The Pharma Innovation Journal 2019; 8(8): 50-62

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(8): 50-62

www.thepharmajournal.com
Received: 01-06-2019
Accepted: 05-07-2019

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Carbamates of sulfathiazole and methyl tryptophanate: Synthesis, antimicrobial activity and docking studies against DNA Gyrase A

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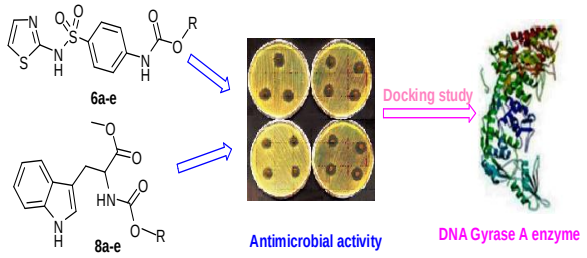
Abstract
It is well demonstrated that bacteria have become resistance hastily and it is considered to be one of the utmost threats to human health. The research on innovative antimicrobial agents explores a great interesting subject matter recently. Therefore, a series of new carbamate derivatives of sulfathiazole 6a-e, a common oral antimicrobial drug and methyl tryptophanate 8a-e, *N*-methyl *α*-amino acid ester containing indole moiety have been synthesized. Structures of the title products were elucidated by spectral analyses like IR, NMR (^{1}H and ^{13}C), mass and elemental compositions. The compounds were evaluated for their *in vitro* antimicrobial activity including minimum inhibitory concentrations (MICs). Whereas, three carbamate derivatives of sulfathiazole 6a, 6b and 6e and one derivative of methyl tryptophanate 8a showed promising antibacterial activity in the range of MIC 3.125-6.25 µg/mL and it is a comparable activity of streptomycin (MIC = 3.125-6.25 µg/mL). Most of the compounds provided potent activity against *E. coli* was equivalent to streptomycin (MIC = 3.125 µg/mL). The title compounds were docked into the active site of *E. coli* DNA Gyrase A enzyme to ensure the binding mode and the results demonstrated that a few compounds showed better binding energies with enzyme than that of standard, streptomycin and associated well to antibacterial activity.

Keywords: Carbamate derivatives, sulfathiazole, methyl tryptophanate, antimicrobial activity, docking

1. Introduction
The infective diseases have become one of the most abundant complications in public health. The research discoveries attempted to give resolutions for infective diseases and the antibiotics are introduced in clinical use to control it [1]. Antibiotics utility has been enriched globally since it uses as modern medicine in invasive surgery and critical treatments like transplantation, cancer, and orthopedic related diseases. The consequence of abundant utility of antibiotics is bacterial resistance and it had a profound impact on the bacteria life on Earth's system [2, 3]. Problems related to currently used antibiotic drug-resistance against many human pathogens like Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Enterococci* (VRE) have reported in the literature [4, 5]. Therefore, it could be fright that the currently existing antibiotic drugs used in the treatment of infectious diseases are inadequate in the long term to protect us [3]. The World Economic Global Risks reports concluded that drug-resistance is one of the greatest threats to human health. Hence, the distinct research is essential for the discovery of new antimicrobial agents which had a clear innovative mechanism of action and it should bring new compounds to overcome the microbial resistance to clinically used drugs.

Peptide-based functionalities like carbamates and amides linkages, because of their high affinity and specificity toward biological functions, are attributed an important strategy in the drug discovery [6-8]. Particularly, carbamates display good chemical and proteolytic stabilities, and can participate in hydrogen bonding through the carboxyl group and the backbone NH. Also, they possessing a unique feature that ability to modulate inter- and intra-molecular interactions with the target enzymes or receptors. Hence, carbamates with substitution on numerous active scaffolds offer opportunities for modulation of biological properties, improvement in stability and pharmacokinetic properties and capability to increase permeability across cellular membranes [9]. Because of these tremendous features, the carbamate



Because of these tremendous features, the carbamate group has become a key structural motif in drug design discovery. Numerous carbamate-bearing molecules have been exploited rapidly in drug design, playing an important role in medicinal chemistry and found in many of approved drugs and pro-drugs in the market [9]. For example, rivastigmine (1), albendazole (2) and felbamate (3) (Figure1) are used in the treatment of alzheimer's disease/Parkinson's, parasitic worm infestations and epilepsy, respectively. In addition, the applications of carbamate derivatives are well reported in various industries, agrochemicals such as pesticides, fungicides, and herbicides, in the polymer industry, and in peptide syntheses [10-12]. Carbamate derivatives have also been reported in numerous biological applications such as inhibitors of HIV, anticancer agents, anticonvulsants, antibacterial agents, antiepileptic's, and enzyme inhibitors [13-19]. Recently, our group synthesized carbamate derivatives of (2'-(1*H*-tetrazol-5-yl)- biphenyl-4-yl) methanamine, carvedilol, and 7-azaindole showed good antimicrobial and antiviral activities, respectively [20-22].

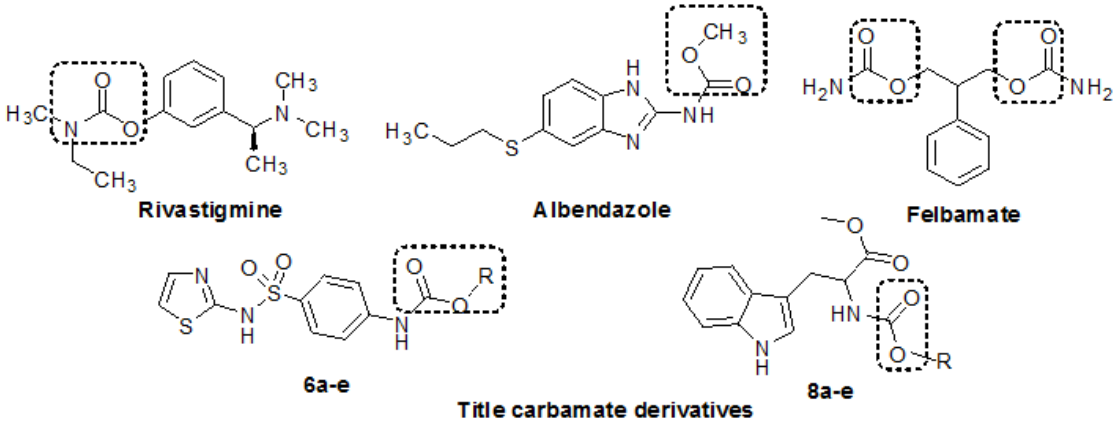


Fig 1: Biologically active carbamate derivatives and title products.

It is well established that sulfonamides possessing functionality, -SO_2-NH- or sulfa drugs are accomplished a prominent role in the history of medicine as the first synthetic antibiotic medications to be used clinically [23]. Since then, thousands of compounds by substituting numerous chemical entities on sulfonamide group were synthesized and the best therapeutic outcomes have been accomplished, including antihypertensive agent bosentan [24], antibacterial [25], antiprotozoal [26], antifungal [27] and anti-inflammatory [28] activities, and over 32 drugs are in clinical use [29], Therefore, the sulfonamide compounds have been considered as key structural motifs in the search for biologically active molecules. Sulfathiazole, chemically *N*-2-thiazolyl sulfanilamide, is a short-acting sulfa drug used as antibiotic until less toxic alternatives were discovered. It has been using still in the treatment of infective diseases in combination with other non-sulfonamide drugs. A number of studies have been made on sulfathiazole to enhance its drug ability and properties, and suitability in other chemotherapeutic use. For example, various alkyl or aryl substituents on the thiazole nucleus of sulfathiazole [30] against microbial strains and 4-{[(2-hydroxy-naphthalen-1-yl)-phenyl-methyl]-amino}-*N*-thiazol-2-yl-benzenesulfonamide against *Staphylococcus aureus* and *Vibrio cholera* with MIC = 4 x 10^{-3} mmol [31] showed potential activity. Furthermore, indole and its derivatives are an important class of heterocyclics, and found in wide variety of biological active scaffolds and naturally occurring compounds which exhibit numerous physiological properties [32, 33]. The amino acid ester, methyl tryptophanate is a significant derivative of indole. Because vital role of tryptophan or its derivatives in protein biosynthesis, the production of important hormones like serotonin [34], antimicrobial and immune regulatory functions of indoleamine 2, 3-dioxygenase [35, 36], and as potential agents in various human-related diseases including cancer, neurodegenerative disorders, and diabetes [37, 38-40], the chemistry tryptophan derivatives have been attained a great topic of research interest in medicinal chemistry.

The structural modification of drugs or drug intermediates or biologically active compounds by assimilating with the class of pharmacophores have become more interest now a day to accomplish new drug lead compounds and potential chemotherapeutic agents [41]. It is also an important approach in the discovery of medicinal important molecules. In such a way, the derivatives of (2'-(1*H*-tetrazol-5-yl)- biphenyl-4-yl) methanamine [20], carvedilol [21], linezolid intermediate [42] and acyclovir [43] synthesized by our group by integrating carbamate functionality and phosphoramidites have exhibited potential antimicrobial and antiviral activities, respectively. As in the part of our continuous research, and considering the overview biological importance of sulfathiazole, methyl tryptophanate and carbamate derivatives, we here in report the synthesis, characterization and biological studies of new series of carbamates of sulfathiazole and methyl tryptophanate using conventional method. The title products were investigated for their antimicrobial activity including minimum inhibitory concentrations and a few compounds are acted as potential antimicrobial agents. Further, the ligand-receptor interactions were understood by carrying out molecular docking studies.

2. Experimental part

2.1 Materials and Methods

The key starting materials like sulfathiazole, methyl

tryptophanate and substituted chloroformates used in the synthesis of title products were procured from Sigma-Aldrich with purity >99% and used directly without any further purification. The analytical grade solvent, tetrahydrofuran (THF) used in the reactions as medium and it was purchased from Spectrochem and other solvents like dichloromethane (DCM), methanol (MeOH) and ethyl acetate (EtOAc) used in the workup procedure and purification techniques were purchased commercially. The progress of the reactions and purity of compounds were examined on Merck, silica plates and the suitable ratio of DCM: MeOH (mostly in the range between 9.2:0.8 v/v to 9.8:0.2 v/v) was used as a mobile phase. The temperature was measured by flexible probe throughout the reaction. The silica gel (200 mesh) procured from Spectrochem was used in column chromatography for purification of the synthesized compounds. Melting points were determined in open capillaries on Guna Digital Melting point apparatus and are uncorrected, and they were expressed in degrees centigrade (ºC). The IR spectra were recorded directly on Bruker Alpha FT-IR spectrophotometer without using KBr pellets and the wave numbers were given in cm^{-1}. The ^{1}H/ $^{[13]}$ C NMR spectra were recorded in $CDCl_3$ on a Bruker 400 MHz spectrophotometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. All chemical shifts were reported in δ (ppm) using TMS as an internal standard and multiplicities are described as the abbreviations: s (singlet), brs (broad singlet), d (doublet), t (triplet), m (multiplet). The elemental analyses were determined on Thermo Finnegan Flash 1112 elemental analyzer. E.S.I mass spectra were recorded on a MLP 2103 mass spectrometer.

2.1. General procedure for the synthesis of title products

2.1.1 Synthesis of carbamates of sulfathiazole

To the solution of sulfathiazole (4) (150 mg, 0.59 mmol) and 4-*N*,*N*-dimethylamino pyridine (DMAP) (86.7 mg, 0.71 mmol) in THF (10 mL) was added 4-nitrophenyl chloroformate (5a) (131.0 mg, 0.65 mmol) dissolved in 3 mL of THF at 10-15ºC. The reaction mixture was heated to 60-65ºC and it was stirred for 5 hours. The progress of a reaction was monitored by TLC using DCM: MeOH (9.2:0.8 v/v) mixture as a mobile phase and it was confirmed the reaction completion. The reaction mixture was filtered-off to remove the salt DMAP.HCl (discarded the salt) and cooled the reaction mass to 20-25ºC. The solvent from the filtrate was removed under vacuum, to obtain the crude residue and it was subjected to column chromatography using DCM: MeOH mixture as a mobile phase. The desired fractions of target product were confirmed together and the solvent was removed under vacuum to obtain 4-nitrophenyl 4-(*N*-thiazol-2-ylsulfamoyl) phenyl carbamate (6a) as pale yellow solid. The same procedure was followed for the preparation of the remaining carbamate analogs of Sulfathiozole 6(b-e)

2.2.2. Synthesis of carbamates Synthesis of methyl tryptophanate

4-Nitrophenyl chloroformate (5a) (153 mg, 0.76 m mol) in THF (5mL) was added to the solution of methyl tyrptophanate **(7)** (150 mg, 0.69 mmol) and 4-*N*,*N*-dimethylamino pyridine (DMAP) (109.6 mg, 0.90 mmol) in THF (10 mL) at 10-15ºC. The reaction mixture was stirred for 10 hours at ambient temperature (25-30ºC). The completion of the reaction was judged based on TLC using DCM: MeOH mixture (9.5:0.5 v/v) as a mobile phase. The reaction mixture was filtered-off to remove the salt DMAP. HCl (discarded the salt) and the solvent from the filtrate was removed under vacuum to obtain the crude residue and it was subjected to column chromatography using DCM: MeOH mixture system as a mobile phase. The combined desired product fractions were evaporated under vacuum to obtain methyl 3-(1*H*-indol-3-yl)-2-((4-nitrophenoxy) carbonylamino) propanoate (8a) as a Pale yellow solid. The same procedure was adopted for the preparation of the remaining carbamate analogs of tryptophanate 8(b-e).

2.3. Spectral data

2.3.1. 4-Nitrophenyl 4-(N-thiazol-2 ylsulfamoyl) phenylcarbamate (6a)

Pale yellow solid; Yield: 89.5%; m.p. 128.1-129.1°C; IR (ν_{max}/cm^{-1}): 3383, 3223 (N-H, str), 2962 (C-H, str), 1741 (C=O, str), 1518 & 1265 (-NO_2, str), 1339 (SO_2, str); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (2H, d, J = 7.6 Hz, Ar-H), 7.45-7.60 (4H, m, Ar-H), 7.05-7.11 (3H, m, Ar-H &thiazole-H), 6.57 (1H, d, J = 9.2 Hz, S-CH in thiazole) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 108.1, 119.3, 123.6, 125.9, 128.5, 133.7, 137.0, 142.6, 145.2, 152.9, 159.2, 170.4 ppm; ESI-MS (m/z): 421.1 ($M+H^+$); Anal. Calcd for: C, 45.71; H, 2.88; N, 13.33. Found: C, 45.68; H, 2.87; N, 13.32.

2.3.2. Methyl 4-(N-thiazol- 2- ylsulfamoyl) phenyl carbamate (6b)

Pale yellow solid; Yield: 83.3%; m.p. 208-212 °C; IR (ν_{max}/cm^{-1}): 3301, 3185 (N-H, str), 2953 (C-H, str), 1719 (C=O, str), 1317 (SO_2, str); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (2H, d, J = 8.0 Hz, Ar-H), 7.49 (2H, d, J = 8.0 Hz, Ar-H), 7.18 (1H, d, J = 9.2 Hz, thiazole-H), 6.55 (1H, d, J = 9.2 Hz, S-CH in thiazole), 3.84 (3H, s, -O-CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 58.4, 110.2, 118.8, 128.4, 134.0, 137.6, 142.9, 154.3, 169.7 ppm; ESI-MS (m/z): 314.05 ($M+H^+$); Anal. Calcd for: C, 42.16; H, 3.54; N, 13.41. Found: C, 42.13; H, 3.51; N, 13.40.

2.3.3. Ethyl 4-(N-thiazol-2-ylsulfamoyl) phenylcarbamate (6c)

Pale yellow solid; Yield: 82.0%; m.p. 208-212 °C; IR (ν_{max}/cm^{-1}): 3299, 3101 (N-H, str), 2976 (C-H, str), 1704 (C=O, str), 1277 (SO_2, str); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (2H, d, J = 8.0 Hz, Ar-H), 7.44 (2H, d, J = 8.0 Hz, Ar-H), 7.15 (1H, d, J = 8.8 Hz, thiazole-H), 6.62 (1H, d, J = 9.2 Hz, S-CH in thiazole), 4.02 (2H, q, J = 8.4 Hz, -O-CH_2-), 1.16 (3H, t, J = 8.4 Hz, -C-CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 62.5, 107.1, 117.7, 123.5, 127.1, 133.2, 138.0, 142.6, 153.3, 169.1 ppm; ESI-MS (m/z): 328.05 ($M+H^+$).

2.3.4. Isobutyl 4-(N-thiazol-2-ylsulfamoyl) pheny lcarbamate (6d)

Pale yellow solid; Yield: 78.4%;m.p. 208-212 °C; IR (ν_{max}/cm^{-1}): 3352, 3109 (N-H, str), 2954 (C-H, str), 1728 (C=O, str), 1295 (SO_2, str); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (2H, d, J = 8.0 Hz, Ar-H), 7.42 (2H, d, J = 8.0 Hz, Ar-H), 7.15 (1H, d, J = 9.2 Hz, thiazole-H), 6.58 (1H, d, J = 9.2 Hz, S-CH in thiazole), 3.97 (2H, d, J = 8.0 Hz, -O-CH_2-), 1.82 (1H, m, -C-CH-), 0.96 (6H, d, 8.0 Hz, -C-$(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.3, 30.1, 68.4, 109.3, 118.0, 123.9, 129.5, 134.1, 137.3, 141.8, 153.1, 170.8 ppm; ESI-MS (m/z): 356.06 ($M+H^+$).

2.3.5. 2, 2, 2-Trichloroethyl 4-(N-thiazol-2-ylsulfamoyl) phenylcarbamate (6e)
Pale yellow solid; Yield: 85.0%;m.p. 208-212 °C; IR (ν_{max}/cm^{-1}): 3304, 3152 (N-H, str), 2945 (C-H, str), 1720 (C=O, str), 1316 (SO_2, str), 1172 (CCl_3, str); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (2H, d, J = 8.4 Hz, Ar-H), 7.43 (2H, d, J = 8.4 Hz, Ar-H), 7.18 (1H, d, J = 8.8 Hz, thiazole-H), 6.57 (1H, d, J = 8.8 Hz, S-CH in thiazole), 4.89 (2H, s, -O-CH_2-CCl_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 73.9, 95.3, 110.2, 116.5, 125.0, 128.7, 132.4, 137.1, 142.0, 153.8, 170.2 ppm; ESI-MS (m/z): 430.0 ($M+H^+$).

2.3.6 Methyl 3-(1H-indol-3-yl)-2-((4-nitrophenoxy) carbonylamino) propanoate (8a)
Pale yellow solid; Yield: 92.6%; m.p. 123.4-124.4°C; IR (ν_{max}/cm^{-1}): 3410, 3326 (N-H, str), 2942 (C-H, str), 1723 (broad, C=O, str), 1519 & 1278 (-NO_2, str); ^{1}H NMR (400 MHz, $CDCl_3$): δ8.19 (2H, d, J = 8.4 Hz, Ar-H), 7.57 (1H, d, J = 7.6 Hz, Ar-H), 7.34 (2H, d, J = 8.4 Hz, Ar-H), 7.14-7.25 (3H, m, Ar-H), 7.04 (1H, s, indole-H), 4.75 (1H, m, -CH-), 3.65 (3H, s, -O-CH_3), 3.31 (2H, m, -CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ29.3, 48.9, 52.3, 111.4, 113.2, 117.2, 118.5, 120.8, 122.3, 123.4, 124.9, 127.5, 140.9, 142.3, 154.2, 155.7, 170.1 ppm; ESI-MS (m/z): 384.1 ($M+H^+$); Anal. Calcd for: C, 59.53; H, 4.47; N, 10.96. Found: C, 59.51; H, 4.46; N, 10.96.

2.3.7 Methyl 3-(1H-indol-3-yl)-2-(methoxycarbonylamino) propanoate (8b)
Pale yellow solid; Yield: 88.0%;m.p. 208-212 °C; IR (ν_{max}/cm^{-1}): 3321 (broad, N-H, str), 3009 (C-H, str), 1700 (broad, C=O, str); ^{1}H NMR (400 MHz, $CDCl_3$): δ7.42 (1H, d, J = 8.0 Hz, Ar-H), 7.27 (1H, d, J = 8.0 Hz, Ar-H), 6.95-7.18 (3H, m, Ar-H, indole-H), 4.47 (1H, m, -CH-), 3.56 (3H, s, -O-CH_3), 3.29(3H, s, -OCH_3), 3.20 (2H, m, -CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ29.0, 48.5, 52.3, 52.6, 110.1, 113.4, 117.0, 118.4, 121.3, 123.6, 126.5, 141.2, 156.3, 169.8ppm; ESI-MS (m/z): 277.09 ($M+H^+$).

2.3.8 Methyl 2-(ethoxycarbonylamino)-3-(1H-indol-3-yl) propanoate (8c)
Pale yellow solid; Yield: 88.5%;m.p. 208-212 °C; IR (ν_{max}/cm^{-1}): 3338 (broad, N-H, str), 2988 (C-H, str), 1699 (broad, C=O, str); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (1H, d, J = 8.0 Hz, Ar-H), 7.38 (1H, d, J = 8.0 Hz, Ar-H), 7.13 (1H, s, indole-H), 7.05-7.13 (2H, m, Ar-H), 4.48 (1H, m, -CH-), 4.05 (2H, q, J = 6.8 Hz, -OCH_2), 3.65 (3H, s, -O-CH_3), 3.10-3.16 (2H, m, -CH_2),1.19 (3H, t, J = 6.8 Hz, -CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ13.5, 28.7, 49.4, 51.8, 58.3, 110.5, 112.9, 118.4, 118.9, 120.5, 122.9, 127.0, 139.7, 157.5, 170.3 ppm; ESI-MS (m/z): 291.1 ($M+H^+$).

2.3.9 Methyl 3-(1H-indol-3-yl)-2-(isobutoxy carbo nylamino) propanoate (8d)
Pale yellow solid; Yield: 85.3%;m.p. 208-212 °C; IR (ν_{max}/cm^{-1}): 3384, 3314 (N-H, str), 2995 (C-H, str), 1738, 1689 (broad, C=O, str); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (1H, d, J = 8.0 Hz, Ar-H), 7.34 (1H, d, J = 8.0 Hz, Ar-H), 7.18 (1H, s, indole-H), 7.1-6.983 (2H, m, Ar-H), 5.23 (1H, m, -CH-), 3.83 (2H, d, J = 6.4 Hz, -OCH_2), 3.66 (3H, s, -O-CH_3), 3.29 (2H, m, -CH_2),1.88 (1H, m, -CH). 0.88 (6H, d, J = 6.8 Hz, -$(CH_3)_2$) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ19.2, 28.04, 28.1, 52.3, 54.5, 70.1, 110.1, 111.2, 118.7, 119.7, 122.3, 122.8, 127.6, 136.2, 156.2, 172.6 ppm; ESI-MS (m/z): 319.4 ($M+H^+$).

2.3.10 Methyl 3-(1H-indol-3-yl)-2-((2, 2, 2-trichloroethoxy) carbonylamino) propanoate (8e)
Pale yellow solid; Yield: 89.2%; m.p. 208-212 °C; IR (ν_{max}/cm^{-1}): 3380, 3296 (N-H, str), 2991 (C-H, str), 1725, 1694 (broad, C=O, str), 1156 (-CCl_3, str); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47 (1H, d, J = 8.0 Hz, Ar-H), 7.35 (1H, d, J = 8.0 Hz, Ar-H), 7.15 (1H, s, indole-H), 7.06 (2H, m, Ar-H), 4.72 (2H, s, -OCH_2), 4.64 (1H, m, -CH-), 3.69 (3H, s, -O-CH_3), 3.26 (2H, m, -CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ28.8, 49.3, 50.9, 68.7, 98.2, 110.2, 113.5, 117.6, 119.0, 120.3, 122.4, 126.1, 139.2, 156.5, 170.5 ppm; ESI-MS (m/z): 393.1 ($M+H^+$).

2.4. Biological activity
2.4.1. Antibacterial activity
Antibacterial activity of the newly synthesized carbamate derivatives of sulfathiazole 6a-e and tryptophanate 8a-e was demonstrated using agar well diffusion method as reported by Murray *et al*.[44] with minor modifications. Streptomycin is an antibiotic as well effective DNA gyrase inhibitor often used to treat numerous bacterial infections; therefore it was used as a standard drug in this study for comparison of activity results. Stock solutions of the title products 6a-e and 8a-e, and standard drug, streptomycin (positive control) were prepared in dimethyl sulfoxide (DMSO) solvent which was used as a negative control, and then the samples were diluted to required concentrations, 50 and 100 µg/mL. The bacterial strains, American Type Culture Collection (ATCC), such as *Klebsiella pneumoniae* (ATCC 700603), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 43300) and *Bacillus subtilis (ATCC 6633)* were collected from Sri Venkateswara University, Department of Microbiology, Tirupati to investigate the antibacterial activity. These strains were sub-cultured previously in appropriate media under gaseous conditions to confirm their purity at 35 °C for 48 h prior to testing of the vehicles. The inoculums of different bacteria were spread over the nutrient agar medium (peptone, beef extract, NaCl, and agar-agar) and wells of bore size (6 mm) were made in the solid medium, after cooling, using a sterile metallic borer. The test samples of synthesized compounds in different concentrations, positive control, and negative control were poured into each cavity of different plates. The culture plates were kept at room temperature for one hour to diffuse the drug in surrounding medium and then incubated at 37 ºC for 24 hours. The diameter of the zone of inhibition formed around the cavities after incubation was accurately measured in mm. The duplicate experiments were performed and taken the average values as the final results and they are tabulated in Table S1.

2.4.2 Antifungal activity
The antifungal activity was demonstrated by following disc diffusion method as we described in the earlier study[45] with modifications. The available fungal strains such as *Fusariumoxysporum* (MTCC-1755), *Aspergillus niger* (MTCC-1881) and *Colletotrichum capsici* (MTCC-2071) were taken to investigate the bio-potency of title products. The standard drug fluconazole was used as positive control for comparison of antifungal activity results. The culture for about 48 h old of selected fungi was mixed with sterile physiological saline and the turbidity was adjusted to the standard inoculum of Mc-Farland scale 0.5 ≈10^6 colony

forming unit (CFU) per mL. This culture with the help of a sterile glass spreader was spread on a surface of the solidified potato dextrose agar (Hi-media) media to attain even distribution of the inoculum. Sterile discs of Whatmann No.1 filter paper of about 8 mm diameter infused in the test samples of different concentrations such as 50 and 100µg/mL, and positive and negative controls are placed on the culture plates. The inoculated Petri plates were incubated for 72 h at 25ºC. The zone of inhibition of fungi around the disc was measured in millimeters as an edge to edge zone of the confluent growth which corresponds to the sharpest edge of the zone. The tests were repeated in duplicate and taken the average values as the final readings and they are given in Table S2.

2.4.3 Minimum inhibitory concentrations

The lowest concentration of test sample showing no growth of inhibition of microorganism is considered to be the minimum inhibitory concentration (MIC). The two-fold serial dilution technique[46] was used to investigate the minimum inhibitory concentrations (MICs) of the active title compounds which were categorized based upon the potential zone of growth of inhibition screened at two different concentrations like 50 and 100 µg/mL. The microorganism suspensions (10^6 CFU/mL), which have been used in the investigation of antibacterial and antifungal activities, were used to inoculate the test compounds in their suitable broth. The test samples of selected active compounds and the standard reference drugs were prepared in proper nutrient broth and their concentrations were adjusted to the concentration range of 50.0, 25.0, 12.5, 6.25 and 3.125 mg/mL. The culture plates of bacteria and fungi were incubated at 37ºC for 24 and 48 h, respectively. Finally, the growth of microorganisms was observed by turbidity measurements and the results are tabulated in Table2.

2.4.4 Docking study

A set of newly synthesized carbamate derivatives of sulfathiazole and tryptophanate and reference standard drugs, streptomycin were evaluated their binding interactions with DNA Gyrase A enzyme (PDB: 3LPX) using the docking module implemented in Pyrx 2010.12. The RCSB Protein Data Bank (PDB) and Pub Chem Data Bank were used to retrieve the three- dimensional structure of DNA Gyrase A (PDB: 3LPX)) and standard drugs such as streptomycin (Pub Chem ID 19649), respectively. Argus Lab 4.0 was used for alienated and geometry optimization of the atomic coordinates of the protein. The chemical structures of desired the title carbamate derivatives were drawn in Chem. Bio Draw and all these structures were converted to energy minimized 3D structures in the pdbqt file format, and the atomic coordinates were generated using Pyrx2010.12. The active binding sites nothing but the coordinates of the ligand in the original target protein grids were analyzed using the Drug Discovery Studio version 3.0 and 3D Ligand Site Virtual tools. In order to get the stable conformers, the protein structures were protonated initially with the addition of polar hydrogen's and then done energy minimization with the MMFF94x force field. Flexible docking was employed and the inhibitor binding site residues were softened and highlighted through the "Site Finder" module implemented in the Pymol software [47-49] The grid dimensions were predicted as ˚ X: 28.27, Y: 27.13, Z: 28.51. The default parameters i.e., placement: triangle matcher, recording 1: London dG and refinement: force field were carried out in the docking study, as well as a maximum of 10 conformations of each compound, were allowed to be saved in a separate database file in a. mdb format. Pymol viewer tool (www.pymol.org), after docking process, used to calculate the binding energy and binding affinity of the protein-ligand complexes were tabulated in Table 3.

3. Results and Discussion

3.1 Chemistry

As a part of our continuous research efforts on the preparation of new biologically active compounds [20-22, 45, 50-52] and considering the biological prominence of sulfathiazole (4), methyl tryptophanate (5), and carbamate derivatives as described above, we have been planned in the present study to understand the antimicrobial potency of carbamate derivatives of sulfathiazole and methyl tryptophanate. The general procedure used in our previous study [20] with modifications was used to prepare carbamate derivatives of sulfathiazole 6a-e and methyl tryptophanate 8a-e and the schematic representation was depicted in Scheme1. Initially, sulfathiazole (4) was treated with 4-nitrophenyl chloroformate (5a) in THF solvent using DMAP as a base at ambient temperature for 10 hours. The high amount of starting material 4 (based on TLC) was remained as an unreacted portion in the reaction mass, therefore the temperature was increased to reflux (60-65ºC) and the reaction was completed within 7.0 hours. Hence, we come to the conclusion that the electron-withdrawing group (-NH-SO_2-) at *para*-position to amine group in compound 4 could impact on the reaction to progress in sluggish at ambient temperature. The reflux condition has been implemented to prepare the rest of all the sulfathiazole carbamate derivatives 6(b-e). In contrast, the intensive impurities/undesired products were observed (Based on TLC) while the reaction was carried out with methyl tryptophanate (7) under the same conditions. The side products could be formed by involving of indole amine or both desired aliphatic amine and undesired indole amine in the reaction at reflux condition. So, the same reaction was carried out at ambient temperature (30-45°C) and the high yield of desired product was observed, however, it took longer reaction time (5-10h). Therefore, this procedure has been followed in the preparation of carbamate derivatives of methyl tryptophanate 8(a-e).

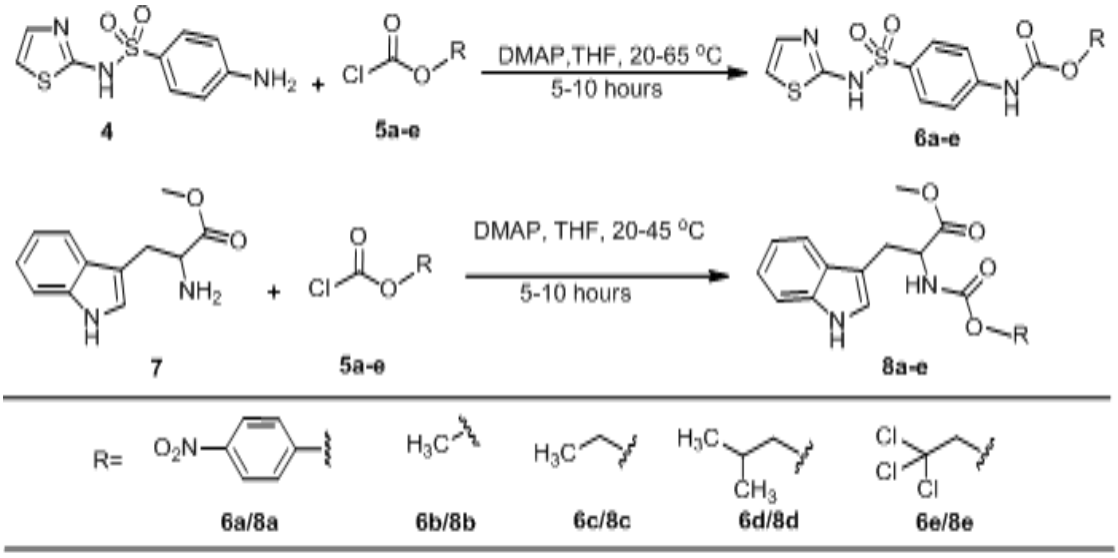
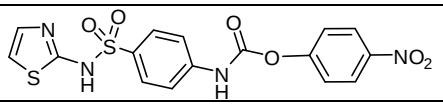
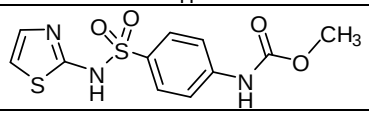
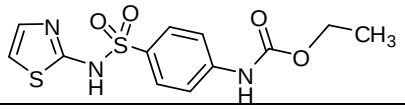
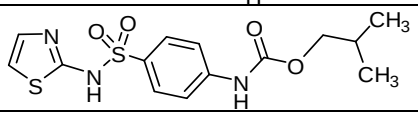
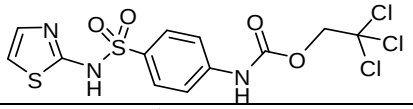
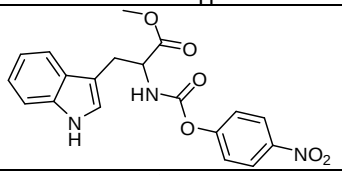
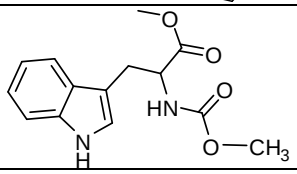
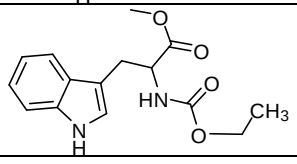
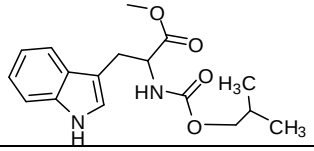
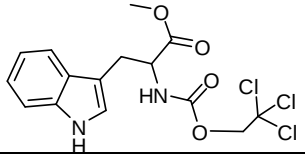


Fig 2: Synthesis of carbamates of sulfathiazole 6a-e and methyl tryptophanate 8a-e.

After completion of the reaction as judged by TLC, the salt, DMAP. HCl formed during the reaction was removed from the reaction mixture by filtration at 45ºC. The filtrate was concentrated under vacuum to obtain crude product and it was subjected to column chromatography using different ratios of MeOH: DCM mixture as the mobile phase to obtain the pure products. The pure products were dried under vacuum at 45-50ºC for 12 hours and these products were used directly in further analysis. The structures, reaction time, yield and melting points range of the title products are tabulated in Table 1.

Table 1: Structure, reaction time, yield and melting points of the title products

Product	Structure	Reaction time (h)	Yield (%)	Melting point (ºC)
6a		6.0	89.5	128.1-129.1
6b		8.0	83.3	157.4-158.4
6c		8.5	82.0	184-185
6d		10.0	78.4	159.6-160.6
6e		8.0	85.0	173.4-174.4
8a		6.0	92.6	123.4-124.4
8b		14.0	88.0	85.4-86.4
8c		10.0	88.5	68.2-69.2

8d		12.0	85.3	83.8-84.8
8e		10.0	89.2	72.4-73.4

3.2 Spectroscopic analysis

The structures of newly synthesized carbamate derivatives 6(a-e) and 8(a-e), after purification, were elucidated using spectroscopic analysis like IR, NMR (^{1}H and ^{13}C), mass, and elemental analyses. In IR spectra of all the title compounds, the intense bands at 3200-3400 cm^{-1} and 1680-1740 cm^{-1} were observed due to –NH and carbonyl (C=O) of carbamate functionality, however, two carbonyl groups are merged and showed one broad band in case of tryptophanate derivatives 8a-e. The CH proton of thiazole ring appeared as a singlet at 6.50-6.90 ppm and the aromatic protons of phenyl ring showed peaks at 7.05-7.75 ppm in ^{1}H NMR spectra of sulfathiazole carbamates 6a-e. The indole protons showed chemical shifts in the range of 6.95-7.60 ppm and aliphatic protons resonated at 4.75-325 ppm in the carbamates of methyl tryptophanate 8a-e. In ^{13}C NMR spectra of all the title products, the carbonyl (-C=O) carbon was appeared at 153-160 ppm and the remaining carbons are resonated at their corresponding positions. The molecular ion peaks in mass spectra displayed corresponding mass of products and the elemental (C H, N) compositions obtained in elemental analyses were coincided to the theoretical compositions of the newly synthesized compounds, and provided further evidence in the structural elucidation.

3.3 Biological activity

The antibacterial and antifungal activities were screened for the newly synthesized carbamate derivatives of sulfathiazole 6a–e and methyl tryptophanate 8a–e to understand their biological potency. The available bacterial strains such as *Staphylococcus aureus* (ATCC 43300), *Bacillus subtilis (ATCC 6633), Escherichia coli* (ATCC 25922) *and Klebsiella pneumoniae* (ATCC 700603), and fungal strains like *Fusarium Oxysporum* (MTCC-1755), *Aspergillus niger* (MTCC-1881) and *Colletotrichum capsici* (MTCC-2071) were used to demonstrate the antibacterial and antifungal activities, respectively. The antimicrobial activity of the tested compounds was assessed by measuring bacterial/fungal growth of zone of inhibition (ZOI) using agar well diffusion method[44] in the screening of antibacterial activity and disc diffusion method [45] in fungal activity investigation. The standard antibiotics such as streptomycin and fluconazole were used as positive controls in antibacterial and antifungal activities, respectively for the comparison of title products activity. The dimethyl sulfoxide (DMSO) used in the preparation of test samples was taken as a negative control and found to exhibit no activity against selected pathogens. The observed antibacterial activity results are tabulated in Table 1 and antifungal activity results in Table 2.

The antibacterial screening results disclosed that all the compounds have exhibited activity at both the concentrations, 50 and 100 µg/mL and most of them are closer to the standard drug, streptomycin. Whereas, the sulfathiazole carbamates such as compound 6a bearing 4-nitrophenyl ring, 6b possessing methyl group and 6e linked with a trichloroethyl group, and methyl tryptophanate carbamate, 8a bearing 4-nitrophenyl ring have shown potential activity against all the tested bacterial strains, and found that their activity was closer to the standard drug, streptomycin. Additionally, compounds 6c against *B. subtilis* (ATCC 6633), 6d against *E. coli* (ATCC 25922) and *S. aureus* (ATCC 43300), 8c against *K. pneumoniae* (ATCC 700603), and 8b, 8d and 8e against *E. coli* (ATCC 25922) exhibited excellent activity. While in comparison, more carbamate derivatives of sulfathiazole exhibited potential antibacterial activity as compared with carbamates of methyl tryptophanate and most of both compound derivatives are more effective agents against *E. coli* (ATCC 25922). Furthermore, substitution of the aromatic entity with the electron-withdrawing group (-NO_2) showed better antibacterial activity than that of aliphatic chain substitution. However, the activity has become diminished upon increasing of aliphatic nature like methyl > ethyl > iso-propyl, and the activity has been enhancing while increased the electron-withdrawing groups on the aliphatic chain (Trichloro ethyl group 6e/8e). Therefore, the results released that electron-withdrawing groups play an important role to exhibit potential antibacterial activity, and could attain better results when substituted aromatic scaffolds possessing electron-withdrawing groups in drug design.

As seen in Table 2, all the compounds exhibited antifungal activity against all the tested fungal strains at both the concentrations 50 and 100 µg/mL, similar to antibacterial activity. In contrast to bacterial activity, very few compounds such as sulfathiazole carbamates 6a bearings 4-nitrophenyl ring and 6c bound with ethyl group, and one methyl tryptophanate derivative 8a bearing 4-nitrophenyl ring showed excellent activity against all the tested fungal strains. Whereas, most of the compounds showed promising activity against fungal strains discretely like compounds 6d and 8b against *F. oxysporium* (MTCC-1755), 6e against *C. capsici* (MTCC-2017), 8d against *A. niger* (MTCC-1881), 8c against *F. oxysporium*(MTCC-1755) and *C. capsici*(MTCC-2017), and 8e against *A. niger* (MTCC-1881) and *C. capsici*(MTCC-2017). Based on the results, the compounds are more active against *F. oxysporium* (MTCC-1755) and *C. capsici* (MTCC-2017) than that of *A. niger* (MTCC-1881), and impotent to predict structure-activity relationship. In whole observations, the title products are more effective against bacterial strains and also good to moderate activity against fungal strains.

Because the excellent bacterial/fungal growth of zone of inhibition (ZOI) exhibited by the title carbamate derivatives 6a-e and 8a-e, our interest has been focused further to screen the minimum inhibitory concentrations (MIC) of the active compounds using the two-fold serial dilution technique[46]. The lowest concentration of target or test sample showing no growth of inhibition of microorganism is considered to be the

minimum inhibitory concentration (MIC) and obtained results were tabulated in Table 2. The compounds 6a, 6b, 6d, 6e, and 8a showed excellent MIC values in the range, 3.125-12.5 µg/mL which are almost equal to the standard drug, streptomycin (3.125-6.25 µg/mL). Particularly, compound 6a (MIC 2.888-5.166 µg/mL) against all the tested bacterial strains, 6b (MIC 5.424 µg/mL) against *K. pneumoniae* (ATCC 700603), *E. coli* (ATCC 25922) and *S. aureus* (ATCC 43300), 6d (MIC6.864 µg/mL) against *E. coli* (ATCC 25922) and *S. aureus* (ATCC 43300), 6e (MIC4.986 µg/mL) against *K. pneumoniae* (ATCC 700603) and *E. coli* (ATCC 25922), 8a (MIC 5.116 µg/mL) against *K. pneumoniae* (ATCC 700603), 8b (MIC 4.082 µg/mL) and 8d (MIC 8.426 µg/mL) against *E. coli* (ATCC 25922) showed activity which are equal to the standard drug. Compound 8a showed promising MIC in the range of 3.912-6.6285 µg/mL against all the tested fungal strains similar to the standard drug, fluconazole (MIC 3.814-6.116 µg/mL). Furthermore, the compounds 6a, 6b and 8a (MIC 5.1µg/mL) against *F. oxysporium* (MTCC-1755), and 8e (MIC 3.814µg/mL) against *A. niger* (MTCC-1881) have shown MIC equal to the standard drug. In overall comparison, most of the compounds showed excellent activity against *E. coli* (ATCC 25922) and *F. oxysporium* (MTCC-1755) equal to the standard drugs, and the compounds 6a and 8a bound with the 4-nitophenyl ring are more potent in both activities.

Table 2: Minimum inhibitory concentrations of the newly synthesized carbamate derivatives 6a-e and 8a-e

Products	Minimum inhibitory concentrations in µg/mL						
	***K. pneumonia* (ATCC 700603)**	***E. coli* (ATCC 25922)**	***S. aureus* (ATCC 43300)**	***B. subtilis* (ATCC 6633)**	***F. oxysporium* (MTCC-1755)**	***A. niger* (MTCC-1881)**	***C. capsici* (MTCC-2017)**
6a	4.164	2.888	4.138	5.166	4.186	3.106	3.812
6b	5.424	6.322	6.844	5.112	5.288	6.144	6.812
6c	8.416	8.222	8.464	7.136	6.928	8.144	7.388
6d	7.312	6.864	8.112	8.146	7.113	8.124	7.914
6e	4.986	3.122	5.608	4.814	4.008	3.014	3.946
8a	5.116	4.814	6.628	4.484	5.006	3.912	4.188
8b	3.212	4.082	6.624	4.814	3.814	4.012	4.116
8c	6.118	7.308	8.200	8.460	7.618	8.422	8.618
8d	8.114	8.426	6.366	7.132	7.882	8.142	7.818
8e	4.158	3.436	7.118	5.212	4.328	4.118	2.928
Std.[a]	4.328	3.146	7.432	5.012	4.116	3.864	4.318
Std.[b]	5.164	5.882	4.118	4.016	3.814	6.116	5.988

Std.[a] – The standard antibiotic, streptomycin was used as positive control in antibacterial activity; Std.[b]– The standard antibiotic, fluconazole was used as positive control in antifungal activity; *K. pneumoniae* (ATCC 700603) - *Klebsiella pneumoniae* (ATCC 700603); *E. coli* (ATCC 25922) - *Escherichia coli* (ATCC 25922); *S. aureus* (ATCC 43300) - *Staphylococcus aureus* (ATCC 43300); *B. subtilis* (ATCC 6633) - *Bacillus subtilis* (ATCC 6633); *F. oxysporium* (MTCC-1755) – *Fusarium Oxysporum* (MTCC-1755); *A.niger* (MTCC-1881)–*Aspergillusniger* (MTCC-1881); *C.capsici* (MTCC-2017)– *Colletotrichum capsici* (MTCC-2071).

3.4. Docking study

Type IIA bacterial topoisomerases, DNA gyrase (Gyrase) is an essential bacterial enzyme that catalyses the ATP-dependent negative super-coiling of double-stranded closed-circular DNA and clinically validated pharmacological target for antibacterial drugs. The function of DNA gyrase is to catalyse the transient break and reunion of the DNA double strand, a process crucial for negative supercoiling or relaxation of positive supercoils in the DNA molecule during its replication. In addition, much attention has been focused on DNA gyrase as the intracellular target of a number of antibacterial agents, including nalidixic acid, novobiocin, ciprofloxacin and streptomycin and as a paradigm for other DNA topoisomerases. Furthermore, the standard antibiotic, streptomycin is an aminoglycoside antibiotic derived from streptomycin griseous with antibacterial activity. Streptomycin irreversibly binds to the 16S rRNA and S12 protein within the bacterial 30S ribosomal subunit. As a result, this agent interferes with the assembly of initiation complex between mRNA and the bacterial ribosome, thereby inhibiting the initiation of protein synthesis. As well as, streptomycin induces misreading of the mRNA template and causes the translational frame shift, thereby results in premature termination. This eventually leads to bacterial cell death.

Computational biology and bioinformatics play a vital role in the process of drug discovery and drug designing. The imperative information about drug-receptor interactions can accomplish while docked the drug molecule with the receptor (target) which is commonly used to identify the binding orientation of drug candidates to their protein targets in order to predict the affinity and activity. The title carbamate derivatives of sulfathiazole 6a-e and methyl tryptophanate 8a-e synthesized in the present study have been exhibited excellent to moderate antimicrobial activity, particularly antibacterial activity on *E. coli*. Therefore, the molecular docking studies were performed on DNA Gyrase A enzyme from *E. coli* (PDB: 3LPX) to understand the mechanism of antibacterial activity of the newly synthesized carbamate derivatives.

Automated docking studies were carried out using the docking module implemented in Pyrx 2010.12 to envisage the antimicrobial data on the structural basis [47]. The RCSB Protein Data Bank (PDB) and Pub Chem Data Bank were used to retrieve the three-dimensional structure of DNA Gyrase A from *E. coli* (PDB: 3LPX) (Figure 2a) and standard drugs such as streptomycin (Pub Chem ID 19649) (Figure 2b), respectively. Argus Lab 4.0.was used for alienated and geometry optimization of the atomic coordinates of the protein and the chemical structures of target compounds were drawn in Chem Bio Draw. The active binding sites nothing but the coordinates of the ligand in original target protein grids were analyzed using the Drug Discovery Studio version 3.0 and 3D Ligand Site Virtual tools. Flexible docking was employed and the inhibitor binding site residues were

softened and highlighted through the "Site Finder" module implemented in the Pymol software. Pymol viewer tool (www.pymol.org), [48, 49] after docking process, used to calculate the binding energy, residual binding interactions, bond length and their bond angles of the protein-ligand complexes were tabulated in Table 3 and the docked view or conformers of the active compounds have depicted in Figure 2c-f.

Table 3: Bonding characterization of the title products 6a-e & 8a-eagainst *E. coli* DNA Gyrase A protein.

Prod.	Rank	Binding energy (K cal/mol)	Residue involved in binding interaction	Bond length (Aº)	Bond angle (º)	Bond type
6a	1	- 7.7	Gly 267 CG…ON	2.6	114.9	H-acc.
			Asn 269 ND…OS	2.3	120.3	H-acc.
			Arg 91 CZ….ON	2.2	121.8	H-acc.
6b	6	-6.6	Asp 297 CA …..HN	2.0	122.4	H-don.
			Lys 270 NZ …..OC	2.1	110.3	H-acc.
			Thr 219 CB ….HN	2.6	84.1	H-don.
6c	8	- 6.5	Asp 297 CA …..HN	2.1	122.4	H-don.
6d	7	- 6.6	Phe 109 CA ….OS	2.2	115.1	H-acc.
			Asn 108 CG …..OS	2.1	121.5	H-acc.
			Leu 264 CA ….ON	2.0	114.7	H-acc.
6e	2	- 7.6	Asp 297 CA ….HN	2.0	122.4	H-don.
			Thr 219 CG …..HN	2.7	110.9	H-don.
			Lys 270 NZ ….OC	2.2	101.0	H-acc.
8a	5	- 6.7	Asp 297 CA …..HN	2.2	122.4	H-don.
			Aeg 518 CZ …..OC	2.8	122.4	H-acc.
8b	4	- 6.9	Asn 169 CB ….HN	2.2	120.7	H-don.
			Arg 91 CZ ….OC	2.4	118.6	H-acc.
8c	9	- 6.4	Asn169 CB ….HN	2.3	101.0	H-don.
			Gly 170 CA ….HN	2.0	122.3	H-don.
8d	10	- 6.3	Asp 297 CA …..HN	2.4	122.4	H-don.
			Leu 264 CA …..OC	2.1	114.7	H-acc.
8e	3	- 6.9	Asn 169 CA …..HN	2.4	120.7	H-don.
			Gly 170 CA …..HN	2.7	122.3	H-don.
			Arg 91 CZ …..OC	2.4	121.8	H-acc.
Std.	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.

Std. – Streptomycin was used as reference standard; H-acc indicates hydrogen accepter; H-don indicates hydrogen donar.

As seen in Table 3 related to the docking results of the newly synthesized carbamate derivatives 6a-e and 8a-e with DNA Gyrase A displayed that carbamate derivatives, 6a (-7.7 Kcal/mol), 6e (-7.6 Kcal/mol), 8b (-6.9 Kcal/mol) and 8e (-6.9 Kcal/mol) showed excellent binding affinity with the enzyme, DNA Gyrase A, which is more than equal to the standard antibiotic, streptomycin (-6.9 Kcal/mol). Furthermore, the same compounds were well associated with *in vitro* antibacterial activity, particularly against *E. coli*. Furthermore, the compounds 6b, 6c, 6d and 8a showed moderate binding energy in the range of -6.5 to -6.7 Kcal/mol.

Sulfathiazole carbamate 6a (Figure 2c), forms three hydrogen bonding interactions with amino acid residues at the active site of the enzyme, DNA Gyrase A. The oxygen atoms of sulfoxide (SO_2) forms one hydrogen bonding interaction with hydrogen of Asn 269, nitrogen atom of nitro group and amide carbamate forms one hydrogen bonding interaction with Gly 267 and Arg 91, respectively. Compound 6e (Figure 2d) forms three hydrogen bonding interaction, whereas nitrogen atom from thiazole ring and carbamate forms hydrogen bonding interactions with Thr 219 and Asp 297, respectively, and one hydrogen bonding interaction of oxygen in carbonyl with Lys 270. In the similar fashion, the methyl tryptophanate derivatives 8b and 8c form each two hydrogen bonding interactions. The nitrogen atom in indole moiety with Asn 169 both in compounds 8b and 8c, oxygen atom of carbonyl group in compound 8b with Arg 91 (Figure 2e), and nitrogen of carbamate in compound 8c Gly 170 (Figure 2f) form hydrogen bonding interactions. The binding modes of compounds 6a, 6e, 8b and 8e, suggesting that they fitted more stably into the DNA Gyrase binding pocket. Hence, the present investigation demonstrates that the synthesized compounds will be the promising next generation antimicrobial drugs, and can be effectively used in the treatment of microbial and other related infections.

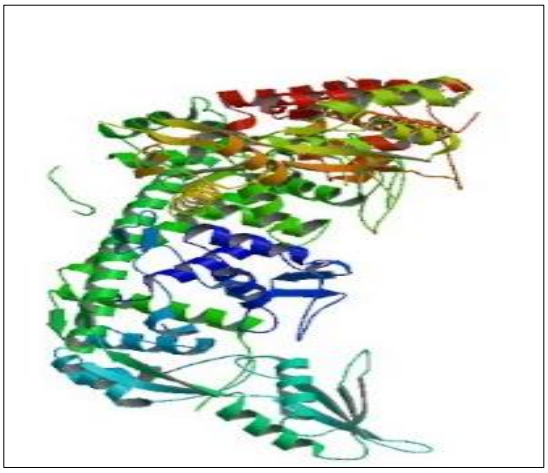


Fig 2a: Three-dimensional structure of DNA Gyrase A (PDB: 3LPX).

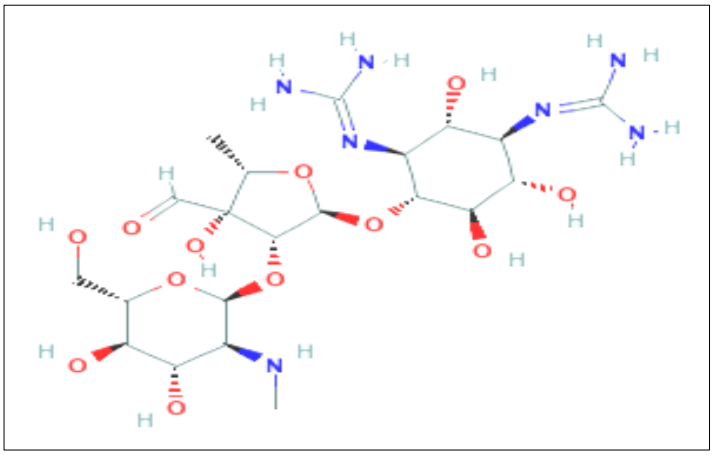


Fig 2b: Structure of reference drug, streptomycin (Pub Chem ID 19649).

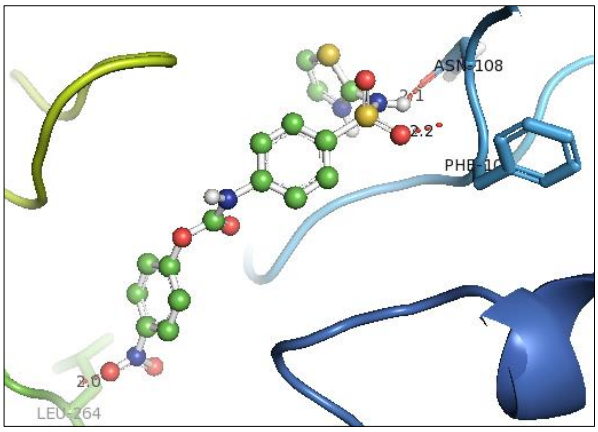


Fig 2c: Docking conformer of compound 6a in the site of DNA Gyrase A.

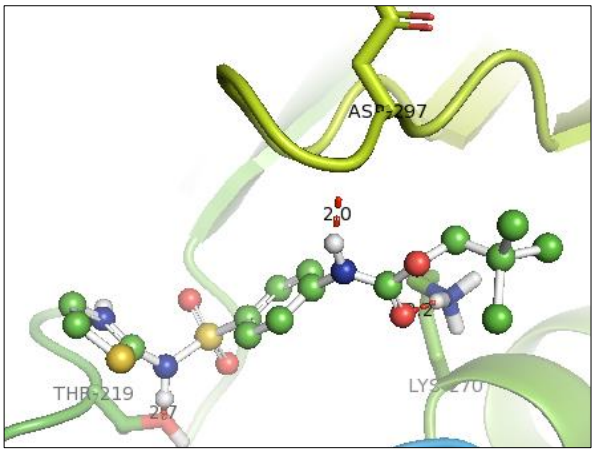


Fig 2d: Docking conformer of compound **6e** in the site of DNA Gyrase A.

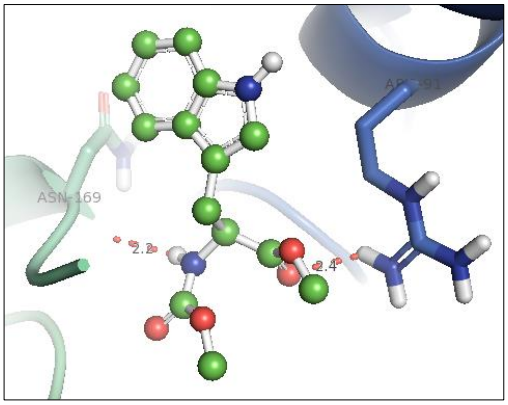


Fig 2e: Docking conformer of compound 8b in the site of DNA Gyrase A

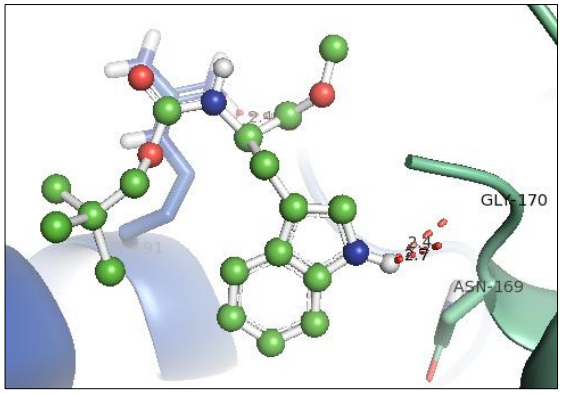


Fig 2f: Docking conformer of compound 8**c** in the site of DNA Gyrase A.

Fig 2: Three dimensional structure of DNA Gyrase A, streptomycin and docking conformers of active newly synthesized compounds.

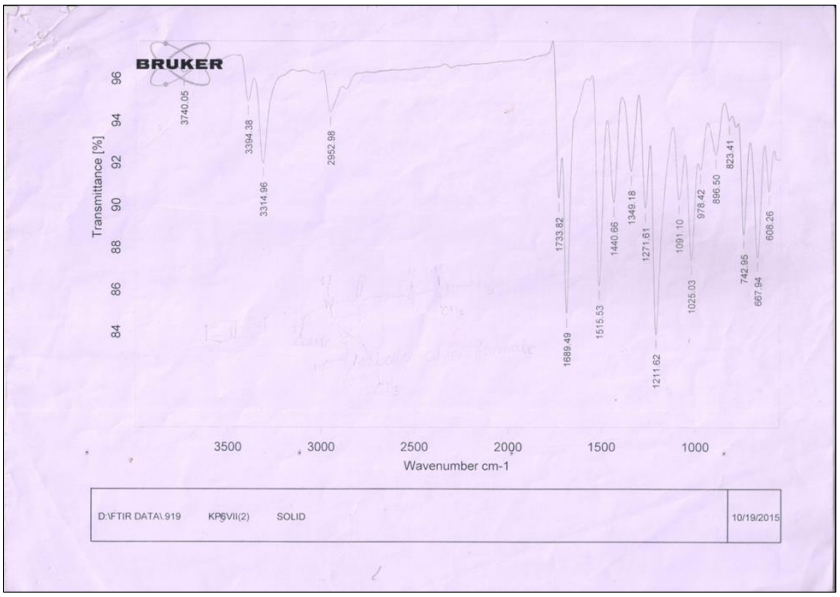


Fig 3: IR Spectra of Methyl 3-(1H-indol-3-yl)-2-(isobutoxycarbonylamino) propanoate (8d)

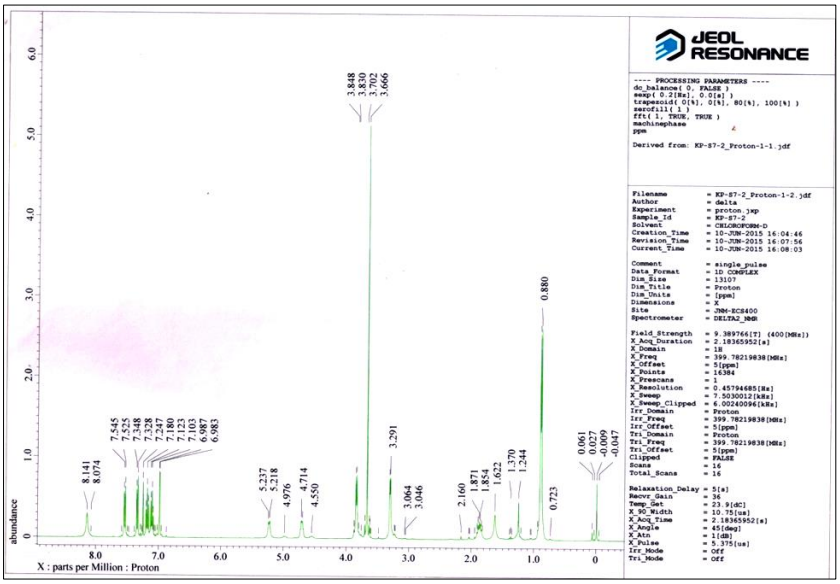


Fig 4: [1]H NMR Spectra of Methyl 3-(1H-indol-3-yl)-2-(isobutoxycarbonylamino)propanoate (8d)

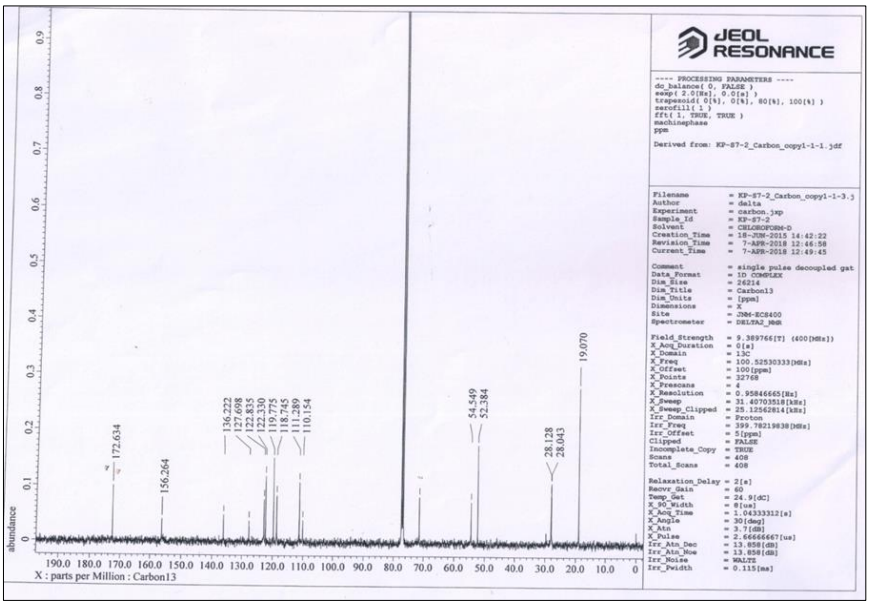


Fig 5: [13]C NMR Spectra of Methyl 3-(1H-indol-3-yl)-2-(isobutoxycarbonylamino) propanoate (8d)

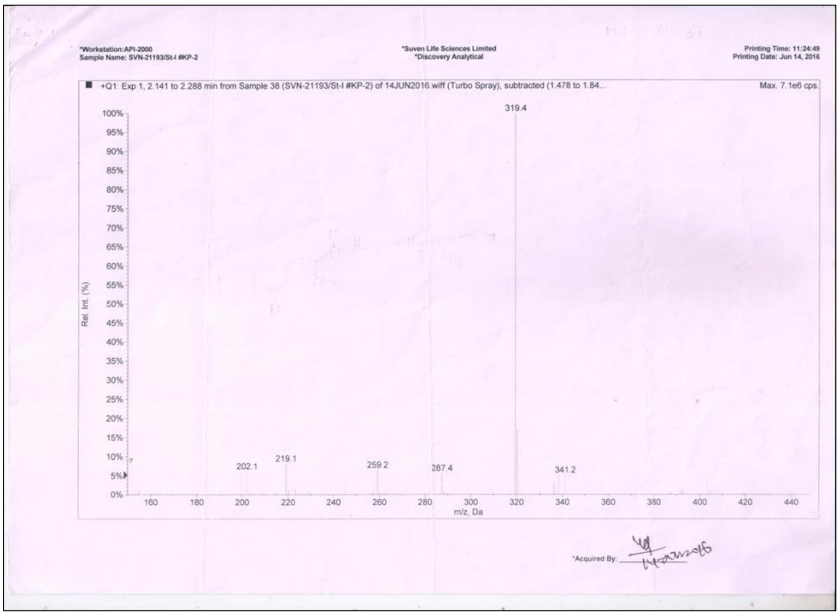


Fig 6: Mass spectra of methyl 3-(1H-indol-3-yl)-2-(isobutoxycarbonylamino) propanoate (8d)

4. Conclusion

The bacteria resistance is one of the utmost threats to human health and the research for the discovery of new antimicrobial agents with stringent mechanistic action explores recently a great interesting subject matter. In the present study, a series of new carbamate derivatives of sulfathiazole 6a-e and methyl tryptophanate 8a-e was accomplished to understand their biological action and characterized well. The compounds were evaluated for their *in vitro* antimicrobial activity against four bacterial strains and three fungal strains including minimum inhibitory concentrations (MICs) for active compounds. Whereas, three carbamate derivatives of sulfathiazole 6a bonded with 4-nitrophenyl ring, 6b bearing with methyl group and 6e bound with 2,2,2-trichloroethyl group and one derivative of methyl tryptophanate 8a bound with 4-nitrophenyl ring showed promising antibacterial activity in the range MIC 3.125-6.25 µg/mL and it is comparable activity of standard drug, streptomycin (MIC = 3.125-6.25 µg/mL). Most of the compounds provided potent activity against *E. coli* and it was equivalent to standard (MIC = 3.125 µg/mL). A few of the compounds showed good to moderate activity against fungal strains. The title compounds were docked into the active site of *E. coli* DNA Gyrase A enzyme to ensure the binding mode of newly synthesized carbamate derivatives. The molecular docking study results demonstrated that a few compounds (6a, 6e, 8b and 8c) showed better binding energies (≥ -6.9 Kcal/mol) with enzyme than that of standard antibiotic, streptomycin (-6.9 Kcal/mol) and the same compounds were associated well with *in vitro* antibacterial activity, particularly on *E. coli* inhibition. Therefore, the present investigation demonstrates that further suitable structural changes in the respect of substitution of new pharmacophore units on synthesized compounds could lead to the promising next generation antimicrobial drugs, and can be effectively used in the treatment of microbial and other related infections.

5. Supporting information

R, 1H, 13C, NMR and Mass spectra for the compound (8d) were given in the supporting information.

6. Acknowledgments

The authors are thankful to Hyderabad Central University, Osmania University and Department of Biochemistry, S. V. University for providing instrumentation facilities to characterize the compounds and biological data, respectively.

7. References

1. Bobbarala V, Vadlapudi V. Int. J. Pharm. Tech. Res, 1 2009, 1115.
2. Fernandes P. Nat. Biotechnol. 2006; 24:14-97.
3. Brown ED, Wright GD, Chem. Rev. 2005; 105:759.
4. Nagai K, Davies TA, Jacobs MR, Appelbaum PC. Antimicrob. Agents Chemother. 2000; 46:1273.
5. Leclerq R, Courvalin P. Agents Chemother. 2006; 46:2727.
6. Conradi RA, Hilgers AR, Ho NF, Burton PS. Pharm. Res. 1992; 9:435.
7. Nielsen PE. Pseudo-peptides in Drug Discovery, Wiley-VCH: Weinheim, Germany, 1992.
8. Sewald N, Jakubke HD. Peptides: Chemistry and Biology, Wiley-VCH: Weinheim, Germany, 2002.
9. Ghosh AK, Brindisi M. J Med. Chem. 2015; 58:2895.
10. Dibenedetto A, Aresta M, Fragale C, Narracci M. Green Chem. 2002; 4:439.
11. Gupte SP, Shivarkar AB, Chaudhari RV. Chem. communm, 2001, 2620.
12. Martin LL, Davis L, Klein JT, Nemoto P, Olsen G E, *et al*. Bioorg. Med. Chem. Lett. 1997; 7:157.
13. Tundo P, McElroy CR, Arico F. Synlett. 2010; 10:1567.
14. Morgan LR, Struck RF, Waud WR. Cancer Chemother. Pharmacol. 2009; 64:829.
15. Deng J, Zhao W, Yang W. React. Funct. Polym. 2006; 67:828.
16. Jung JC, Avery MA. Tetrahedron Lett. 2006; 47:7969.
17. Gattinoni S, Simone CD, Dallavalle S, Bioorg. Med. Chem. Lett. 2010; 20:4406.
18. Meara JAO, Jakalina A, La Plante S, Bonneau PR, Coulombe R, *et al*. Bioorg. Med. Chem. Lett. 2007; 17:33-62.
19. Kratky M, Vinsova J, Bioorg. Med. Chem. 2016; 24:13-22.
20. Narasaiah T, SubbaRao D, Rasheed S, Madhava G,

Srinivasulu D, Brahma Naidu P *et al*. Der Pharmacia Lettre. 2012; 4:854.
21. Lakshmi Reddy SV, Thaslim Basha SK, Naresh K, Naga Raju C, Der Chemica Sinica. 2013; 4:127.
22. Sudhamani H, Thaslim Basha SK, Chandra Reddy SM, Sreedhar B, Adam S, Naga Raju C, Res. Chem Intermed. 2016; 46:7471.
23. Montanaro A. Immunol. Allergy Clin. North am. 1998; 18:843.
24. Kanda Y, Kawanishi Y, Oda K, Sakata T, Mihara S, Asakura K *et al*. Bioorg. & Med. Chem. 2001; 9:897.
25. Stokes SS, Albert R, Buurman Ed T, Andrews B, Shapiro AB, Green OM, *et al*. Bioorg. & Med. Chem. Lett. 2012; 22:7019.
26. Chibale K, Haupt H, Kendrick H, Yardley V, Saravanamuthu A, Fairlamb AH *et al*. Bioorg. & Med. Chem. Lett. 2001; 11:2655.
27. Rahavi EI, Camoutsis C, Zoumpoulakis P, Geronikaki A, Soković M, Glamocilija J *et al*. Bioorg. & Med. Chem. 2002; 16:1150.
28. Kennedy JF, Thorley M. Pharmaceutical Substances 3rd Ed. Kleeman A, Engel J, Kutscher B and Reichert D Thieme: Stuttgart, 1999.
29. Kolaczek A, Fusiarz L, Lawecka J, Branowska D, Chemik. 2014; 68:620.
30. Ganapathi K, Shirsat V, Deliwala CV. Proc. Indian Acad. Sci.16 A 126; (b) Backer J and De Jonge J, Rec. Trav. Chim. 1941; 60:495.
31. Figueroa LV, Diaz FC, Camacho AL, Garcia EC, Pool EG, Lopez MR *et al*. Int. J Pharm Tech. Res. 2013; 5:12-47.
32. Bergman J, Koch E, Pelcman B. Tetrahedron. 1995; 51: 5631.
33. Ninomiya I. J. Nat. prod. 1992; 55:541.
34. Takikawa O. Biochem Biophys Res Commun. 2005; 338:12.
35. Schmidt SK, Siepmann S, Kuhlmann K, Meyer HE, Metzger S, Pudelko S *et al*. PLoS ONE. 2012; 7:44797.
36. Nakashima H, Uto Y, Nakata E, Nagasawa H, Ikkyu K, Hiraoka N *et al*. Bioorg. Med. Chem. 2008; 16:8661.
37. Van Baren N, Van den Eynde BJ. Cancer Immunol Res. 2015; 3:978.
38. Lovelace MD, Varney B, Sundaram G, Lennon MJ, Lim CK, Jacobs K *et al*. Neuropharmacol. 2017; 112:373.
39. Herrera R, Manjarrez G, Hernandez J. Nutr. Neurosci. 2005; 8:57.
40. Fernstrom MH, Fernstrom JD. Life Sci. 1993; 52:907.
41. Xu K, Wang P, Xu X, Chu F, Lin J, Zhang Y *et al*. Res. Chem. Intermed. 2015; 41:1385.
42. Janakiramudu DB, Subba Rao D, Srikanth C, Madhusudana S, Murthy PS, Nagalakshmidevamma M *et al*. Res. Chem. Intermed. 2018; 44:469.
43. SubbaRao D, Madhava G, ThaslimBasha SK, Janardhan A, Madhu Sudhana S, Narasimha G *et al*. Med. Chem. Res. 2017; 26:999.
44. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken HR. Manual of Clinical Microbiology 6th Ed. ASM Press, Washington DC, 1995, 15-18.
45. SubbaRao D, Madhava G, Naga Raju C, Balaji M, Madhusudhana S, Usha Rani A. Med. Chem. Res. 2016; 25:751.
46. Jones RN, Barry AL, Gaven TL, Washington JA, Lennette EH, Balows A *et al*. Manual of Clinical Microbiology (Ed.), American Society of Microbiology, Washington DC, 1984, 972
47. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS *et al*. J Comput. Chem. 2009; 30:2785.
48. TerHaar E, Coll JT, Austen DA, Hsiao HM, Swenson L, Jain J, Nat. Struct. Biol. 2001; 8:593.
49. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM *et al*. J Comput, Chem. 2004; 25:1605.
50. Subba Rao D, Srinivasulu D, Rajasekhar D, Naga Raju C. Chin. Chem. Lett. 2013; 24:759.
51. Subba Rao D, Rasheed S, Thaslim Basha SK, Naga Raju C, Naresh K. Der Pharma Chemica, 2013; 5:61.
52. Munichandra Reddy S, SubbaRao D, Madhava G, Venkatesh M, Gnana Kumari P, Naga Raju C. J Chem. Sci. 2016; 128:1303.