Indian Journal of Chemical Technology
Vol. 28, September, 2021 pp. 612-617

Michael addition mediated domino cyclization of hydrazide embedded pyrazolyl derivatives: Biological and its molecular docking examinations

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Received 07 September 2020; accepted 01 September 2021

An easing and efficiently synthesized biologically and pharmacologically active quinolinyl- tethered pyrazoline molecules in the presence of chalcones, have been obtained from condensation of corresponding aldehydes and ketones, through concomitant utilization of aryl hydrazide as useful starting substrates underwent Michael-addition followed by domino cyclization. The final products have been obtained in excellent yields, and they have been well characterized using FTIR, ^{1}H, ^{13}C NMR and mass spectral analyses. Moreover, all the newly synthesized molecules have been examined for the antimicrobial and anticancer activities by MTT assay as well as molecular docking studies.

Keywords: Anticancer, Chalcone, Domino, Molecular docking, Pyrazoline, Quinoline, Michael-addition, Antimicrobial

Synthesis of heterocyclic compounds particularly the nitrogen containing motifs plays a significant role in the establishment of diverse biological active molecules[1]. Chalcone, acts as a simple chemical scavenger of numerous naturally occurring compounds which are broadly existing in vegetables, fruits, teas, and other plants. In addition, these quinoline- appended pyrazoline motifs have been utilized for the synthesis of wide variety of compounds with useful pharmacological activities *viz*., antiviral, anti-inflammatory, anti HIV, antimicrobial, anticancer, antitumor, anticonvulsant, antimalarial, anti-amoebic and antioxidant activities[2-6]. These molecules can be embedded into nanocarriers for targeted delivery and can be used as a nano drug delivery system[7,8]. Some of the representative compounds are used as fluorescent dyeing agent, electroluminescent materials, chemical switches and semiconductors[9]. Many of the synthetic strategies have been employed for an efficient construction of different quinoline tethered pyrazoline molecular architectures. Fascinatingly, the Michael-addition mediated domino cyclization strategy for the synthesis of our final (quinoline containing pyrazoline based) products is one of the facile and expedient routes. However, the domino synthesis is an imperative strategy in which a process of involving two or more bond forming reactions usually C-C bonds under the identical conditions without addition of reagents and catalysts for subsequent results is thus functionality formed in the previous process. Adopting this protocol in our present study, we have established the synthesis of quinoline containing pyrazoline scaffolds from chalcone through aldol condensation followed by Michael- addition mediated reactions.

Experimental Section

Materials

Chemicals were obtained for the construction of products from Sigma-Aldrich Chemicals (USA) and Merck (India).

Physical-chemical characteristics

The melting points were obtained using capillary method. The FT-IR spectral data was obtained in Perkin Elmer instrument at the wavelength range of 4000-400 cm^{-1}. The ^{1}H /^{13}C NMR spectral data evidenced at the range of 500 MHz, and 125 MHz correspondingly in deutriated chloroform at BRUKER AV III spectrometer in TMS (internal standard). The chromatographic (TLC) analysis was conducted on coated silica plates through spot identifications with an aid of UV lamp and iodine chamber.

Synthesis of (E)-3-(2-Chloro-8-methylquinolin-3-yl)-1-phenylprop-2-en-1-one compounds (3)

A stirred solution of substituted 4-methyl acetophenone (2) (0.01 mmol), with 8-methyl-2-

chloroquinoline-aldehyde (1) (0.01 mmol) in ethanol solvent (20 mL) was kept in a RB flask and then kept in an ice bath. After, NaOH solution of 10 mL (40%) was added in drops and constantly stirred for half an hour. The mixture was continued for 2–3 h at room temperature, further it was kept back in a refrigerator until it thickened. Then crude mass was poured in to cold water (40 mL). Furthermore, the resulting products were then filtered and washed thoroughly with cold water. Eventually, we had obtained the pure product of title compound **3** in 91% yield[10] (Scheme 1).

Synthesis of (5-(2-chloro-8-methylquinolin-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl) (pyridin-4-yl) methanone compounds (5a)

A stirred solution of quinoline comprising chalcones 3 (1 mmol) and pyridine-4-carboxylic acid hydrazide 4a (1 mmol) in ethanol (15 mL) solvent was kept in 100ml RB flask, further the catalytic amount of AcOH was added and allowed to reflux at 4 h. After completion of reaction through successive monitoring using TLC analysis, the crude reaction mass was poured into ice cold water. The result was pale yellowish solid which was filtered and washed with hot ethanol[11]. The isolated yield of the pure product 5a was 89% (Scheme 1).

(5-(2-Chloro-8-methylquinolin-3-yl)-3-p-tolyl-4, 5-dihydro-1H-pyrazol-1-yl)(3-methoxyphenyl) methanone (5b)

A stirred solution of quinoline comprising chalcones 3 (1 mmol) and 3-methoxybenzohydrazide 4b (1 mmol) in ethanol (10 mL) solvent kept in 100 mL RB flask, further the catalytic amount of AcOH was added and allowed to reflux at 4 h. After completion of reaction through successive monitoring using TLC analysis, the crude reaction mass was poured into ice cold water. The resulted pale yellowish solid thus obtained was filtered and washed with hot ethanol[11]. The isolated yield of the pure product 5b was 85% (Scheme 1).

(5-(2-Chloro-8-methylquinolin-3-yl)-3-p-tolyl-4,5-dihydro-1H-pyrazol-1-yl)(furan-2-yl)methanone (5c)

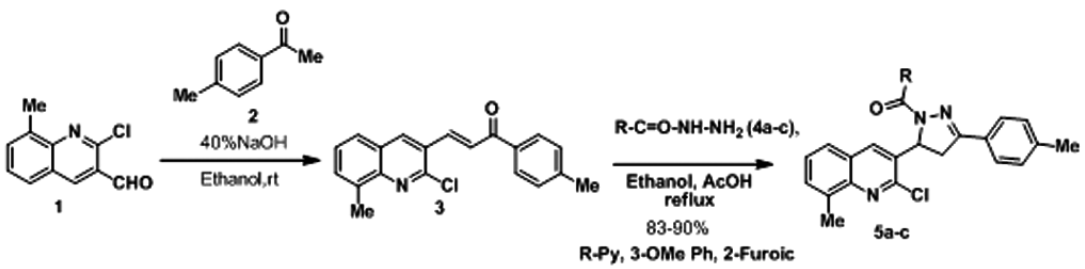
A stirred solution of quinoline comprising chalcones 3 (1 mmol) and furan-2-carbohydrazide 4c (1 mmol) in ethanol (20 mL) solvent were kept in 100 mL RB flask. Then catalytic amount of AcOH was added and allowed to reflux at 4 h. After completion of reaction, through successive monitoring using TLC analysis, the crude reaction mass was poured into ice cold water. The resulted pale yellowish solid thus obtained was filtered and washed with hot ethanol[9]. The isolated yield of the pure product 5c was 87% (Scheme 1).

(E)-3-(2-Chloro-8-methylquinolin-3-yl)-1-p-tolylprop-2-en-1-one (3)

FTIR (KBr, cm^{-1}): 3065 (Aromatic CH), 2855, 2923 (Aliphatic CH),1732 (C=O), 1602 (C=C); ^{1}H NMR (500MHz, $CDCl_3$): δ 8.44-7.32 (Aromatic-H, 10H), 2.76 (Quinoline methyl-H,3H), 2.45 (Aromatic methyl-H, 3H) ppm; ^{13}C NMR (125MHz, $CDCl_3$) δ 189.44, 149.37, 147.12, 144.15, 139.28, 136.77, 136.41, 135.09, 131.67, 129.48, 128.36, 128.86, 128.26, 127.74, 127.44, 127.06, 126.15, 125.94, 21.76, 17.78.

(5-(2-Chloro-8-methylquinolin-3-yl)-3-p-tolyl-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-4-yl)methanone (5a)

Nature of the compound : Brown colour solid, Yield : 89 %, M. P : 124-125 °C, R_f : 0.63 (H: EtOAc/1:1), FTIR (KBr, cm^{-1}) 3056, 3023 (Aromatic CH), 2973, 2924 (Aliphatic CH), 1646 (C=O), 1565 (C=C), 1338 (C=N); ^{1}H NMR (500MHz, $CDCl_3$): δ 8.57 (d, *J*.=.5Hz, 2H), 7.73 (d, *J*.=.10Hz, 2H), 7.54 (d, *J*.=.10Hz,2H), 7.31 (d, *J*.=.10Hz, 2H), 7.18-7.15 (m, 4H), 5.68 (dd, $J_{1,2}$= 10Hz, 15Hz, 1H), 3.73 (dd, $J_{1,2}$=10Hz, 15Hz, 1H), 3.14 (dd, $J_{1,2}$=15Hz, 20Hz, 1H), 2.36 (s, 6H) ppm; ^{13}C NMR (125MHz, $CDCl_3$): δ 164.31 (C=O), 154.87, 149.72, 141.58, 138.57, 137.76, 136.91,128.07, 127.15, 126.29, 123.64, 61.03 (Pyr-CH), 41.54 (Pyr-CH_2), 15.76 (Me) ppm; HRMS(ESI): Anal.Calcd. for ($C_{26}H_{21}ClN_4O$) (M+): 440.1404, found:440.0857



Scheme 1 — Construction of quinoline tethered pyrazoline frameworks

(5-(2-Chloro-8-methylquinolin-3-yl)-3-p-tolyl-4,5-dihydro-1H-pyrazol-1-yl)(3-methoxyphenyl)methanone (5b)

Nature of the compound : Brown Colour solid, Yield : 85 %, M. P : 102-103°C, R_f : 0.52 (H: EtOAc/1:1), FTIR (KBr, cm^{-1}) 3054, 3026 (Aromatic CH), 2927 (Aliphatic CH), 1646 (C=O), 1596 (C=C), 1339 (C=N); ^{1}H NMR (500MHz, $CDCl_3$): δ 7.72-7.70 (m, 2H), 7.65 (d, *J* = 10Hz, 1H), 7.55 (s, 1H), 7.50 (d, *J* = 10Hz, 1H), 7.41-7.29 (m, 4H), 7.20 (t, *J* = 10Hz, 1H), 7.07 (t, *J* = 10Hz, 2H), 6.03 (dd, $J_{1,2}$= 10Hz, 15Hz, 1H), 3.83 (s, 3H), 3.81 (dd, $J_{1,2}$= 5Hz, 15Hz, 1H), 3.38 (dd, $J_{1,2}$= 5Hz, 15Hz, 1H), 2.48 (s, 6H) ppm; ^{13}C NMR (125MHz, $CDCl_3$): δ 166.28 (C=O), 159.06, 154.69, 140.10, 137.49, 135.37, 131.25, 130.56, 128.82, 128.70, 127.56, 126.80, 122.68, 121.66, 120.59, 119.75, 117.58, 114.93, 109.34, 60.83 (Pyr-CH), 40.63 (Pyr-CH_2), 15.63 (Me) ppm; HRMS(ESI): Anal.Calcd. for ($C_{28}H_{24}ClN_3O_2$) (M+): 469.1557, found: 469.0482.

(5-(2-Chloro-8-methylquinolin-3-yl)-3-p-tolyl-4,5-dihydro-1H-pyrazol-1-yl)(furan-2-yl)methanone(5c)

Nature of the compound : Brown Colour solid, Yield : 87 %, M. P : 115-116°C, R_f : 0.58 (H: EtOAc/1:1), FTIR (KBr, cm^{-1}): 3047, 3028 (Aromatic CH), 2924 (Aliphatic CH), 1649 (C=O), 1596 (C=C), 1339 (C=N); ^{1}H NMR (500MHz, $CDCl_3$): δ 7.80-7.77 (m, 2H), 7.68 (dd, *J*= 5Hz, 1H), 7.58 (m, 1H), 7.46-7.43 (m, 3H), 7.29 (dd, *J*= 10Hz, 1H), 7.18-7.14 (m, 1H), 7.04-7.00 (m, 1H), 6.55-6.54(m, 1H), 5.96 (dd, $J_{1,2}$= 5Hz, 15Hz, 1H), 3.88 (dd, $J_{1,2}$= 5Hz, 15Hz, 1H), 3.38 (dd, $J_{1,2}$= 5Hz, 20Hz, 1H), 2.42 (s, 6H) ppm; ^{13}C NMR (125MHz, $CDCl_3$): δ 163.59 (C=O), 155.98, 155.40, 145.58, 139.86, 131.21, 130.73, 128.94, 126.85, 121.68, 120.53, 119.71, 119.23, 111.66, 109.41, 61.42 (Pyr-CH), 41.12 (Pyr-CH_2), 15.68 (Me) ppm; HRMS(ESI): Anal.Calcd. for ($C_{25}H_{20}ClN_3O_2$) (M+1): 429.1244, found: 430.1605.

Microbial activity

Antibiotic sensitivity was tested against the synthesized compounds in the selected Gram- positive and negative organisms. The pure pyrazoline products were synthesized chemically and its antibacterial activity was analyzed by disc diffusion method against selected microbes. The selected organisms were cultured in nutrient broth and the OD of the each organism was checked in their log phase (OD 0.8 to 0.9), 0.1 mL of each culture was spread on nutrient agar plate aseptically (Sterile swap technique). The synthesized 50µM concentration of the quinoline pyrazoline compounds was sterilized aseptically under UV illumination (5 to 10 min) to maintain the sterility of the compound. The aseptically prepared paper disc was impregnated in to the synthesized compounds for 5 to 10 min to attain the maximum absorption and each paper disc with compound was placed in different region in the selected bacterial lawn to assess the antibiotic activity. The prepared discs were aseptically placed on bacterial lawn and incubated at 27±2°C for 48 h for observation[12].

Anticancer activity

Production of HeLa cell suspension

A subculture of HeLa cell lines in Dulbecco's Modified Eagle's Medium (DMEM) was trypsinized separately, after discarding the culture medium. To the disaggregated cells in the flask 25 mL of DMEM with 10% FBS was added. The cells were suspended through the medium by gentle passage with the pipette and the cells homogenized.

Cells seeding

The homogenized cell suspension (1 mL) was added to each well of a 24 well culture plate along with different concentration of tested sample (0 to 200 µg/mL) and incubated at 37°C in a humidified CO_2 incubator with 5% CO_2. After 48 h incubation the cells were observed under an inverted tissue culture microscope and 80% confluence of cells cytotoxicity assay was subjected[13].

Cytotoxicity assay

The assay was carried out using (3- (4, 5-dimethyl thiazol-2-yl) -2, 5-diphenyltetrazoliumbromide (MTT), in which it is cleaved by mitochondrial succinate dehydrogenase and reductase of viable cells, yielded a measurable purple product formazan. This formazan production is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity. After 48 h incubation the wells were added with MTT and left for 3 h in room temperature. All wells were removed from the content using pipette and 100µl SDS in DMSO were added to dissolve the formazan crystals, the absorbance read in Lark LIPR-9608 micro plate reader at 540 nm[14].

Molecular docking

Theoretical view of the pyrazoline compounds was established by using molecular docking studies. The three dimensional (3D) structures of the COX-2 (PDB ID: 6COX) were obtained from protein data bank. Auto Dock 4.2 suited programs which utilizes the Lamarckian Genetic Algorithm (LGA) which was used for the study[15]. Before performing docking, hydrogen atoms, charges were added to this crystal structures as required

in the Lamarckian Genetic Algorithm. The chemical structures were not available in the pubchem database. So, we used Chemdraw to draw the compound structure and all these ligands were prepared for molecular docking studies using Auto Dock Tools (ADT). The grid size along the x-, y-, z- axes was set to 126 Å, 126 Å and 126 Å, the grid spacing was set as 0.475 Å, the grid centre was set as 29.117 Å, 29.128 Å, and 0.448 Å for COX-2. The auto docking parameters used were as follows: GA population size = 150; maximum number of energy evaluations = 250000; GA crossover mode = two points. The lowest binding energy conformer was searched out of 20 different conformers for each docking simulation, and the resultant one was used for further analysis. The docked conformations were viewed using PyMoL[16] (http://www.pymol.org) software package.

Results and Discussion

The 8-methyl quinolone derived chalcone compound **3** associated FTIR spectrum showed the characteristic stretching absorption band of C=C, C=O, aromatic CH, aliphatic CH and C-Cl functionality at 1600, 1660, 3043, 2985, and 830 cm^{-1} respectively. The ^{1}H NMR spectrum of 8-methyl quinolone chalcone compound **3** showed the aromatic methyl quinoline attached methyl proton at 2.45 ppm and 2.77 ppm as singlet. The olefinic two protons showed peaks at 8.21 ppm and 7.62 as doublet with the coupling constant of J = 15 Hz. The aromatic (ten) protons showed the corresponding peaks at 8.44-7.26 ppm as multiplet. Subsequently, the ^{13}C NMR spectrum of 8-methyl Quinolinechalcone compound **3** showed the carbonyl carbon peak at 189.48 ppm. Along with this the quinolone based methyl and aromatic methyl carbon peaks presented at 21.72 ppm and 17.73 ppm accordingly.

The 8-methyl quinoline-pyrazoline compound 5a of FTIR spectrum showed the aromatic and aliphatic stretching band at 3056, 3023, 2973 and 2924 cm^{-1}. The carbonyl stretching absorption at 1646 cm^{-1}, C=C 1590cm^{-1}, and the C=N 1338 cm^{-1} respectively. ^{1}H NMR spectrum of 8-methyl Quinoline Pyrazoline compound 5a showed aromatic methyl six protons at 2.36 ppm singlet. The five membered Pyrazoline methine and methylene protons is ABX type, so it showed in three doublet of doublet at 5.68, 3.73, and 3.14 ppm and the coupling constant J = 10 Hz, 15 Hz and 20 Hz respectively. ^{13}C NMR spectrum of 8-methyl Quinoline-pyrazoline compound 5a showed methine and methylene carbon at 41.54 ppm, 61.03 ppm, respectively. The aromatic methyl carbon peak showed at 15.76ppm. Mass spectrum of the compound 5a found 440.0857. The ^{1}H and ^{13}C NMR spectrum of compounds 5b and 5c gives supplementary. The mass values of compounds 5b and 5c were found as 469.0482 and 430.1605 respectively.

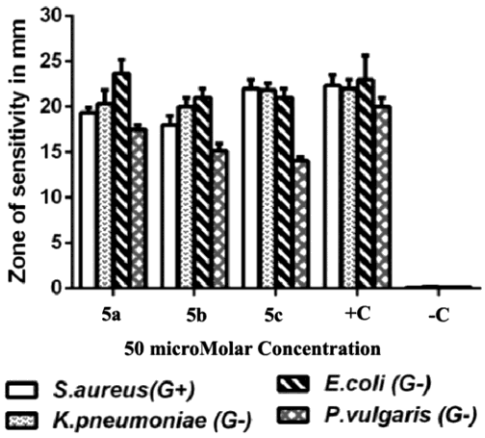


Fig. 1 — Antimicrobial activity profiles of synthesized quinoline-pyrazoline compounds on selected microorganism.

The synthesized compounds pyridine, 2-methoxy and 2-furoic substituted aresensitive to both G+ and G- organisms, those compounds were shown (Fig. 1) in various series of zone of inhibition (in mm) on disc diffusion method. Hence, we concluded that, these synthesized compounds can be used as effective as α-pinene and pyrrole[17-19] compounds towards antimicrobial activities for the prevention of bacterial diseases.

Anticancer activity

The anticancer and docking results were summarized in Table 1. It was observed that quinoline-pyrazoline derivative of compound 5a containing pyridine, which showed the most potent inhibitory activity in HeLa cell line. Among them, compound 5b and 5c displayed the good activity, because furan ring and methoxy group substitutes to phenyl ring presented in the 3rd position.

Molecular docking

Theoretical study of synthetic pyrazoline compounds 5a, 5b and 5c with bio macromolecules provides insight into the preferred binding location and also helps to validate experimental observations. In this work Auto Dock-based blind docking was performed to investigate the exact binding location for all synthetic compounds at the active site of COX-2. During the docking process, the lowest binding energy conformer was searched out of 20 different conformers for each docking simulation and the resultant one conformer was used for further analysis.

Molecular docking of quinoline-pyrazoline compounds with COX-2 (PDB: 6COX)

The docking simulation of synthesized compounds tightly bound with the active site of the COX-2. The docking summary of compounds with COX-2 is listed in Table 1. Upon the examination of docking features in which the compound **5a** had the lowest binding energy (-11.78 kcal/mol) and established the two hydrogen bonding between the 3rd nitrogen atom of pyridine ring with charged negative residue of ARG44 (bond distance 2.2 Å), the 6th nitrogen atom of quinoline ring had polar residue of ASN39 (bond distance 3.4 Å). Polar and charged negative ASN34, GLN42, CYS41,ASN43, GLN461, GLU46, ARG469, GLU465 were the binding site of COX-2 protein (PDB: 6COX) and LYS468. Furthermore the following residues were mainly involved in hydrophobic interactions (Fig. 2 and 3).

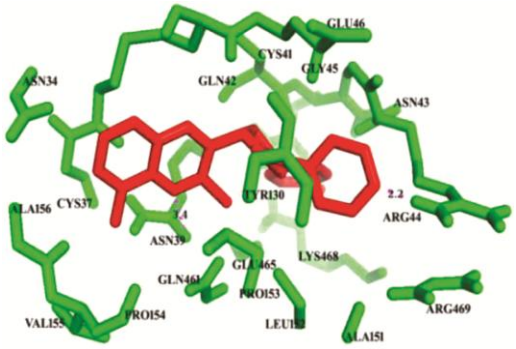


Fig. 2 — Binding mode of compound 5a.

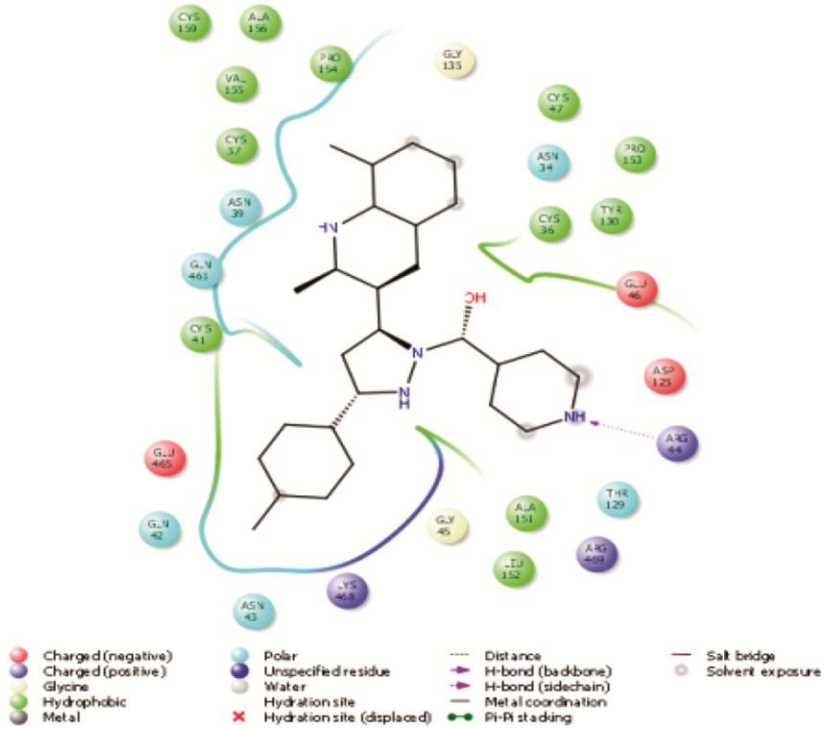


Fig. 3 — Binding interaction of the compound 5a in binding pocket of COX-2.

Table 1 — Anticancer and molecular docking data of the quinoline-pyrazoline compounds.

Comp	B. E[a] (ΔG) kcal/mol	No. of H bonds	Hydrogen bonds between the atoms of compounds and amino acid of 6COX		Distance (Å)	[b]HeLa ([c]IC_{50} µg/mL)
			Amino acid	Atom of compounds		
5a	-11.78	2	ARG 44(2HH1), ASN 39(OD1)	N6, N3	2.2, 3.4	24.62
5b	-11.23	2	GLY 135(O), CYS 47(HN)	N1, O1	2.5, 1.8	49.09
5c	-10.86	4	ASN 34 (1HD2), GLY 135(O), CYS 47(O, HN)	N2, O1, O7, O7	2.3, 2.8, 2.4, 2.6	36.50
CisPlatin						13.60

a = Binding energy, b = IC_{50} is the requisite concentration to inhibit 50 % growth of cell, c = HeLa cancer cell line (human cervical).

Conclusion

In the present study, the quinoline-pyrazoline compounds were synthesized along with *in vitro* and *in silico* studies for examination. The structures proposed for all the final compounds were confirmed by spectroscopic characterizations. Besides, these compounds were tested against microbial pathogens and found that they posses potential antimicrobial activity. 5a, 5b and 5c were evaluated for anticancer properties in HeLa cell line. The compound 5a, 5b and 5c have also exhibited significant anticancer activity. The molecular docking study of the compounds was carried out for the better understanding of the drug-receptor interaction. IC_{50} values of all the active compounds are comparable with standard drug Cis-Platin. All the compounds possessed with the required binding energy to dock itself with the binding pocket of COX-2 ranging from -11.78 to -10.86 kcal/mol. The anticancer activities of these compounds are fully supported by the *in silico* molecular docking study.

Acknowledgement

One of the authors, S. Muniraj is thankful for the financial support by the University Grant Commissions (UGC), Hyderabad-500001, under the project grants no.F MRP-5615/15(SERO/UGC), Link no: 5615, Comcode: TNMD013, January 2015. The authors are grateful to The Convener of ICACSEM-2020 for selecting our work for manuscript preparation and publication. The authors also thank Sophisticated Analytical Instrumentation Facility (SAIF), IIT Madras for analytical support.

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