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Research Article

Screening and Evaluation of antibacterial and antifungal activities of substituted Pyrazole moieties, Oxadiazoles and Imidazol ethanones

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In this paper we concentrate on antibacterial and antifungal activities of synthesized quinoline derivatives containing pyrazole moieties, oxadiazole and imidazol ethanones.The respective clinical strain was spread separately on the Mueller-Hinton broth medium for antibacterial activity and Sabouraud dextrose agar (SDA) broth for antifungal activity. Then 2 µL of test organism suspension was added and incubated at 37ºC for 24 hr. for bacteria and 48 hr. for fungi studies. The drugs Ofloxacin and Fluconazole were used as standards for comparison of antibacterial and antifungal activities respectively. The Minimum Inhibitory Concentration (MIC) was the lowest concentration of test compound that inhibit the visible growth of the organism and was determined in triplicates and mean values were taken.

Keywords: Pyrazole, oxadiazoles and imidazol ethanones, SDA, biological activity.

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CHEMISTRY RESEARCH ARTICLE

Screening and Evaluation of antibacterial and antifungal activities of substituted Pyrazole moieties, Oxadiazoles and Imidazol ethanones

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ABSTRACT

In this paper we concentrate on antibacterial and antifungal activities of synthesized quinoline derivatives containing pyrazole moieties, oxadiazole and imidazol ethanones.The respective clinical strain was spread separately on the Mueller-Hinton broth medium for antibacterial activity and Sabouraud dextrose agar (SDA) broth for antifungal activity. Then 2 µL of test organism suspension was added and incubated at 37ºC for 24 hr. for bacteria and 48 hr. for fungi studies. The drugs Ofloxacin and Fluconazole were used as standards for comparison of antibacterial and antifungal activities respectively. The Minimum Inhibitory Concentration (MIC) was the lowest concentration of test compound that inhibit the visible growth of the organism and was determined in triplicates and mean values were taken.

Keywords: Pyrazole, oxadiazoles and imidazol ethanones, SDA, biological activity.

1. INTRODUCTION

One of the major causes for the progress of chemistry of benzimidazoles, oxadiazoles, pyrazoles and pyrazolin-5-ones is the association of these moieties with various biological activities. There are some examples of drugs containing these nuclei being used for the treatment of various diseases. Considering the scope of these derivatives in the drug discovery and their importance in medicinal field, the present investigation is focused on the synthesis and biological screening of title molecules. Some new routes for the synthesis of these derivatives have been developed and the target molecules have been screened for biological activities. Some of the synthesized compounds exhibited potent biological activities.

2. MATERIALS AND METHOD

The antibacterial and antifungal activities of the synthesized compounds were examined by cup plate agar disc diffusion method[1] against the following bacterial strains: *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and fungi *Aspergillus niger, Ustilago maydis,* as compared to the standard drugs Gentamicin and Nystatin for bacterial and fungal growth respectively.

Petri dishes, Whatman No.1 filter paper, autoclave, micropipette, DMSO, Gentamicin, Nystatin, agar-agar, beef extract, peptone, sodium chloride, dextrose, distilled water, bacterial and fungal cultures. The antimicrobial activities of synthesized compounds were carried by disc diffusion method using nutrient agar medium (NAM) for bacterial and potato dextrose agar (PDA) medium for fungal cultures respectively. NAM was prepared with beef extract (3 g), peptone (5 g), NaCl (5 g) and agar-agar (15 g) in 1000 mL distilled water and pH was adjusted to 7.0. PDA was prepared by adding dextrose (20 g), agar-agar (15 g) to potato infusion (1000 mL) and pH was adjusted to 5.5. Potato infusion was made by boiling 200 grams of sliced potatoes in distilled water for 30 minutes and then filtered through Whatman No.1 filter paper and filtrate was made up to 1 litre with distilled water. Both the media were sterilized in an autoclave at 121ºC, 15 lbs pressure for 30 minutes. After sterilization, 20 mL o both media were poured into petri dishes in a laminar air flow and allowed to solidify.

After solidification, the NAM was inoculated with 100 µL of desired bacteria and PDA was inoculated with 100 µL of desired fungi.

Compounds were dissolved in DMSO with a concentration of 100, 500, 1000 µg/mL and Whatman No.1 filter paper discs were placed in the solution and kept for one minute. After drying, the disks were placed on NAM and PDA inoculated with bacteria or fungi and NAM plates were incubated at 37°C and PDA plates at 30°C. Zone of inhibition was measured after 24 hr. and compared with standard drugs Gentamicin and Nystatin for bacterial and fungal growth respectively. The experiments were repeated thrice and mean values of the radius of zone of inhibition were measured.

Test tubes (10mL), micropipette, Whatman No.1 filter paper, DMSO, Oflaxacin, Fluconazole, Mueller-Hinton broth[7-8], Sabouraud dextrose agar (SDA) broth, distilled water, bacterial and fungal cultures. Following common standard strains were used for screening of antibacterial and antifungal activities: *Staphylococcus aureus*, *Escherichia coli* and fungi *Aspergillus niger*. DMSO was used as diluent to get desired concentration of synthesized compounds to test upon standard bacterial strains. Each synthesized compound was diluted for obtaining 2000 µg/mL concentration, as a stock solution. In primary screening 1000 µg/mL concentrations of the synthesized compounds were taken. The synthesized compounds found active in this primary screening were further tested in a second set of dilution against all microorganisms. The compounds found active in primary screening were similarly diluted to obtain 500, 200, 100, 87.5, 75, 62.5, 50, 37.5, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.19 and 0.09 µg/mL and 2 m of these solutions were taken in test tubes. The highest dilution showing at least 99% inhibition zone was taken as MIC. The results of this were much affected by the size of the inoculums. The test mixture should contain 10^8 microorganism/mL. The Minimum Inhibitory Concentration of the compounds was determined by broth dilution method.

The respective clinical strain was spread separately on the Mueller-Hinton broth medium for antibacterial activity and Sabouraud dextrose agar (SDA) broth for antifungal activity. Then 2 µL of test organism suspension was added and incubated at 37ºC for 24 hr. for bacteria and 48 hr. for fungi studies. The drugs Ofloxacin and Fluconazole were used as standards for comparison of antibacterial and antifungal activities respectively. The Minimum Inhibitory Concentration (MIC) was the lowest concentration of test compound that inhibit the visible growth of the organism and was determined in triplicates and mean values were taken.

3. RESULTS AND DISCUSSION

Antibacterial and antifungal activities of 4-(substitutedbenzylidene)-3-methyl-1-(2- (quinolin-8-yloxy) acetyl)-1*H*-pyrazol-5(4*H*)-ones 5a-i:

The Minimum Inhibitory Concentration of the compounds **5a-i** was determined by Serial broth dilution method. The drugs Ofloxacin and Fluconazole were used as standards for comparison of antibacterial and antifungal activities respectively. The results are presented in Table 3.6.

The newly synthesized quinoline derivatives containing pyrazole moiety were found potent in the concentration range 100 – 50 µg/mL compared to the standard drugs, 0.19 µg/mL for Ofloxacin and 1.56 µg/mL for Fluconazole. The compounds 5e and 5h were more potent against *Staphylococcus ureus* and 5c and 5f have moderate potencies. Compound **5a, 5b, 5d, 5g** and **5i** were weakly potent towards *S. aureus.* Compound **5i** was more potent towards *Escherichia coli* and **5a, 5b, 5d** and **5h** have moderate potencies. Compounds **5c, 5e, 5f** and **5g** were weakly potent towards *E. coli.*

Antibacterial and antifungal activities of 5-((2-(substituted phenoxymethyl) -1*H* benzo[*d*]imidazol-1-yl) methyl)-1, 3, 4 oxadiazole-2-thiols (6a-f):

The antimicrobial activities of synthesized compounds **3a-f, 4a-f, 5a-f** and **6a-f** were carried by disc diffusion method using nutrient agar medium (NAM) for bacterial and potato dextrose agar (PDA) medium for fungal cultures respectively. The results are presented in Table 3.1 & 3.2.

Compounds **5a, 5b, 5c, 5d, 6a, 6d, 6e** and **6f** were found moderately potent and compounds **3a-f**, **4a-f** and **6c** were found weakly potent. Compounds **5a, 5d, 5e** and **5f** have potencies that were equal to Gentamicin against *Pseudomonas aeruginosa*. Compounds **5b** and **6a-f** were found moderately potent and compounds **3a-f** and **4a-f** were found weakly potent. Compounds **5e** and **5f** have potencies that were equal to Gentamicin against *Salmonella typhi*. Compounds **5a, 5b, 5c, 5d, 6a, 6b, 6d, 6e** and **6f** were found moderately potent and compounds **3a-e**, **4a-e** and **6c** were found weakly potent.

Finally we conclude that the substituted phenoxymethyl group placed at the position-2 of benzimidazole moiety influence the biological activities very significantly. Further the present investigations reveal that a hydrazide moiety at position-1 of benzimidazole moiety enhance the antibacterial activity much more than an oxadiazole ring indicated by the results that compounds **5a-f** investigated presently are more potent than the compounds **6a-f**.

Antibacterial and antifungal activities of 1-(substituted 3, 5-diphenyl-4, and 5-dihydro-1*H*-pyrazol-1-yl)-2-(quinolin-8-yloxy) ethanones (7a-o):

The Minimum Inhibitory Concentration of the compounds **7a-o** was determined by Serial broth dilution method. The drugs Ofloxacin and Fluconazole were used as standards for comparison of antibacterial and antifungal activities respectively. The results are presented in Table 3.5.

The newly synthesized quinoline derivatives containing pyrazole moiety were found potent in the concentration range 100 – 62.5 µg/mL compared to the standard drugs, 0.19 µg/mL for Ofloxacin and 1.56 µg/mL for Fluconazole.

Compound **7n** was weakly potent towards *Staphylococcus aureus.* Compound 7e was more potent towards *Escherichia coli* and **7d, 7i, 7j, 7n** and **7o** have moderate potencies. Compounds **7a, 7b, 7c, 7f, 7g, 7h, 7k, 7l** and **7m** were weakly potent towards *E. coli.*But only few compounds showed significant antifungal inhibition with **7d, 7n** and **7o** being more potent whereas **7e, 7i** and **7j** were weakly potent towards *Aspergillus niger.*

Antibacterial and antifungal activities of 3-methyl-4-(2-substituted phenylhydrazono)-1-(2-(2-((p-tolyloxy)methyl)-1*H*-benzo[*d*]imidazol-1 yl)acetyl)-1*H*-pyrazol-5(4*H*)-ones (10a-i) and 1-(3,5-dimethyl-4-(substituted phenyldiazenyl)-1*H*-yrazol-1-yl)-2-(2-((p-tolyloxy)methyl)-1*H*-benzo[*d*]imidazol-1-yl) ethanones (11a-i):

The antimicrobial activities of synthesized compounds **(10a-i)** and **(11a-i)** were carried by disc diffusion method using nutrient agar medium (NAM) for bacterial and potato dextrose agar (PDA) medium for fungal cultures respectively. The results are presented in Table 3.3 & 3.4.

Compounds **10b, 10c, 10e, 11b, 11g** and **11h** have potencies that were equal to Gentamicin against *S. aureus.* Compounds **10a, 10i, 11a, 11c** and **11i** were found weakly potent. Compounds **10d, 10f, 10g, 11d** and **11f** were found to be more potent than other compounds against *Bacillus subtilis* as compared to the control Gentamicin. Compounds **10b, 10c, 10e, 10h, 10i, 11b, 11e** and **11g** have potencies that were equal to Gentamicin against *Bacillus subtilis.*

Compounds **10a, 11a, 11c, 11h** and **11i** were found weakly potent. Compounds **10c, 10d, 10e, 10f, 11d, 11e, 11f** and **11g** were more potent against *Escherichia coli* and compounds **10h, 10i, 11b, 11c, 11h** and **11i** have potencies that were equal to Gentamicin against *Escherichia coli.* Compounds **10a** and **11a** were found weakly pot Compounds **10c, 10f, 10g, 11d, 11e, 11f** and **11g** were more potent against *Pseudomonas aeruginosa* whereas the compounds **10b, 10d, 10e, 10h, 10i, 11g** and **11h** were moderately potent. Compounds **10a, 11a, 11h** and **11i** were weakly potent against *Pseudomonas aeruginosa*.

4. CONCLUSION

Antibacterial and anti-fungal activities of various compounds from results we conclude that, The compounds showed significant antifungal inhibition with **5e** and **5d** being more potent and **5c, 5f** and **5g** were weakly potent towards *Staphylococcus aureus* The investigations revealed that compounds **5e** and **5f** have potencies that were equal to Gentamicin against *S. aureus, Bacillus subtilis* and *E. coli*. The compounds **7a, 7b, 7c, 7g, 7h, 7k, 7l** and **7m** were more potent against *Staphylococcus aureus* and **7d, 7e, 7f, 7i, 7j** and **7o** have moderate potencies. Compounds **10f, 10g, 11d, 11e** and **11f** were found to be more potent than other compounds against *S. aureus* as compared to the control Gentamicin. The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. But only few compounds exhibit significant antifungal inhibition.

Table 3.1 *Invitro* antibacterial activity results

Compound	Radius of zone of inhibition, (mm) measured after 24 hr. (concentrations in µg/mL)														
	Gram-positive organisms						Gram-negative organisms								
	Stapylococcus aureus			*Bacillus subtilis*			*Escheritia coli*			*Pseudomonas aeruginosa*			*Salmonella typhi*		
	1000	500	100	1000	500	100	1000	500	100	1000	500	100	1000	500	100
3a	2.4	1.4	0.3	2.3	1.3	0.3	2.3	1.2	0.3	2.1	1.6	0.4	2.0	1.3	0.5
3b	2.4	1.3	0.2	2.1	1.1	0.2	2.1	1.1	0.3	2.1	1.6	0.3	1.8	1.2	0.3
3c	2.3	1.4	0.2	2.1	1.2	0.3	2.0	1.0	0.2	1.9	1.5	0.2	1.7	1.2	0.2
3d	2.3	1.4	0.3	2.3	1.2	0.3	2.3	1.2	0.3	2.0	1.5	0.2	1.9	1.3	0.4
3e	2.6	1.6	0.4	2.6	1.5	0.4	2.5	1.3	0.4	2.1	1.6	0.4	2.1	1.4	0.5
3f	2.8	1.8	0.4	2.7	1.6	0.4	2.8	1.5	0.5	2.3	1.8	0.5	2.1	1.5	0.6
4a	2.3	1.5	0.3	2.4	1.6	0.3	2.0	0.9	0.3	2.1	1.1	0.3	1.8	1.0	0.3
4b	2.3	1.5	0.2	2.1	1.4	0.2	1.8	0.8	0.2	2.0	1.1	0.3	1.7	0.9	0.3
4c	2.1	1.4	0.2	2.2	1.4	0.2	1.7	0.6	0.1	1.9	1.0	0.3	1.6	0.9	0.2
4d	2.2	1.4	0.3	2.3	1.5	0.3	1.9	0.9	0.2	2.1	1.2	0.4	1.8	1.0	0.3
4e	2.4	1.6	0.3	2.5	1.5	0.3	2.0	1.0	0.3	2.1	1.3	0.4	1.9	1.1	0.3
4f	2.5	1.6	0.4	2.6	1.9	0.4	2.1	1.1	0.3	2.2	1.4	0.5	1.9	1.2	0.4
5a	3.7	2.1	0.7	3.9	2.2	0.5	3.7	2.0	0.6	3.5	1.8	0.6	2.9	1.9	0.6
5b	3.8	2.2	0.6	3.5	2.0	0.4	3.6	1.8	0.5	3.5	1.6	0.5	2.8	1.9	0.5
5c	3.5	1.9	0.6	3.4	1.8	0.5	3.5	2.0	0.6	3.4	1.5	0.6	2.6	1.8	0.6
5d	3.6	2.1	0.5	3.8	2.2	0.6	3.6	2.1	0.6	3.5	1.9	0.7	2.9	2.1	0.7
5e	4.0	2.9	0.9	4.2	2.8	0.8	3.8	2.2	0.8	3.6	2.0	0.7	3.2	2.2	1.1
5f	4.2	3.0	1.1	4.3	2.9	1.2	4.0	2.5	1.2	3.8	2.2	1.0	3.2	2.3	1.2
6a	3.1	2.1	0.5	3.2	1.9	0.5	2.9	1.7	0.5	3.0	1.9	0.6	2.6	1.6	0.6
6b	3.0	2.1	0.4	2.9	1.7	0.3	2.7	1.3	0.4	2.9	1.8	0.4	2.5	1.6	0.4
6c	2.8	1.6	0.2	2.8	1.6	0.3	2.5	1.4	0.3	2.8	1.6	0.2	2.1	1.4	0.2
6d	2.9	1.8	0.4	3.1	1.8	0.4	2.8	1.6	0.4	3.0	1.8	0.5	2.4	1.5	0.3
6e	3.2	2.1	0.7	3.3	2.0	0.8	3.2	1.8	0.5	3.1	2.0	0.8	2.7	1.6	0.5
6f	3.4	2.3	0.9	3.5	2.1	0.8	3.4	1.9	0.8	3.2	2.2	0.9	2.8	1.8	0.6
Gentamicin	4.1	3.1	1.1	4.3	2.9	1.1	4.0	2.3	1.2	3.6	2.1	0.7	3.2	2.3	1.2
DMSO	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0

Table 3.2 *Invitro* antifungal activity results

Compound	Radius of zone of inhibition, (mm) measured after 24 hr. (Concentrations in µg/mL)					
	Fungi					
	Aspergillus niger			*Ustilago maydis*		
	1000	500	100	1000	500	100
3a	NA	NA	NA	NA	NA	NA
3b	NA	NA	NA	NA	NA	NA
3c	NA	NA	NA	NA	NA	NA
3d	NA	NA	NA	NA	NA	NA
3e	NA	NA	NA	NA	NA	NA
3f	NA	NA	NA	NA	NA	NA
4a	NA	NA	NA	NA	NA	NA
4b	NA	NA	NA	NA	NA	NA
4c	NA	NA	NA	NA	NA	NA
4d	NA	NA	NA	NA	NA	NA
4e	NA	NA	NA	NA	NA	NA
4f	NA	NA	NA	NA	NA	NA
5a	NA	NA	NA	NA	NA	NA
5b	NA	NA	NA	NA	NA	NA
5c	NA	NA	NA	NA	NA	NA
5d	NA	NA	NA	NA	NA	NA
5e	1.2	0.5	0.2	NA	NA	NA
5f	1.4	0.8	0.4	0.8	0.5	0.1

6a	NA	NA	NA	NA	NA	NA
6b	NA	NA	NA	NA	NA	NA
6c	NA	NA	NA	NA	NA	NA
6d	NA	NA	NA	'NA	NA	NA
6e	NA	NA	NA	NA	NA	NA
6f	1.2	0.5	0.2	NA	NA	NA
Nystatin	3.7	1.8	0.4	2.8	1.2	0.3
DMSO	0.0	0.0	0.0	0.0	0.0	0.0

NA – No activity

Table 3.4 *Invitro* antifungal activity results

Compound	*Radius of zone of inhibition, (mm) measured after 24 hr. (concentrations in µg/mL)*					
	Fungi					
	Aspergillus niger			*Ustilago maydis*		
	1000	500	100	1000	500	100
5	NA	NA	NA	NA	NA	NA
10a	NA	NA	NA	0.9	0.4	NA
10b	NA	NA	NA	NA	NA	NA
10c	NA	NA	NA	NA	NA	NA
10d	2.8	1.2	NA	2.5	1.2	0.6
10e	NA	NA	NA	NA	NA	NA
10f	1.6	0.5	NA	NA	NA	NA
10g	NA	NA	NA	NA	NA	NA
10h	NA	NA	NA	NA	NA	NA
10i	NA	NA	NA	NA	NA	NA
11a	NA	NA	NA	1.5	0.9	0.3
11b	NA	NA	NA	0.5	0.2	NA
11c	NA	NA	NA	NA	NA	NA
11d	2.1	1.0	0.3	NA	NA	NA
11e	NA	NA	NA	NA	NA	NA
11f	NA	NA	NA	2.0	0.9	0.3
11g	NA	NA	NA	NA	NA	NA
11h	NA	NA	NA	NA	NA	NA
11i	NA	NA	NA	NA	NA	NA
Nystatin	3.7	1.8	0.4	2.8	1.2	0.3
DMSO	0.0	0.0	0.0	0.0	0.0	0.0

NA – No activity

Table 3.5 Minimum Inhibitory Concentration (MICs) of 1-(substituted 3, 5-diphenyl-4, 5-dihydro-1*H*-pyrazol-1-yl)-2-(quinolin-8-yloxy ethanones 7a-o

S. No.	Minimum Inhibitory Concentration (Concentration in µg/mL)			
	Compound	Gram-positive *Staphylococcus aureus*	Gram-negative *Escherichia coli*	Fungi *Aspergillus niger*
1	7a	62.5	100	NA
2	7b	75	87.5	NA
3	7c	75	87.5	NA
4	7d	87.5	75	75
5	7e	87.5	62	87.5
6	7f	87.5	100	NA
7	7g	75	87.5	NA
8	7h	75	87.5	NA
9	7i	87.5	75	87.5
10	7j	87.5	75	100
11	7k	62.5	100	NA
12	7l	75	87.5	NA
13	7m	75	87.5	NA
14	7n	100	75	75
15	7o	87.5	75	75
16	Ofloxacin	0.19	0.16	
17	Fluconazole			1.56

Table 3.6 Minimum Inhibitory Concentration (MICs) of 4-(substituted benzylidene)-3-methyl-1-(2-(quinolin-8-yloxy) acetyl)-1*H*-pyrazol-5(4*H*)-ones 5a-i

S.No	Minimum Inhibitory Concentration (Concentration in µg/mL)			
	Compound	Gram-positive *Staphylococcus aureus*	Gram-negative *Escherichia coli*	Fungi *Aspergillus*
1	5a	100	87	_
2	5b	100	75	_
3	5c	75	100	100
4	5d	100	75	_
5	5e	62.5	100	75
6	5f	75	100	100
7	5g	100	100	100
8	5h	50	75	_
9	5i	100	62.5	62.5
10	Ofloxacin	0.19	0.19	
11	Fluconazole			1.56

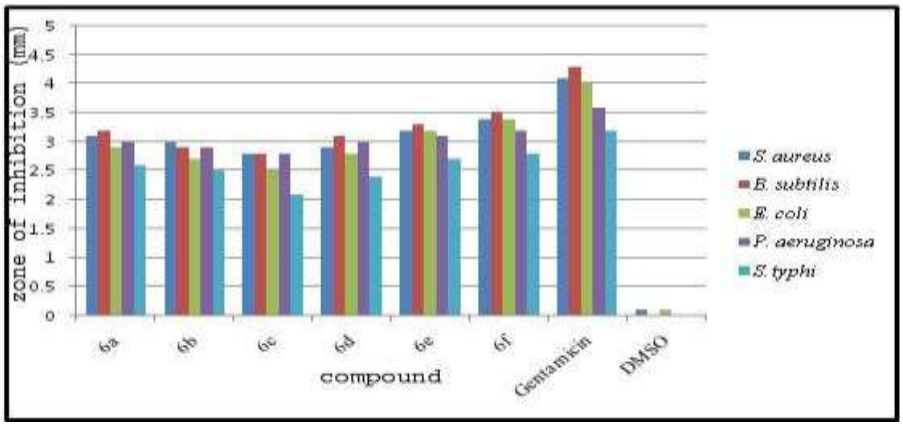

Figure 3.1 Histogram showing antibacterial activity of compounds 6a-f at a concentration of 1000 µg/mL

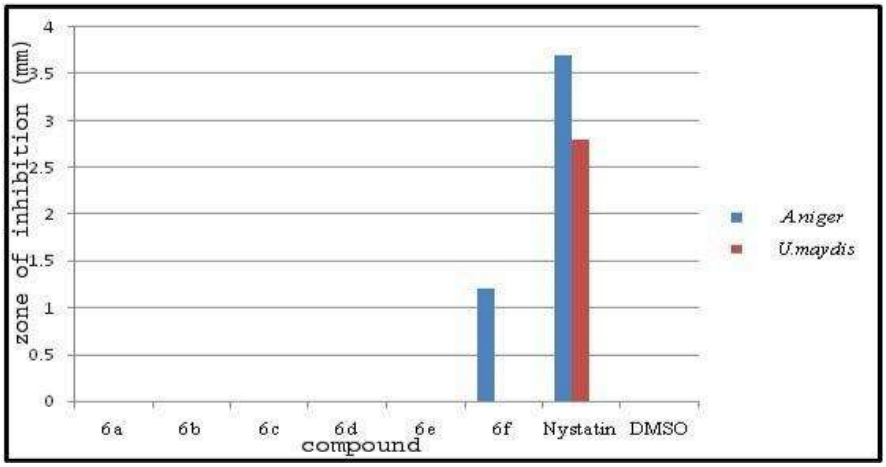

Figure 3.2 Histogram showing antifungal activity of compounds 6a-f at a concentration of 1000 µg/mL

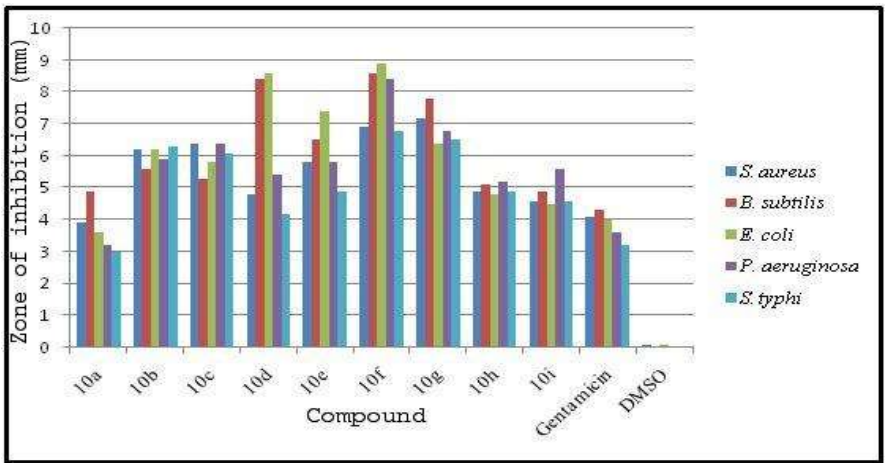

Figure 3.3 Histogram showing antibacterial activity of compounds 10a-i at a concentration of 1000 µg/mL

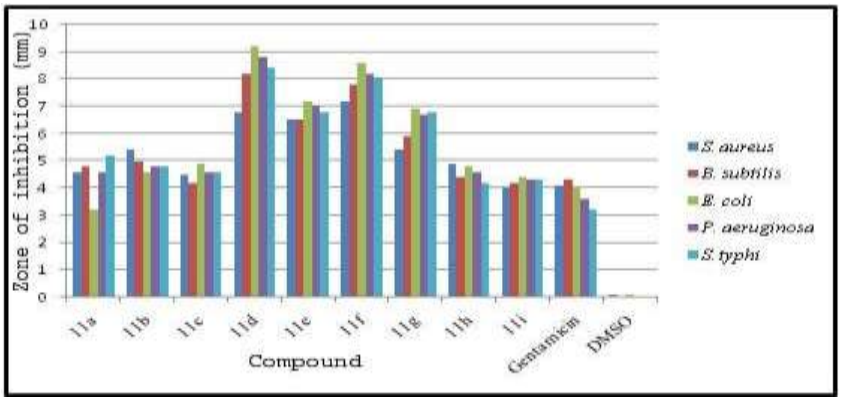

Figure 3.4 Histogram showing antibacterial activity of compounds 11a-i at a concentration of 1000 µg/mL

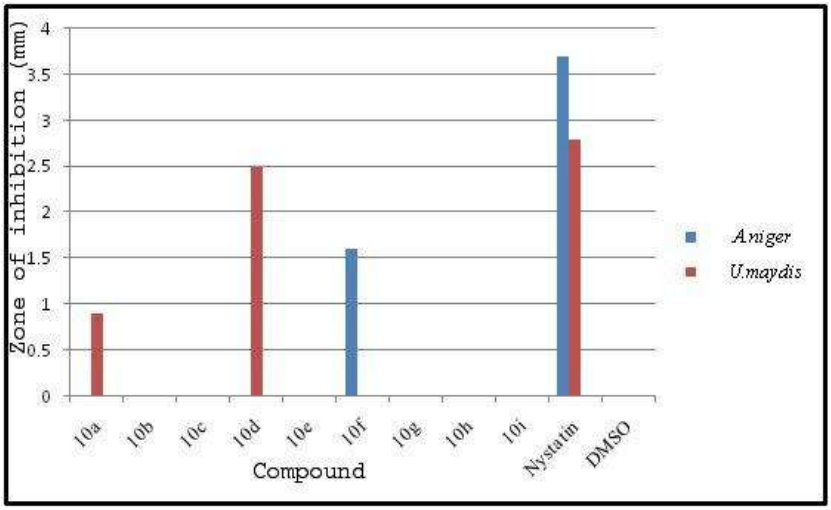

Figure 3.5 Histogram showing antifungal activity of compounds 10a-i at a concentration of 1000 µg/mL

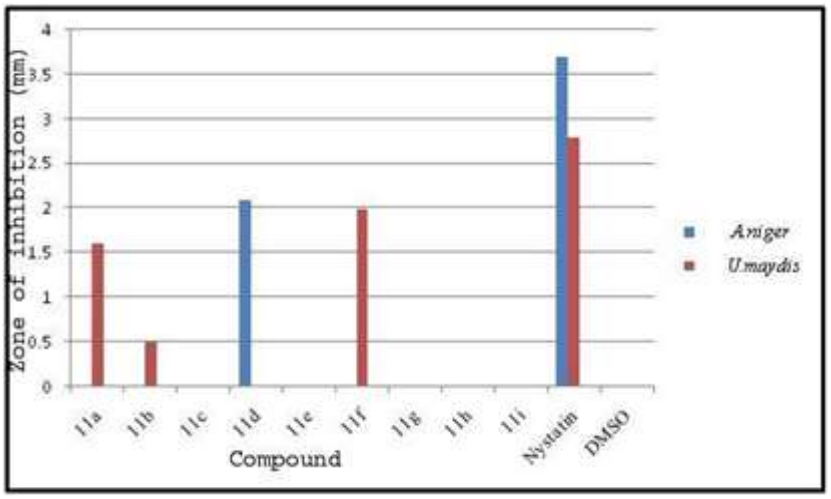

Figure 3.6 Histogram showing antifungal activity of compounds 11a-i at a concentration of 1000 µg/mL

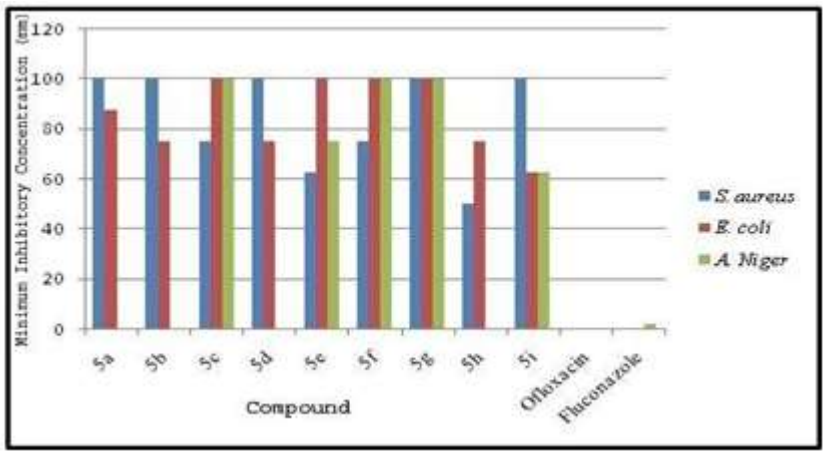

Figure 3.7 Histogram showing antimicrobial activity of compounds 5a-i

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