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Synthesis, Biological Evaluation and Docking Study of Etodolac-Triazole Conjugate

Papigani Neeraja[1], Suryapeta Srinivas[2], Venkanna Banothu[3], Khagga Mukkanti[4], Pramod Kumar Dubey[5] and Sarbani Pal[6*#]

[1]Department of Chemistry, DVR College of Engineering & Technology, Kashipur (Village), Sangareddy District, Telangana- 502285, India.
[2]Alembic pharmaceuticals ltd, 2700 MN Park, Genome Valley,Turkapally Village, Shameerpet Medchal, Malkajgiri – 500101,India.
[3]Department of biotechnology, JNTUH, Hyd-500085, India.
[4]IST JNTUH, Hyd-500085, India.
[5]Department of Chemistry, NIPER, Hyd- 500037, India.
[6]Department of Chemistry, MNR Degree & PG College, Kukatpally, Hyd-500085, India.

Authors' contributions

This work was carried out in collaboration between all authors. Authors PN and SS designed Scheme, literature survey, bench work, analysis of molecules and wrote the first draft of the manuscript. Author VB managed biological study. Authors KM, PKD and SP guided the literature survey, in scheme, preparation of manuscript preparation. All authors read and approved the final manuscript.

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ABSTRACT

A new set of 15 compounds containing etodolac moiety and triazole ring were prepared by CuAAC reaction in moderate to high yield. All the synthesized compounds were purified by chromatographic techniques and characterized by spectral data IR, ^{1}H and ^{13}C NMR and mass spectrometry. The newly derived compounds were screened for their anti-bacterial activities against one gram-positive (*S. aureus*) and two gram-negative (*E. coli, K. pneumoniae*) bacteria using an agar-well diffusion method. Most of the compounds showed good to moderate antibacterial activities. Especially compound 4e having good activities against all the strains. The

**Corresponding author: E-mail: sarbani277@yahoo.com;*
 # ORCiD number 0000-0003-1730-0782

compound 4e displayed significant inhibitory potential with MIC 25 µg/mL against all the strains. The potential DNA gyrase inhibitory activity of this compound was investigated by using molecular docking studies carried out using Autodock Vina software. The compound 4e showed the lowest ΔG_{bind} results (-7.7 Kcal/mol, -7.9Kcal/mol). The cytotoxic activity of the obtained compounds was determined using A549 cancer cell line by a MTT assay. They displayed promising activity against the human lung cancer cell line. Especially 4o, 4b, 4d shown lowest IC_{50} values.

Keywords: NSAID; etodolac; 1,2,3-triazole; antibacterial and anticancer activity; molecular docking.

1. INTRODUCTION

In the past decades bacteria have emerged remarkable causing severe infections that are extremely difficult to treat and are leading cause of high mortality rates worldwide. There is a pressing need for the discovery of new drugs. Additionally, studies indicated that some of the commonly used NSAIDS that fight pain, fever and inflammation may have the ability to kill bacteria as well [1,2]. Etodolac is the one of the cyclooxygenase-2 inhibitors which belongs to the NSAIDS agents. Etodolac derivatives possess attractive biological properties including antibacterial, anticancer, and antimicrobial activities etc. [3-8].

Triazoles which are an important class of heterocyclic compounds, have been studied for over a century and continue to attract considerable attention because of many biological activities [9-21]. We anticipated that integration of structural features of etodolac Fig. 1 and 1,2,3-triazole Fig.1 in a single molecule entity as represent by A Fig.1 might be beneficial for the identification of the new biologically active molecules, compounds with dual activity (antibacterial, anticancer) are beneficial because of following reasons, it can kill bacterial infection in immune suppressive cancer patients [22-24] hence compounds having dual activity are of considerable therapeutic interest. In continuation of our research [25,26] in the field of synthesis of small library of biologically active hybrid molecules we designed a new templet A which contains etodolac -1,2,3 triazole moiety. The new template A was used to generate a library of 15 molecules that were evaluated for their anti-cancer and antibacterial activities in vitro. Herein, we report our preliminary results of this study. Our objective was not only to achieve a rapid synthesis of compounds based on A but also to establish mild and environmentally benign reaction conditions.

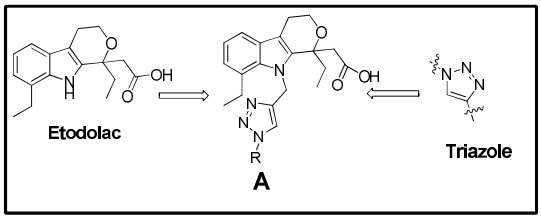


Fig. 1. New template A for the generation of library of small molecules with potential dual activity

2. EXPERIMENTAL SECTION

2.1 Chemistry

2.1.1 General methods

Melting points were determined by open glass capillary method on a cintex melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ACF-300 machine or a varian 300 or 400 MHz spectrometer using DMSO-d_6, with reference to tetramethylsilane as an internal reference. Mass spectra were recorded on a Jeol JMC D-300 instrument by using electron ionization at 70eV. All reactions were monitored by TLC (thin layer chromatography) on pre-coated silica gel plates. Column chromatography was performed by using silica gel (100-200 mesh, SRL, India) [10-20 times (by weight) of the crude product].

Commercially available chemicals were used directly without any further purification. Melting points were checked by open glass capillary method by using Cintex melting point apparatus. IR spectra was recorded by Perkin-Elmer IR spectrometer using KBr pellets. Bruker ACF-300 machine or a Varian 300 or 400 MHz spectrometer were used to record ^{1}H and ^{13}CMR tetramethylsilane as an internal reference using $CDCl_3$ as solvent. Jelol JMC D-300 mass spectrometer was used to record mass spectra of newly synthesized compounds. Progress of

reactions were monitored routinely by TLC (thin layer chromatography) in a regular intervals. Column chromatography was used to purify crude final compounds using silica gel (100-200 mesh, SRL, India).

2.1.2 Procedure for synthesis of substrates alkyne 2 and azide 3

In order to synthesize our hybrid molecule A, we planned to constructs triazole ring by CUAAC reaction [27,28]. Accordingly we planned to synthesize required substrates. We decided to prepare terminal alkyne by functional group modification of NSAID etodolac.

2.1.2.1 Procedure for synthesis of 2-(1,8-diethyl-9-(prop-2-yn-1-yl)-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetic acid (2)

To a stirred solution of 2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetic acid (1) (990mg, 3.48 mmol) in DMF (80 mL) added potassium carbonate (957mg, 6.96 mmol) lot wise at 5-10 ^{0}C, stirred the contents for 5min, and followed by propargyl bromide (490mg, 4.17 mmol) addition, this reaction mixture was maintained for 4 hrs, at RT, the progress of the reaction was monitored at regular intervals by checking TLC, after completion of the reaction, reaction mixture was quenched in a crushed ice, thus solid was separated, filtered the solid under vacuum, and washed with n-hexane dried the solid under vacuum for 4hrs, the compound 2 purified by column chromatography by using silica gel (100-200 mesh) with MeOH/DCM to get desired product. Mobile phase: (Methanol: DCM 1:9). Yield: 91%.

2.1.2.2 General procedure for synthesis of Azide 3

After successful synthesis of etodolac based terminal alkyne 2, fifteen organic azides 3 (a-o) were prepared from primary amines by following two different pathways [29].

After synthesizing terminal alkyne and azide, we assembled by successful implementation of CUAAC reaction [27, 28].

2.1.3 General procedure for synthesis of 4

A mixture of terminal alkyne 2 (250mg, 0.76 mmol), copper sulphate pentahydrate (50 mg, 0.20 mmol) and sodium ascorbate (40 mg, 0.20 mmol) in DMF (3 mL) was stirred vigorously for 30 mins. Then azide 3 (0.76 mmol) was added to the above reaction mixture. The progress of the reaction was monitored by checking TLC at regular interval. After completion of the reaction, the reaction mixture was quenched in crushed ice. The solid separated was filtered, dried, and purified by column chromatography on silica gel using dichloromethane/methanol to give desired product. Mobile phase: (Methanol: DCM 1:9). Yield: 78-90%.

2.1.4 2-(1,8-Diethyl-9-(prop-2-yn-1-yl)-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetic acid (2)

Off white colored solid; m.p.:140-142^{0}C; R_f ; 0.60 (Methanol : DCM 1:9); MS *m/z* 407.20 ($M+1^+$, 100%); IR (v_{max} in cm^{-1}) : 3390, 3307, 3279, 2962, 2934, 1714, 1618, 1497, 1196, 1173, 652, 528, 557, 478. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.89 (s, 1H), 7.36 (d, 7.6Hz, 1H), 7.08-7.00 (m, 2H), 4.07-3.91 (m, 2H), 3.09-3.04 (m, 1H), 2.98-2.94 (m, 1H), 2.90-2.80 (m, 3H), 2.77-2.72 (m, 1H), 2.47 (t, 2.4Hz, 1H), 2.19-1.99 (m, 2H), 1.36 (t, 8Hz, 3H), 0.84 (t, 7.6Hz, 3H) . ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.97, 135.32, 134.50, 126.645, 126.16, 120.52, 119.69. 116.01, 108.65, 77.19, 75.28, 74.64, 60.68, 52.33, 42.82, 30.73, 24.22, 22.40, 13.84, 7.62.

2.1.5 2-(9-((1-(2-((2,4-Dimethylphenyl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetic acid (4a)

Pale yellow colored solid; m.p.:142-145^{0}C; R_f ; 0.59 (Methanol : DCM 1:9); MS *m/z* 530.27 ($M+1^+$, 100%); IR (v_{max} in cm^{-1}) : 3332, 3307, 2925, 1712, 1617, 1595, 1509, 1339, 1300, 1175, 1110, 855, 795, 749, 690, 495. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.97 (s, 1H), 7.99 (s, 1H), 7.53 (s, 1H), 7.47 (d, 8.4Hz, 1H), 7.35 (d, 7.6Hz, 1H), 7.08-7.01 (m, 1H), 7.01-6.94 (m, 3H), 5.22 (d, 4.4Hz, 2H), 5.06 (s, 2H), 4.04-3.89 (m, 2H), 3.04-2.99 (m, 1H), 2.94-2.92 (m, 1H), 2.88-2.77 (m, 3H), 2.73-2.68 (m, 1H), 2.25 (s, 3H), 2.06 (s, 3H), 1.97-1.92 (m, 1H), 1.33-1.31 (m, 3H), 0.79 (t, 7.6Hz, 3H) . ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.89, 163.18, 143.17, 135.96, 135.51, 134.57, 131.79, 131.33, 129.99, 127.28, 126.73, 126.21, 125.24, 123.37, 120.52, 119.70, 116.02, 108.73, 74.93, 0.68, 57.75, 53.34, 43.19, 31.19, 24.20, 22.31, 20.89, 17.52, 13.88, 7.64.

2.1.6 2-(9-((1-(2-((2,6-Dimethylphenyl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetic acid (4b)

Off white colored solid; m.p.:139-141^{0}C; R_f : 0.57 (Methanol : DCM 1:9); MS *m/z* 530.27 ($M+1^+$, 100%); IR (v_{max} in cm^{-1}) : 3333, 3308, 2925, 1712, 1617, 1594, 1510, 1338, 1300, 1175, 1110, 858, 795, 752, 690, 495. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.93 (s, 1H), 7.60-7.59 (m, 2H), 7.35 (d, 7.6Hz, 1H), 7.12-7.05 (m, 3H), 7.02-7.00 (m, 3H), 5.25 (d, 4.8Hz, 2H), 5.10 (s, 2H), 4.05-3.91 (m, 2H), 3.05-3.01 (m, 1H), 2.92-2.88 (m, 1H), 2.86-2.78 (m, 3H), 2.74-2.69 (m, 1H), 2.08 (s, 6H), 1.99-1.92 (m, 2H), 1.33 (t, 7.6Hz, 3H), 0.79 (t, 7.6Hz, 3H) . ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.94, 163.40, 143.29, 135.48, 135.15, 134.54, 132.39, 128.34, 127.95, 126.69, 126.19, 125.00, 120.53, 119.71, 116.03, 108.75, 74.85, 60.67, 57.74, 53.05, 43.17, 31.15, 24.21, 22.31, 18.19, 13.87, 7.63.

2.1.7 2-(9-((1-(2-((2-Chlorophenyl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetic acid (4c)

Off white colored solid; m.p.:138-140^{0}C; R_f : 0.59 (Methanol : DCM 1:9); MS *m/z* 537.20 ($M+1^+$, 100%); IR (v_{max} in cm^{-1}) : 3332, 3308, 2926, 1711, 1618, 1594, 1509, 1339, 1300, 1175, 1110, 857, 795, 753, 690, 495. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.87 (s, 2H), 7.56 (s, 1H), 7.42-7.33 (m, 3H), 7.24-7.21 (m, 2H), 7.08-6.99 (m, 2H), 5.24 (s, 2H), 5.07 (s, 2H), 4.03-3.94 (m, 2H), 3.04-3.00 (m, 1H), 2.92-2.88 (m, 1H), 2.86-2.79 (m, 3H), 2.72-2.69 (m, 1H), 2.11-1.90 (m, 2H), 1.30 (t, 7.2Hz, 3H), 0.79 (t, 6.8Hz, 3H) . ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.94, 162.91, 143.17, 137.93, 135.43, 134.74, 134.52, 130.09, 126.67, 126.20, 125.33, 125.25, 120.54, 120.24, 119.73, 118.08, 116.04, 108.81, 74.87, 60.66, 57.68, 53.37, 43.17, 31.23, 24.18, 22.28, 13.83, 7.64.

2.1.8 2-(9-((1-(2-((3-Chlorophenyl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetic acid (4d)

Pale yellow colored solid; m.p.:141-143^{0}C; R_f : 0.60 (Methanol : DCM 1:9); MS *m/z* 537.20 ($M+1^+$, 100%); IR (v_{max} in cm^{-1}) : 3333, 3308, 2926, 1711, 1619, 1594, 1509, 1340, 1300, 1175, 1110, 857, 795, 755, 695, 495. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.84 (s, 2H), 7.60 (s, 1H), 7.55 (s, 1H), 7.36-7.18 (m, 4H), 7.09-6.99 (m, 3H), 5.25 (s, 2H), 5.07 (s, 2H), 4.04-3.90 (m, 2H), 3.05-3.01 (m, 1H), 2.93-2.89 (m, 1H), 2.87-2.77 (m, 3H), 2.73-2.67 (m, 1H), 2.14-1.93 (m, 2H), 1.34-1.32 (m, 3H), 0.79 (t, 67.2Hz, 3H) . ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.94, 162.91, 143.17, 137.93, 135.45, 134.53, 134.75, 130.10, 126.67, 126.20, 125.35, 125.26, 120.55, 119.73, 118.08, 116.04, 108.82, 74.87, 60.66, 57.69, 53.37, 43.17, 31.23, 24.18, 22.28, 13.83, 7.64.

2.1.9. 2-(9-((1-(2-((4-Chlorophenyl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetic acid (4e)

Off white colored solid; m.p.:139-141^{0}C; R_f : 0.58 (Methanol : DCM 1:9); MS *m/z* 537.20 ($M+1^+$, 100%); IR (v_{max} in cm^{-1}) : 3332, 3309, 2926, 1712, 1617, 1594, 1509, 1338, 1300, 1175, 1110, 857, 795, 753, 690, 495. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.87 (s, 2H), 7.56 (s, 1H), 7.42-7.34 (m, 3H), 7.24-7.21 (m, 2H), 7.08-6.99 (m, 2H), 5.22 (s, 2H), 5.06 (s, 2H), 4.02-3.93 (m, 2H), 3.03-2.99 (m, 1H), 2.91-2.88 (m, 1H), 2.85-2.78 (m, 3H), 2.71-2.69 (m, 1H), 2.12-1.91 (m, 2H), 1.30 (t, 7.2Hz, 3H), 0.79 (t, 6.8Hz, 3H) . ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.94, 162.91, 143.17, 137.93, 135.43, 134.75, 134.53, 130.09, 126.67, 126.20, 125.35, 120.55, 120.25, 119.73, 118.08, 116.04, 108.82, 74.87, 60.66, 57.69, 53.37, 43.17, 31.23, 24.18, 22.28, 13.83, 7.63.

2.1.10 2-(1,8-Diethyl-9-((1-(2-((2-nitrophenyl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetic acid (4f)

Pale yellow colored solid; m.p.:140-145^{0}C; R_f : 0.60 (Methanol : DCM 1:9); MS *m/z* 547.23 ($M+1^+$, 100%); IR (v_{max} in cm^{-1}) : 3332, 3309, 2925, 1712, 1618, 1594, 1510, 1338, 1300, 1175, 1110, 856, 795, 753, 690, 496. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.37 (s, 1H), 8.87 (s, 1H), 8.65 (d, 7.2Hz, 1H), 8.12 (d, 8Hz, 1H), 7.66-7.62 (m, 1H), 7.49 (s, 1H), 7.32 (d, 7.6Hz, 1H), 7.26-7.19 (m, 1H), 7.03 (t, 7.2Hz, 1H), 6.96 (d, 6.8Hz, 1H), 5.31 (d, 6.8Hz, 2H), 5.16 (d, 8.8Hz, 2H), 4.06-3.93 (m, 2H), 2.96-2.88 (m, 2H), 2.85-2.77 (m, 3H), 2.74-2.69 (m, 1H), 2.15-1.94 (m, 2H), 1.30 (t, 7.2Hz, 3H), 0.80 (t, 7.6Hz, 3H) . ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.06, 163.91, 143.66, 136.62, 136.07, 135.52, 134.47, 133.24, 126.59, 126.19, 125.89, 125.01, 124.53, 122.14, 120.44, 119.66, 115.99, 108.78, 74.85, 60.65, 57.77, 53.61, 43.16, 31.31, 24.17, 22.30, 13.81, 7.63.

2.1.11 2-(1,8-Diethyl-9-((1-(2-((3-nitrophenyl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetic acid (4g)

Pale yellow colored solid; m.p.:141-145^{0}C; R_f : 0.60 (Methanol : DCM 1:9); MS *m/z* 547.23 (M+1$^+$, 100%); IR (v_{max} in cm^{-1}) : 3332, 3307, 2925, 1712, 1618, 1594, 1510, 1339, 1300, 1175, 1109, 856, 795, 753, 690, 496. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.28 (s, 1H), 8.82 (s, 1H), 8.41-.40 (m, 1H), 7.94-7.86 (m, 2H), 7.58 (s, 1H), 7.45-7.41 (m, 1H), 7.35-7.33 (m, 1H), 7.08-6.98 (m, 2H), 5.25 (s, 2H), 5.15 (s, 2H), 4.03-3.91 (m, 2H), 3.04-3.00 (m, 1H), 2.93-2.89 (m, 1H), 2.85-2.76 (m, 3H), 2.73-2.68 (m, 1H), 2.12-1.92 (m, 2H), 1.30-1.29 (m, 3H), 0.79 (t, 7.2Hz, 3H) . ^{13}C NMR ($CDCl_3$, 100 MHz) δ171.91, 163.41, 148.43, 143.18, 138.16, 135.40, 134.53, 129.97, 126.67, 126.21, 125.74, 120.55, 119.75, 119.62, 116.05, 114.92, 108.84, 74.95, 60.67, 57.62, 53.19, 43.15, 31.25, 24.15, 22.27, 13.81, 7.65.

2.1.12 2-(1,8-Diethyl-9-((1-(2-((4-nitrophenyl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetic acid (4h)

Off white colored solid; m.p.:140-145^{0}C; R_f : 0.62 (Methanol : DCM 1:9); MS *m/z* 547.23 (M+1$^+$, 100%); IR (v_{max} in cm^{-1}) : 3332, 3307, 2925, 1712, 1618, 1594, 1510, 1339, 1300, 1175, 1109, 856, 795, 753, 690, 496. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.39 (s, 1H), 8.79 (s, 1H), 8.13 (d, 8.8Hz, 2H), 7.67 (d, 7.6Hz, 1H), 7.08-6.98 (m, 2H), 5.25 (s, 2H), 5.13 (s, 2H), 4.01-3.90 (m, 2H), 3.04-3.00 (m, 1H), 2.93-2.90 (m, 1H), 2.84-2.75 (m, 3H), 2.72-2.67 (m, 1H), 2.12-1.92 (m, 2H), 1.29 (t, 7.6Hz, 3H), 0.80 (t, 6.8Hz, 3H) . ^{13}C NMR ($CDCl_3$, 100 MHz) δ171.88, 163.45, 143.97, 143.16, 142.92, 135.38, 134.53, 126.66, 126.20, 125.66, 125.03, 120.57, 119.79, 119.58, 116.07, 108.86, 74.94, 60.66, 57.59, 53.21, 43.17, 31.25, 24.15, 22.25, 13.82, 7.66.

2.1.13 2-(1,8-Diethyl-9-((1-(2-oxo-2-(phenylamino)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetic acid (4i)

Off white colored solid; m.p.:138-143^{0}C; R_f : 0.58 (Methanol : DCM 1:9); MS *m/z* 502.24 (M+1$^+$, 100%); IR (v_{max} in cm^{-1}) : 3333, 3308, 2925, 1712, 1618, 1594, 1510, 1338, 1300, 1175, 1110, 859, 795, 752, 690, 495. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.93 (s, 1H), 8.74 (s, 1H), 7.58 (s, 1H), 7.45 (d, 7.6Hz, 1H), 7.34 (d, 7.6Hz, 3H), 7.26 (t, 8Hz, 2H), 7.11-7.04 (m, 2H), 7.00-6.98 (m, 1H), 5.21 (d, 3.6Hz, 2H), 5.06 (s, 2H), 4.02-3.91 (m, 2H), 3.02-2.98 (m, 1H), 2.91-2.87 (m, 1H), 2.86-2.76 (m, 3H), 2.71-2.67 (m, 1H), 2.11-1.91 (m, 2H), 1.30 (t, 7.6Hz, 3H), 0.78 (t, 7.6Hz, 3H) . ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.91, 163.08, 142.98, 136.89, 135.50, 134.58, 129.09, 126.73, 126.23, 125.42, 125.20, 120.51, 120.23, 119.69, 116.02, 108.76, 74.49, 60.67, 57.71, 53.26, 43.14, 31.19, 24.19, 22.29, 13.85, 7.65.

2.1.14 2-(1,8-Diethyl-9-((1-(2-oxo-2-(o-tolylamino)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetic acid (4j)

Pale yellow colored solid; m.p.:139-145^{0}C; R_f : 0.59 (Methanol : DCM 1:9); MS *m/z* 516.26 (M+1$^+$, 100%); IR (v_{max} in cm^{-1}) : 3332, 3308, 2926, 1711, 1617, 1594, 1510, 1338, 1300, 1175, 1110, 859, 794, 753, 690, 495. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.88 (s, 1H), 7.91 (s, 1H), 7.75 (d, 7.6Hz, 1H), 7.51 (s, 1H), 7.34 (d, 7.6Hz, 3H), 7.26-7.13 (m, 2H), 7.10-7.06 (m, 2H), 7.02-7.00 (m, 1H), 5.25 (s, 2H), 5.08 (s, 2H), 4.03-3.92 (m, 2H), 3.06-3.02 (m, 1H), 2.95-2.93 (m, 1H), 2.89-2.82 (m, 3H), 2.74-2.69 (m, 1H), 2.11 (s, 3H), 2.00-1.93 (m, 2H), 1.33 (t, 7.2Hz, 3H), 0.80 (t, 7.2Hz, 3H) . ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.92, 162.85, 143.35, 135.45, 134.52, 130.67, 126.84, 126.68, 125.99, 125.19, 122.71, 120.57, 119.75, 116.04, 74.87, 60.67, 57.74, 53.60, 43.21, 31.22, 24.21, 22.29, 17.53, 13.87, 7.63.

2.1.15 2-(1,8-Diethyl-9-((1-(2-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetic acid (4k)

Off white colored solid; m.p.:140-145^{0}C; R_f : 0.61 (Methanol : DCM 1:9); MS *m/z* 490.20 (M+1$^+$, 100%); IR (v_{max} in cm^{-1}) : 3332, 3308, 2925, 1594, 1510, 1338, 1300, 1175, 1110, 859, 795, 755, 690, 495. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.97 (s, 1H), 8.09 (d, 7.2Hz, 1H), 7.80-7.70 (m, 3H), 7.52 (d, 7.6Hz, 1H), 7.34 (d, 7.6Hz, 1H), 7.04-6.95 (m, 2H), 5.36 (s, 2H), 4.07-3.93 (m, 2H), 3.11-3.07 (m, 1H), 2.98-2.94 (m, 1H), 2.88-2.71 (m, 4H), 2.15-1.96 (m, 2H), 1.33 (t, 7.2Hz, 3H), 0.82 (t, 7.2Hz, 3H) . ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.12, 143.12, 135.58, 134.52, 133.93, 131.62, 128.65, 126.64, 126.17, 125.68, 125.07,

120.45, 119.62, 115.97, 108.69, 74.82, 60.67, 57.74, 43.21, 31.07, 24.22, 22.34, 13.83, 7.63.

2.1.16 2-(1,8-Diethyl-9-((1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetic acid (4l)

Pale yellow colored solid; m.p.:140-145^{0}C; R_f : 0.60 (Methanol : DCM 1:9); MS *m/z* 490.20 ($M+1^+$, 100%); IR (v_{max} in cm^{-1}) : 3332, 3308, 2925, 1594, 1510, 1338, 1300, 1175, 1110, 859, 795, 755, 690, 495. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.79 (s, 1H), 8.33-8.30 (m, 1H), 8.08-8.06 (m, 1H), 7.89 (s, 1H), 7.74 (t, 8.4Hz, 1H), 7.32 (d, 7.6Hz, 1H), 7.02-6.93 (m, 2H), 5.38-5.30 (m, 2H), 4.07-3.94 (m, 2H), 3.12-3.07 (m, 1H), 2.97-2.93 (m, 1H), 2.86-2.79 (m, 3H), 2.75-2.69 (m, 1H), 2.18-1.96 (m, 2H), 1.32 (t, 7.6Hz, 3H), 0.83 (t, 7.2Hz, 3H) . ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.02, 143.99, 137.43, 135.35, 134.49, 131.02, 126.53, 126.15, 125.97, 123.41, 121.40, 120.54, 119.68, 116.01, 115.30, 108.86, 74.87, 60.69, 57.69, 43.25, 31.25, 24.18, 22.30, 13.82, 7.66.

2.1.17 2-(1,8-Diethyl-9-((1-(2-((4-methoxy-2-nitrophenyl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetic acid (4m)

Off white colored solid; m.p.:140-145^{0}C; R_f : 0.61 (Methanol : DCM 1:9); MS *m/z* 577.24 ($M+1^+$, 100%); IR (v_{max} in cm^{-1}) : 3332, 3308, 2926, 1711, 1617, 1594, 1510, 1338, 1300, 1175, 1110, 859, 795, 755, 690, 495. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.10 (s, 1H), 8.01 (s, 1H), 7.56 (d, 3.2Hz, 1H), 7.51 (s, 1H), 7.32 (d, 7.6Hz, 3H), 7.26 (s, 1H), 7.22-7.1 (m, 1H), 7.01 (t, 7.2Hz, 1H), 6.96-6.94 (m, 1H), 5.36-5.27 (m, 2H), 5.20-5.09 (m, 2H), 4.06-3.93 (m, 2H), 3.85 (s, 3H), 3.13-3.09 (m, 1H), 2.96-2.92 (m, 1H), 2.85-2.78 (m, 3H), 2.74-2.69 (m, 1H), 2.15-1.94 (m, 2H), 1.31 (t, 7.6Hz, 3H), 0.80 (t, 7.2Hz, 3H) . ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.14, 163.55, 155.82, 143.65, 135.53, 134.43, 126.57, 126.18, 125.01, 123.67, 123.14, 120.41, 119.64, 115.95, 108.95, 108.74, 74.82, 60.65, 57.76, 55.95, 53.56, 43.12, 31.26, 24.16, 22.31, 13.81, 7.62.

2.1.18 2-(1,8-Diethyl-9-((1-(2-((4-(methoxycarbonyl)phenyl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetic acid (4n)

Off white colored solid; m.p.:142-145^{0}C; R_f : 0.60 (Methanol : DCM 1:9); MS *m/z* 560.25 ($M+1^+$, 100%); IR (v_{max} in cm^{-1}) : 3333, 3307, 2926, 1711, 1617, 1595, 1510, 1338, 1300, 1175, 1110, 859, 795, 755, 690, 495. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.08 (s, 1H), 8.89 (s, 1H), 7.96 (d, 8.8Hz, 2H), 7.58-7.54 (m, 3H), 7.34 (d, 7.6Hz, 1H), 7.08-6.98 (m, 2H), 5.23 (s, 2H), 5.09 (s, 2H), 4.02-3.92 (m, 2H), 3.87 (s, 3H), 3.03-2.99 (m, 1H), 2.92-2.88 (m, 1H), 2.85-2.76 (m, 3H), 2.72-2.67 (m, 1H), 2.12-1.92 (m, 2H), 1.31 (t, 7.2Hz, 3H), 0.79 (t, 7.2Hz, 3H) . ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.85, 166.52, 163.22, 143.09, 141.17, 135.46, 134.56, 130.82, 126.69, 126.22, 125.47, 120.53, 119.72, 119.29, 116.03, 108.80, 74.94, 60.66, 57.66, 53.28, 52.16, 43.17, 31.24, 24.16, 22.17, 13.83, 7.64.

2.1.19 2-(1,8-Diethyl-9-((1-(4-methoxy-2-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetic acid (4o)

Off white colored solid; m.p.:142-145^{0}C; R_f : 0.60 (Methanol : DCM 1:9); MS *m/z* 520.22 ($M+1^+$, 100%); IR (v_{max} in cm^{-1}) : 3332, 3308, 2926, 1594, 1510, 1338, 1300, 1175, 1110, 859, 795, 755, 690, 495. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.01 (s, 1H), 7.69 (s, 1H), 7.58 (s, 1H), 7.40-7.32 (m, 2H), 7.25-7.23 (m, 1H), 7.04-6.96 (m, 2H), 5.35 (s, 2H), 4.05-4.03 (m, 2H), 3.95 (s, 3H), 3.10-3.06 (m, 1H), 2.97-2.93 (m, 1H), 2.88-2.83 (m, 3H), 2.75-2.71 (m, 1H), 2.15-1.96 (m, 2H), 1.32 (t, 7.2Hz, 3H), 0.81 (t, 6.8Hz, 3H) . ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.14, 161.01, 145.18, 142.83, 135.62, 134.53, 129.46, 126.67, 126.18, 125.56, 122.78, 120.45, 119.60, 119.39, 115.97, 110.65, 108.65, 74.83, 60.67, 57.81, 56.47, 43.22, 31.05, 24.24, 22.35, 13.85, 7.64.

2.2 Biology

All the newly synthesized 15 compounds (4a-4o) based on the hybrid frame work A Fig. 1 were tested antibacterial and anticancer activities as planned.

2.2.1 Antibacterial activity

Antibacterial activities of our compounds were tested against one gram-positive (*S. aureus*) and two gram-negative (*E. coli & K. pneumoniae*) bacteria using an agar-well diffusion method [30, 31] and amoxicillin was used as a standard compound in this assay.

2.2.1.1 Test organisms and culture condition

Three organisms including two Gram-negative (*E. coli*, *K. pneumoniae*) and one Gram-positive

(*S. aureus*) were used for the determination of antibacterial activity of our newly synthesized compounds. All three bacterial strains were donated by Department of Microbiology, Osmania General Hospital, Hyderabad. Standard microbiological methods were used to check purity of all bacterial strains. Mueller Hinton Agar (MHA) slants were used to store bacterial stock culture and temperature was well maintained by 4^0C.

2.2.1.2 Determination of antibacterial activity

Antibacterial activities of test compounds was performed by an agar-well diffusion method [30, 31]. Amoxicillin was taken as the positive references at a concentration of 0.1 mg per50 µL. DMSO was used as a negative control. The bacterial strains were reactivated from stock cultures by transferring into Mueller-Hinton broth and incubating at 37^0C for 18 hrs. A final inoculums containing 10^6 colonies forming units (1 x 10^6 CFU/mL) was added aseptically to MHA medium and poured into sterile petri dishes. Test compounds were dissolved in DMSO to prepare solution. 0.1 mg /50 µL was added to wells (8 mm in diameter) punched on agar surface. Plates were incubated overnight at 37 ºC and diameter of inhibition zone (DIZ) around each well was measured in mm. Each experiment was repeated 3 times (triplicates).

The anti-bacterial activity was investigated by determining the minimum inhibitory concentrations (MICs). MIC of compounds was assessed using the broth microdilution method [32]. Each test compound was dissolved in dimethyl sulfoxide (DMSO, Fisher Chemicals) to give a stock solution. Minimum Inhibitory Concentration (MIC), is the lowest concentration of an anti-microbial growth that will inhibit the visible growth of a microorganism after overnight incubation.

2.2.1.3 Compound preparation

Compounds were weighed individually 1mg and dissolved in methanol for final stock concentration as 1 mg/mL as same sample, standard amoxicillin solution were prepared.

2.2.1.4 Culture preparation

Loop of culture was inoculated in 3 mL of nutrient broth and incubate 37 ^{0}C for overnight in shaking incubator.

2.2.1.5 Inoculum preparation

From overnight grown culture, 20 µL of culture was taken and inoculated in 1.5 mL of nutrient broth and added different concentration of compound and incubated at 37 ^{0}C for overnight in an incubator.

2.2.1.6 Result

After 24 hrs of compound treatment, tubes were observed, and results were noted.

2.2.2 Anticancer activity

All the newly synthesized compounds possessing antibacterial activities were tested in *vitro* against human cancer cell line. i.e. A549 (lung adeno carcinoma epithelial cell line), using colorimetric MTT assay and doxorubicin as standard drug. Based on results of our previous studies when tested against noncancerous human embryonic kidney cells *i.e*. HEK293 none of our compounds showed any significant effects indicating their selectivity towards the growth inhibition of cancer cells [26]. So we decided to check anticancer activity against lung cancer as NSAID triazole conjugate showed good anticancer activity against lung cancer cell line [12-14, 26].

DMEM (Dulbecco's modified Eagles medium), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], trypsin, EDTA Phosphate Buffered Saline (PBS) and were purchased from Sigma Chemicals Co. (St. Louis, MO) and Fetal Bovine Serum (FBS) were purchased from Gibco. 25 cm ^{2} and 75 cm^2 flask and 96 well plated purchased from Eppendorf India.
The Cancer cell line A549 was purchased from NCCS, Pune and the cells were maintained in DMEM supplemented with 10 % FBS and the antibiotics penicillin/streptomycin (0.5 mL^{-1}), in atmosphere of 5% CO_2 /95% air at 37 ^{0}C.

2.2.3 MTT assay for cytotoxicity

2.2.3.1 Preparation of test compound

With media make up the final concentration to 1 mg / mL and the cells were treated with series of concentrations from 10 to 100 µg /mL.

2.2.3.2 Principle

MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol- 2-

yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The assay depends both on the number of cells present and on the assumption that dead cells or their products do not reduce tetrazolium. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, dark purple colored formazan crystals. The cells are then solubilized with a DMSO and the released, solubilized formazan reagent is measured spectrophotometrically at 570 nm.

2.2.3.3 Procedure

Cell viability was evaluated by the MTT Assay [33] with three independent experiments with six concentrations of compounds in triplicates. Cells were trypsinized and perform the tryphan blue assay to know viable cells in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0 X 10^3 cells / well in 100 µL media in 96 well plate culture medium and incubated overnight at 37^0C. After incubation, take off the old media and add fresh media 100 µL with different concentrations of test compound in wells in 96 plates. After 48 hrs, Discard the drug solution and add the fresh medic with MTT solution (0.5 mg / mL^{-1}) was added to each well and plates were incubated at 37 ^{0}C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50 % values is generated from the dose-response curves for each cells using with origin software.

% Inhibition = [(Control – Treatment) / Control] X 100

2.3 Docking

To predict interaction of synthesized compounds with targets Molecular docking studies were performed. DNA-gyrase cleavage complex of S. aureus (Gram-positive bacteria) with PDB_ID:5CDQ and the cleavage complex of topoisomerase Top. IV of K. pneumonia (Gram-negative bacteria) with PDB_ID: 5EIX were carried out using Autodock Vina software [34, 35] open source molecular docking software. Based on antibacterial activity of our synthesized compounds 4e showed best activity.so therefore 4ewas selected molecular docking studies

2.3.1 Docking method

Molecular docking studies of molecule 4e into the crystal structures of DNA-gyrase cleavage complex of S. aureus (Gram-positive bacteria) with PDB_ID: 5CDQ and the cleavage complex of topoisomerase Top. IV of K. pneumonia (Gram-negative bacteria) with PDB_ID: 5EIX were carried out using Autodock Vina software [27, 28] open source molecular docking software. We have generated a grid box with desired parameters around the active site of DNA-gyrase cleavage complex of S. aureus (PDB_ID: 5CDQ) as centre: x=40.123, y= -46.732, z= 64.933 and grid box size: x=22, y=36, z=26. We have generated a grid box with desired parameters around the active site of topoisomerase Top. IV of K. pneumonia (PDB_ID: 5EIX) as centre: x= 183.18, y=-28.952, z= -7.879 and grid box size: x=22, y=32, z=32. We generated 20 conformations in each docking output by using advanced Genetic algorithm method in Vina. Protein/DNA complex and molecule input preparations and docking output analysis were carried out using MGLTools-1.5.6 software.

3. RESULTS AND DISCUSSION

3.1 Chemistry

In order to synthesize triazole molecules required substrates i.e. alkyne 2 and azide 3 were prepared as shown in scheme 1.

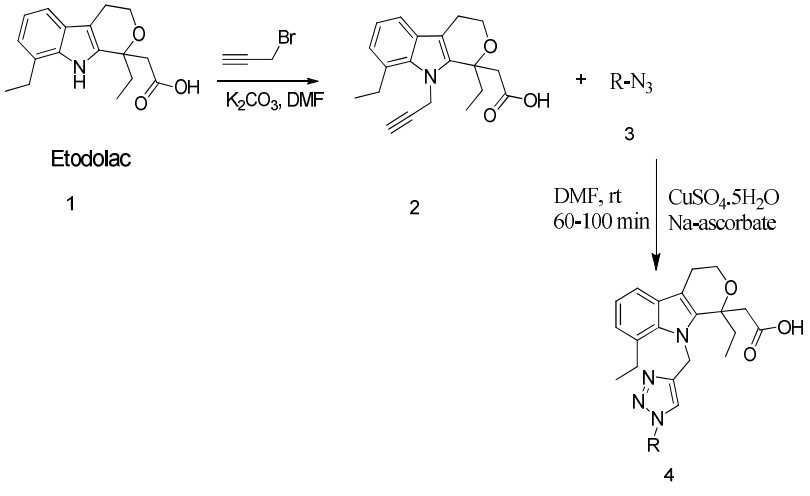
In the first step regioselective *N*-propargylation of the indole ring of Etodolac 1 was performed using propargyl bromide, potassium carbonate in dimethyl formamide solvent to get terminal alkyne 2. *N*-propargylation was confirmed by the Kaiser and bromophenol tests [36, 37]. The product isolated as white solid with 91% of yield.

Organic azides 3 were prepared from primary organic amines by following two different pathways [29].

The terminal alkyne 2 was coupled with series of azides 3 (a-o) in the presence of $CuSO_4.5H_2O$ and sodium ascorbate at RT using DMF as solvent to afford desired target molecules 4 in 78-90% yield.

The partial representation of 1H and 13C NMR spectral data of compound 4h are presented in Fig. 2. This compound possesses one –NH group that appeared at 3318 cm-1 in the corresponding IR spectra. The carbonyl group appeared at 1720 cm-1 in the IR spectra.

The appearance of a doublet at δ 5.31 in ^{1}HNMR spectrum Fig. 2 of 4h was due to the $–CH_2$ group attached to the triazole nitrogen and appearance of singlet at δ 7.49 in ^{1}HNMR spectrum Fig. 2 of 4h was due to triazole ring hydrogen. Appearance of δ 172.07 and δ 163.91 in ^{13}CNMR spectrum of 4h was due to carbonyl of COOH group and carbonyl of amide. Appearance of δ 57.77 was due to carbon attached to nitrogen of triazole ring. Fig. 2 represents chemical shift values of carbon and hydrogens.



3a R=2,4-di(Me)$C_6H_3NHCOCH_2$
3b R= 2,6-di(Me)$C_6H_3NHCOCH_2$
3c R= 2-Cl$C_6H_4NHCOCH_2$
3d R= 3-Cl$C_6H_4NHCOCH_2$
3e R= 4-Cl$C_6H_4NHCOCH_2$
3f R= 2-$NO_2C_6H_4NHCOCH_2$
3g R= 3-$NO_2C_6H_4NHCOCH_2$
3h R= 4-$NO_2C_6H_4NHCOCH_2$
3i R=$C_6H_5NHCOCH_2$
3j R=2-$CH_3C_6H_4NHCOCH_2$
3k R= 2-$NO_2C_6H_4$
3l R= 3-$NO_2C_6H_4$
3m R= 4MeO-2-$NO_2C_6H_3NHCOCH_2$
3n R= 4-$COOCH_3C_6H_4NHCOCH_2$
3o R= 4MeO-2-$NO_2C_6H_4$

4a R=2,4-di(Me)$C_6H_3NHCOCH_2$ (81%, 90mins)
4b R= 2,6-di(Me)$C_6H_3NHCOCH_2$ (78%, 80mins)
4c R= 2-Cl$C_6H_4NHCOCH_2$ (85%, 90mins)
4d R= 3-Cl$C_6H_4NHCOCH_2$ (89%, 90mins)
4e R= 4-Cl$C_6H_4NHCOCH_2$ (86%, 80mins)
4f R= 2-$NO_2C_6H_4NHCOCH_2$ (90%, 90mins)
4g R= 3-$NO_2C_6H_4NHCOCH_2$ (88%, 90mins)
4h R= 4-$NO_2C_6H_4NHCOCH_2$ (85%, 80mins)
4i R=$C_6H_5NHCOCH_2$ (79%, 100mins)
4j R=2-$CH_3C_6H_4NHCOCH_2$ (81%, 80mins)
4k R= 2-$NO_2C_6H_4$ (90%, 90mins)
4l R= 3-$NO_2C_6H_4$ (81%, 80mins)
4m R= 4MeO-2-$NO_2C_6H_3NHCOCH_2$ (86%, 100mins)
4n R= 4-$COOCH_3C_6H_4NHCOCH_2$ (85%, 90mins)
4o R= 4MeO-2-$NO_2C_6H_4$ (79%, 90mins)

Scheme 1. Synthesis novel compounds from terminal alkyne 2 and azide 3 based on CUAAC reaction

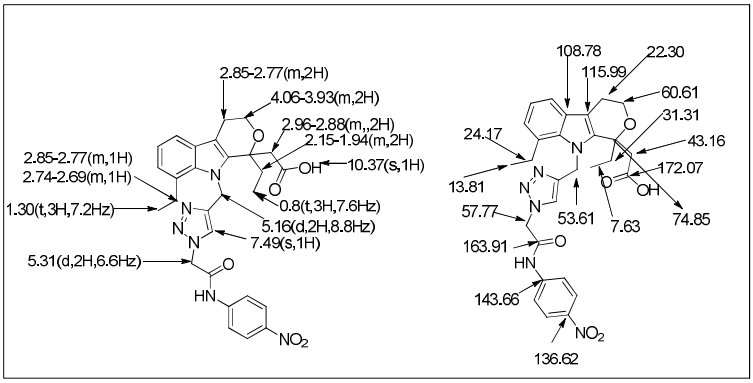


Fig. 2. Important ^{1}HNMR (LHS) and ^{13}CNMR (RHS) signals, observed in the NMR spectra of 4h (solvent used $CDCl_3$)

Table 1. Antibacterial activities of compound 4

Compound codes	Zone of inhibition[a] (mm) and MIC[b] (µg/mL) of selected compounds		
	S. aureus **(G+ve)**	***E. coli*** **(G-ve)**	***K. pneumoniae*** **(G-ve)**
4a	13 ± 0.31 (100)	13 ± 0.51 (>200)	13 ± 0.51 (150)
4b	13 ± 0.54 (150)	14 ± 0.18 (150)	12 ± 0.25 (>200)
4c	15 ± 0.65 (50)	14 ± 0.35 (100)	13 ± 0.16 (>200)
4d	15 ± 0.12 (50)	14 ± 0.63 (150)	15 ± 0.43 (50)
4e	16 ± 0.96 (25)	15 ± 0.87 (25)	16 ± 0.92 (25)
4f	12 ± 0.14 (>200)	12 ± 0.25 (>200)	14 ± 0.45 (50)
4g	13 ± 0.61 (150)	11 ± 0.10 (>200)	13 ± 0.24 (150)
4h	12 ± 0.19 (>200)	12 ± 0.16 (>200)	12 ± 0.19 (>200)
4i	13 ± 0.68 (150)	14 ± 0.49 (150)	14 ± 0.43 (100)
4j	12 ± 0.10 (>200)	14 ± 0.23 (150)	15 ± 0.75 (100)
4k	12 ± 0.27 (>200)	12 ± 0.10 (>200)	15 ± 0.55 (25)
4l	11 ± 0.19 (>200)	15 ± 0.84 (100)	13 ± 0.32 (150)
4m	12 ± 0.36 (>200)	13 ± 0.18 (150)	15 ± 0.45 (25)
4n	11 ± 0.11 (>200)	14 ± 0.32 (50)	14 ± 0.38 (100)
4o	13 ± 0.23 (150)	13 ± 0.15 (150)	15 ± 0.67 (25)
Amoxycillin	28 ± 0.35 (25)	29 ± 0.25 (25)	31 ± 0.23 (25)

[a]Zone of inhibition was calculated for stock solution at 0.4 mg/ 50 µl; [b]Minimal inhibitory concentration (MIC) values of the particular compounds are given in brackets;
S. aureus = Staphylococcus aureus; E. coli = Escherichia coli; K. pneumoniae = Klebsiella pneumonia; and Data are means (n = 3) ± Standard deviation of three replicates

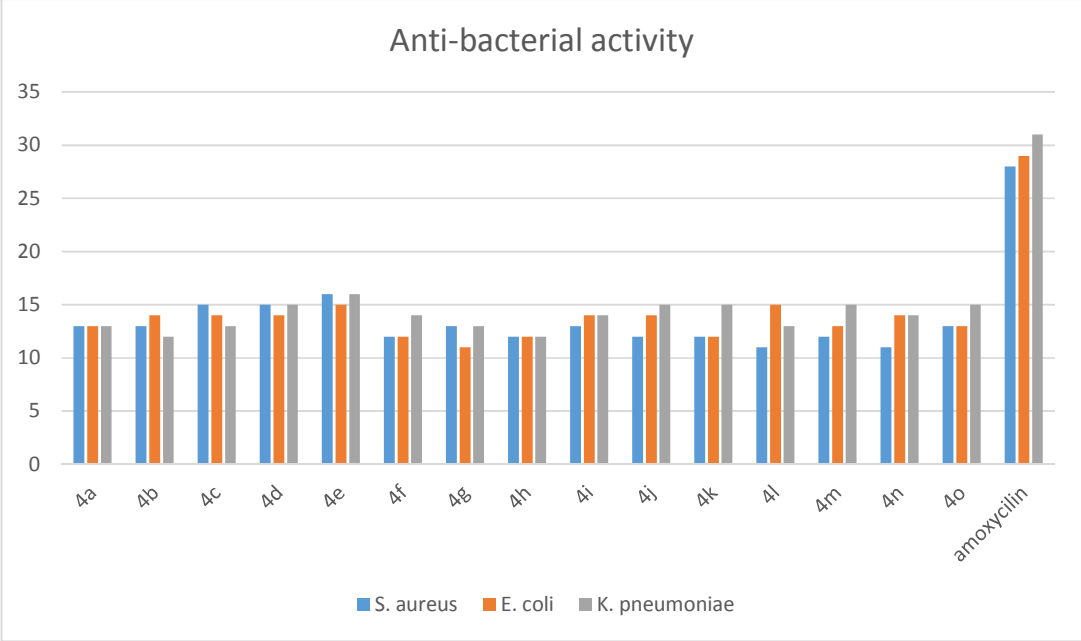


Fig. 3. Graphical representation of anti-bacterial activity of 4 against *S. aureus, E. coli and K. pneumoniae*

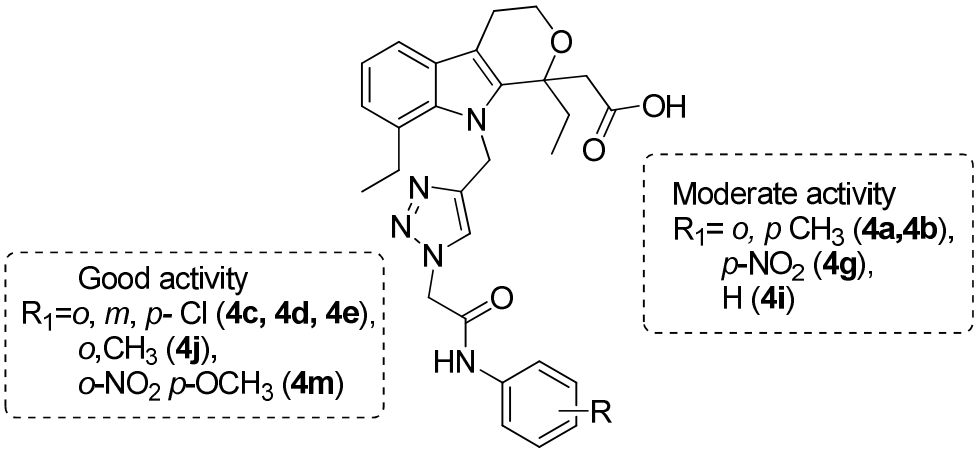


Fig. 4. SAR study of anti-bacterial activity of compound 4

3.2 Biology

3.2.1 Antibacterial activity

All the newly 15 synthesized compounds were screened for their antibacterial activities against one gram-positive (*S. aureus*) and two gram-negative (*E. coli, K. pneumoniae*) bacteria using an agar-well diffusion method and amoxicillin was used as a standard compound in this assay. The results i.e. the Zone of inhibition (mm) and MIC values (µg/mL) obtained are presented in Table 1. Graphical representation of antibacterial activities of the synthesized compounds 4 are shown in Fig. 3. While most of the compounds showed good to moderate activities against all strains in comparison with standard amoxycillin. Among them the compound 4e was identified as the most promising one because of its good activity against both Gram-positive and Gram-negative bacterial species with MIC of 25 µg / mL across all the strains (comparable to Amoxycillin). And also compounds 4c, 4d, 4j, 4k, 4m and 4o shows good activity against Gram-positive and Gram-negative bacteria. The compounds 4k, 4m and 4o displayed significant inhibitory potential with MIC 25 µg/mL against *K. pneumoniae* bacterial strain. The Structure Activity Relationship (SAR) summary of anti-bacterial activities of compound 4 is presented in Fig. 4 indicated that the activity varied with the change of position and nature of "R" group present on the aromatic ring connected to the triazole a -$NHCOCH_2$- linker. For example, good activity was because of the R group presented *ortho*-methyl, chloro, nitro and *para, meta*-nitro moiety whereas activity was moderate when R group was at *ortho*-position representing a methyl and *meta*-position representing nitro.

3.2.2 Anti-cancer activity

All the synthesized compounds possessing antibacterial activities were tested in *vitro* against human cancer cell line using colorimetric MTT assay and doxorubicin as standard drug. Compounds with promising (>50%) activities were taken for the determination of IC_{50} values, results of the active compounds are presented in Table 2. And graphical representation of anti-cancer activity of compound 4 shown in Fig. 5. 4b, 4d, 4h and 4o showed good activity against lung cancer cell line. 4j and 4l showed moderate activity. The SAR summary of anti-cancer activities of compound 4 against A549 cell line is presented in Fig. 6. For example, good activity was observed when the R group contains *meta*-chloro or *ortho*-nitro moiety whereas activity was reduced when R group was at *ortho*-position representing methyl group indicated that the activity varied with the change of position and nature of "R" group present on the aromatic ring connected to the triazole either via (i) a -NHCOCH2- linker or (ii) directly. Considering the former class good activity was observed in case of di-Me, *p*-NO_2, and *m*-Cl group whereas activity was moderate to low in case of o-Me. in case of later class good activity was noted for *o*-NO_2 *p*-OMe substituent whereas activity was moderate due to *m*-Cl group.

3.3 Molecular Docking Studies Analysis

The binding affinity of 4e molecule towards DNA-gyrase cleavage complex of *S. aureus* and topoisomerase Top. IV of *K.*

pneumonia organism's DNA binding site surrounded by the protein residues were analyzed using molecular docking studies. The binding affinity of the molecule showing strong interaction energies with the DNA active site and the results were shown in the Table 3.

The crystal structure of the DNA-gyrase contains an inhibitor Moxifloxacin intercalation in the E and F chains of DNA. The DNA binding protein with A and C chains are stabilizing the binding of the DNA with the bound Moxifloxacin. The molecule 4e docked in the intercalation location of DNA of the complex. The 4e molecule is stabilized by hydrogen bonds, hydrophobic and Pi-Pi interactions. The DG2010 residue of the F chain formed hydrogen bond with oxygen in the tricyclic ring of ligand. Other hydrogen bond formed between DG2009 residue of F chain and nitrogen on the triazole ring. The aromatic rings present in ligand is stabilized by strong Pi-Pi interactions with DC-2012 and DA-2013 residues from E chain and DG-2009, DG-2010 and DC-2011 residues from F-chain. Further this ligand also showed hydrophobic interactions with Gly-459, Asn-476 and DT8 residues in the active site.

Table 2. Cytotoxicity (IC_{50} values) of the compound 4 against A549 cell line

Compound	IC_{50}(µg/mL)
	A549
4b	42.91
4d	46.19
4h	51.36
4j	56.40
4l	60.76
4o	32.92
Doxorubicin	4.39

Table 3. Binding affinity of the compound 4e

PDB_ID	Binding affinity (in kcal/mol)
5CDQ	-7.7
5EIX	-7.9

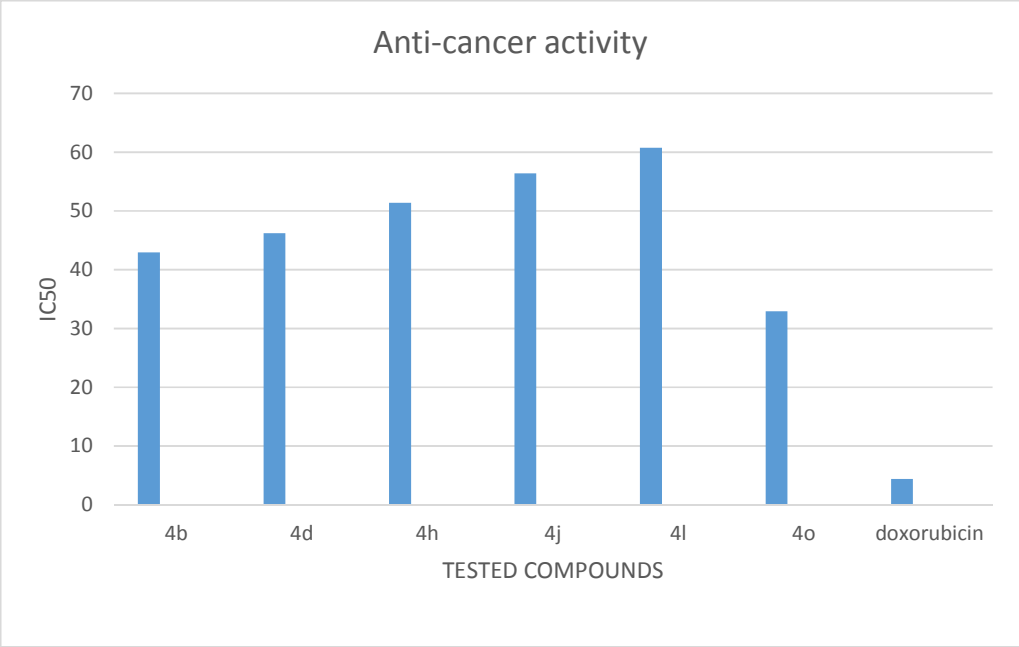


Fig. 5. Graphical representation of Anti-cancer activity of compound 4

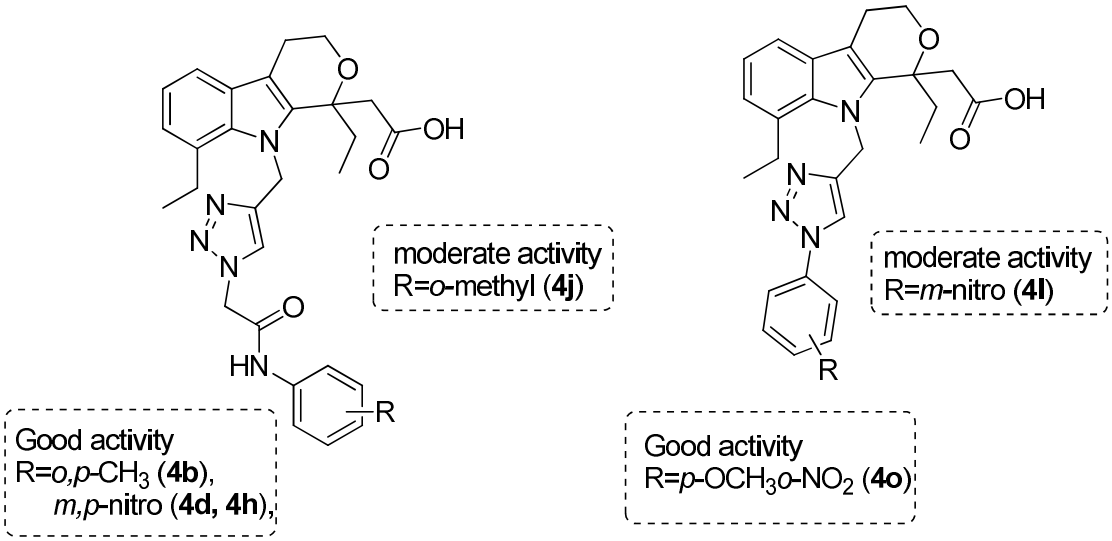


Fig 6. SAR study of anti-cancer activity of compound 4 against A549 Cell line

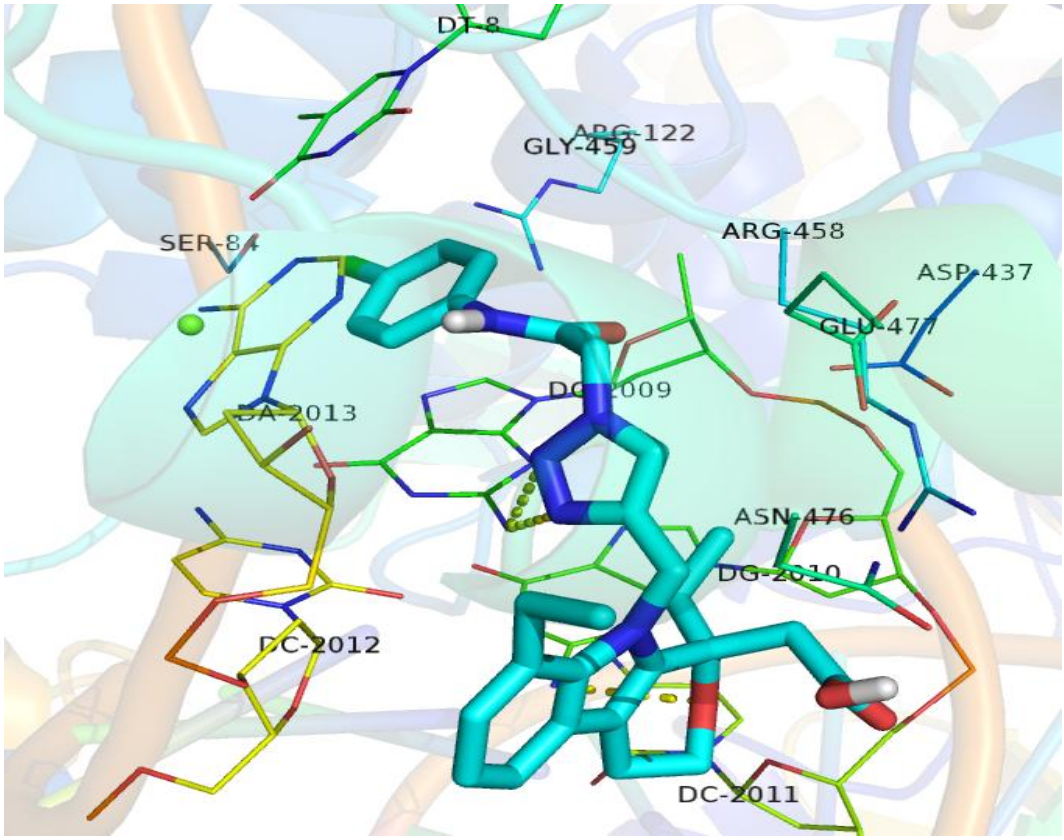


Fig. 7. Molecule 4e docked in the active site of the DNA-gyrase cleavage complex of S. aureus (PDB_ID: 5CDQ). Inhibitor molecule shown in aqua colour stick style and the DNA side chains and amino acid side chains are shown in line style

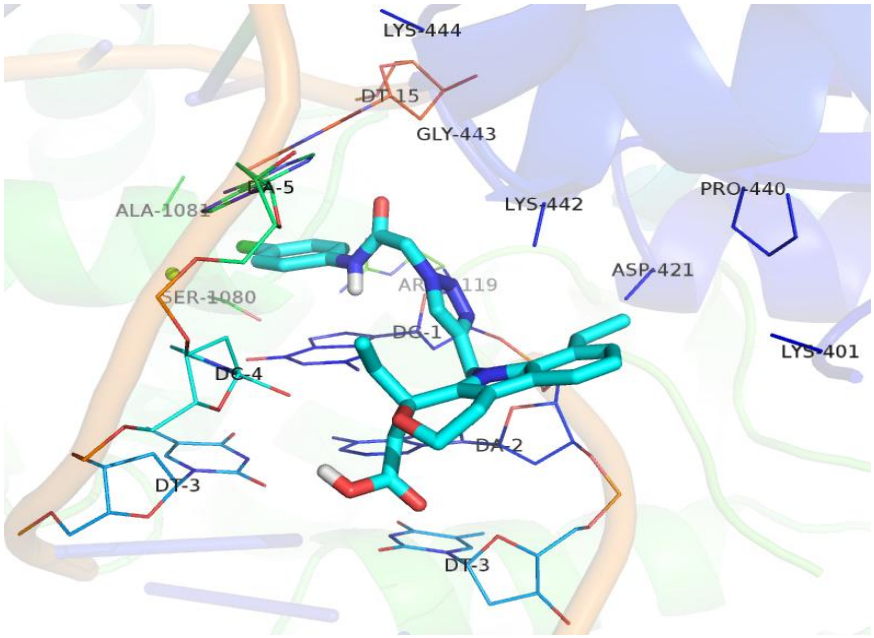


Fig. 8. Molecule 4e docked in the active site of the topoisomerase Top. IV of K. pneumonia (PDB_ID: 5EIX). Inhibitor molecule shown in aqua colour stick style and the DNA side chains and amino acid side chains are shown in line style

The crystal structure contains an inhibitor Levofloxacin intercalation with the E, F and I chains of DNA. The DNA binding proteins are A and G chains stabilizing the binding of the DNA with the bound Levofloxacin. The molecule 4e docked in the intercalation location of DNA of the topoisomerase, Top. IV complex. The molecule bound to the DNA stabilized by mainly hydrophobic and Pi-Pi interactions. Ligand showed Pi-Pi interactions between aromatic ring and both DG-1 residue from F chain and DT15 residue from H chain. Further, hydrophobic interactions formed with DA-2 and DT-3 residues from F chain and DA-5 residue from I chain in the active site. The interaction of DNA bases and the amino acid side-chains proteins are also shown in Fig. 8.

4. CONCLUSIONS

We have described design and synthesis of a small library of new compounds having NSAID etodolac and triazole moieties of potential biological significance. These molecules were conveniently prepared for the first time in the good yields. Some of the compounds showed moderate dual activity when tested against three bacterial strains and A549 lung cancer cell line. Docking study was also performed to know mechanism of enzyme inhibition of newly synthesized and target enzymes. These compounds find useful applications in synthesis of etodolac based library of compounds which can show promising biological activity.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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