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Short Communication

Antimicrobial Activity of some quinazoline heterocycles

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ABSTRACT

Derivatives of Quinazoline heterocycles possess a broad spectrum of pharmacological action. This class of compounds is known to be subjected to a large variety of structural modifications in order to synthesize derivatives with different biological properties. A series of new Quinazoline heterocycles are synthesized from chalcone**.** The characterization was done by IR, NMR and mass spectral data. The screening of antimicrobial activity of these synthesized compounds were done *in vitro* against some four Gram positive and four Gram negative strains of bacteria as well as four fungal strains in Dimethylformamide.

Keywords: Pyrazolo quinazolines, Antibacterial and antifungal activity, DMF.

INTRODUCTION

Quinazoline heterocycles are common in a variety of biologically important natural products and have caused universal concerns due to their widely and distinct biopharmaceutical activities [1]. These motifs have been reported to have several biological and pharmacological properties such as antitumor [2-3], antioxidant [4], antimicrobial [5], and anti-obesity [6]. A large number of quinazoline derivatives have been synthesized to large number of useful drugs and to design more useful medicines. These compounds exhibit good antimicrobial activity against both Gram negative and Gram positive microbes. Microbial studies were undertaken to test the inhibitory effect of the synthesized molecules. The present work includes synthesis, characterization, and antimicrobial studies of these compounds. Many researchers have worked on QSAR study of Pyrazolo quinazolines [7-9]. In the present work, some new Quinazoline heterocycles are synthesized from chalcones and their antimicrobial efficacy is worked out in dimethylformamide (DMF).

EXPERIMENTAL

Materials: Reagent grade chemicals were used without further purification. The purity of the synthesized compounds was checked by Thin Layer Chromatography.

Synthesis:

Synthesis of (different chalcones) Int.-I: Equimolar mixture of α-tetralone and different substituted benzaldehydes in ethanol were refluxed for 1.5 h in presence of catalytic amount of potassium hydroxide. The completion of reaction was confirmed by analytical thin layer chromatography (TLC) (Performed on aluminum coated plates Gel $60F_{254}$ (E. Merck)) using (7:3-Hexane: Ethyl acetate) as mobile phase. After completion of reaction, the reaction mass was cooled and the resulting solid was filtered, washed with water and dried under vacuum to give crude product. The obtained crude product was purified by adding suitable solvent (diethyl ether) to remove colored, non polar impurity by scratching/stirring. The product was then allowed to settle down and the above solution was decanted. The procedure was repeated 3-4 times to remove impurities (tituration). The purity of Int.-I was 0.995 in mole fraction as determined by gas chromatography.

Synthesis of ((5-amino-3-(methylthio)-1H-pyrazole-4-carbonitrile) Int.-II: A mixture of malanonitrile (0.01 mmol) and dry K_2CO_3 (0.012 mmol) were stirred in dry DMF at room temperature for 30 min. To this reaction mixture, 0.02 mole of carbon disulphide was added drop wise and the resulting solution was stirred for 2.5 hrs at room temperature. The solution was then

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cooled at 0 to 5^0C. To this cooled solution, 0.02 mole dimethyl sulphate was added and the solution was again stirred for 5-6 hrs at room temperature. The progress of the reaction was monitored by thin layer chromatography. After completion of the reaction, it was poured into crushed ice to give solid product. The resulting solid was filtered, washed with cold water and dried under vacuum to give crude product. Equimolar solution of this crude product and hydrazine hydrate in isopropyl alcohol (IPA) was refluxed for 30 min. The reaction mixture was then poured into crushed ice. The resulting solid was filtered, washed with water and dried under vacuum to give product. The obtained crude product was purified by tituration with hexane and was used in the next step without further purification.

Synthesis of pyrazolo quinazoline derivatives: An equimolar mixture of Int-I (chalcones) and Int-II (5-amino-3-(methylthio)-1H-pyrazole-4-carbonitrile) were refluxed in n-butanol for 4-5 hrs. The completion of reaction was confirmed by Thin Layer Chromatography using (6:4- Hexane: Ethyl acetate) as a mobile phase. The reaction mixture was then allowed to cool and the resulting solid was filtered, washed with diethyl ether to remove impurities. The procedure was repeated 3-4 times to free the product from impurities. All the reaction schemes are given below.

The characterization of all these compounds was done by IR, NMR and mass spectral data. The IR spectra were recorded on Shimadzu FT-IR-8400 instrument using KBr pellet method. The Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique. ^{1}H NMR and was determined in DMF solution on a Bruker Ac 400 MHz spectrometer. The physical constants of all the 10 synthesized compounds (KC-1 to KC-10) are given in Table 1. Spectral data of all the synthesized compounds are given below.

Antimicrobial activity:

Microorganisms tested: The studied microorganisms were obtained from National Chemical Laboratory (NCL), Pune, India. The microorganisms were maintained at 4°C. The Gram positive bacteria studied were *Staphylococcus aureus* ATCC29737 (*SA*), *Corynebacterium rubrum* ATCC14898 (*CR*), *Listeria monocytogens* ATCC19112 (*LM*), *Bacillus cereus* ATCC11778 *(BC)*; Gram negative bacteria were *Pseudomonas aeruginosa* ATCC27853 (*PA*), *Escherichia coli* NCIM2931 (*EC*), *Klebsiella pneumoniae* NCIM2719 (*KP*), *Salmonella typhimurium* ATCC23564 (*ST*) and Fungi were *Candida albicans* ATCC2091 (*CA*), *Cryptococcus neoformans* NCIM3542 (*CN*), *Candida glabrata* NCIM3448 (*CG*), *Candida epicola* NCIM3367 (*CE*). The organisms were maintained on nutrient agar and MGYP medium (Hi Media, India) for bacteria and fungi respectively, at 4°C and sub-cultured before use. The microorganisms studied are clinically important ones causing several infections and food spoilage.

Agar well diffusion method: *In vitro* antimicrobial activity of the different Pyrazolo quinazolines was studied against pathogenic microbial strains by the agar well diffusion method [10]. Mueller Hinton No. 2 / Sabouraud dextrose agar (Hi-media) was used for the antibacterial and antifungal susceptibility test respectively. The different Pyrazolo quinazolines were dissolved in 100% DMF to give a concentration of 20 mg ml^{-1}. The Mueller Hinton agar / Sabouraud dextrose agar was melted and cooled to 48-50°C and a standardized inoculum (1.5×10^8 CFU/ ml, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile Petri dishes; wells (8.5 mm) were prepared in the seeded agar plates. The test compound (100 µl) was introduced into the well. The plates were incubated overnight at 37°C and 28°C for 24 h and 48 h respectively, for bacteria and fungi. DMF was used as negative control. The microbial growth was determined by measuring the diameter of the zone of inhibition and the mean values are presented with ± SEM.

RESULTS AND DISCUSSION

The IR, NMR and mass spectra of synthesized compounds (KC 1-10) are as follows:

Spectral Data:

KC-1:

7-(4-chlorophenyl)-10-(methylthio)-5,6,7,9-tetra hydrobenzo[h]pyrazolo[5,1-b]quinazoline-11-carbonitrile

IR(cm^{-1}, KBr): 3475.85 (-NH (sec.) str.), 3049.56 (Ar-H str.), 2924.18(-CH2 sym. str.), 2227.86 (-CN str.), 1664.62(C=C str. α,β unsaturated 6-member ring), 1604.83(-NH bending vib. Secondary amine), 1381.08 (-CH bending.), 1315.50(C-N (sec) bending.), 1242-1010(C-H in plane bending, phenyl ring), 767.69 (C-H str. 5-adjecent c atoms), 767.69(C-Cl str.),

^{1}H NMR (DMSO-d_6) δ(ppm): 2.400 (3H, singlet, -CH3), 1.785-2.750 (4H, multiplet, C-H), 6.068 (1H, singlet, C-H), 7.216-7.704 (8H, multiplet C-H), 10.139 (1H, singlet, -NH).

Mass: (m/z) =404.09

KC-2:

7-(4-methoxyphenyl)-10-(methylthio)-5,6,7,9-tetrahydrobenzo[*h*]pyrazolo[5,1-*b*]quinazoline-11-carbonitrile

IR(cm-1, KBr): 3077.11 (Ar-H asym. str.), 1658.38 (C=N str), 2945.40-2843.17 (CH2 str. Of cyclohexanone ring), 1597.11(C=C str.), 1090.95 (C-O-C sym. str. 954.80 (ring str. in cyclohexanone), 1210.45 (C-O-C asym. Str.) 2814.11(C-H str. Alkane)

^{1}H NMR (DMSO-d_6) δ(ppm):

2.396 (3H, singlet,-CH3), 3.696 (3H, singlet-OCH3), 1.798-2.757(4H, multiplet, C-H), 5.937 (1H, singlet, C-H), 6.896-7.691 (8H, multiplet C-H), 10.009 (1H, singlet, N-H).

Mass: (m/z) =400.50

KC-3:

7-(4-fluorophenyl)-10-(methylthio)-5,6,7,9-tetrahydrobenzo[h]pyrazolo[5,1-b]quinazoline-11-carbonitrile

IR(cm^{-1}, KBr): 3479.70 (-NH (sec.) str.), 3037.99 (Ar-H str.), 2918.40(-CH2 sym. str.), 2227.86 (-CN str.), 1666.55 (C=C str. α,β unsaturated 6-member ring), 1599.04 (-NH bending vib. Secondary amine), 1381.08 (-CH bending.), 1319.08 (C-N (sec) bending.), 1242-1010(C-H in plane bending, phenyl ring), 1093.67 (C-F str.), 725.26 (C-H str. 5-adjecent c atoms),

^{1}H NMR (DMSO-d_6) δ(ppm): 2.419 (3H, singlet, -CH3), 1.791-2.767 (4H, multiplet, C-H), 5.987 (1H, singlet, C-H), 7.148-7.684 (8H, multiplet C-H), 10.251 (1H, singlet, -NH).

Mass: (m/z) =388.46

KC-4:

7-(4-bromophenyl)-10-(methylthio)-5,6,7,9-tetrahydrobenzo[h]pyrazolo[5,1-b]quinazoline-11-carbonitrile

IR(cm^{-1}, KBr): 3257.88 (-NH (sec.) str.), 3047.63 (Ar-H str.), 2929.97 (-CH2 sym. str.), 2227.86 (-CN str.), 1653.05 (C=C str. α,β unsaturated 6-member ring), 1604.83 (-NH bending vib. Secondary amine), 1383.01 (-CH bending.), 1315.50 (C-N (sec) bending.), 1242-1010(C-H in plane bending, phenyl ring), 723.33 (C-H str. 5-adjecent c atoms), 582.52 (C-Br str.),

^{1}H NMR (DMSO-d_6) δ(ppm): 2.422 (3H, singlet, -CH3), 1.799-2.787 (4H, multiplet, C-H), 5.993 (1H, singlet, C-H), 7.210-7.815 (8H, multiplet C-H), 10.247 (1H, singlet, -NH).

Mass: (m/z) =449.37

KC-5:

7-(3,4-dimethoxyphenyl)-10-(methylthio)-5,6,7,9-tetrahydrobenzo[h]pyrazolo[5,1-b]quinazoline-11-carbonitrile

IR(cm^{-1}, KBr): 3236.66 (-NH (sec.) str.), 3007.12 (Ar-H str.), 2929.97 (-CH2 sym. str.), 2224.40 (-CN str.), 1666.55(C=C str. α,β unsaturated 6-member ring), 1604.83(-NH bending vib. Secondary amine), 1383.09 (-CH bending.), 1334.78 (C-N (sec) bending.), 1242-1010(C-H in plane bending, phenyl ring), 702.11 (C-H str. 5-adjecent c atoms), 731.05(C-H in plane bending),

^{1}H NMR (DMSO-d_6) δ(ppm): 2.451 (3H, singlet,-CH3), 3.708 (3H, singlet-OCH3), 4.023 (3H, singlet –OCH3), 1.798-2.757(4H, multiplet, C-H), 5.981 (1H, singlet, C-H), 7.002-7.758 (8H, multiplet C-H), 10.087 (1H, singlet, -NH).

Mass: (m/z) =430.52

KC-6:

7-(4-cyanophenyl)-10-(methylthio)-5,6,7,9-tetrahydrobenzo[h]pyrazolo[5,1-b]quinazoline-11-carbonitrile

IR(cm^{-1}, KBr): 3310.18 (-NH (sec.) str.), 2990.17 (Ar-H str.), 2896.57 (-CH2 sym. str.), 2227.12 (-CN str.), 1666.55(C=C str. α,β unsaturated 6-member ring), 1597.64(-NH bending vib. Secondary amine), 1381.13 (-CH bending.), 1289.74 (C-N (sec) bending.), 1242-1010(C-H in plane bending, phenyl ring), 712.87 (C-H str. 5-adjecent c atoms), 729.67(C-H in plane bending),

^{1}H NMR (DMSO-d_6) δ(ppm): 2.434 (3H, singlet,-CH3), 1.705-2.724(4H, multiplet, C-H), 5.598 (1H, singlet, C-H), 7.220-7.945 (8H, multiplet C-H), 10.136(1H, singlet, -NH).

Mass: (m/z) =395.48

KC-7:

7-(3-chlorophenyl)-10-(methylthio)-5,6,7,9-tetrahydrobenzo[h]pyrazolo[5,1-b]quinazoline-11-carbonitrile

IR(cm^{-1}, KBr): 3387.87 (-NH (sec.) str.), 2967.46 (Ar-H str.), 2894.57 (-CH2 sym. str.), 2224.40 (-CN str.), 1667.99(C=C str. α,β unsaturated 6-member ring), 1590.12(-NH bending vib. Secondary amine), 1357.23 (-CH bending.), 1309.47 (C-N (sec) bending.), 1242-1010(C-H in plane bending, phenyl ring), 699.24 (C-H str. 5-adjecent c atoms), 724.51(C-H in plane bending), 767.69(C-Cl str.),

^{1}H NMR (DMSO-d_6) δ(ppm): 2.400 (3H, singlet,-CH3), 1.785-2.750 (4H, multiplet, C-H), 6.068 (1H, singlet, C-H), 7.216-7.704 (8H, multiplet C-H), 10.139 (1H, singlet, -NH).

Mass: (m/z) =404.92

KC-8:

7-(3-methoxyphenyl)-10-(methylthio)-5,6,7,9-tetrahydrobenzo[h]pyrazolo[5,1-b]quinazoline-11-carbonitrile

IR(cm^{-1}, KBr): 3337.71 (-NH (sec.) str.), 3015.87 (Ar-H str.), 2897.12 (-CH2 sym. str.), 2227.97 (-CN str.), 1666.55(C=C str. α,β unsaturated 6-member ring), 1615.92(-NH bending vib. Secondary amine), 1397.54 (-CH bending.), 1337.56 (C-N (sec) bending.), 1242-1010(C-H in plane bending, phenyl ring), 709.57 (C-H str. 5-adjecent c atoms), 724.69(C-H in plane bending),

^{1}H NMR (DMSO-d_6) δ(ppm): 2.447 (3H, singlet,-CH3), 3.699 (3H, singlet-OCH3), 1.771-2.810(4H, multiplet, C-H), 6.017 (1H, singlet, C-H), 7.087-7.826 (8H, multiplet C-H), 10.110 (1H, singlet, -NH).
Mass: (m/z) =400.14

KC-9:
7-(3-bromophenyl)-10-(methylthio)-5,6,7,9-tetrahydrobenzo[h]pyrazolo[5,1-b]quinazoline-11-carbonitrile
IR(cm^{-1}, KBr): 3337.69 (-NH (sec.) str.), 2967.43 (Ar-H str.), 2937.65 (-CH2 sym. str.), 2229.70 (-CN str.), 1666.55(C=C str. α,β unsaturated 6-member ring), 1591.25(-NH bending vib. Secondary amine), 1403.17 (-CH bending.), 1312.36 (C-N (sec) bending.), 1242-1010(C-H in plane bending, phenyl ring), 699.14 (C-H str. 5-adjecent c atoms), 737.45(C-H in plane bending),
^{1}H NMR (DMSO-d_6) δ(ppm): 2.402 (3H, singlet,-CH3), 1.695-2.699(4H, multiplet, C-H), 5.947 (1H, singlet, C-H), 6.986-7.724 (8H, multiplet C-H), 10.116 (1H, singlet, -NH).
Mass: (m/z) =449.37

KC-10:
10-(methylthio)-7-(p-tolyl)-5,6,7,9 tetrahydrobenzo[h]pyrazolo[5,1-b]quinazoline-11-carbonitrile
IR(cm^{-1}, KBr): 3314.25 (-NH (sec.) str.), 2987.34 (Ar-H str.), 2945.67 (-CH2 sym. str.), 227.64 (-CN str.), 1666.55(C=C str. α,β unsaturated 6-member ring), 1593.14(-NH bending vib. Secondary amine), 1374.28 (-CH bending.), 1340.05 (C-N (sec) bending.), 1242-1010(C-H in plane bending, phenyl ring), 698.47 (C-H str. 5-adjecent c atoms), 742.36(C-H in plane bending),
^{1}H NMR (DMSO-d_6) δ(ppm): 2.432 (3H, singlet,-CH3), 3.825(3H, singlet-CH3), 1.802-2.799(4H, multiplet, C-H), 6.024 (1H, singlet, C-H), 7.108-7.809 (8H, multiplet C-H), 10.074 (1H, singlet, -NH).
Mass: (m/z) =384.50

^{1}H-NMR spectra:
The ^{1}H NMR spectra of compound KC-2 is shown in Figure 1. Residual peak of DMSO is shown as a singlet at 3.327δppm. The peak of S-CH_3 is shown around 2.396 δppm. Protons of cyclo hexane ring are shown in aliphatic region at the range 1.798-2.757 δppm. Protons of –O-CH_3 are near about 3.696 δppm as singlet. Chiral proton is clearly shown at 5.937 δppm which is proof of ring cyclization. Aromatic protons of two phenyl rings are shown in the aromatic range between 6.896-7.691δppm with their appropriate multiplicity. Double doublet is clearly shown at the range of 7.179-7.210 which is confirmation of attachment of –Cl group at p-position. One proton is at 10.009 δppm as a singlet gives confirmation of pyrazole ring. Due to attachment of one proton with nitrogen, this is shown at highly deshielded region.
The antibacterial activity of Pyrazolo quinazolines derivatives showed a differential activity against the bacterial strains investigated (Fig: 2). In DMF, KC-7 showed more inhibition than other bases (Fig. 2a). Against *C. rubrum*, only KC-3, KC-4, KC-7 and KC-8 showed activity whereas against *L. monocytogens*, KC-4, KC-8, KC-9 and KC-10 showed activity. *B. cereus* was the most susceptible bacteria, getting inhibited by 8 out of 10 tested compounds.

Antimicrobial Activity: The antibacterial activity against Gram negative bacteria was meager (Fig. 2b). None of the tested 10 compounds could kill *E. coli* and *P. aeruginosa* and thus they were the resistant bacteria. *S. typhimurium* was the most susceptible bacteria, getting inhibited by 7 out of 10 tested compounds. *K. pneumoniae* got inhibited by KC-3, KC-4 and KC-10. KC-3 inhibited both *S. typhimurium* and *K. pneumoniae* with zone of inhibition nearly 11mm. Over all, it can be concluded that the synthesized compounds showed more antibacterial activity towards Gram positive bacteria. Similar results were reported by Bhalu et al. (2014) [11] for dihydropyrano[c]chromenes derivatives. KC-4 showed best activity. This may be because of the side chain 4-Br. None of the compounds showed antifungal activity. In a study by Moteriya et al. [12], similar results were seen. It is reported that antimicrobial activity is dependent on the solvent used, molecular structure and the bacterial strain used [13] and hence antimicrobial activity should also be checked with other solvents. Therefore, it can be concluded that these synthesized compounds can be explored for the treatment of infectious diseases caused by Gram positive bacteria.

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Reaction scheme:

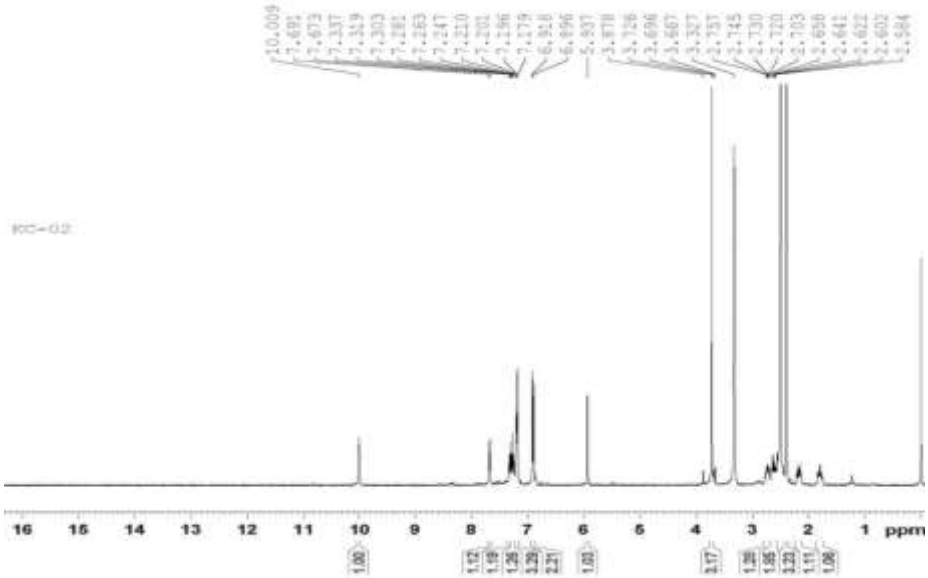

Fig: 1 NMR spectra of compound KC 2

Table 1: Physical constants of synthesized compounds

Compound. Code	Substitution R	M.F.	M.W.	Yield (%)
KC-1	-4-Cl	$C_{22}H_{17}ClN_4S$	404.09	78
KC-2	-4-OCH_3	$C_{23}H_{20}N_4OS$	400.14	79
KC-3	-4-F	$C_{22}H_{17}FN_4S$	388.46	78
KC-4	-4-Br	$C_{22}H_{17}BrN_4S$	449.37	76
KC-5	-3,4-diOCH_3	$C_{24}H_{22}N4O_2S$	430.52	71
KC-6	-4-CN	$C_{23}H_{17}N_5S$	395.48	60
KC-7	-3-Cl	$C_{22}H_{17}ClN_4S$	404.92	77
KC-8	-3-OCH_3	$C_{23}H_{20}N_4OS$	400.14	76
KC-9	-3-Br	$C_{22}H_{17}BrN_4S$	449.37	71
KC-10	-4-CH_3	$C_{23}H_{20}N_4S$	384.50	64

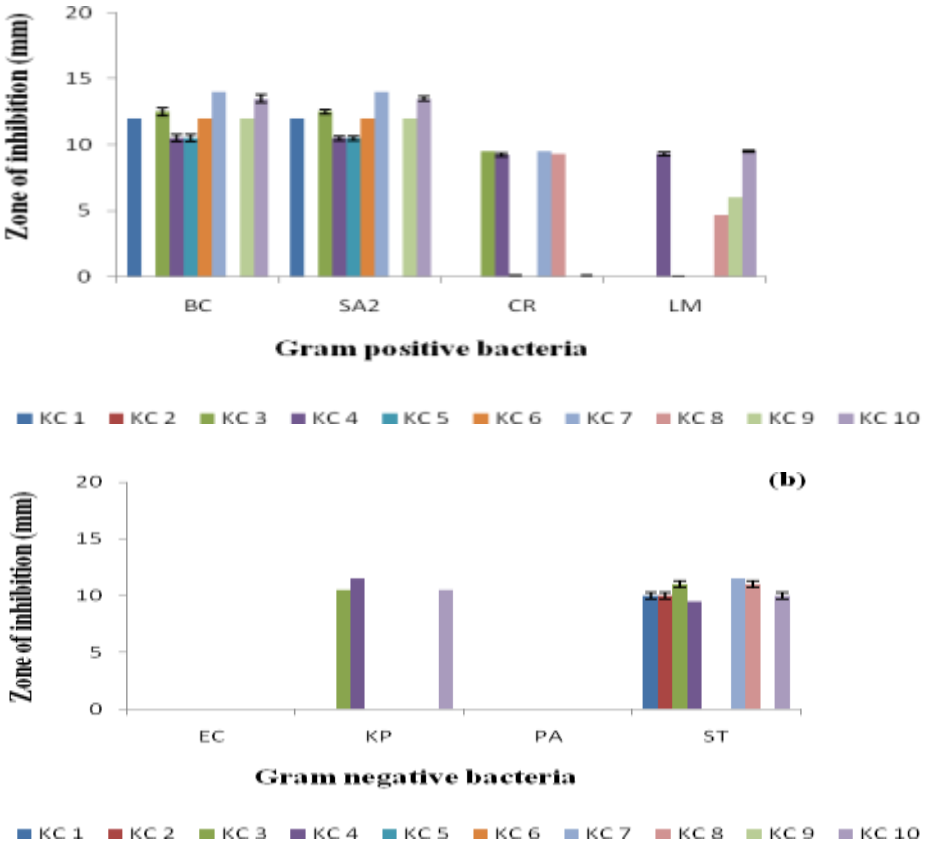

Fig 2: Antibacterial activity of compounds dissolved in DMF against Gram positive (a) and Gram negative bacteria (b)

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