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Synthesis and biological evaluation of 3-substituted 1,2-benzisoxazole derivatives for antimicrobial activity

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ABSTRACT

Several 3-substituted 1,2-benzisoxazole derivatives were synthesized from treatment of 4-amino-2-hydroxy acetophenone by different aldehyde substitution with the hope that different substituent formed at the third position show significant antimicrobial activity. 4-amino-2-hydroxy acetophenone 1 on treatment with different aldehydes 2a-e in sodium hydroxide afford 3a-e. Compounds 3a-e on oximation yields 4a-e. Compounds 4a-e on treatment with silica gel and sodium carbonate affords 5a-e. The resultant 3-substituted 1,2-benzisoxazole derivatives have been characterized by spectral data. The compounds 5a-e have been screened for their antimicrobial activity. Compounds 5a, 5b and 5e exhibit good antimicrobial activity.

Key words: 3-substituted 1,2-benzisoxazole, aldehydes, antimicrobial activity.

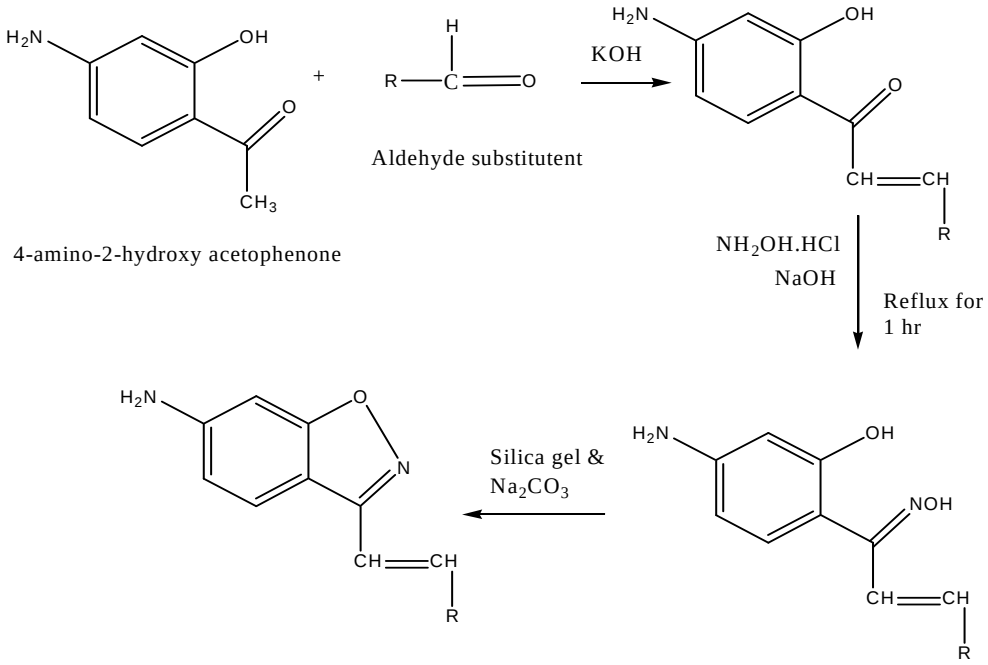
INTRODUCTION

1,2-benzisoxazole derivatives known to have important biological activities and are useful in different activities. 1,2-benzisoxazole derivatives have recently attracted attention as an important class of heterocyclic compounds in the field of drugs and pharmaceuticals. These compounds are widely used as analgesic [1], anticonvulsant [2,3], antipsychotic [4,5], and as antimicrobial [6] agents.

In view of the diverse type of biological activity it was thought worthwhile to prepare the title compound i.e., 3-substituted 1,2-benzisoxazole derivatives have been synthesized by the treatment of 4-amino 2-hydroxy acetophenone with different aldehyde substitutent and evaluated for the antimicrobial activity with the hope that differant substituent formed at third position may prove to be biologically active.

The general synthetic strategy employed to prepare the benzisoxazole derivatives was based on Claisen-Schmidt condensation **(Scheme I)** [7]. A series of five benzisoxazole derivatives (5a-e)

were prepared by condensing various aldehyde substitutent and substituted acetophenone to form the expected compounds, using 4-amino-2-hydroxy acetophenone 1 on treatment with different aldehydes 2a-e in sodium hydroxide afford 3a-e. Compounds 3a-e on oximation yields 4a-e. Compounds 4a-e on treatment with silica gel and sodium carbonate affords 5a-e. The starting materials ie., 1 and 2a-e were commercially available.



Scheme I: Scheme for the synthesis of 3-propene 1,2-benzisoxazole derivatives

MATERIALS AND METHODS

All melting points were determined on a veego melting point apparatus. The homogemecity of all the compounds was checked by TLC on silica gel coated plates. IR spectra (KBr) were recorded on FTIR spectrophotometer. ^{1}H NMR spectra were recorded in $CDCl_3$ on 3oo MHz instrument using TMS as internal standatd. (chemical shift in δ,ppm).

General procedure for the preparation of compounds 3a-e

A mixture of 4-amino-2-hydroxy acetophenone (0.01M) and substituted aldehyde (20% 0.04 M) was dissolved in 10 mL ethanol. To this mixture 10 mL of 40 % KOH was added, the reaction mixture was stirred and kept at RT for 24 hr. Then the reaction mixture was poured over crushed ice and contents were acidified with concentrated hydrochloric acid. Product thus obtained was purified by recrystallization from 75% acetic acid.

General procedure for the preparation of compounds 4a-e

Compound 3 (0.01M) was dissolved in 10 mL of ethyl alcohol. To this 0.05 M of sodium hydroxide (in10 mL water) and 0.015 M of hydroxylamine hydrochloride (in 10 mL water) were added. Contents were refluxed for 1 hr and the reaction mixture was left overnight. Mixture was poured in cold water and contents were acidified with hydrochloric acid. Product thus obtained was purified by recrystallization from 75% ethanol.

General procedure for the preparation of compounds 5a-e

Compound 4 (0.01M) was dissolved in dichloromethane (5mL) in another beaker silica gel (10g) of 60-120 mesh treated with 0.02M of sodium carbonate solution stirred and dried. This reaction mixture was transferred to solution 4 and solvent was evaporated on water bath at 50º C. The reaction mixture was cooled to RT & product was extracted with dichloromethane (20 mL), solvent was evaporated by warming on water bath. Residue was treated with 5% NaOH in order to remove unreacted oxime. The obtained compounds 5a-e was purified by recrystallization from ethanol.

Antimicrobial activity

The compounds 5a-e were screened for their antimicrobial activity against E. coli, B. subtilis, P. aeruginosa, S. typhae, S. cohini and S. aureus by agar agar well diffusion method [8]. All the results were compared to standard antibiotic gentamicin, an aminoglycoside (broad spectrum). Results are presented in table 5.2 to 5.15 and figure 5.1 to 5.13.

For the antimicrobial activity, the compounds were dissolved in absolute DMSO (3mg/ml). Further dilutions of the compounds were prepared at the required quantities of 50 and 100 µg/ml concentrations. The standard drugs were also prepared at 50 and 100µg/ml concentration.

Antimicrobial activity of test compounds in 50µg\ml concentration

In the present investigation of antimicrobial activity of synthesized compounds 5a-e, it was observed that all the samples were showing potential antimicrobial activity. Table 5.1 shows the zone of inhibition of test samples against various microorganisms (in 50µg\ml concentration).

Biostatical Interpretation (One Way ANOVA)

The results are presented as means $\pm$ SD (standard deviation) of synthesized compounds and standard drug gentamicin against various microorganism.. Statistical analysis were performed using one way analysis of variance (ANOVA) followed by bonferroni test for multiple comparisons, using primer of biostatistics. P values <0.05 were considered to be significant.

Table 5.1: Zone of Inhibition of test samples against various microorganisms

Test Sample*	Zone of Inhibition (mm)**					
	E.Coli	*B.Subtilis*	*P.Aeruginosa*	*S.Typhae*	*S. Cohini*	*S. aureus*
Gent	36±0.82	31.25±0.957	22±1.414	30±1.63	28.75±0.957	28.75±0.96
5a	23 ± 1.41	13±1.15	19±0.82	15.75±1.26	17.75±0.96	20.25±2.06
5b	22±0.82	18±1.63	16.25±1.71	18±1.63	18.5±1.73	20±1.63
5c	17±1.41	14±1.63	12.75±1.5	10.5±0.58	16±1.63	15.5±1.29
5d	16.75±0.5	11.5±1.29	12.5±0.58	13.5±1.29	11.25±0.96	13±1.55
5e	19.25±1.71	16.25±1.26	15±0.82	17±0.82	18±1.41	17±0.82

*** Data is presented in mean ±SD; * n=4; p<0.05 was considered as level of significance*

In case of E. coli, it was observed that 5a was having maximum antimicrobial potential. The effect of 5c, 5d and 5e was significantly less ($p<0.05$) as compared to 5a. Although 5b was showing less zone of inhibition as compared to 5a but the variation was non-significant ($p>0.05$). The effect of 5a was significantly less ($p<0.05$) as compared to gentamicin, the standard drug used in the present study. The comparison between zone of inhibition of the test compounds and E. coli are shown in figure 5.1.

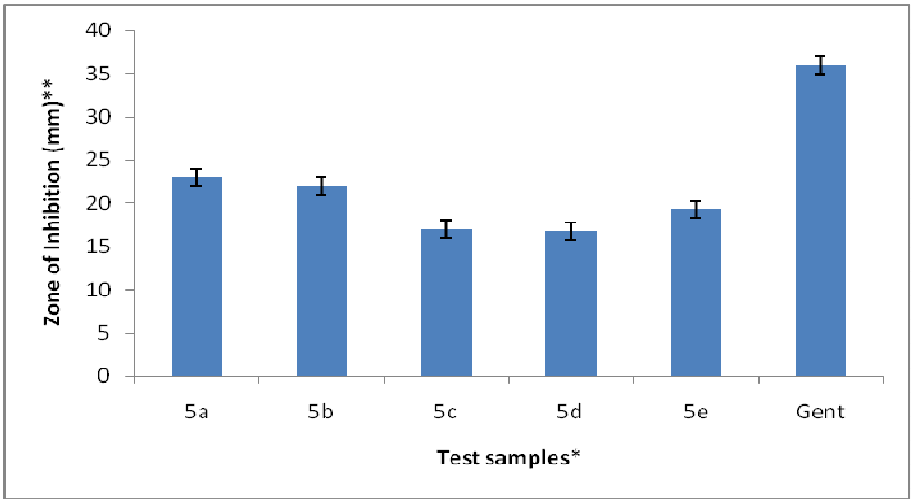


Fig 5.1: Antimicrobial activity of test compounds against E. coli

When zone of inhibition due to different compounds was compared for B. subtilis, it was found that 5b was having maximum activity. Although in this case the compound 5b was significantly less effective ($p<0.05$) as compared to gentamicin. Compound 5e was less effective than 5b but this difference was non-significant ($p>0.05$). All other samples i.e., 5a, 5c and 5d were significantly less effective than 5b ($p<0.05$). The comparison between zone of inhibition of the test compounds and B. subtilis are shown in figure 5.2.

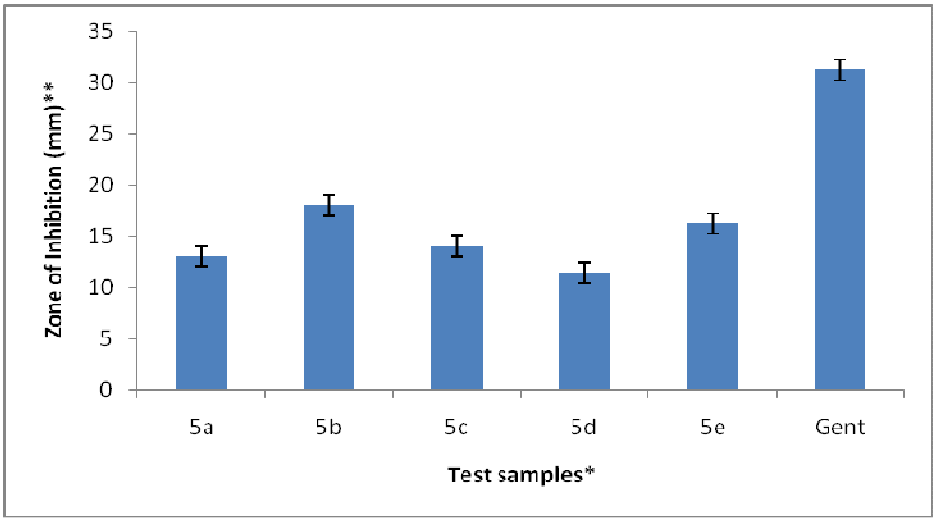


Fig 5.2: Antimicrobial activity of test compounds against B. subtilis

In case of P. aeruginosa, it was observed that 5a was having maximum antimicrobial potential. Although in this case the compound 5a was significantly less effective ($p<0.05$) as compared to gentamicin. The compound 5b was less effective than 5a but this difference was non-significant ($p>0.05$). All other compounds i.e., 5c, 5d and 5e were significantly less effective than 5a ($p<0.05$). The comparison between zone of inhibition of the test compounds and B. subtilis are shown in figure 5.3.

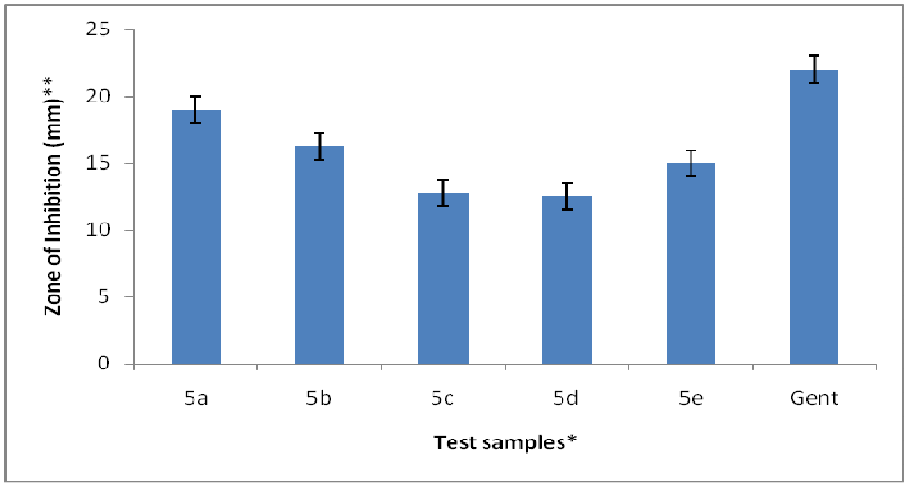


Fig 5.3: Antimicrobial activity of test compounds against P. aeruginosa

When zone of inhibition due to different compounds was compared for S. typhae, it was found that 5b was having maximum activity. Although in this case the compound 5b was significantly less effective ($p<0.05$) as compared to gentamicin. Compound 5a was less effective than 5b but this difference was non-significant ($p>0.05$). All other samples i.e., 5c, 5d and 5e were significantly less effective than 5b ($p<0.05$). The comparison between zone of inhibition of the test compounds and S. typhae are shown in figure 5.4.

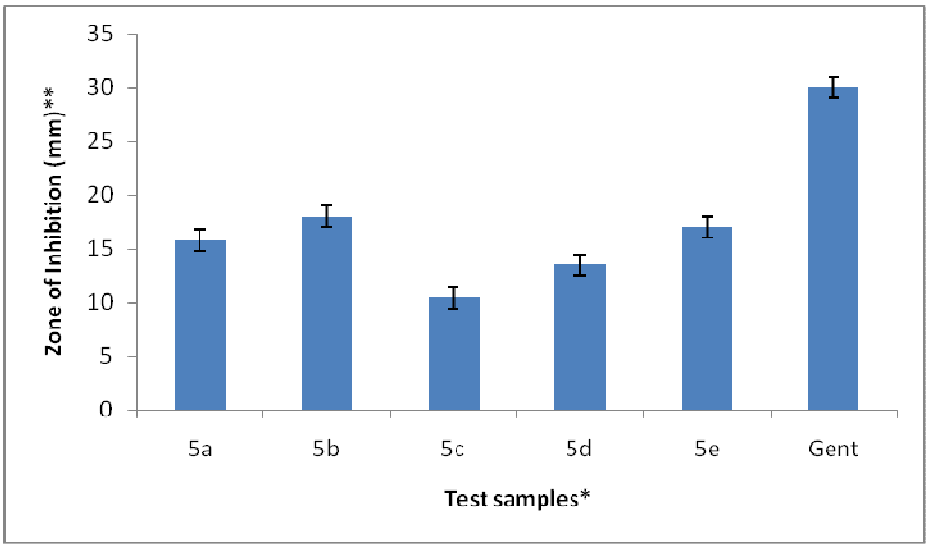


Fig 5.4: Antimicrobial activity of test compounds against S. typhae

When zone of inhibition due to different compounds was compared for S. cohini, it was found that 5b was having maximum activity. Although in this case the compound 5b was significantly less effective ($p<0.05$) as compared to gentamicin. Compound 5c was less effective than 5b but this difference was non-significant ($p>0.05$). All other samples i.e., 5a, 5d and 5e were significantly less effective than 5b ($p<0.05$). The comparison between zone of inhibition of the test compounds and B. subtilis are shown in figure 5.5.

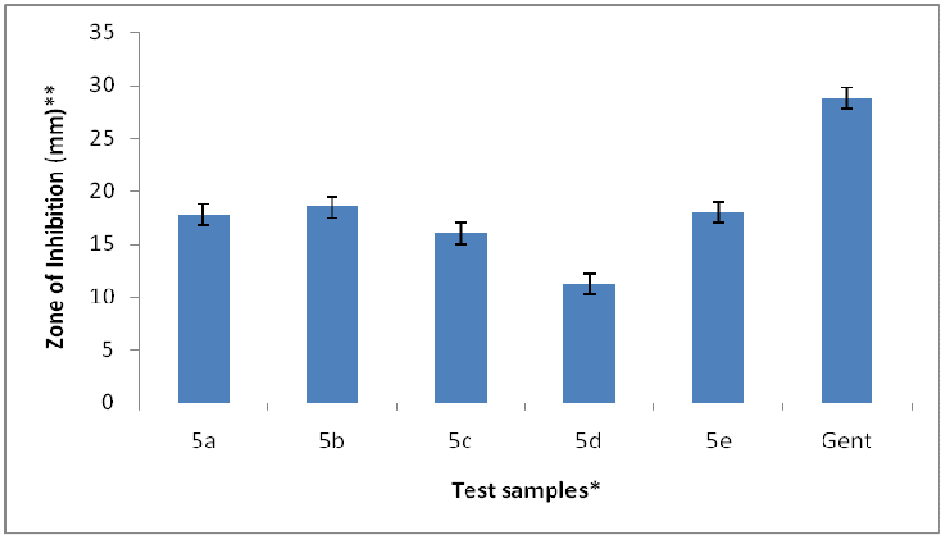


Fig 5.5: Antimicrobial activity of test compounds against S. cohini

In case of S. aureus, it was observed that 5a was having maximum antimicrobial potential. The effect of 5b, 5c and 5d was significantly less ($p<0.05$) as compared to 5a. Although 5e was showing less zone of inhibition as compared to 5a but the variation was non-significant ($p>0.05$). The effect of 5a was significantly less ($p<0.05$) as compared to gentamicin. The comparison between zone of inhibition of the test compounds and E.coli are shown in figure 5.6.

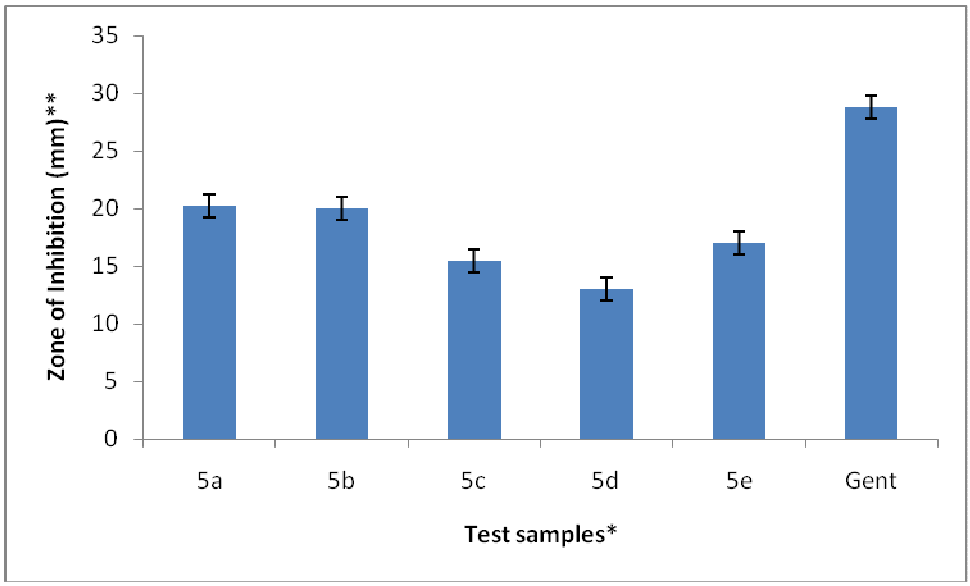


Fig 5.6: Antimicrobial activity of test compounds against S. aureus

Antimicrobial activity of test compounds in 100µg\ml concentration

In the present investigation of antimicrobial activity of synthesized compounds 5a, 5b, 5c, 5d and 5e for E. coli, B. subtilis, P. aeruginosa, S. typhae, S. cohini and S. aureus, it was observed that all the samples were showing potential antimicrobial activity. Table 5.2 shows the zone of inhibition of test samples against various microorganism (in 100µg\ml concentration).

5.3.1 Biostatical Interpretation (One Way ANOVA)

The results are presented as means $\pm$ SD (standard deviation) of synthesized compounds and standard drug gentamicin against various microorganism.. Statistical analysis were performed

using one way analysis of variance (ANOVA) followed by bonferroni test for multiple comparisons, using primer of biostatistics. P values <0.05 were considered to be significant.

Table 5.2: Zone of Inhibition of test samples against various microorganisms

Test Sample*	Zone of Inhibition (mm)**					
	E.Coli	***B.Subtilis***	***P.Aeruginosa***	***S.Typhae***	***S. Cohini***	***S. aureus***
Gent	33.75±0.95	32±0.81	24±1.82	29.25±0.95	29±1.15	29±0.81
BI-1	22.5±1.29	18±0.95	18.75±0.95	16.75±0.95	18.75±1.70	19.25±1.89
BI-2	21.75±0.95	17.25±1.70	18±1.29	18±1.63	17.75±0.95	19.75±1.70
B-3	18.25±0.95	14.25±1.70	16.75±1.5	15.25±0.95	16±1.63	14.75±0.95
BI-4	16.75±0.5	12±1.41	17±2.38	14±1.41	11.5±1.29	13.75±0.95
BI-5	19.5±1.73	16.5±1.29	15.5±0.95	15.25±0.95	15.5±0.57	14.75±0.5

In case of E. coli, it was observed that 5a was having maximum antimicrobial potential. The effect of 5c, 5d and 5e was significantly less ($p<0.05$) as compared to 5a. Although 5b was showing less zone of inhibition as compared to 5a but the variation was non-significant ($p>0.05$). The effect of 5a was significantly less ($p<0.05$) as compared to gentamicin, the standard drug used in the present study. The comparison between zone of inhibition of the test compounds and E.coli are shown in figure 5.7.

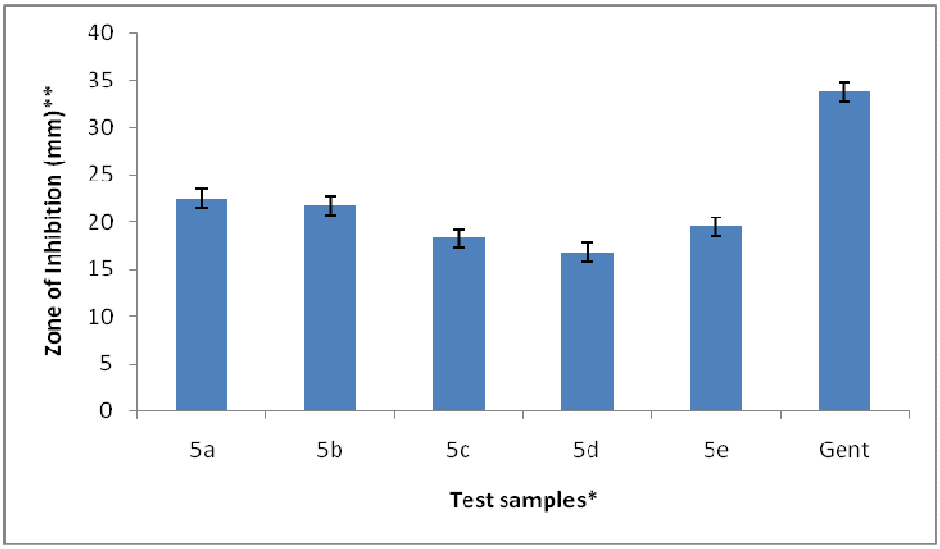


Fig 5.7: Antimicrobial activity of test compounds against E. coli

When zone of inhibition due to different compounds was compared for B. subtilis, it was found that 5a was having maximum activity. Although in this case the compound 5a was significantly less effective ($p<0.05$) as compared to gentamicin. Compound 5e was less effective than 5a but this difference was non-significant ($p>0.05$). All other compounds i.e., 5b, 5c and 5d were significantly less effective than 5a ($p<0.05$). The comparison between zone of inhibition of the test compounds and B. subtilis are shown in figure 5.8.

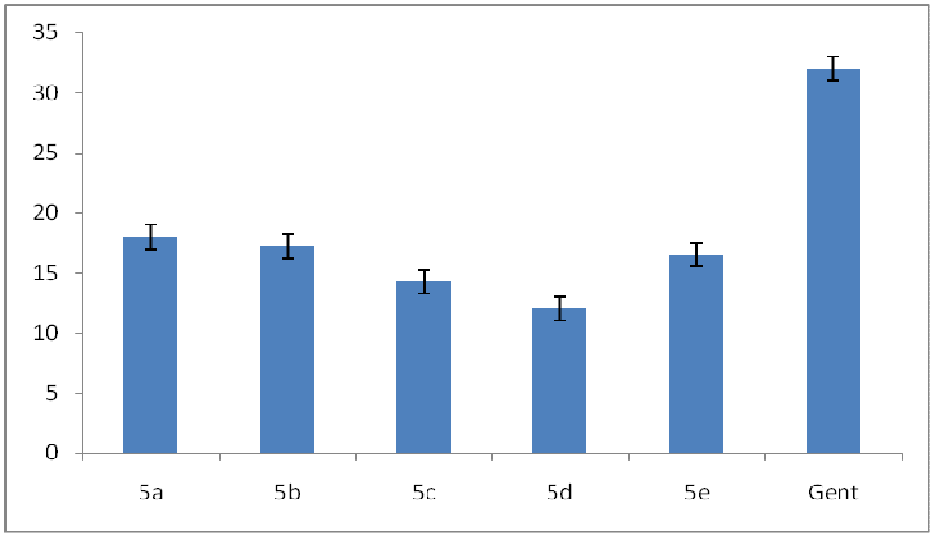


Fig 5.8: Antimicrobial activity of test compounds against B. subtilis

In case of P. aeruginosa, it was observed that 5a was having maximum antimicrobial potential. Although in this case the compound 5a was significantly less effective ($p<0.05$) as compared to gentamicin. The compound 5e was less effective than 5a but this difference was non-significant ($p>0.05$). All other compounds i.e., 5b, 5c and 5d were significantly less effective than 5a ($p<0.05$). The comparison between zone of inhibition of the test compounds and B. subtilis are shown in figure 5.9.

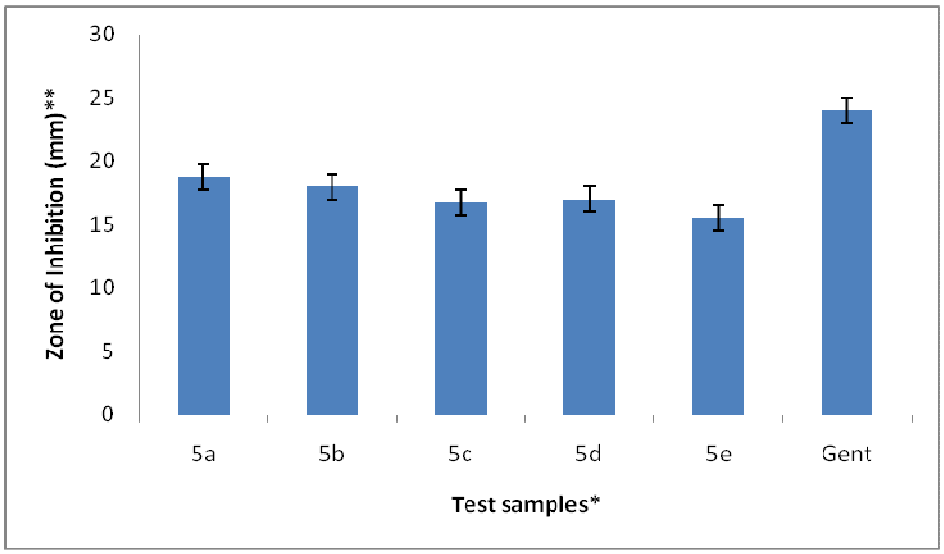


Fig 5.9: Antimicrobial activity of test compounds against P. aeruginosa

When zone of inhibition due to different compounds was compared for S. typhae, it was found that 5b was having maximum activity. Although in this case the compound 5b was significantly less effective ($p<0.05$) as compared to gentamicin. Compound 5c was less effective than 5b but this difference was non-significant ($p>0.05$). All other samples i.e., 5a, 5d and 5e were significantly less effective than 5b ($p<0.05$). The comparison between zone of inhibition of the test compounds and S. typhae are shown in figure 5.10.

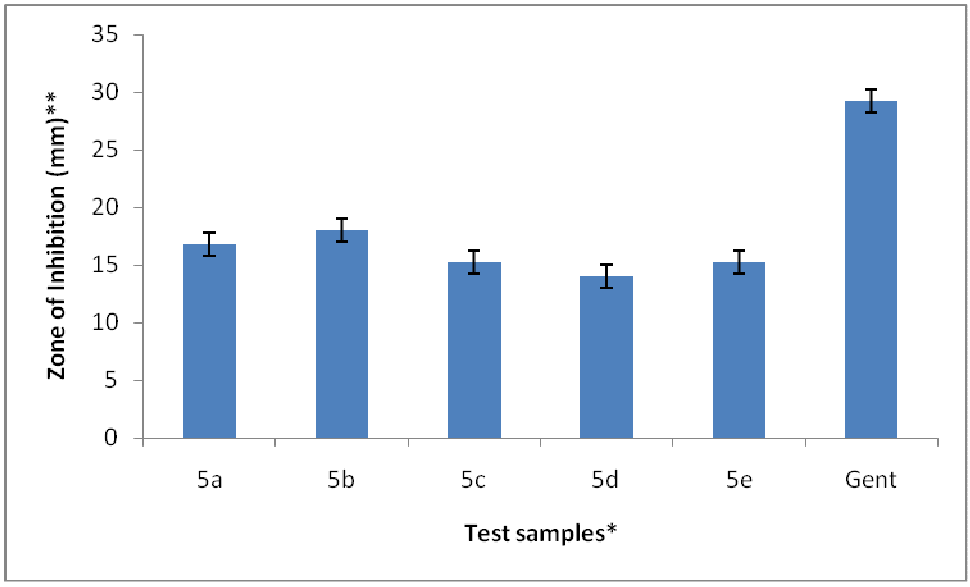


Fig 5.10: Antimicrobial activity of test compounds against S. typhae

When zone of inhibition due to different compounds was compared for S. cohini, it was found that 5a was having maximum activity. Although in this case the compound 5a was significantly less effective ($p<0.05$) as compared to gentamicin. Compound 5c was less effective than 5a but this difference was non-significant ($p>0.05$). All other samples i.e., 5b, 5d and 5e were significantly less effective than 5b ($p<0.05$). The comparison between zone of inhibition of the test compounds and B. subtilis are shown in figure 5.11.

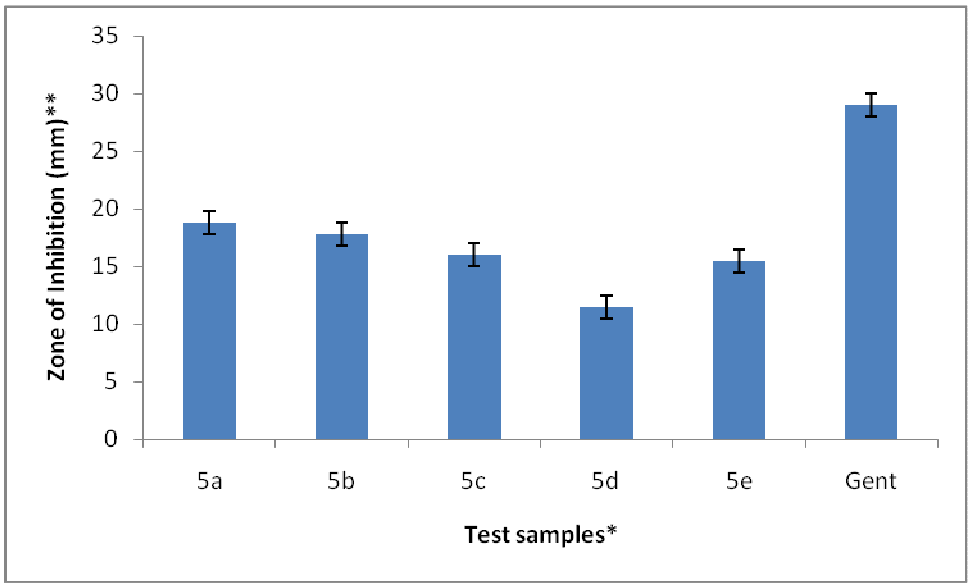


Fig 5.11: Antimicrobial activity of test compounds against S. cohini

In case of S. aureus, it was observed that 5b was having maximum antimicrobial potential. The effect of 5c, 5d and 5e was significantly less ($p<0.05$) as compared to 5b. Although 5a was showing less zone of inhibition as compared to 5b but the variation was non-significant ($p>0.05$). The effect of 5b was significantly less ($p<0.05$) as compared to gentamicin, the standard drug used in the present study. The comparison between zone of inhibition of the test compounds and E.coli are shown in figure 5.12.

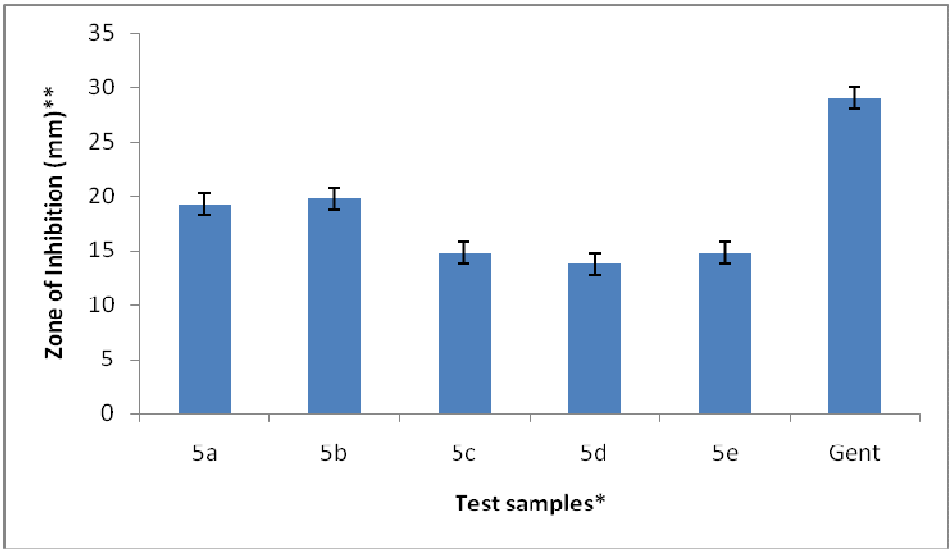
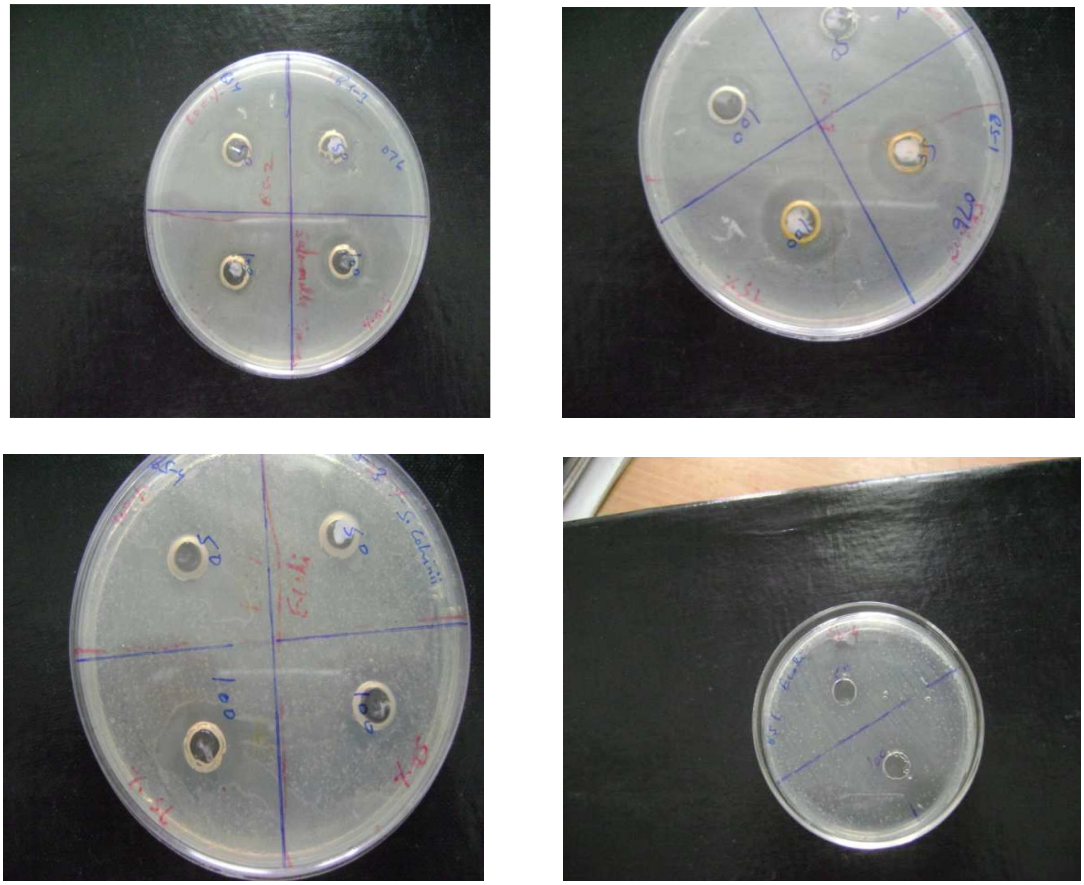


Fig 5.12: Antimicrobial activity of test compounds against S. aureus



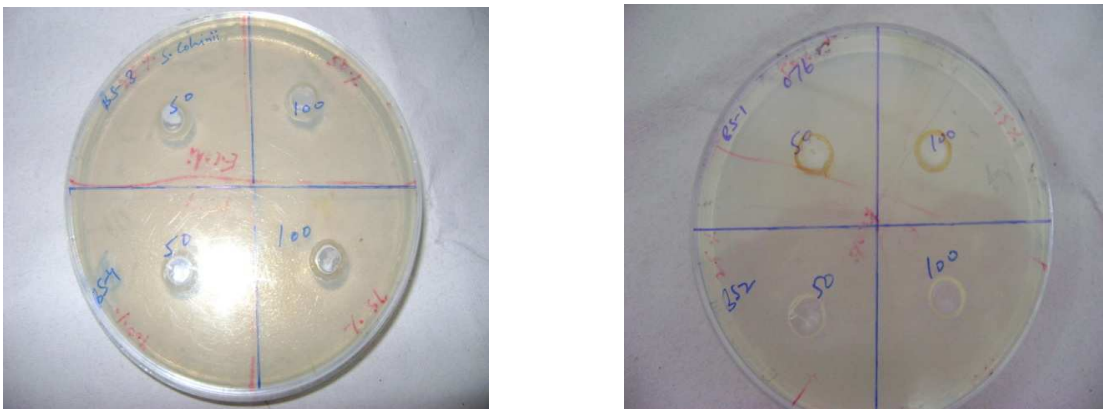


Fig 5.13: The petriplates showing zone of inhibition by well diffusion method.

RESULTS AND DISCUSSION

The benzisoxazole derivatives were synthesized by Claisen Schmidt condensation reaction and the products were confirmed by chromatographic and spectral data. The melting point of all compounds was observed different from ingredients melting point which was confirmed the synthesis of product. The purity of synthesized compounds was checked by observing single spot on TLC plate. All synthesized compounds was gave only single spot. It means all synthesized compounds were obtained in pure form. The melting point of all synthesized compounds are given in Table 5.3. The structure of synthesized compounds was determined by spectral analysis. The λmax of synthesized compounds was observed at range between 220-280nm. This range of λmax was showed the presence of α, β-unsaturated carbonyl moiety. IR spectrum of 5a-e showed a strong band near 1530-1550 cm^{-1} indicates the presence of >C=N of isoxazole ring. IR spectrum showed two strong absorptions at 3020 and 1620 cm^{-1} indicates the presence of C-H stretching and C=C stretching of aromatic ring respectively. A band near 2800-2900 and 3100 cm^{-1} indicates the presence of C-H stretching and C= C Stretching of aliphatic ring respectively. A band near 3373 cm^{-1} shows N-H stretching. The structure is further supported by ^1H NMR which shows the presence of amino NH peak at δ 4.0. It also shows the presence of ethylenic double bond, a peak in the region δ 7.6 (s, H, $-CH_A=CH$) and δ 2.3-2.8 (q1 $^1HCH=CH_B$). Mass spectroscopy helps to find the molecular weight of the synthesized compounds. The benzisoxazole derivatives showed the molecular ion peak that equivalent to the molecular weight of proposed compound. Hence m/z value confirms the molecular weight of the respective synthesized compounds. The anti-microbial activity of all synthesized compounds (5a-e) were studied against E.coli, Salmonella typhi, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and S. cohini by agar well diffusion method.

Table 5.3: Physicochemical properties of a series of benzisoxazole derivatives

S. No.	Compound	R-moiety	Appearance	Molecular formula	Yield (%)	Melting point range (°C)
1	BI-1	Acetaldehyde	Brownish crystalline	$C_{10}H_{10}N_2O$	52.4	165-168
2	BI-2	4-fluoro benzaldehyde	Yellowish crystalline	$C_{15}H_{11}FN_2O$	58.8	160-162
3	BI-3	pyridine 2-carbaldehyde	Brownish crystalline	$C_{14}H_{11}N_3O$	62.1	152-156
4	BI-4	4-methoxy benzaldehyde	Brown	$C_{16}H_{14}N_2O_2$	56.7	158-160
5	BI-5	Benzaldehyde	Brown	-	60	148-149

CONCLUSION

In conclusion, we have discovered a novel series of 3-substituted 1,2-benzisoxazole and evaluated them for the antimicrobial activity invitro. E. coli, P. aeruginosa, S. typhae are gram negative bacteria and S. aureus, B. subtilis, S. cohini are gram positive bacteria. All the synthesized compounds were showing significant antimicrobial activity against all the test microorganisms. Among the tested compounds, 5a and 5b had found to be shown relatively better antimicrobial activity. The effect of 5c, 5d and 5e was significantly less ($p<0.05$) as compared to that of 5a and 5b. Although the effect of 5a and 5b were significantly less ($p<0.05$) as compared to gentamicin, the standard drug used in the present study. Thus from the above experiment it can be concluded that all test compounds possess broad spectrum antibiotic property.

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