci* (VRE) (Gram-positive); *Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa* and *Enterobactor* species (Gram-negative) set a clinical challenge and become a threat to the survival of man on this planet. Man cannot be the ultimate species of appreciation unless he undermines these invisible conquerors of the world. To sustain mankind on earth one must be cautious of i. the existence of superbugs ii. the spreading of resistance world-wide iii. the tie-up of death and resistance and iv. the need to design and develop novel "magic bullets".

The fundamental strategy to eradicate the pathogenic agents banks on its kind. The antibiotics can be *bactericidal* (kill bacteria) or *bacteriostatic* (stop bacteria from multiplying) based on their mode of action. The antibiotics can also be of *narrow-spectrum* (effective against only a few types of bacteria) or *broad-spectrum* (effective against a wide range of bacteria inclusive of Gram-positive and Gram-negative bacteria) based on their bioactivity over the range of pathogens. Gram-positive infections are treated with drugs that i. inhibit the topoisomerase IV ii. inhibit the folate synthesis iii. inhibit the cell wall synthesis and iv. disrupt the plasma membrane while Gram-negative infections with drugs that i. inhibit the protein synthesis and ii. inhibit the DNA or RNA synthesis.

Antimicrobial agents can be classified as i) antibacterials (synthetic origin), ii) antibiotics (microbial origin), iii) antivirals, iv) antiprotozoals, v) antifungals and vi) antiparasitic drugs. Many of the antivirals, antiprotozoals, antifungals and antiparasitic drugs are of synthetic or semisynthetic origin. The microbial originated antibiotics include penicillins, cephalosporins, macrolides, glycopeptides, tetracyclines, aminoglycosides, etc,. The synthetic antibacterials include ansamycins, abyssomycins, depsipeptides, fluoroquinolones, lantibiotics, moenomycin, oxazolodinones, pleuromutilins, streptogramins, sulfonamides, etc. Penicillins, cephalosporins, monobactams, carbapenems find therapeutic value which are on constant drug designing to combat the pathogens of time.

The expedition beyond the frontiers of drugs today ended up with new molecular frame works. For example, TK-666, a potent synthetic inhibitor of bacterial thymidylate kinase was developed by AstraZenica. Platensimycin, a potent inhibitor of bacterial β-ketoacyl-ACP synthase type I/II was isolated from *Streptomycis platensis* by Merck.[3] Klebdelomycin, an inhibitor of DNA gyrase A was isolated from *Klebdilosporangium species* by Merck.[4]

Oxidation can be a chemical reaction that can produce reactive oxygen species (ROS) such as H_2O_2, HOCl, HOBr, ROOH, HNO_2, ozone O_3, singlet oxygen O_2, free radicals like oxygen radical, $O_2\cdot$, superoxide radical anion, $O_2\cdot^-$, hydroxyl radical, $\cdot OH$, perhydroxyl radical, $\cdot OOH$, alkoxy radical, $RO\cdot$, peroxy radical, $ROO\cdot$, and reactive nitrogen species (NOS) nitric oxide, $NO\cdot$, Nitrogen dioxide, $NO_2\cdot$ and peroxynitrite radical, $ONOO\cdot$ leading to chain reactions that may damage cellular components such as proteins (enzyme inhibition, denaturation, degradation), lipids (lipid peroxidation) and DNA (mutations, cancer). An antioxidant is a molecule that prevents the oxidation of other molecules. Antioxidants terminate these chain reactions. Antioxidants are used for two different groups of materials: industrial chemicals added to products to prevent oxidation and natural chemicals found in food and body tissues which exert beneficial health benefits as well as redox signaling. It's a paradox in metabolism that oxygen is required for the existence of life on the Earth and it damages the living cells. When the body fails to balance or counteract or detoxify the harmful effects of free radicals by means of antioxidants oxidative stress results. Oxidative stress leads to many pathophysiological conditions which include neurodegenerative disease such as Alzeimer's diseases such as Parkinson's disease, neurodegeneration in motor neuron diseases, the pathologies caused by diabetes, rheumatoid arthritis, gene mutations and cancers, fragile X syndrome, chronic fatigue syndrome, heart and blood vessel disorders, heart attack, heart failure, atherosclerosis and

inflammatory diseases. Many antioxidant enzymes such as catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase, superoxide dismutase, etc, protect DNA from oxidative stress. It is found that heavy metals like lead induce oxidative stress by i. producing ROS and ii. Depleting cellular antioxidant reserves.[5] Dietary antioxidants, vitamin A, vitamin C, vitamin E and β-carotene, glutathione (mother of antioxidants), flavonoids, catechins, polyphenols, selenium, etc, are found to exert a beneficial health effect if treated with.[6] For treating lead toxicity vitamin C, vitamin E, methionine, N-acetyl cysteine, zinc, selenium, α-lipoic acid, pyridoxine or taurine are combined with chelators such as $CaNa_2EDTA$ and DMSA.[7]
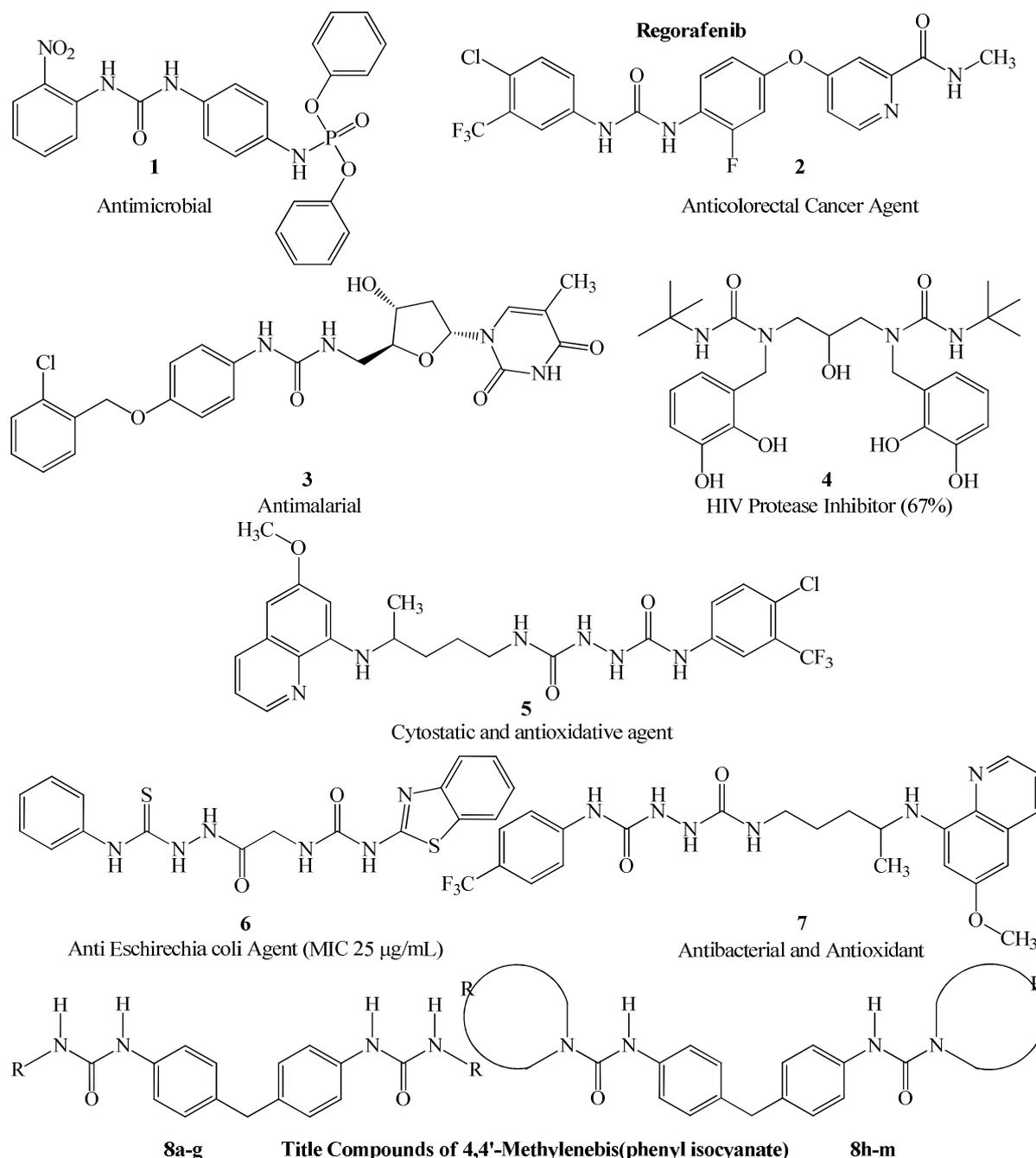

Figure 1. Bioactive urea/*bis*urea derivatives

As the virulent pathogen modifies its genetic constitution to evade antibiotic inhibition and acquires defense mechanisms to become resistant to antimicrobials there is always a unique reason to explore new antimicrobials to sustain human race and to avoid miserable ailments due to pathophysiology. The quest for exploring new frontiers of antimicrobials has bloomed emphatically

for the past two decades or so, goaded by brewing threat of microbial resistance. As our armory of "magic bullets" is getting old and less efficient only a few original replacements have become available. A number of reasons, chilling R & D costs, highly competitive market and the inability to identify ingenious antimicrobial targets, have rephrased in to a proliferative occurrence of nosocomial infective agents which no longer responsive to I or II line of antimicrobials. Deteriorating efficacy of therapeutic arbitration extends across all kinds of microbial pathology, weather caused by virus, bacteria, fungi or parasites and new diagnostic tools and therapeutic arms and ammunition are very much needed to decipher this perplexing puzzle.

One such promising niche of antimicrobials and antioxidants is urea derivatives. Urea derivatives have been widely used as anticancer agents;[8] as an anticolorectal cancer agent;[9] cytotoxic agents, anti-HIV-1 agents and antimicrobial agents;[10] as Antiepileptic agents;[11] as telomeric and genomic G-quadruplex DNA interacting agents;[12] as superoxide scavenging agents;[13] as hypoglycemic and antibacterial agents;[14] as antihyperglycaemic agents;[15] as antidiabetic agents;[16] as anti-inflammatory and antimicrobial agents (**1**);[17] as antiulcer agents (inhibitors of H+/K+-ATPase);[18] as antiproliferative agents against A375P human melanoma cell lines;[19] as strong and broad-spectrum antiproliferative agents against different cancer types;[20] as cytostatic and antioxidative agents;[21] as anti-TB or antimycobacterial agents;[22] and as antibacterial agents[23] as antimicrobial agents.[24]

Regorafenib, (**2**) a urea derivative and a broad-spectrum kinase inhibitor targeting the RAS/RAF/MEK/ERK pathway has been approved by FDA to treat metastatic colorectal cancer (CRC) and gastrointestinal stromal tumors.[25]

Yet *bis*urea derivatives also proved to be as biological potential of hybrid molecules with anti-fungal, anti-TB, anti-malarial (**3**), anti-inflammatory and anti-cancer activities.[26] They have also been HIV protease inhibitors (**4**);[27] cytostatic and antioxidative agents (**5**);[21] antiproliferative agents;[28] anti-leukemia agents;[29] anticonvulsants;[30] Raf kinase inhibitors in human cancer cell lines;[31] antimicrobial agent against *E. coli* (**6**);[32] antibacterial and antioxidative agents (**7**)[33] and thymidine phosphorylase inhibitors.[34] Amazingly *tris*urea derivatives induced apoptosis in human cancer cells.[35]

In the light of MDR nature of urea moiety in regorafenib and antibacterial nature of several urea derivatives, as a choice and chance to explore for new niches of anti-infective agents the authors have selected a new simple template molecule, 4,4′-Methylene*bis*(phenyl isocyanate) and crafted it to meet the need (synthesis of **10a-g & 10h-m**) in a simple efficient nucleophilic addition reaction.

2. Materials and Methods

2.1. Chemicals and Apparatus

All the chemicals were purchased from Sigma-Aldrich and Merck and were used as such without further purification. The solvents used for spectroscopic studies and other physical studies were reagent grade and were purified further by literature methods.

The melting points were determined on *EZ*-Melt Automated Melting Point Apparatus equipped with Digital Imaging Processing Technology of Stanford Research Systems using open capillary method and are uncorrected.

The IR spectra were recorded on a Bruker Alpha-Eco ATR-FTIR (Attenuated Total Reflection – Fourier Transform Infrared) Interferometer with single reflectionsampling module equipped with ZnSe Crystal and reported in reciprocal centimeters (cm^{-1}).

^1H and ^{13}C NMR spectra were recorded in DMSO-d_6 solvent on a Bruker FT-NMR 400 MHz spectrometer operating at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR. The ^1H and ^{13}C NMR chemical shifts were referenced to tetramethyl silane and reported in delta, δ values in ppm. Multiplicities were abbreviated as s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), m (multiplet) and br s (broad singlet).

The mass spectra were recorded on LCMS-2010A Shimadzu mass spectrometer equipped with electron spray ionization (ESI) source at atmospheric pressure in positive ion mode. A 10^{-5} M solution of **10a** and **10m** were prepared in HPLC grade DMSO separately. Each solution was infused in to the mass spectrometer at a rate of 6 μL/min. The optimized conditions set for ESI were as shown: dry N_2 flow rate 12 L/min. at 200 ºC; the nebulizer N_2 was operated at 35 psi; capillary voltage was 4000 V; the skimmer was at 65 V; and the fragmenter was maintained at a voltage of 90 V. Quantification was performed in selected ion monitoring (SIM) mode using target ions *m/z* 374 for **10a** and *m/z* 450 for

10m. The CHN Analysis was carried on Flash EA 1112 Series Thermo Finnigan for **10a** and **10m** compounds.

2.2. Synthesis of Acyclic and Alicyclic bisurea derivatives of 4,4'-Methylenebis(phenyl isocyanate)

To a mixture of 4,4'-methylenebis(phenyl isocyanate) (**8**) (0.001 mol, 0.250 g) and 0.6 mL of triethyl amine (TEA) in THF (10 mL) contained in a 50 mL RB flask, 2-aminoethanol (**9a**) (0.002 mol, 0.122 g, 0.12 mL) was added drop-wise at 10-15 °C with constant stirring. Later the reaction mixture was stirred at 45-50 °C for 1 hr. The progress of the reaction was monitored by TLC. The product, **10a** was filtered off as it was insoluble in THF and washed with a little methanol to get pure compound in excellent yield. The compounds were further purified using column chromatography. The same procedure was adopted for the synthesis of other title compounds **10b-m** using different substituted acyclic amines, **9b-g** and alicyclic amines, **9h-m** as depicted in Scheme 1.
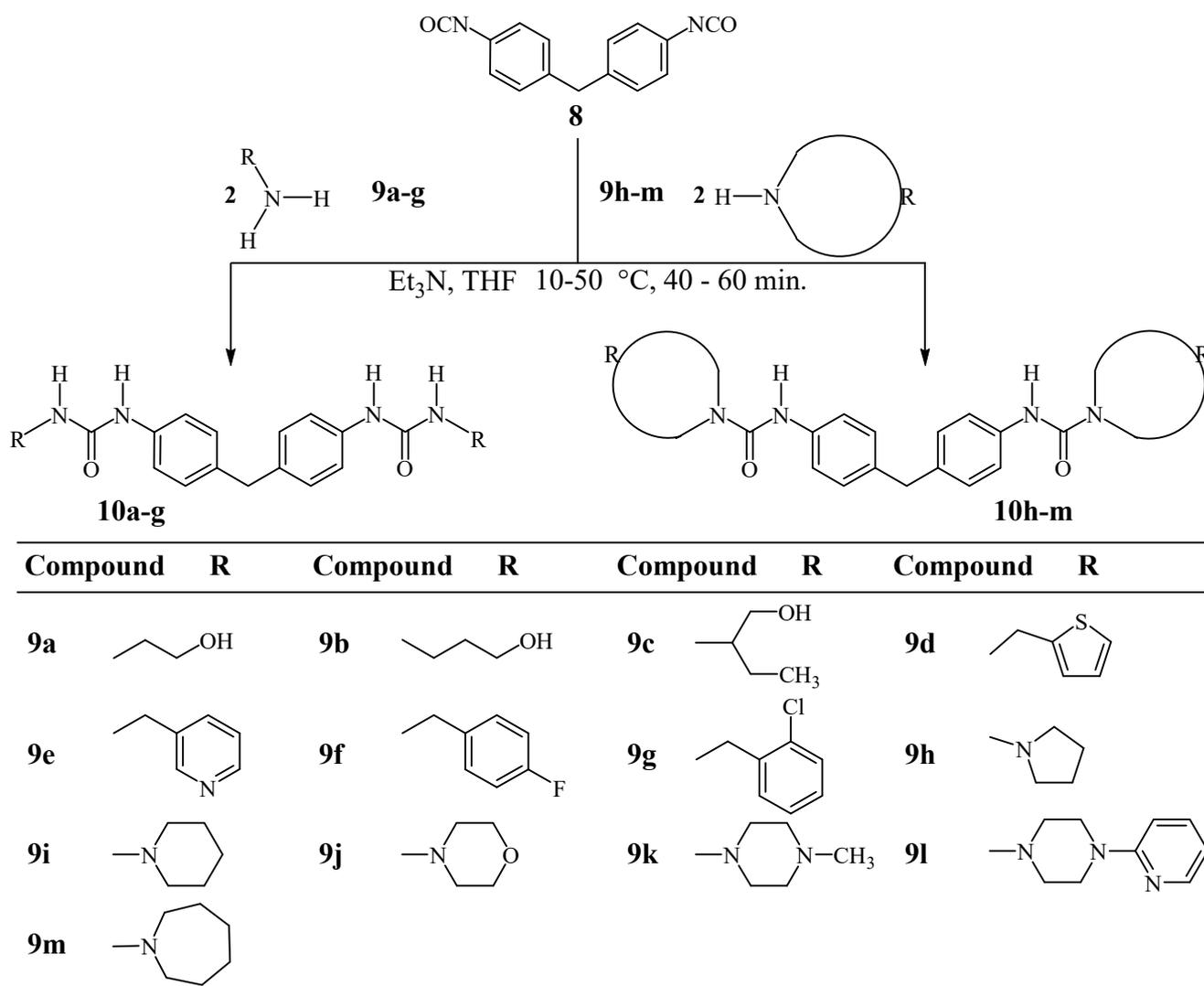
Scheme 1. Synthesis of new bisurea derivatives of 4,4'-Methylenebis(phenyl isocyanate).

2.3. Spectral Data

1,1'-(methylenebis(4,1-phenylene))bis(3-(2-hydroxyethyl)urea) (**10a**): Pale yellow solid, yield:89%, mp: 167.3 °C; IR (cm^{-1}): 3314 (O-H and N-H str), 3030 (Ar C-H str), 2939 (ali. Methylene C-H str), 2880 (Ar-CH$_2$-Ar C-H str), 1629 (C=O str), 1571 (C=C str), 1246 (C-N str), 1026 (C-O str), 811 (*p*-disub. Benzene C-H str); ^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ: 8.69 (2H, s, N-H-7, 7'), 7.27-7.00 (8H, dd,

J = 8.0 Hz, J = 8.4 Hz, Ar H-3, 5, 3', 5', 2, 6, 2', 6'), 6.43 (2H, t, J = 4.8 Hz, N-H-9, 9'), 3.92 (2H, s, OH-12, 12'), 3.73 (2H, s, benzylic H-0, 0'), 3.43 (4H, t, J=5.6 Hz, 11, 11, 11', 11'), 3.14 (4H, t, J = 5.6 Hz, H-10, 10, 10', 10');13C NMR (100 MHz, DMSO-d_6) δ: 155.6 (C-8, C-8'), 138.5 (C-4, C-4'), 134.2 (C-2, C-2', C-6, C-6'), 128.8 (C-1, C-1'), 118.4 (C-3, C-3', C-5, C-5'), 79.2 (C-11, C-11'), 60.5 (C-10, C-10'), 41.9 (benzylic C-0, C-0'). LCMS *m/z (%)*: 374 (M+D)$^+$ (100 %), 373 (M+H)$^+$, 372 M$^+$; Anal. Calcd for $C_{19}H_{24}N_4O_4$: C, 61.28; H, 6.50; N, 15.04; O, 17.18%. Found: C, 61.36; H, 6.54; N, 14.89; O, 17.21%.

1,1'-(methylenebis(4,1-phenylene))bis(3-(3-hydroxypropyl)urea) (**10b**): White solid, yield: 93%, mp: 211.4 ºC; IR (cm^{-1}): 3413 (O-H str), 3320 (N-H str), 3041 (Ar C-H str), 2936 (ali. Methylene C-H str), 2876 (Ar-CH$_2$-Ar C-H str), 1630 (C=O str), 1560 (C=C str), 1304 (C-N str), 1057 (C-O str), 917 (O-H bend), 813 (*p*-disub. Benzene C-H str); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.27 (2H, s, NH-7, 7'), 8.20-7.94 (8H, dd, J = 7.6 Hz, J = 7.6 Hz, Ar-H-3, 3', 5, 5', 2, 2', 6, 6'), 6.99 (2H, s, N-H-9, 9'), 5.42 (2H, s, O-H-13, 13'), 4.67 (2H, s, benzylic H-0, 0˙), 4.38-4.30 (8H, m, H-12, 12, 12', 12',10, 10, 10', 10'), 4.06 (4H, t, J = 5.6 Hz, H-11, 11, 11', 11').

1,1'-(methylenebis(4,1-phenylene))bis(3-(1-hydroxybutan-2-yl)urea) (**10c**): Pale yellow solid, yield:91%, mp: 178.9 ºC; IR (cm^{-1}): 3482 (O-H str), 3292 (N-H str), 3076 (Ar C-H str), 2951 (ali. CH$_3$ C-H str), 2890 (ali. Methyne C-H str), 2873 (Ar-CH$_2$-Ar C-H str), 1627 (C=O str), 1559 (C-C str), 1400 (C-H bend), 1294, 1225 (C-N str), 1047 (C-O str), 822 (*p*-disub. Benzene C-H str); 1H NMR (400 MHz, DMSO-d_6) δ: 8.53 (2H, s, N-H-7, 7'), 7.27-7.00 (8H, dd, J = 8.4 Hz, ArH-3, 5, 3', 5', 2, 6, 2', 6'), 6.08 (2H, s, N-H-9, 9'), 4.78 (2H, s, O-H-12, 12'), 3.73 (2H, s, benzylic H-0, 0˙, 6, 6˙), 3.33 (4H, t, J = 5.2 Hz, H-11, 11, 11', 11'), 1.57 (2H, m, H-10, 10'), 1.38 (4H, m, H-13, 13, 13', 13'), 0.87 (6H, t, J = 7.6 Hz, H-14, 14, 14, 14', 14', 14').

1,1'-(4,4'-methylenebis(4,1-phenylene))bis(3-thiophen-2-yl-methyl)urea) (**10d**): White solid, yield: 97%, mp: 238.2 ºC; IR (cm^{-1}): 3324 (N-H str), 3043 (Ar C-H str), 2912 (ali. CH$_2$ C-H str), 2838 (Ar-CH$_2$-Ar C-H str), 1630 (C=O str), 1563 (C=C str), 1415 (C-H bend), 1300, 1236 (C-N str), 819 (*p*-disub. Benzene C-H str), 690 (C-S str); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.47 (2H, s, N-H-7, 7'), 7.38 (2H, d, J = 4.4 Hz, Ar-H-13, 13'), 7.31-7.05 (8H, dd, J = 8.4 Hz, J = 8 Hz, Ar-H-3, 5, 3', 5', 2, 6, 2', 6'), 6.98 (2H, d, J = 5.2 Hz, Ar-H-15, 15'), 6.96 (2H, t, J = 3.6 Hz, Ar-H-14, 14'), 6.62 (2H, t, J = 6 Hz, N-H-9, 9'), 4.46 (4H, d. J = 5.6 Hz, H-10, 10, 10', 10'), 3.77 (2H, s, benzylic H-0, 0').

2.3.5. 1,1'-(methylenebis(4,1-phenylene))bis(3-(pyridin-3-ylmethyl)urea) (**10e**): White solid, yield: 96%, mp: 210.0 ºC; IR (cm^{-1}): 3302 (N-H str), 3033 (Ar C-H str), 2906 (ali. CH$_2$ C-H str), 1631 (C=O str), 1552 (C=C str), 1412 (C-H bend), 1298, 1229 (C-N str), 854 (*p*-disub. Benzene C-H str); 1H NMR (400 MHz, DMSO-d_6) δ: 8.69 (2H, s, N-H-7, 7'), 8.53 (2H, d, J = 1.6 Hz, Ar-H-12, 12'), 8.46-8.44 (2H, dd, J = 1.2 Hz, J = 1.2 Hz, Ar-H-14, 14'), 7.71 (2H, d, J = 8 Hz, Ar-H-16, 16'), 7.36 (2H, t, J = 4.8 Hz, Ar-H-15, 15'), 7. 33-7.04 (8H, dd, J = 8.4 Hz, J = 8.4 Hz, J = 8.4 Hz, Ar-H-3, 5, 3', 5', 2, 6, 2', 6'), 6.85 (2H, t, J = 6 Hz, N-H-9, 9'), 4.32 (4H, d, J = 6 Hz, H-10, 10'), 3.76 (2H, s, benzylic H-0, 0˙).

1,1'-(methylenebis(4,1-phenylene))bis(3-(4-fluorobenzyl)urea) (**10f**): White solid, yield: 97%, mp: 240.7 ºC; IR (cm-1): 3298 (N-H str), 3036 (Ar C-H str), 2927 (ali. CH$_2$ C-H str), 1620 (C=O str), 1555 (C=C str), 1413 (C-H bend), 1299, 1214 (C-N str), 1099 (C-F str), 821 (*p*-disub. Benzene C-H str); 1H NMR (400 MHz, DMSO-d6) δ: 8.49 (2H, s, N-H-7, 7'), 7.33-7.28 (8H, dd, J = 8.4 Hz, J = 7.6 Hz, Ar-H-3, 3', 5, 5', 12, 12', 16, 16'), 7.15 (4H, protons couple with F to give a triplet, t, J = 8.8 Hz, Ar-H-13,13', 15, 15'), 7.05 (4H, d, J = 8.4 Hz, Ar-H-2, 6, 2', 6'), 6.59 (2H, t, J = 5.2 Hz, N-H-9, 9'), 4.26 (4H, d,J = 5.6 Hz, H-10, 10'), 3.74 (2H, s, benzylic H-0, 0˙).

1,1'-(4,4'-methylenebis(4,1-phenylene))bis(3-(2-chlorobenzyl)urea) (**10g**): White solid, yield: 95%, mp: 257.3 ºC; IR (cm-1): 3307 (N-H str), 3031 (Ar C-H str), 2917 (ali. CH$_2$ C-H str), 1631 (C=O str),

1575 (C=C str), 1421 (C-H bend), 1289, 1237 (C-N str), 818 (*p*-disub. Benzene C-H str), 748 (*o*-disub. benzene C-H str), 653 (C-Cl str); ^{1}H NMR (400 MHz, DMSO-d6) δ: 8.59 (2H, s, N-H-7, 7'), 7.46-7.04 (16H, m, Ar-H-13, 13', 3, 5, 3', 5', 15, 15', 14, 14', 16, 16', 2, 6, 2', 6'), 6.62 (2H, t, J = 5.6 Hz, N-H-9, 9'), 4.36 (4H, d, J = 6.0 Hz, H-10, 10'), 3.76 (2H, s, benzylic H-0, 0'); 13C NMR (100 MHz, DMSO-d6) δ: 155.1 (C-8, C8'), 138.1 (C-11, C-11'), 137.3 (C-4, C-4'), 134.4 (C-12, C-12'), 131.9 (C-2, C-2', C-6, C-6'), 129.0 (C-13, C13'), 128.8 (C-16, C-16'), 128.7 (C-14, C-14'), 128.5 (C-1, C-1'), 127.1 (C-15, C-15'), 117.8 (C-3, C-3', C-5, C-5'), 40.7 (benzylic C-0, C-0'), 40.1 (C-10, C-10').

N,N'-(methylenebis(4,1-phenylene))bis(pyrrolidine-1-carboxamide) (**10h**): White solid, yield: 98%, mp: 217.8 °C; IR (cm-1): 3307 (N-H str), 3034 (Ar C-H str), 2960 (ali. CH$_2$ C-H str), 2866 (ring CH$_2$ C-H str), 1637 (C=O str), 1590 (C=C str), 1516 (aromatic ring str), 1412 (C-H bend), 1301, 1236 (C-N str), 805 (*p*-disub. Benzene C-H wag); ^{1}H NMR (400 MHz, CDCl$_3$) δ: 7.30-7.04 (8H, dd, J = 8.4 Hz, J = 8.0 Hz, Ar-H-3, 5, 3', 5', 2, 6, 2', 6'), 6.18 (2H, s, N-H-7, 7'), 3.84 (2H, s, benzylic H-0, 0'), 3.43 (8H, t, J = 6.8 Hz, 10,10, 13, 13, 10', 10',13', 13'), 1.95-1.90 (8H, m, H-11, 11, 12, 12, 11',11', 12', 12').

N, N'-(methylenebis(4,1-phenylene))bis(piperidine-1-carboxamide) (**10i**): Pale yellow solid, yield: 99%, mp: 202.5 °C; IR (cm-1): 3317 (N-H str), 3039 (Ar C-H str), 2925 (ali. CH$_2$ C-H str), 2853 (ring CH$_2$ C-H str), 1636 (C=O str), 1521 (aromatic ring str), 1420 (C-H bend), 1304, 1237 (C-N str), 806 (*p*-disub. Benzene C-H wag); ^{1}H NMR (400 MHz, DMSO-*d6*) δ: 8.33 (2H, s, N-H-7, 7'), 7.34-7.01 (4H, dd, J = 7.6 Hz, J = 8.8 Hz, Ar-H-3, 3', 5, 5', 2, 2', 6, 6'), 3.77 (2H, s, benzylic H-0, 0'), 3.75 (8H, s, H-10, 10, 10', 10', 14, 14, 14', 14'), 1.54 (4H, s, H-12, 12, 12', 12'), 1.46 (8H, s, H-11, 11, 11', 11', 13, 13, 13',13'); ^{13}C NMR (100 MHz, DMSO-*d6*) δ: 154.9 (C-8, C-8'), 138.5 (C-4, C4'), 134.9 (C-2, C-2', C-6, C-6'), 128.8 (C-1, C-1'), 119.7 (C-3, C-3'), 118.2 (C-5, C-5'), 44.6 (C-10, C-10', C-14, C-14'), 40.1 (benzylic C-0, C-0'), 25.4 (C-11, C-11' , C-13, C-13'), 24.0 (C-12, C-12').

N,N'-(methylenebis(4,1-phenylene))bis(morpholine-4-carboxamide) (**10j**): White solid, yield: 98%, mp: 241.5 °C; IR (cm^{-1}): 3334 (N-H str), 3037 (Ar C-H str), 2968 (ring CH$_2$ (near O) C-H str), 2927 (ali. CH$_2$ C-H str), 2857 (ring CH$_2$ (near N) C-H str), 1640 (C=O str), 1520 (aromatic ring str), 1417 (C-H bend), 1302, 1245 (C-N str), 1113 (ring C-O-C str), 805 (*p*-disub. Benzene C-H wag); ^{1}H NMR (400 MHz, DMSO-*d6*) δ:8.45 (2H, s, N-H-7, 7'), 7.35-7.04 (8H, dd, J = 8.4 Hz, J = 8.4 Hz, Ar-H-3, 5, 3', 5', 2, 6, 2', 6'), 3.77 (2H, s, benzylic H-0, 0'), 3.59 (8H, t, J = 4.4 Hz, 10, 10, 14, 14, 10', 10', 14', 14'), 3.40 (8H, t, J = 5.2 Hz, H-11, 11, 13, 13, 11',11', 13', 13').

N,N'-(methylenebis(4,1-phenylene))bis(4-methylpiperazine-1-carboxamide) (**10k**): White solid, yield: 99%, mp: 210.5 °C; IR (cm^{-1}): 3329 (N-H str), 3041 (Ar C-H str), 2935 (ali. CH$_2$ C-H str), 2853 (ring CH$_2$ (near N) C-H str), 2793 (CH$_3$ C-H str), 1636 (C=O str), 1516 (aromatic ring str), 1410 (C-H bend), 1296, 1234 (C-N str), 808 (*p*-disub. Benzene C-H wag); ^{1}H NMR (400 MHz, DMSO-*d6*) δ: 8.62 (2H, s, N-H-7, 7'), 7.55-7.23 (8H, dd, J = 8.0 Hz, J = 8.0 Hz, Ar-H-3, 5, 3', 5', 2, 6, 2', 6'), 3.97 (2H, s, benzylic H-0, 0'), 2.70 (8H, s, 10, 10, 14, 14, 10', 10', 14', 14'), 2.49 (8H, s, H-11, 11, 13, 13, 11',11', 13', 13'), 2.38 (6H, s, 15, 15, 15, 15', 15', 15').

N,N'-methylenebis(4,1-phenylene))bis(4-(pyridine-2-yl)piperazine-1-carboxamide) (**10l**): White solid, yield: 97%, mp: 213.8 °C; IR (cm-1): 3336 (N-H str), 3004 (Ar C-H str), 2937 (ali. CH$_2$ C-H str), 2848 (ring CH$_2$ (near N) C-H str), 1648 (C=O str), 1596 (C=N str), 1531, 1486 (arom**2**atic ring str), 1420 (C-H bend), 1307, 1229 (C-N str), 818 (*p*-disub. Benzene C-H wag); 1H NMR (400 MHz, DMSO-d6) δ: 8.52 (2H, s, NH-7, 7'), 8.13 (2H, d, J = 4.0 Hz, Ar-H-17, 17'), 7.56 (2H, t, J = 7.6 Hz, Ar-H-19, 19'), 7.38-7.05 (8H, dd, J = 8.4 Hz, J = 8.0 Hz, Ar-H-2, 2', 3, 3', 5, 5', 6, 6'), 6.86 (2H, d, J = 8.4 Hz, Ar-H-20, 20'), 6.67 (2H, t, J = 5.6 Hz, Ar-H-18, 18'), 3.78 (2H, s, benzylic H-0, 0'), 3.53 (16H, s, H-10, 10, 10', 10', 11, 11, 11', 11', 13, 13, 13', 13', 14, 14, 14', 14').

N,N'-(methylenebis(4,1-phenylene))bis(azepane-1-carboxamide)(**10m**): White solid, yield: 97%, mp: 219.4 °C; IR (cm-1): 3321 (N-H str), 3032 (Ar C-H str), 2920 (ali. CH$_2$ C-H str), 2856 (ring CH$_2$ C-H str), 1637 (C=O str), 1514 (aromatic ring str), 1416 (C-H bend), 1301, 1246 (C-N str), 802 (*p*-disub.

Benzene C-H wag); ^{1}H NMR (400 MHz, CDCl$_3$) δ: 7.29-7.05 (8H, dd, J = 8.4 Hz, J = 8.0 Hz, Ar-H-3, 5, 3', 5', 2, 6, 2', 6'), 6.27 (2H, s, N-H-7, 7'), 3.86 (2H, s, benzylic H-0, 0´), 3.49 (8H, t, H-10, 10, 15, 15, 10', 10', 15', 15',), 1.76 (16H, m, H-11, 11, 12, 12, 13, 13, 14, 14, 11',11', 12', 12', 13', 13', 14', 14'); ^{13}C NMR (100 MHz, CDCl$_3$) δ: 155.3 (C-8, C-8'), 137.4 (C-4, C-4'), 135.9 (C-2, C-2', C-6, C-6'), 129.3 (C-1, C-1'), 120.1 (C-3, C-3', C-5, C-5'), 46.7 (C-10, C-10', C-15, C-15'), 40.6 (benzylic C-0, C-0'), 28.7 (C-11, C-11', C-14, C-14'), 27.3 (C-12, C-12' , C-13, C-13'). LCMS *m/z (%)*: 450 (M+D)$^+$ (100 %); Anal. Calcd for C$_{27}$H$_{36}$N$_4$O$_2$: C, 72.29; H, 8.09; N, 12.49; O, 7.13%. Found: C, 72.15; H, 8.16; N, 12.41; O, 7.28%.

2.4. Antibacterial Activity

Primary antimicrobial screening study by whole cell growth inhibition assay method, using the title compounds at a single concentration, in duplicate (n=2) was done. The inhibition of growth is measured against five bacterial species and two fungi by Community for Open Antimicrobial Drug Discovery (CO-ADD), a global open-access screening initiative of The University of Queensland's Institute for Molecular Bioscience which is funded by the Wellcome Trust and The University of Queensland, Australia to uncover significant and rich chemical diversity held outside of corporate screening collections and provides unencumbered free antimicrobial screening for any interested academic researcher. CO-Add has been recognized as a novel approach in the fight of superbugs.

2.4.1. Sample Preparation

Samples were provided by the collaborator and stored frozen at -20 °C. Samples were prepared in DMSO and water to a final testing concentration of 32 μg/mL, in 384-well, non-binding surface plate (NBS) for each bacterial/fungal strain, and in duplicate (n=2), and keeping the final DMSO concentration to a maximum of 1% DMSO. All the sample-preparation was done using liquid handling robots. Compounds that showed solubility issues during stock solution preparation are detailed in the data sheet.

2.4.2. Antimicrobial Assay Procedure

All bacteria were cultured in Cation-adjusted Mueller Hinton broth (CAMHB) at 37 ºC overnight. Then a sample of each culture was diluted 40-fold in fresh broth and incubated at 37 ºC for 1.5-3.0 h. The resultant mid-log phase cultures were diluted (CFU/mL measured by OD$_{600}$), then added to each well of the compound containing plates, giving a cell density of 5x10^5 CFU/mL and a total volume of 50 μL. All the plates were covered and incubated at 37 ºC for 18 h without shaking.

2.4.3. Antifungal Assay Procedure

Fungi strains were cultured for three days on Yeast Extract-Peptone Dextrose (YPD) agar at 30 ºC. A yeast suspension of 1x10^6 to 5x10^6 CFU/mL (as determined by OD$_{530}$) was prepared from five colonies. The suspension was subsequently diluted and added to each well of the compound containing plates giving a final cell density of fungi suspension of 2.5x10^3 CFU/mL and a total volume of 50 μL. All plates were covered and incubated at 35 ºC for 24 h without shaking.

2.4.4. Antibiotic Standards Preparation and Quality Control

Colistin and vancomycin were used as positive bacterial inhibitor standards for Gram-negative and Gram-positive bacteria, respectively. Fluconazole was used as a positive fungal inhibitor standard. The antibiotics were provided in 4 concentrations, with two above and two below its MIC value and plated in to the first 8 wells of column 23 of the 384-well NBS plates. The quality control (QC) of the assays was determined by the antimicrobial controls and the Z'-factor (using positive and negative controls). The tested strains and positive controls are listed in supporting information. All antibiotic and antifungal controls displayed inhibitory values within the expected range. For inhibitory results for controls please contact the CO-ADD at support@co-add.org.

2.5. Antioxidant Activity

The beneficial effects of fruits, vegetables, beverages and newly synthesized drug molecules on human health have been attributed to their antioxidant activities. Therefore, antioxidant activity of food products is recognized as one of the important parameters in determining their functional values. Until now, antioxidant activity has been measured by various chemical and biological methods; 1. β-Carotene Bleaching (BCB) Test 2. Thiobarbituric Acid Reactive Species (TBARS) Assay 3. DPPH (1,1-diphenyl-2-picrylhydrazyl) Radical Scavenging Assay 4. FRAP The ferric reducing antioxidant Power) assay and 5. Phycoerythyrin Assay. The present study employed DPPH Radical Scavenging Assay to assess the antioxidant activity of the title compounds.[36]

2.5.1. Antioxidant Activity by DPPH Scavenging Assay

Antioxidant potential of the chemicals was estimated using modified DPPH free radical scavenging assay in 96 micro-well flat plates (Tepe *et al.*, 2005; Maya *et al.,* 2014). Stock solutions of the chemicals were prepared as 1 mg/mL in DMSO. Each well was filled in with 200 μL chemical in DMSO starting from 100 μg/mL down to the lowest 20 μg/mL. Then 5μL of the DPPH solution (2.5 mg/mL in methanol) was added to each well. After keeping the plate in the darkness for 30 minutes, the optical density (OD) of each well was read using Tecan Infinite M 200 micro plate reader at wavelength 517 nm. All the tests were performed in triplicate (n=3). All the values are reported in Mean + SD. Percentage of inhibition was calculated using the formula:

$$\%Inhibition = \frac{OD\ (DPPH - Sample)}{OD\ (DPPH)} x100$$

The radical scavenging activity was examined and compared with other natural antioxidant, ascorbic acid which was used as positive control. Table 2 explains the % inhibition of DPPH at different concentrations.

2.6. Molecular Docking Studies

The DNA gyrase is an important bacterial enzyme that catalyzes the ATP-dependent negative super-coiling of double-stranded closed-circular DNA. Gyrase belongs to a class of enzymes known as topoisomerases which are involved in the control of topological transitions of DNA. From an enzymological stand point it is of inherent interest how gyrase influences the topological state of DNA molecules. In addition, more studies were focused on DNA gyrase as the intracellular target of a number of antibacterial agents and as a paradigm for other DNA topoisomerases (Ter et al., 2001).[37]

Streptomycin, an antibiotic derived from *Streptomyces griseus*, irreversibly binds to the 16S rRNA and S12 protein within the bacterial 30S ribosomal subunit. Consequently this reagent interferes with the assembly of initiation complex between mRNA and the bacterial ribosome, thereby inhibiting the initiation of protein synthesis. In addition, streptomycin induces misreading of the mRNA template and causes translational frame shift and results in premature termination which ultimately leads to bacterial cell death (Petersen *et al.,* 2004).[38]

2.6.1. Materials and Methods: Accession of Target Protein

The three-dimensional structure of DNA gyrase A (PDB: 3LPX) and the reference drugs such as Streptomycin (Pub Chem ID 19649) was downloaded from the RCSB protein Data Bank. The atomic coordinates of the protein was estranged and geometry optimization was done using Argus Lab 4.0.1. The enzyme 3LPX and the reference drug, Streptomycin (Pub Chem ID 19649) were shown in Figure 2 (A&B).[39]
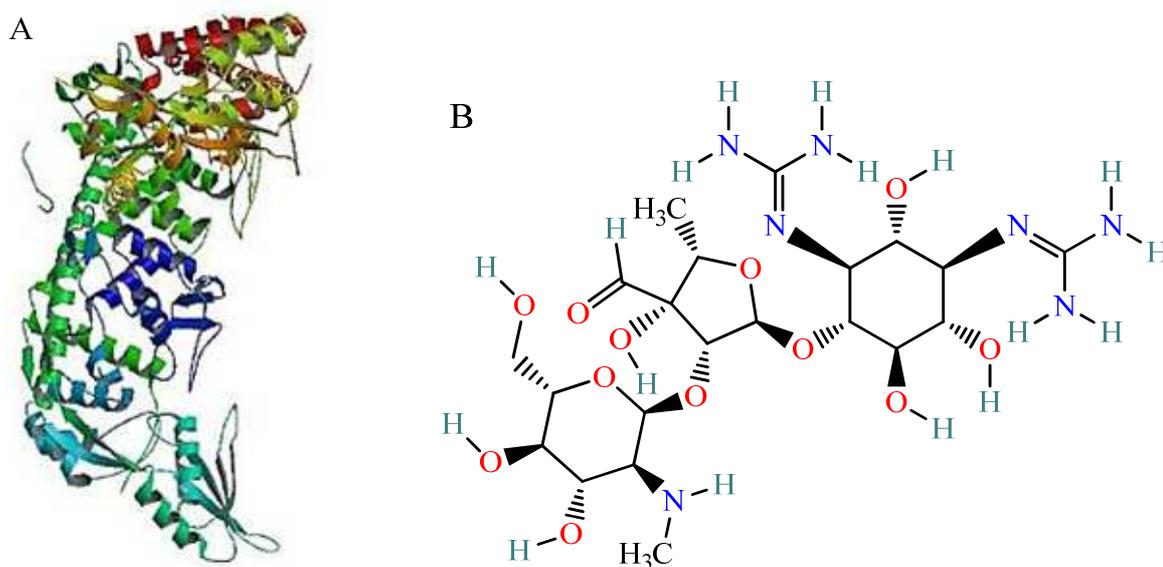
Figure 2. A. DNA Gyrase A (*E. coli*)   **B.** Streptomycin-PC ID 19649

2.6.2. Ligand Preparation

The chemical structures of the compounds were drawn using ChemBioDraw and converted all the ligands into Pdbqt file format and atomic coordinates were generated using Pyrx 2010.12

2.6.3. Analysis of Target Active Binding Sites

The active sites are the coordinates of the ligand in the original target protein grids, and these active binding sites of target protein were analyzed using the Drug Discovery Studio version 3.0 and 3D Ligand Site virtual tools.

2.6.4. Molecular Docking Analysis

Molecular docking studies were carried against DNA gyrase A protein with the title compounds, **10a-m** and the reference drug, streptomycin using the docking module implemented in Pyrx 2010.12. Initially the protein structures were protonated with the addition of polar hydrogens, followed by energy minimization with the MMFF94x force field, in order to get the stable conformer of the proteins. Flexible docking was employed, the inhibitor binding site residues were and highlighted through the "site finder" module implemented in the Pymol software. The grid dimensions were predicted as X: 28.27, Y: 27.13, Z: 28.51 for Aromatase respectively. The docking was carried out with the default parameters i.e., placement: triangle matcher, recording 1: London dG, refinement force field and a maximum of 10 conformations of each compound were allowed to be saved in a separate data base file in a mdb format. After the docking process, the binding energy and binding affinity of the protein ligand complexes were calculated using Pymol viewer tool (www.pymol.org).

2.6.5. Structural Analysis and Visualization

Protein and ligand interactions were analysed and visualized through Pymol viewer tool (www.pymol.org).

3. Results and Discussion

3.1. Chemistry

A new series of bisurea derivatives of 4,4'-methylene*bis*(phenyl isocyanate) were synthesized by simple nucleophilic substitution using different acyclic and alicyclic amines one after another which were added drop wise at 10-15 °C in the presence of a base, TEA in THF and later at 45-50 °C with constant stirring for 1 h as depicted in Scheme 1. The resulting insoluble *bis*urea derivatives **10a-m** were filtered off and further purified by washing with methanol.

The structures of the title compounds were established by FT-IR, ^{1}H, ^{13}C NMR, mass spectra and elemental analysis. In IR spectra, stretching bands at 3482-3314 cm^{-1}, 3336-3302 cm^{-1}, 3076-3004 cm^{-1}, 2960-2906 cm^{-1} and 2866-2848 cm^{-1} belong to O-H, N-H, aromatic C-H, aliphatic C-H and C-H of ring CH$_2$ stretching respectively. Absorption bands at 1648-1620 cm^{-1} represent C=O stretching of *bis*urea derivatives. The wave numbers at 1307-1289 and 1246-1214 cm-1 correspond to C-N stretching and 1057-1026 cm-1 correspond to C-O stretching. In ^{1}H NMR spectra of the *bis*urea derivatives, O-H proton resonated at 5.42-3.92 ppm. N-H proton signal appeared at 9.27-8.33 and 6.99-6.08 ppm. The aromatic protons of phenyl, pyridinyl, thiophenyl, fluorobenzyl and chlorobenzyl rings appeared at 8.53-6.67 ppm. The methylene protons of pyrrolidine, piperidine, morpholine, piperazine, azepane moieties appeared at 3.75-1.76 ppm. Aliphatic methyl(ene) protons resonated in the region 4.46-0.87 ppm. The ^{13}C NMR spectral data obtained for the compounds, **10a, 10g, 10i** and **10m** stood as additional structural support for the title compounds. The carbonyl carbon (C-8, 8') of the urea moiety resonated in the region 155.6-154.9 ppm and that of methylenic carbon (C-0, 0') common to the two phenyl groups resonated between 41.9-40.1 ppm. The LCMS and CHN analysis data obtained for **10a** and **10m** were in good agreement with the calculated values, and thus provided the additional support for the title compounds.

3.2. Antimicrobial Activity

Table 1. Antimicrobial activity with % inhibition of the title compounds **10a-m**

Compound Code	*Sa*	*Ec*	*Kp*	*Pa*	*Ab*	*Ca*	*Cn*	Conc.
10a	39.27	-3.35	19.41	21.49	0.04	12.27	-56.75	32 ug/mL
10b	39.25	2.92	1.09	24.99	4.87	2.27	-57.49	32 ug/mL
10c	27.37	1.53	11.19	20.89	0.62	-0.17	-38.07	32 ug/mL
10d	38.55	-5.58	5.00	23.05	-3.62	6.29	-66.42	32 ug/mL
10e	43.02	3.89	18.06	24.80	11.12	3.50	7.56	32 ug/mL
10f	46.23	4.52	20.19	24.92	14.31	4.25	9.30	32 ug/mL
10g	44.01	4.02	19.46	24.10	12.76	4.59	8.24	32 ug/mL
10h	30.22	0.09	16.99	24.43	5.12	-2.82	-36.92	32 ug/mL
10i	21.66	-3.07	6.07	19.72	-3.13	19.82	2.44	32 ug/mL
10j	29.19	1.12	19.30	17.98	12.02	2.61	10.23	32 ug/mL
10k	42.05	3.67	18.91	19.47	17.22	2.71	6.67	32 ug/mL
10l	51.83	5.16	22.24	23.73	21.84	24.16	9.24	32 ug/mL
10m	48.46	4.68	20.20	25.74	18.03	14.16	9.32	32 ug/mL

Sa: Staphylococcus aureus; *Ec: Escherichia coli;* *Kp: Klebsiella pneumonia;* *Pa: Pseudomonas aeruginosa;* *Ab: Acinetobacter baumannii;* *Ca: Candida albicans;* *Cn: Cryptococcus neoformans;* **Conc:** *Concentration*

The in vitro primary antimicrobial inhibition of growth screening was studied against 5 bacteria and 2 fungi by whole cell growth inhibition assays, using the title compounds at a single concentration, in duplicate (n=2) . All the title compounds showed moderate antimicrobial activity against the selected resistant pathogens at a concentration of 32.0 µg/µL (Table 1).

3.3. Antioxidant Activity

All the title compounds showed moderate antioxidant activity by DPPH method. Among the title compounds **10h** and **10m** showed the highest and **10e** and **10d** exhibited noteworthy antioxidant activity (Table 2).

Table 2. Antioxidant activity of the title compounds **10a-m** with % of Inhibition values

Compound Code	% of inhibition of DPPH [Mean ± SD] at different concentrations (µg/mL)				
	20	40	60	80	100
10a	31.17±1.12	33.99±1.12	38.72±0.53	39.86±0.48	47.33±0.07
10b	31.02±0.60	31.25±0.57	32.55±0.49	33.16±0.47	33.31±0.36
10c	29.19±0.30	36.66±1.16	39.18±0.56	40.07±1.00	43.37±1.03
10d	30.11±0.57	38.72±1.09	41.31±0.44	42.76±0.51	43.60±0.64
10e	30.26±0.48	35.29±0.64	37.73±1.05	42.30±1.01	57.55±0.82
10f	30.34±0.89	31.71±0.45	33.38±0.64	35.59±0.22	40.63±0.49
10g	28.96±0.42	35.44±0.73	36.97±1.22	37.04±0.45	38.57±1.25
10h	31.48±1.40	37.27±0.57	43.90±0.38	45.58±1.34	55.34±0.46
10i	31.10±1.30	32.70±0.43	38.34±0.54	39.79±0.83	45.58±1.16
10j	30.56±1.04	33.54±1.17	36.28±0.92	40.32±1.12	42.91±0.48
10k	31.55±0.59	35.14±1.02	41.46±0.87	42.00±0.90	43.07±0.17
10l	27.82±0.80	30.41±0.65	37.58±0.85	40.78±0.63	46.80±0.82
10m	35.90±1.09	37.73±0.75	42.76±0.24	47.79±0.66	52.74±0.80
Standard (Ascorbic acid)	85.82±1.40	87.2±0.94	89.18±0.94	89.25±0.32	89.25±0.32

*All the values are reported in Mean + SD (n=3).

3.4. Molecular Docking Studies

In order to provide strength to the synthesized compounds, docking analysis was carried out for compounds **10a-m** with selective pharmacological target such as DNA Gyrase A. protein of *E. coli* which is a suitable target for anti-bacterial activity. The crystal structure of DNA Gyrase A (PDB id: 3LPX) was retrieved from the protein data bank and the reference drug Streptomycin (**PC ID 19649)** from PubChem DrugBank. The docking results of DNA Gyrase A showed that the compounds, **10a-m** have significant binding modes when compared with the control drug, Streptomycin (-6.9 kcal/mol). Among the 13 compounds, **10l, 10f, 10m, 10g, 10e** and **10k** showed highest binding affinity towards DNA Gyrase A. The H-bonds, binding affinities and energy profiles of compounds **10a-m** along with reference drug towards the active site amino acids of the enzyme are summarized in Table 3.

Table 3. Bonding characterization of title compounds **10a-m**

Compound Code	Rank	Binding energy (kcal/mol)	Binding interaction	Bond Length (Å)	Bond Angle (º)	Bond Type
10a	12	- 7.1	Lys270 HZ…OC	1.9	110.3	H- acc
			Lys 270 HZ…OC	2.0	109.6	H- acc
			Asp 297 CA…HN	1.8	122.2	H- don
			Gly 110 OC…HN	1.9	121.7	H- don
10b	13	- 7.0	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
			Thr 219 CA…HO	2.0	119.9	H-don
10c	11	- 7.4	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
10d	10	- 8.0	Gly 105 CA…HN	2.0	124.2	H- don
			Asp 104 CG…HN	2.4	118.7	H- don
			Glu 263 CD…NH	2.6	116.7	H- acc
10e	5	- 8.8	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
10f	2	- 9.0	Gly 105 CA…HN	2.3	124.2	H- don
10g	4	- 8.8	Gly 110 OC…HN	2.0	123.1	H- don
			Gly 110 CA…HN	2.3	121.7	H- don
10h	9	- 8.4	Gly 110 OC…HN	2.3	121.7	H- don
			Met 301 HN…OC	2.1	122.2	H- acc
10i	8	- 8.4	Glu 263 CD…NH	2.6	116.7	H- acc
			Gly 110 OC…HN	2.4	121.3	H- don
10j	7	- 8.5	Arg 518 NE…OC	2.4	119.2	H- acc
			Asp 297 OC... HN	2.6	121.4	H- don
10k	6	-8.7	Lys 270 NZ….OC	2.3	110.3	H- acc
			Asp 297 CA….HN	2.0	122.4	H- don
10l	1	-9.7	Lys 270 NZ….OC	2.3	110.3	H- acc
			Pro 218 CA….HN	2.7	123.9	H- don
			Glu 263 CD….HN	2.1	116.7	H- don
10m	3	-9.0	Gly 110 CA….HN	2.4	121.4	H-don
*STM	R	- 6.9	Arg 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

*Streptomycin (STM), the reference drug against *E. coli* DNA Gyrase A protein

The binding modes of compounds **10l, 10f, 10m, 10g, 10e** and **10k** (Figure 3) suggested that they fitted more stably in to the DNA Gyrase A binding site (active site). Hence the present

investigation demonstrated that the synthesized compounds would be the promising next generation anti-microbial drugs, which could be effectively used in the treatment of microbial infections.
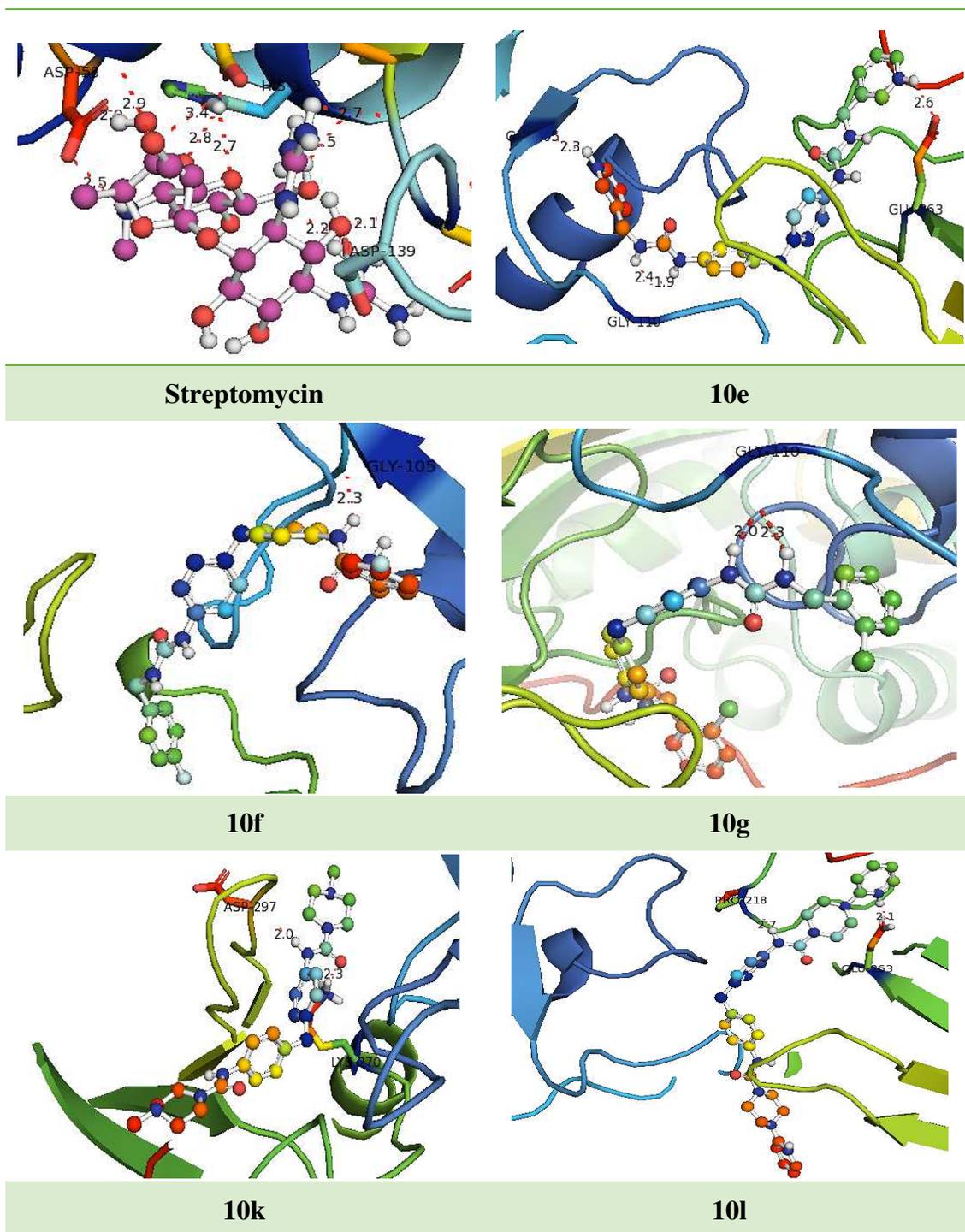

Figure 3. Graphical representation of ligand binding modes with DNA gyrase A protein

4. Conclusion

In conclusion, the fabricated title compounds were synthesized in a simple method with ease in high yields and evaluated their antibacterial activity. The in vitro combating potentialities of the title compounds against 5 bacterial and 2 fungal pathogens and their antioxidant activity revealed that all the title compounds were moderate antimicrobials and moderate antioxidants. However, the binding modes and binding energies of the compounds **10e, 10f, 10g, 10k, 10l** and **10m** in molecular docking

studies, surprisingly suggested that they could be the promising next generation antimicrobials for treating infectious diseases.

Conflict of Interest

The authors confirm that this article content has no conflict of interest.

Supporting Information

Full experimental details, FTIR, ^{1}H, ^{13}C NMR and Mass spectra; CHN analysis of **10a** and **10m**; antimicrobial screening and antioxidant activity evaluation with references; tables and figures are furnished in Supplementary Information which accompanies this paper on http://www.acgpubs.org/OC.

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