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Synthesis, characterization and antimicrobial activity of pyrazolo chalcone compounds

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ABSTRACT

Some new pyrazolo chalcone compounds were synthesized and their structure characterization was done by spectroscopic techniques such as FT-IR, ^{1}H NMR and mass. Screenings of all these synthesized compounds were done *in vitro* against some bacterial and fungal strains in dimethyl sulphoxide and *N*,*N*-dimethyl formamide using agar well diffusion method. It is observed that *N*,*N*-dimethyl formamide is good solvent for these compounds in selected strains.

***Keywords*:** Pyrazolo chalcone compounds, Dimethyl sulphoxide, *N*,*N*-dimethyl formamide, Gram positive bacteria, Gram negative bacteria, Fungai

1. INTRODUCTION

The discovery of antimicrobials provides a noble way for better health for millions of people around the word. The development of resistance to current antimicrobial therapy continues to stimulate the search for more effective agents, the increasing the clinical importance of drug resistant and bacterial pathogens has lent additional urgency to microbiological research and development of novel biologically active compounds. Therefore, the synthesis of new lead structures and chemical entities for the development of antimicrobial agents is an important task in medical field. The biologically active compounds play important role in food industry, agriculture, biopharmaceutical and healthcare [1-5].

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Further, biological activity of synthesized compounds is very much useful for identification, synthesis and development of new biological ingredient which can be used as medicine. Biological activity describes intrinsic properties of compounds which depend on its structure.

Chalcone compounds occupy a central position among those molecules that makes life possible. Chalcones, with donor π-acceptor moieties separated by a keto vinyl group are known well effective chemical materials. These compounds are extensively used in pharmacology, biology, electronics, material sciences [6-8] etc. It is also used for the preparation of photorefractive polymers, nonlinear materials, chromophore sensors, fluorescent probes for determination of metal ions, sensing of DNA and in the study of photo-alignment layer of liquid crystal displays [9, 10]. These compounds are also known for their diverse nature of activity such as anti-cancer [11-13], anti-inflammatory [14, 15], anti-ulcer [16, 17], anti-microbial [18-23], anti-HIV [24, 25], anti-tubercular [26-28], anti-cancer [29-31], anti- anti-platelets [32-34], anti-viral effect [35-37], etc.

2. EXPERIMENTAL SECTION

A methanolic solution of different 1-(1H-benzo[d]imidazole-2-yl] ethan-1-one (0.01 mole), pyrazolo aldehyde (0.008-0.007 mole) and potassium hydroxide (0.04 mole) was stirred at room temperature for 1 hr. The completion of reaction was confirmed by analytical thin layer chromatography (TLC) (Performed on aluminium coated plates Gel $60F_{254}$ (E. Merck)) using (0.6:0.4-Hexane:Ethyl acetate) as mobile phase. After completion of reaction, the reaction mass was poured in to crushed ice and the resultant solid was filtered, washed with methanol to remove unreacted reagents and dried under vacuum to give crude product.

The reaction scheme for the synthesis of compounds is given in Figure 1.

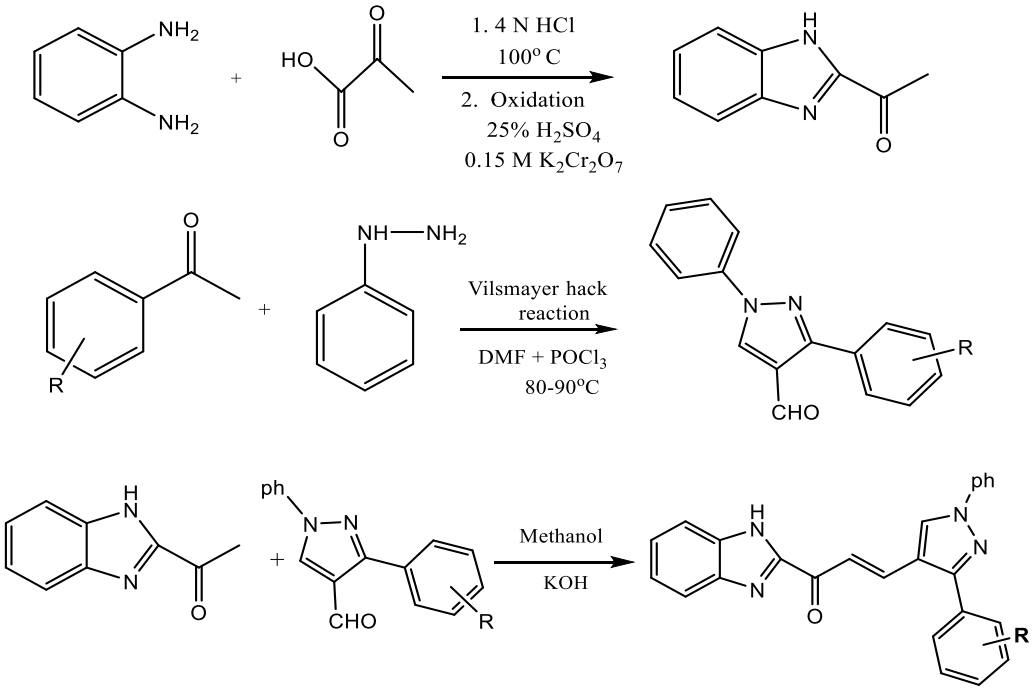


Figure 1. Reaction scheme for the formation of pyrazolo chalcone compounds.

2. 1. Structure confirmation

The structures of synthesized crystallized compounds were confirmed by FTIR, ^{1}HNMR and mass spectral data. IR spectra were recorded on Shimandzu FT-IR-8400 instrument.^{1}H NMR spectra were taken on a Bruker ADVANCE II 400 using DMSO-d_6 and mass spectra were determined using direct inlet probe on a GCMS-QP-2010 mass spectrometer. (Figure 2 to 4) shows IR, ^{1}H NMR and mass spectra of AH-1.

Overall, eight compounds are synthesized and the IUPAC names of these compounds are:

AH-1: (E)-1-(1H-benzo[d]imidazole-2-yl)-3-(3-methxyphenyl-1H-pyrazol-4-yl)prop-2-en1one

AH-2: (E)-1-(1H-benzo[d]imidazole-2-yl)-3-(1, 3-diphenyl -1H-pyrazol-4-yl) prop-2-en-1one

AH-3: (E)-1-(1H-benzo[d]imidazole-2-yl)-3-(3-(4-chlorophenyl)-phenyl-1H-pyrazol-4-yl) prop-2-en-1one

AH-4: (E)-1-(1H-benzo[d]imidazole-2-yl)-3-(3-(3,4-dimethoxyphenyl)-1-phenyl-1H-pyrazol-4-yl) prop-2-en-1one

AH-5: (E)-1-(1H-benzo[d]imidazole-2-yl)-3-(3-(4-fluorophenyl-1H-pyrazol-4-yl)pop-2-en-1one

AH-6: (E)-1-(1H-benzo[d]imidazole-2-yl)-3-(3-(2-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl) prop-2-en-1one

AH-7: (E)-1-(1H-benzo[d]imidazole-2-yl)-3-(3-(4-methxyphenyl)-1-phenyl-1H-pyrazol-4-yl) prop-2-en-1one

AH-8: (E)-1-(1H-benzo[d]imidazole-2-yl)-3-(3-(3-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl) prop-2- en-1one

AH-9: (E)-1-(-1H-benzo[d]imidazol-2-yl)-3-(-3-(3-bromophenyl)-1-phenyl-1H-pyrazol-4-yl) prop-2-en-1-one

AH-10: **(**E)-1-(1H-benzo[d]imidazole-2-yl)-3-(3-(3-bromophenyl)-1-phenyl-1H-pyrazol-4-yl) prop-2-ene-1-one

AH-11: (E)-1-(1H-benzo[d]imidazole-2-yl)-3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl) prop-2-en-1-one

AH-12: (E)-1-(1H-benzo[d]imidazole-2-yl)-3-(3-(2,5-dichloroophenyl)-1-phenyl-1H-pyrazol-4-yl)prop-2-en-1-one

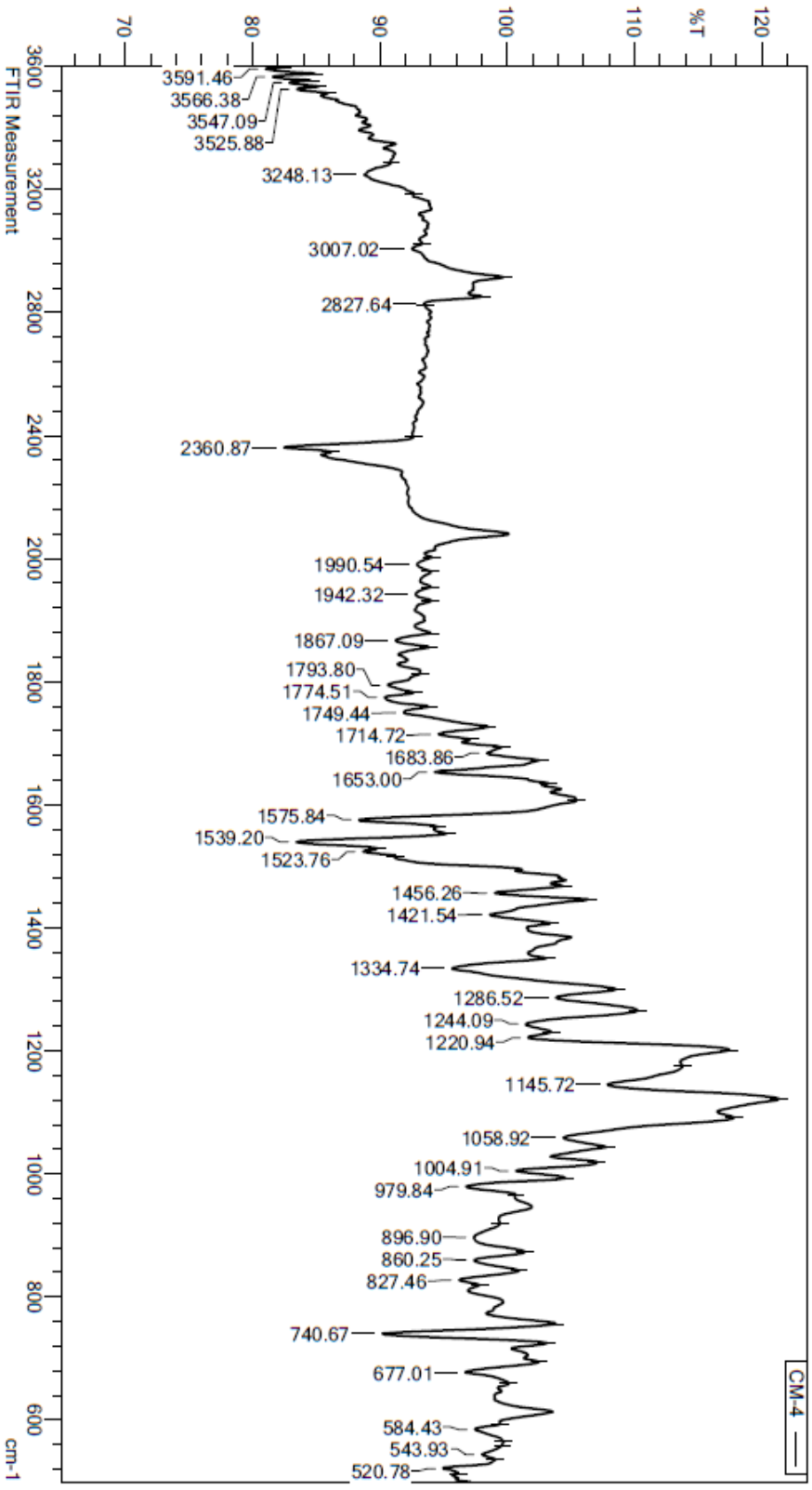


Figure 2. IR spectrum of AH-1

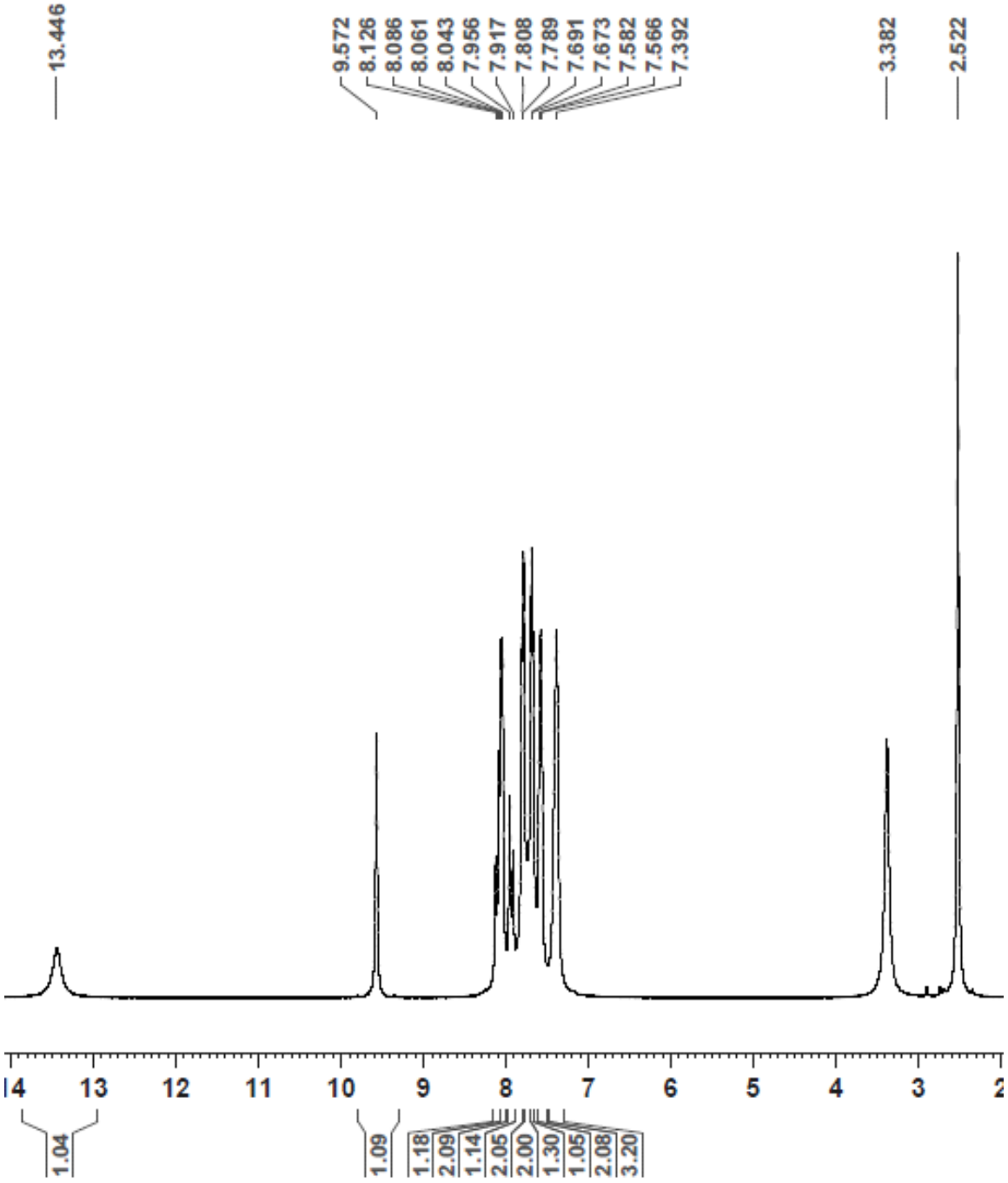


Figure 3. ^{1}H NMR spectrum of AH-1

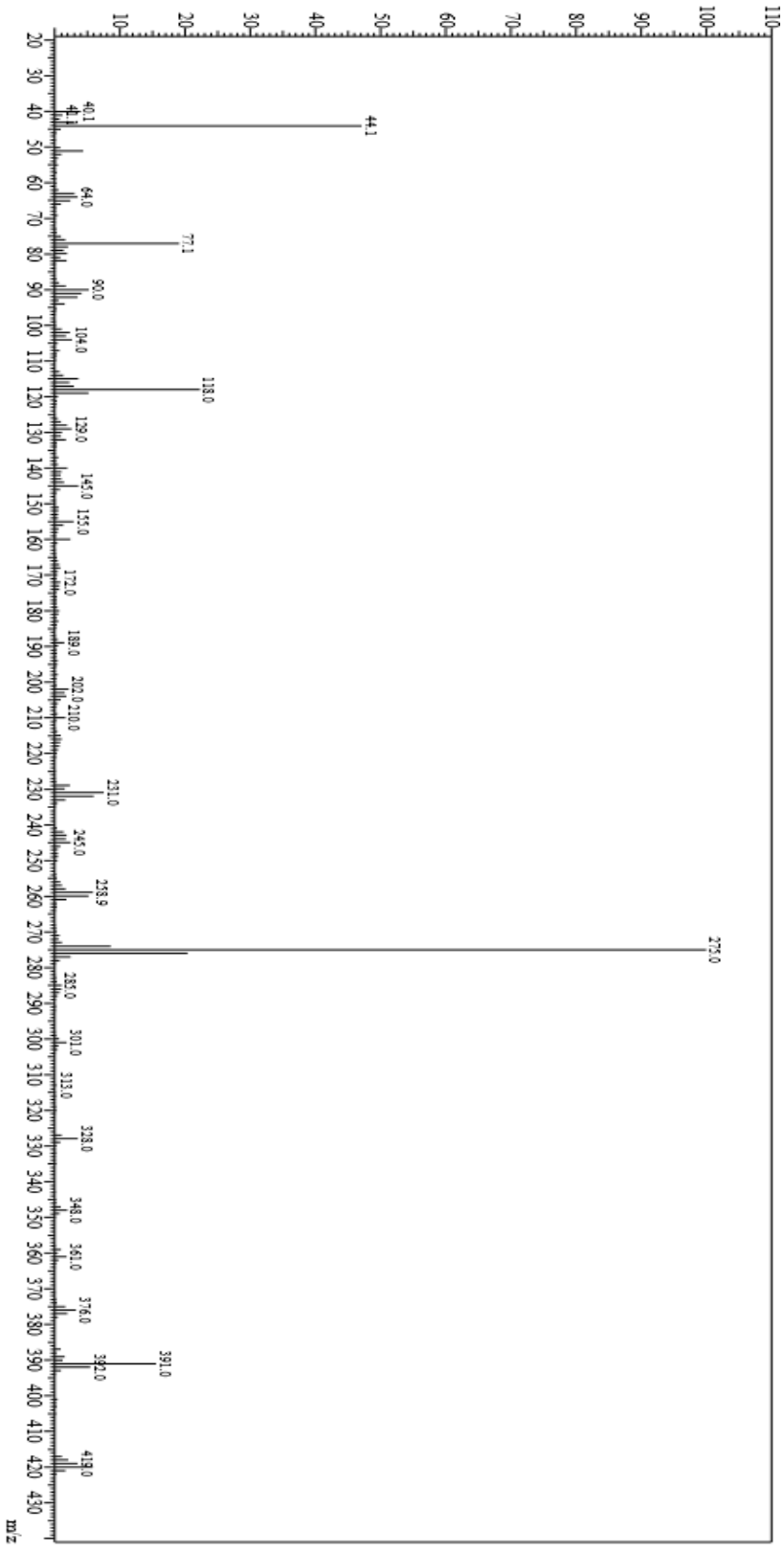


Figure 4. Mass spectrum of AH-1

2. 2. Preparation of Solutions of compounds

All the synthesized compounds were purified by re crystallization method. The DMF and DMSO were of AR grade supplied by Spectrochem Pvt. Ltd. (Mumbai, India) and were purified according to the standard procedure [38]. The antimicrobial and antifungal activities of all the synthesized compounds were studied in DMF and DMSO by agar well diffusion method. For all the compounds, solution of 20 mg/ml concentration was prepared in DMF and DMSO.

2. 3. Agar well diffusion method

In vitro antimicrobial activities of all the synthesized compounds were determined using standard agar well diffusion assay [39]. Mueller Hinton agar and Sabouraud dextrose agar media was used for antibacterial and antifungal activity respectively. Molten Mueller Hinton agar/ Sabouraud dextrose agsar (40-42 °C) were seeded with 200 µl of inoculum (1×10^8 cfu (colony forming unit) / ml) and poured into Petri dishes. The media were allowed to solidify and wells were prepared in the seeded agar plates with the help of a cup borer (8.5 mm). 100 µl of compound solution in DMSO / DMF was added into the sterile 8.5 mm diameter well.

The plates were incubated at 37 °C and 28 °C for 24 and 48 h for bacteria and fungi, respectively. Pure DMSO and DMF solvents were used as a negative control. The antimicrobial activity was assayed by measuring the diameter of the zone of inhibition formed around the well in millimetres. The experiment was done in triplicate and the average values of zone of inhibition were reported.

3. RESULTS AND DISCUSSION

Table 1 shows the physical parameters and different substitution groups of the synthesized compounds.

Table 1. Physical constants of pyrazolo chalcone derivatives.

Compound Code	Substitution R	Molecular formula	Molecular Weight	Yield (%)	R_f * Value
AH-1	3-OCH_3	$C_{26}H_{20}N_4O_2$	419.00	80	0.84
AH -2	-H	$C_{25}H_{18}N_4O$	389.90	80	0.84
AH -3	4-Cl	$C_{25}H_{17}ClN_4O$	424.11	80	0.80
AH -4	3, 4-di-OCH_3	$C_{27}H_{22}N_4O_3$	450.00	81	0.71
AH -5	4-F	$C_{25}H_{17}FN_4O$	408.44	85	0.88
AH -6	2-Cl	$C_{25}H_{17}ClN_4O$	424.11	86	0.80

AH -7	4- OCH_3	$C_{26}H_{20}N_4O_2$	420.16	89	0.88
AH -8	3-NO_2	$C_{25}H_{17}N_5O_3$	435.00	83	0.83
AH -9	4-NO_2	$C_{25}H_{17}N_5O_3$	435.13	83	0.88
AH -10	3-Br	$C_{25}H_{17}BN_4O$	400.15	82	0.81
AH -11	4-Br	$C_{25}H_{17}BN_4O$	400.15	84	0.81
AH -12	2, 5-di-Cl	$C_{25}H_{17}Cl_2N_4O$	458.07	86	0.71

* 0.6:0.4-Hexane: Ethyl acetate

3. 1. Spectral Data

AH-1

IR (cm^{-1}): 3148.13 (alkene CH-str.), 2362.87 (-NH), 3160 (C-H str. Ar. ring), 1776.51 (-CO str. in –CO-CH=CH-), 1575.84 (Ar-C=C str.), 1244.09-1220.94 (C-O-C str.), 979.84 (trans –CH=CH-), 740.67, 700.2 (m-OCH_3). **^{1}H NMR (DMSO-d_6) δ(ppm):** 3.868 (s, 3H, -OCH_3), 7.256-7.363 (d, 1H, *J*=42.8 Ar-CH), 7.363-7.261 (d, 2H, *J*=40.8 Hz, Ar-CH), 7.435-7.398 (dd, 1H, *J*=14.8 Ar-CH), 7.502-7.538 (dd, 2H, *J*=14.4 Ar-CH), 7.570-7.502 (d, 1H, *J*=27.2 Ar-CH), 7.610-7.570 (t, 3H, *J*=8, Ar-CH), 7.858-7.838 (d, 1H, *J*=8.00 Ar-CH), 8.109-7.995 (m, 4H, Ar-CH), 9.580 (s, 1H, Ar-CH), 13.434 (s, 1H, -NH). **MS: (m/z):** 419.00

AH-2

IR (cm^{-1}): 3234.62 (alkene C-H str. of aromatic ring), 1774.51 (-CO str. in –CO-CH=CH-), 1537.27 (Ar-C=C str.), 1219.01 (-C-O str.), 979.84 (trans –CH=CH-), 684.73-717.52 (mono substituted Ar. ring). **^{1}H NMR (DMSO-d_6) δ(ppm):** 7.38-7.32 (t, 1H, *J*=7.6 Htz Ar-CH), 7.363-7.326 (t, 2H, *J* =7.2 Htz Ar-CH), 7.627-7.547 (m, 6H, Ar-CH), 7.731-7.713 (d, 2H, *J*=7.2 Ar-CH), 7.863-7.843 (d, 1H, *J* =8.0 Hz, Ar-CH), 8.052 (d, 1H, Ar-CH), 8.129-8.072 (m, 3H, *J* = 16 Ar-CH), 9.602 (s, 1H, Ar-CH), 13.445 (S, 1H, -NH). **MS: (m/z):** 389.90

AH-3

IR (cm^{-1}): 3195.86 (alkene CH-str.), 1774.51 (-CO str. in –CO-CH=CH-), 1537.27 (Ar-C=C str.), 1595.98 (alkene C-H bending), 1219.01 (-C-O str.), 978.84 (trans–CH=CH-), 860.35 (p-Cl).**^{1}H NMR (DMSO-d_6) δ(ppm):** 7.416-7.39 (d, 2H, *J*=10.4 Ar-CH), 7.433 (s, 1H, Ar-CH), 7.60- 7.57 (t, 2H, *J*=8.48, Ar-CH), 7.905-7.588 (m, 4H, *J* = 8.12 Hz, Ar-CH), 7.945-7.905 (d, 2H, *J*=16 Ar-CH), 8.116-8.041 (dd, 4H, *J*=30 Ar-CH), 9.53 (s, 1H, Ar-CH), 13.490-13.452 (Broad singlet, 1H, -NH). **MS: (m/z):** 424.11

AH-4

IR (cm^{-1}): 3255.84 (alkene CH-str.), 1774.51 (-CO str. in –CO-CH=CH-), 1575.84 (Ar-C=C str.), 1434.74 (alkene C-H bend.), 1244.09-1220.94 (-C-O str.), 976.84 (trans –CH=CH-), 845.25, 775.15, 715 (3, 4-diOCH_3). **^{1}H NMR (DMSO-d_6) δ(ppm):** 3.88-387 (s, 6H, -OCH_3),

7.438-7.865 (m, 6H, Ar-CH), 7.865-7.569 (t, 3H, Ar-CH), 7.865-7.846 (d, 1H, *J*=7.6 Ar-CH), 8.114-8.022 (m, 4H, Ar-CH), 9.553 (s, 1H, Ar-CH), 13.462 (s, 1H, NH**). MS: (m/z):** 450.00

AH-5

IR (cm^{-1}): 3254.76 (alkene CH-str.), 1776.51 (-CO str. in –CO-CH=CH-), 1545.27 (Ar-C=C str.), 1600.01 (alkene C-H bending), 1222.01 (-C-O str.), 977.91 (trans -CH=CH-), 863.25 (p-F). **^{1}H NMR (DMSO-d$_6$) δ(ppm):** 7.472-7.382 (m, 5H, CH), 7.607-7.588 (t, 2H, *J*=7.6 Htz), 7.59-7.57 (m, 2H, Ar-CH), 7.903-7.750 (t, 2H, *J*=15.6 Htz), 7.943-7.903 (d, 2H, *J*=16 Ar-CH), 8.122-8.044 (t, 4H, Ar-CH), 9.596 (s, 1H, Ar-CH), 13.441 (s, 1H, -NH). **MS: (m/z):** 408.44

AH-6

IR (cm^{-1}): 3248.13 (alkene CH-str.), 1785.51 (-CO str. in –CO-CH=CH-), 1575.84 (Ar-C=C str.), 1590.40 (alkene C-H bending), 1245.09-1221.94 (-C-O str.), 977.50 (trans –CH=CH-), 749.50 (O-Cl). **^{1}H NMR (DMSO-d$_6$) δ(ppm):** 7.39 (s, 3H, Ar-CH), 7.582-7.566 (d, 2H, *J*=6.4 Ar-CH), 7.691-7.673 (d, 1H, *J*=7.2 Ar-CH), 7.956-7.691 (m, 6H, Ar-CH), 8.126-7.917(d, 3H, *J*=83.6 Ar-CH), 9.572 (s, 1H, Ar-CH), 13.446 (s, 1H, NH). **MS: (m/z):** 424.11

AH-7

IR (cm^{-1}): 3248.13 (alkene CH-str.), 1774.51 (-CO str. in –CO-CH=CH-), 1575.84 (Ar-C=C str.), 1334.74 (alkene C-H bending), 975.00 (trans–CH=CH-), 860.25 (p-OCH_3). **^{1}H NMR (DMSO-d$_6$) δ(ppm):** 3.863 (s, 3H, -OCH_3), 7.17-7.15 (d, 2H, *J* = 8.0 Htz, Ar-CH), 7.42-7.38 (t, 3H, Ar-CH), 7.60-7.56 (t, 2H, Ar-CH), 7.658-7.58 (s, 3H, Ar-CH), 7.92 (s, 1H, Ar-CH), 7.96 (s, 1H, Ar-CH), 8.07-8.03 (m, 3H, Ar-CH), 9.56 (s, 1H, Ar-CH), 13.427 (s, 1H, NH). **MS: (m/z):** 419.00

AH-8

IR (cm^{-1}): 3245 (alkene CH-str.), 1774.51 (-CO str. in –CO-CH=CH-), 1653.00, 1683.86, 1573.91 (Ar-C=C str.), 980.14 (trans–CH=CH-), 740.67, 715.59 (m-NO_2). **^{1}H NMR (DMSO-d$_6$) δ(ppm):** 7.45-7.35 (m, 3H, Ar-CH), 7.61-7.57 (m, 4H, Ar-CH), 7.73-7.72 (d, 1H, *J*=4.0 Ar-CH), 7.87-7.85 (d, 1H, *J*=8.0 Ar-CH), 8.16-7.94 (m, 5H, Ar-CH), 8.45-8.42 (d, 1H, *J*=16, Ar-CH), 9.63-9.59 (d, 1H, *J*=16 Ar-CH), 13.465 (s, 1H, -NH). **MS: (m/z):** 435.00

AH-9

IR (cm^{-1}): 3255.84 (alkene CH-str.), 2360.87 (-C-N aromatic), 1774.51 (carbonyl str. in –CO-CH=CH-), 1653.00, 1598.99, 1653.00 (Ar-C=C str.), 988.50 (trans alkene -CH=CH-), 740.67, 715.59 (p-nitro). **^{1}H NMR (DMSO-d$_6$) δ(ppm):** 7.436-7.386 (m, 3H, Ar-CH), 7.617-7.594 (m, 4H, *J*=7.6 Htz), 8.428-7.948 (m, 6H, Ar-CH), 8.449-8.428 (d, 2H, *J*=8.4), 9.624 (s, 1H, Ar-CH), 13.437 (s, 1H, -NH). **MS: (m/z):** 435.13

AH-10

IR (cm^{-1}): 3247 (alkene CH-str.), 2299.25 (-C-N aromatic), 1774.81 (carbonyl str. in –CO-CH=CH-), 1653.00, 1683.96, 1573.99 (Ar-C=C str.), 979.97 (trans alkene –CH=CH-), 741.67, 716.59 (p-NO_2). **^{1}H NMR (DMSO-d$_6$) δ(ppm):** 7.397-7.325 (m, 4H, Ar-CH), 7.610-7.591 (m, 3H, Ar-CH), 7.741-7.721 (m, 2H, Ar-CH), 8.801-7.850 (m, 6H, Ar-CH), 9.591 (s, 1H, Ar-CH), 13.431 (s, 1H, -NH). **MS: (m/z):** 400.15

AH-11

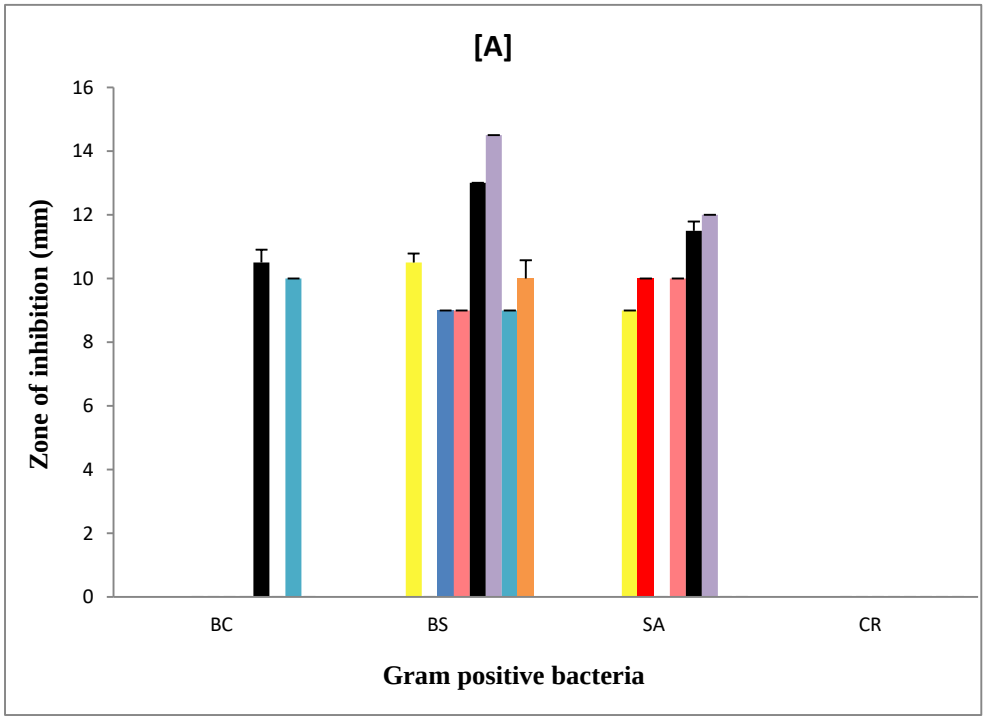
IR (cm^{-1}): 3251.84 (alkene CH-str.), 2354.87 (-C-N aromatic), 1772.51 (carbonyl str. in –CO-CH=CH-), 1651.00, 1597.96, 1651.00 (Ar-C=C str.), 977.80 (trans alkene –CH=CH-), 738.67, 713.59 (m-Br). **^{1}H NMR (DMSO-d$_6$) δ(ppm):** 7.451-7.384 (m, 5H, Ar-CH), 7.607-7.569 (t, 2H, J = 7.6 Htz), 7.785-7.751 (m, 4H, Ar-CH), 7.944-7.905 (d, 1H, *J* =15.6, Ar-CH), 8.122-8.044 (m, 3H, Ar-CH), 9.224 (s, 1H, Ar-CH), 9.597 (s, 1H, -NH). **MS: (m/z):** 400.15

AH-12

IR (cm^{-1}): 3256.99 (alkene CH-str.), 2361.60 (-C-N-aromatic), 1774.51 (carbonyl str. in –CO-CH=CH-), 1576.84 (Ar-C=C str.), 1334.74 (alkene -CH bending), 846.25, 776.15, 715.15 (2, 4-diCl). **^{1}H NMR (DMSO-d$_6$) δ(ppm):** 7.481-7.395 (m, 4H, Ar-CH), 7.678-7.589 (t, 2H, *J*=7.6 Htz), 7.812-7.778 (m, 5H, Ar-CH), 7.945-7.909 (s, 1H, Ar-CH), 8.144-8.055 (m, 3H, Ar-CH), 9.608 (s, 1H, -NH). **MS: (m/z):** 458.07

3. 2. Antimicrobial activity:

Figure 5 [A] shows zone of inhibition against Gram positive bacteria in DMSO and DMF solutions for all compounds. In DMSO (as in Figure 5 [A]), it is observed that against *Bacillus cereus* (BC), only AH-9 and AH-11 exhibited inhibition. Other compounds had no effect on this bacterial strain. The inhibition for AH-9 was slightly more than that for AH-11. For *Bacillus subtilis* (BS), AH-10 exhibited maximum inhibition which is followed by AH-9. AH-7, AH-8 and AH-11 showed minimum inhibition. AH-1 to AH-4 and AH-6 had no effect against BS. Against *Staphylococcus aureus* (SA), again AH-10 exhibited maximum inhibition which is followed by AH-9. AH-1 to AH-4, AH-7, AH-11 and AH-12 had no effect. None of the studied compounds had any effect on *Corynebacterium rubrum* (CR).



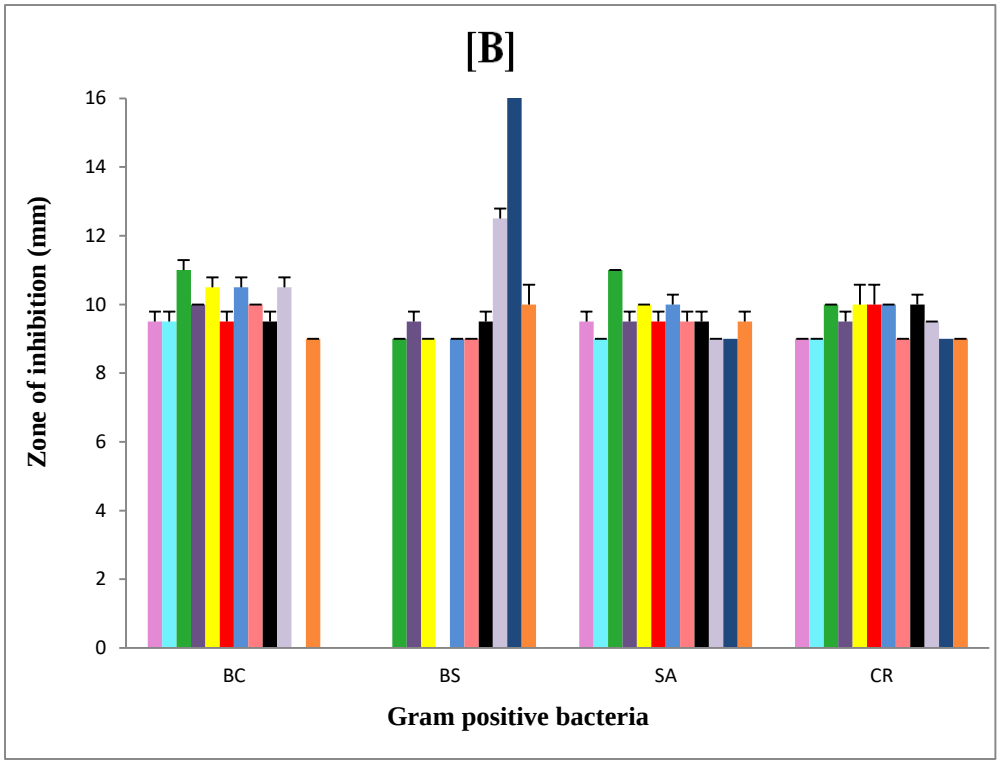


Figure 5. Zone of inhibition of pyrazolo chalcone compounds against Gram positive bacteria in [A] DMSO and [B] DMF
(■): AH-1; (■): AH-2; (■): AH-3; (■): AH-4; (■): AH-5; (■): AH-6; (■): AH-7; (■): AH-8; (■): AH-9; (■): AH-10; (■): AH-11; (■): AH-12

Figure 5 [B] shows zone of inhibition of compounds against gram positive bacteria in DMF. Against *Bacillus cereus* (BC), except AH-11, all other compounds exhibited inhibition and inhibition is maximum for AH-3. For *Bacillus subtilis* (BS), AH-11 showed maximum inhibition which is followed by AH-10. AH-3, AH-5, AH-7 and AH-8 had same minimum inhibition. AH-1, AH-2 and AH-6 had no effect on this bacterial strain. All the compounds could affect *Staphylococcus aureus* (SA) and *Corynebacterium rubrum* (CR). For *Staphylococcus aureus*, AH-3 showed slightly high inhibition than other compounds which exhibited more or less same inhibition. However, for *Corynebacterium rubrum*, AH-3, AH-5, AH-6, AH-7 and AH-9 exhibited same inhibition which is slightly higher than other compounds.

Thus, the inhibition of these compounds against Gram positive bacteria in DMSO and DMF solutions suggests that inhibition depends on compound structure, solvent and bacterial strain. Comparison of Figure 5 [A] and [B] shows that more compounds could inhibit the selected bacteria in DMF than those in DMSO. So, DMF is good solvent for inhibition for the studied compounds. All the studied compounds have the same central moiety but different substitution groups as shown in Table 1. Thus, for a particular strain, a particular group had more effect on a particular strain than other.

So, in DMSO, against BC, only 4-NO_2 (as in AH-9) and 4-Br (as in AH-11) had been effective. Other substitution groups had no effect. For BS, 3-Br as in AH -10 showed maximum effect. 4-OCH_3 (in AH-7), 3-NO_2 (AH-8) and 4-Br (AH-11) also affect this strain whereas other groups had no effect at all against BS. Against SA, again 3-Br caused maximum inhibition. However, for CR, none of the group was effective in DMSO.

Out of these four bacterial strains, CR is most resistant bacteria whereas BS is most susceptible bacteria in DMSO.

In DMF, almost all the groups could affect the selected strains to same extent especially SA and CR. Against BC, 4-Br (AH-11) had no effect whereas other groups exhibited inhibition and inhibition is maximum for AH-3 whereas for BS, 4-Br showed maximum inhibition. Some other groups such as 4-Cl (in AH-3), 4-F (in AH-5), 4-OCH_3 (in AH-7), 3-NO_2 (in AH-8) and 3-Br (in AH-10) also had some effect on BS. All the substitution groups of studied compounds could affect SA and CR. For SA, 4-Cl had slightly high inhibition than other groups. Overall, in DMF, all the four selected bacteria are susceptible.

These findings also suggest that the position of group also affect the inhibition against bacteria. As discussed above, against BC, AH-9 containing 4-NO_2 and AH-11 containing 4-Br were effective but AH-8 having 3-NO_2 and AH-10 having 3-Br had no effect at all. Similar findings are also observed for other bacterial strains also. 4-Cl group had no effect but 2-Cl could inhibit SA.

Further, number of groups plays an important role. AH-1 and AH-7 has 3-OCH_3 and 4-OCH_3 groups respectively whereas AH-4 contains 3, 4-diOCH_3 groups. It is observed that when methoxy group is at 4^{th} position, it is most effective as observed against BS in DMSO whereas AH-1 (having 3-OCH_3) and AH-4 (having 3, 4-diOCH_3) were not effective at all. This suggests that methoxy groups at these positions are not affective. This confirms the effect of position and number of groups on inhibition.

Figure 6 [A] shows zone of inhibition for compounds against some gram negative bacterial strains in DMSO. It is observed that against *Escherichia coli* (EC), only AH-10, AH-11 and AH-12 showed inhibition. The order of inhibition is AH-11 (4-Br) > AH-10 (3-Br) >AH-12 (2, 5-dichloro). Other compounds had no effect. *Klebsiella pneumonia* (KP) is not affected by any of the synthesized compounds. Against *Pseudomonas aeruginosa* (PA), only AH-8, AH-11 and AH-12 exhibited inhibition and inhibition is maximum for AH-12. Thus, for PA, 2, 5-dichloro group (in AH-12) is most effective. For *Salmonella typhimurium* (ST) also, only few compounds AH-2, AH-3, AH-11 and AH-12 had effect. Thus, for ST, -H, 4-Cl, 4-Br and 5-dichloro groups are effective. Thus, in DMSO, KP is more resistant bacteria.

The zone of inhibition against gram negative bacteria in DMF is shown in Figure 6 [B]. For EC, all compounds exhibited inhibition and AH-3 showed maximum inhibition. There is slight change in inhibition among other compounds and the order is: AH-3 > AH-5 > AH-1 AH-4 AH-6 AH-7 AH-8 AH-9 > AH-12 > AH-2 AH-10 AH-11. Against KP, except AH-11, all other compounds had inhibition and AH-10 exhibited maximum inhibition. The minimum inhibition is observed by AH-1 and AH-2 containing 3-OCH_3 and –H group respectively. Thus, 4-Br (as in AH-11) is not effective whereas 3-Br (as in AH-10) is most effective. This again proves that position of group affect inhibition. Against both PA and ST, all the compounds showed inhibition and for both these strains AH-11 containing 4-Br group had maximum inhibition.

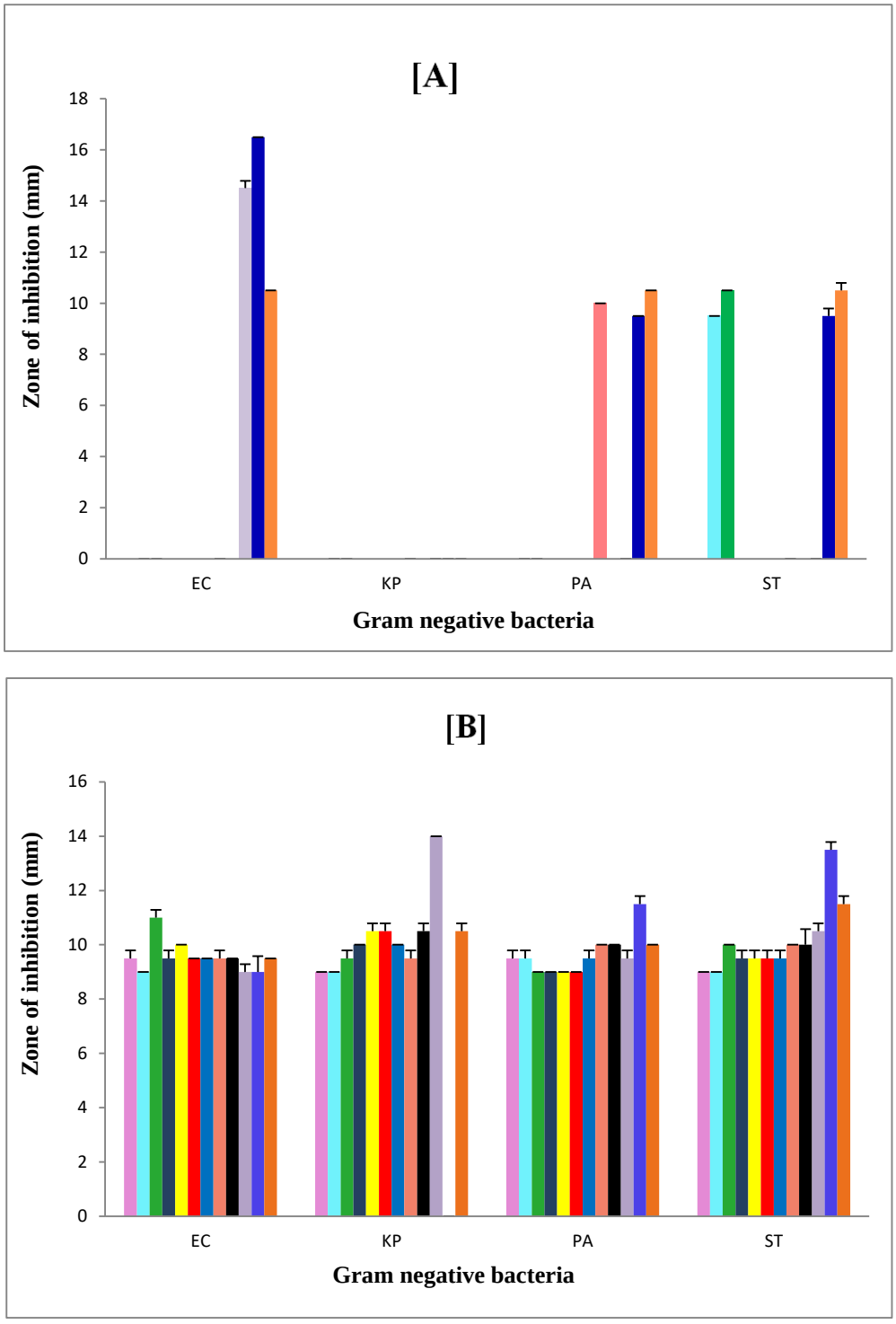


Figure 6. Zone of inhibition of pyrazolo chalcone compounds against Gram negative bacteria in [A] DMSO and [B] DMF.
(■): AH-1; (■): AH-2; (■): AH-3; (■): AH-4; (■): AH-5; (■): AH-6; (■): AH-7; (■): AH-8; (■): AH-9; (■): AH-10; (■): AH-11; (■): AH-12

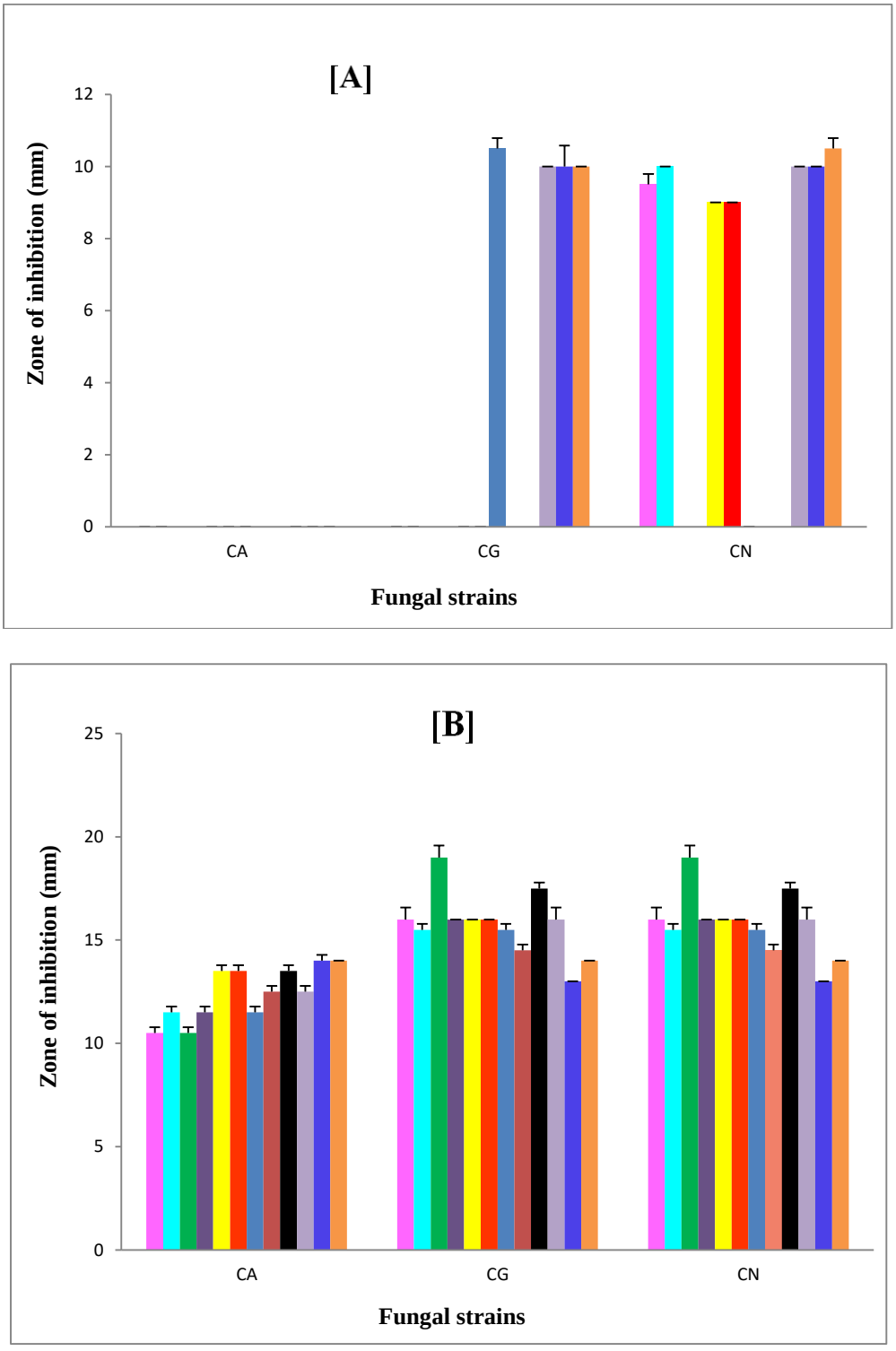


Figure 7. Zone of inhibition of pyrazolo chalcone compounds against fungai in [A] DMSO and [B] DMF.

For gram negative bacterial strains also, DMF is better solvent as compared to DMSO.

Figure 7 [A] shows zone of inhibition against some fungal strains in DMSO and DMF. In DMSO (Figure 7 [A]), not a single compound could inhibit *Candida albicans* (CA). For *Candida glabrata* (CG), AH-7 containing 4-OCH_3 group showed maximum inhibition. AH-10, AH-11 and AH-12 had almost equal minimum inhibition. Other compounds had no effect on CG. For *Cryptococcus neoformans* (CN), AH-12 exhibit maximum inhibition. Few other compounds such as AH-2, AH-5, AH-6, AH-10 and AH-11 also exhibited inhibition. Rest of the compounds had no effect at all. So, for CN, 2,5-dichloro is most effective group. The other groups like 4-F, 2-Cl, 3-Br and 4-Br also had effect on CN. This suggests that for this fungal strain, most of the halogen containing compounds are effective. In DMSO, (CA) is the most resistant bacteria.

Figure 7 [B] shows zone of inhibition against selected fungal strains in DMF. All the studied compounds could inhibit the selected three fungal strains. For CA, AH-11 and AH-12 exhibits maximum. For CG and CN, highest inhibition is exhibited by AH-3. Thus, for CA, both 4-Br and 2,5-dichloro are most effective whereas for other two fungal strains, CG and CN, 4-Cl is most effective. Thus, in DMF, all the three selected strains are susceptible. For fungal strains also, DMF is good solvent for the studied compounds.

4. CONCLUSION

The inhibition depends on strain, substitution, position of substitution and solvent. The studied compounds are more effective against selected bacterial and fungal strains in DMF than those in DMSO.

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