Journal of Cell and Tissue Research Vol. 10(2) 2243-2250 (2010)
ISSN: 0974- 0910 *(Available online at www.tcrjournals.com)* Original Article

EVALUATIONS OF ANTIMICROBIAL ACTIVITY OF SOME PHARMACOLOGICAL IMPORTANT DIHYDROPYRIMIDINES COMPOUNDS*

JOSHI, K. K.,[1]? AKBARI, J.[2] AND JOSHI, H. S.[2]

[1]Department of Biotechnology, V.V. P. Engineering College, Rajkot 360 005; [2]Department of Chemistry, Saurashtra University, Rajkot 360 005. E. mail: krishnaonline06@gmail.com

Received: April 2, 2010: Revised: June 1, 2010: Accepted: June 12, 2010

Abstract: Antibiotics are the products of microbes that in dilute solution inhibit or kill other organisms. Antimicrobial agents include antibiotics produced by microbes and similar synthetic compounds that have the same effect. Naturally occurring antibiotics may be modified to give semi synthetic derivatives. In present study the effectiveness of a new synthetic compounds of dihydropyrimidines against a panel of pathogenic bacteria including Gram-positive and Gram-negative species, aerobes, facultative anaerobes, and obligate anaerobes have been analyzed. Pathogenic strains were isolated or obtained from the local pathology laboratory. Compounds were also subjected to find out its potency using broth dilution assay in combination with antibiotics. Study shows several of the synthetic compounds of dihydropyrimidines show antimicrobial activity and worth material for further investigation.

Key words: Dihydropyrimidines compounds, Antimicrobials

INTRODUCTION

Animicrobials are the substances that kill (microbicidal) or inhibit (microbistatic) multiplication or growth of microbes such as bacteria, fungi, or viruses or prevent their pathogenic actions. They differ in their physical, chemical, and pharmacological properties, antibacterial spectrum of activity and in their mechanism of action

Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism. Of course, in today's common usage, the term antibiotic is used to refer to almost any drug that cures a bacterial infection. Antimicrobials include not just antibiotics, but synthetically formed compounds as well. Penicillin represented the first true antibiotic: a substance produced by one microorganism that, in very small amounts, inhibits or kills other microorganisms.

However, the future effectiveness of antimicrobial therapy is somewhat in doubt because of bacterial resistance. Currently, bacterial resistance is combated by the discovery of new drugs. However, microorganisms are becoming resistant more quickly than new drugs are being found. Thus, future research in antimicrobial therapy may focus on finding how to overcome resistance to antimicrobials, or how to treat infections with alternative means. Bacteria are good targets for the activity of antimicrobial substances. Aspects of their metabolism are significantly different from that of humans. Antibiotics may act upon bacterial reactions that are not found in human cells. This provides the basis for the selective toxicity of antibiotics, affecting the bacteria but not the human host.

This research work is a part of Ph. D Thesis carried out in Department of Biochemistry, Saurashtra University, Rajkot from 2005 to 2009.

Table 1- Physical data and structure of DHPM A series: N-(4-clorophenyl)-6-methyl-4-aryl-2-thioxo- 1, 2, 3, 4-tetrahydropyrimidine-5-carboxamide

Code	Substitution R	Molecular Formula	Molecular Weight	Melting Point ?C	Rf Value	% Yield
DHPM A1	H	$C_{18}H_{16}ClN_3OS$	357.85	272-273	-	53
DHPM A2	4-OCH_3	$C_{19}H_{18}ClN_3O_2S$	387.88	240-241	-	52
DHPM A3	2-OCH_3	$C_{19}H_{18}ClN_3O_2S$	387.88	235-236	-	48
DHPM A4	3-NO_2	$C_{18}H_{15}ClN_4O_3S$	402.85	262-264	-	41

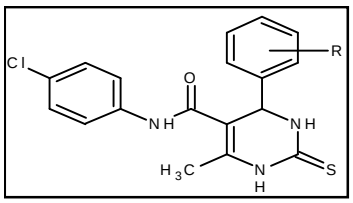


Table 2- Physical data and structure of DHPM B series: 3-isopropyl-4-aryl-1,4,5,7-tetrahydro- pyrazolo[3,4-d] pyrimidin-6-ones.

Code	Substitution R	Molecular Formula	Molecular Weight	Melting Point ?C	Rf Value	% Yield
DHPM B1	4-OCH_3	$C_{14}H_{16}N_4O_2$	286.32	271-273	-	24
DHPM B2	3,4-$(OCH_3)_2$	$C_{16}H_{20}N_4O_3$	316.35	251-253	-	18
DHPM B3	3-NO_2	$C_{14}H_{15}N_5O_3$	301.30	255-256	-	31

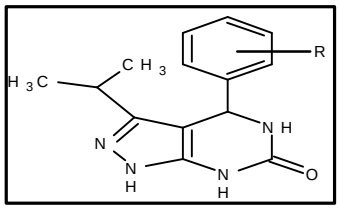


Table 3: Physical data and structure of DHPM C series: N-(4-chlorophenyl)-6-isopropyl-4-aryl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidine-carboxamide

Code	Substitution R	Molecular Formula	Molecular Weight	Melting Point ?C	Rf Value	% Yield
DHPM C1	H	$C_{20}H_{20}ClN_3OS$	385.91	280-282	0.53	27
DHPM C2	4-OCH_3	$C_{21}H_{22}ClN_3O_2S$	415.93	265-267	0.62	45
DHPM C3	3-NO_2	$C_{20}H_{19}ClN_4O_3S$	430.90	278-280	0.71	36
DHPM C4	4-Cl	$C_{20}H_{19}Cl_2N_3OS$	420.35	245-246	0.65	42
DHPM C5	3,4-$(OCH_3)_2$	$C_{22}H_{24}ClN_3O_3S$	445.96	251-253	0.68	51
DHPM C6	2,5-$(OCH_3)_2$	$C_{22}H_{24}ClN_3O_3S$	445.96	255-257	0.69	46
DHPM C7	2-OCH_3	$C_{21}H_{22}ClN_3O_2S$	415.93	278-279	0.60	55
DHPM C8	2-OH	$C_{20}H_{20}ClN_3O_2S$	401.90	280-281	0.70	38

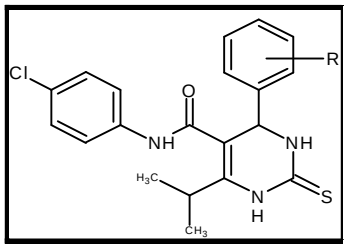
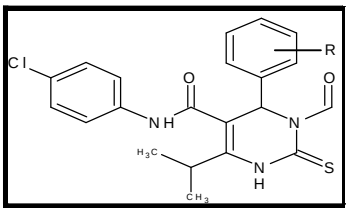


Table 4 - Physical data and structure of DHPM D series: N-(4-chlorophenyl)-3-formyl-6-isopropyl-4-aryl—thioxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxamide

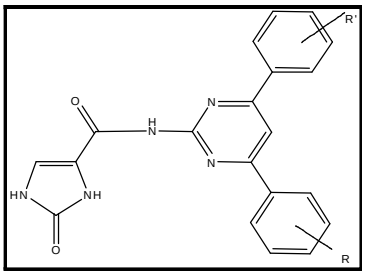
	Substitution R	Molecular Formula	Molecular Weight	Melting Point ?C	Rf Value	% Yield
DHPM D1	4-OCH_3	$C_{22}H_{22}ClN_3O_3S$	443.94	273-275	0.69	78
DHPM D2	4-Cl	$C_{21}H_{19}Cl_2N_3O_2S$	448.36	251-253	0.63	83
DHPM D3	H	$C_{21}H_{20}ClN_3O_2S$	413.92	288-290	0.54	72



Antimicrobial agents include antibiotics and synthetic compounds that have the same effect. Naturally occurring antibiotics may be modified to give semi synthetic derivatives. The development of antibiotics was carried out in parallel with the search for chemical antibacterial agents: artificial compounds that inhibit or kill microbes. The term antibiotic originally described only those formulations derived from living organisms but is now applied also to synthetic antimicrobials, such as the sulfonamides these often differ from their parent compound in their antimicrobial activity or their pharmacological properties. In the past decade dihydropyrimidines (DHPMs) and their derivatives have attracted considerable interest because of their promising activity as calcium channel blockers, antihypertensive agents, α- 1a-antagonists and neuropeptide Y (NPY) antagonists [1]. Fused pyrimidines are used in a variety of agrochemicals, natural and veterinary products [2-3]. Pyrimidine derivatives and heterocyclic annulated pyrimidines exhibit a wide variety of interesting biological effects such as antiproliferative[4], antiviral[5], antitumor[6], anti-inflammatory[7], antibacterial [8], antifungal[9],

Table 5: Physical data and structure of DHPM F series: *N*-(4, 6-diphenylpyrimidin-2-yl)-2-oxo-2, 3-dihydro-1*H*-imidazole-4-carboxamides

Codes	Substitution		Molecular formula	Molecular weight	Melting point (^{O}C)	Rf value	% of Yield
	R	R'					
DHPM F1	H	H	$C_{20}H_{15}N_5O_2$	357.36	146-148	0.43	43
DHPM F2	4-CH_3	4-F	$C_{20}H_{13}F_2N_5O_2$	393.34	212-214	0.47	54
DHPM F3	4-F	4-CH_3	$C_{21}H_{16}FN_5O_2$	389.38	196-198	0.52	48
DHPM F4	4-OCH_3	4-OCH_3	$C_{21}H_{17}N_5O_3$	398.36	166-168	0.57	48
DHPM F5	H	3,4,5-triOCH_3	$C_{23}H_{21}N_5O_5$	447.44	136-138	0.47	53
DHPM F6	3-NO_2	2-Cl	$C_{20}H_{13}ClN_6O_4$	36.80	156-158	0.49	45
DHPM F7	4-Cl	2-OH	$C_{20}H_{14}ClN_5O_3$	407.80	210-212	0.50	46
DHPM F8	2-Cl	2,5-di-OCH_3	$C_{22}H_{18}ClN_5O_4$	451.86	234-236	0.56	53
DHPM F9	4-Cl	2,5-di- OCH_3	$C_{22}H_{18}ClN_5O_4$	452.86	230-234	0.58	57
DHPM F10	3-NO_2	2,5-di- OCH_3	$C_{22}H_{18}N_6O_6$	462.41	198-200	0.41	43
DHPMF11	4-Br	4-OCH_3	$C_{21}H_{16}BrN_5O_3$	466.28	256-258	0.63	48
DHPM F12	H	4-CN	$C_{21}H_{14}N_6O_2$	382.37	178-180	0.47	51
DHPM F13	4-Cl	4-Cl	$C_{20}H_{13}ClN_5O_2$	426.25	226-228	0.40	*46*
DHPM F14	4-F	4-OCH_3	$C_{21}H_{16}FN_5O_3$	409.38	144-148	0.54	*52*
DHPM F15	4-Br	4-CH_3	$C_{21}H_{16}BrN_5O_2$	450.28	246-248	0.61	*55*
DHPM F16	H	4-OCH_3	$C_{22}H_{19}N_5O_4$	387.39	212-216	0.55	*46*
DHPM F17	4-F	H	$C_{20}H_{14}FN_5O_2$	375.35	208-212	0.63	*54*
DHPM F18	4-Cl	H	$C_{20}H_{14}ClN_5O_2$	391.81	186-188	0.46	*41*
DHPM F19	4-Br	H	$C_{20}H_{14}BrN_5O_2$	436.26	162-164	0.32	*56*
DHPM F20	4-CH_3	H	$C_{21}H_{17}N_5O_2$	371.39	188-190	0.49	*47*
DHPM F21	4-OCH_3	2-Cl	$C_{21}H_{16}ClN_5O_3$	421.83	266-268	0.54	*53*
DHPM F22	4-OCH_3	4-CN	$C_{22}H_{16}N_6O_3$	412.42	172-174	0.53	56



antitubercular[10], and antihistaminic[11] activities. Various substitutated pyrimidines are synthesized and examined for their antibacterial activity [12-14].

We embarked on the antibacterial activity of compounds having dihydropyrimidine moieties embedded in a joint molecular framework to improve specificity and efficacy of the scaffold against microorganisms.

MATERIALS AND METHODS:

Dihydropyrimidines compounds: The Dihydropyrimidines compounds (DHPM) of series A (N-(4-clorophenyl)-6-methyl-4-aryl-2-thioxo- 1, 2, 3, 4-tetrahydropyrimidine-5-carboxamide), DHPM B series (3-isopropyl-4-aryl-1,4,5,7-tetrahydro-pyrazolo[3,4-d] pyrimidin-6-ones), DHPM C series (N-(4-chlorophenyl)-6-isopropyl-4-aryl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidine-carboxamide), DHPM D series (N-(4-chlorophenyl)-3-formyl-6-isopropyl-4-aryl—thioxo-1,2,3,4-tetrahydro-5-pyrimidine-carboxamide) and DHPM F series (*N*-(4, 6-diphenylpyrimidin-2-yl)-2-oxo-2, 3-dihydro-1*H*-imidazole-4-carboxamides) were synthesized in Department of Chemistry, Saurashtra University, Rajkot by Akbari [15]. Details of these compounds are give in tables 1 to 5 along with their formulae.

Test microorganisms: Six microbial strains were obtained from American Type Culture Collection (ATCC) and 2 strains were clinically isolated, obtained from local pathology laboratory, Rajkot. Followings microbes were studied viz., a*) E. coli* ATCC 8739; b) *E. aerogens* ATCC 13048; c*) B. spinzizenii* ATCC 6633; d) *S. aureus* ATCC 5923; e) *P. auroginosa* ATCC 27853; f) DH5α (*E. coli)* (ampicilline resistant); g) *S. aureus* clinically isolated from pus h) *P. auroginosa* clinically isolated from sputum

Media used: Nutrient broth was used for the growth of the organisms. Composition of Nutrient Broth was i). peptic digest of animal tissue- 5 gm/liter, ii), sodium chloride - 5 gm/liter, iii), beef extract -1.5 gm/liter, iv), yeast extract - 1.5 gm/litre, pH between 7.2-7.4, at 25 ºC. N-agar plates were used for the primary screening of antimicrobial.

Preparation of compounds (samples) for antibacterial assay in primary screening: 100 mg/ 10ml concentration was made (solvent used were DMF/DMSO) and 100µl of this stock solution was then used for assay.

Microbiological assay used for primary screening:A loopful of isolated colony from plate was inoculated in 25ml of N-broth in a conical flask &

inoculated at room temperature on rotary shaker for 24 hours to activate the test culture. The Agar Dilution Method for determining antimicrobial susceptibility is a well-established technique [16]. The antimicrobial agent is incorporated into the agar medium with each plate containing a different concentration of the agent. The inocula can be applied rapidly and simultaneously to the agar surface using inoculum replicating apparatus [17-18].

N-agar media (Hi-media) was used and Petri dishes were made . Young culture of bacteria (100µl) was spreaded on it with spread plate method for better isolation of organism. On N-agar medium a ditch was made with the help of cup-borer (1.1 cm). The test compound was introduced into well and plates were then incubated overnight at 37°C. The experiment was performed under strict aseptic conditions. Microbial growth was determined by measuring the diameter of zone of inhibition in cm.

Primary antimicrobial screening of compounds: Agar dilution method. Well diameter: 1.1cm; Incubation hours: 24 hrs; Stock Concentration: 100mg/10ml; Working Concentration: 100µg/ 10µl; Inoculum added: 100 µl. (All values were measured in centimeter), (NT= Not Tested), Solvent: Dimethyl Formamide/DMSO.

RESULTS

Result of DHPM A series of compound (figure 1) showed that all the compounds were very effective against *B. spinzizenii* at the concentration of 100µg/ 10µl, zone diameter in the range of 0.9-2.7 cm. *E.aerogens* was sensitive to compound A3 (2.0 cm) zone diameter.$DH_5\alpha$ a ampicillin resistant strain of *E. coli* showed significant zone of inhibition against all of the compound in the range of 2.2 to 1.6 cm. Compare to the ATCC strain of *P. auroginosa,* clinical isolate showed less susceptibility against all the compounds in the range of 1.0 to 1.5 zone of inhibition except compound DHPM A2 showed similar inhibition to ATCC as well as clinical isolate strain. *S. aureus* showed good zone of inhibition against all the compounds. Compare to ATCC strain clinical isolate *S. aureus* showed even the similar result with good zone diameter in cm.

Figure 2 represents B series compounds of DHPM. Study shows that B1, B2, B3 and B9 were not at all affecting the growth of organism *E. coli, E. aerogens, B. spinizinenii* and $DH_5\alpha$ at the concentration of 100 µg/ml. *E. aerogens* also showed moderate susceptibility against comounds B4, B5, B6, B7, and B8. *B. spinzizenii* sensitive to the compound B4 and B5. But it showed less susceptibility toward compound B7 and B8. $DH_5\alpha$ showed moderate zone diameter against comound B5, B7 and B8. *P. aurogenosa* showed moderate susceptibility against compounds B2 and B3 and B4 compare to that with clinical isolate which also shows very moderate zone inhibition against compound B4 and B5. But it was suceptiable to compounds B7 and B8. In case of *S. aureus* ATCC strain showed more sensitivity than clincal isolate.

The result of DHPM C series are presented in Figure 3. It is evident from the data that C3 showed moderate to good zone diameter against the organisms. It showed good zone of inhibition against organism *E. aerogens* and *P. auroginosa*. *P.auroginosa* ATCC strain was moderate to good inhibited by all this series of compounds in terms of zone inhibition 1.2 to 2.9 in cm. compare it with clinical isolated strain which was also inhibited by C5, C6 and C7 with significant zone of inhibition. *S. aureus* was inhibited by compound C7, C8 and C9 with significant zone inhibition 2.0, 1.9 and 2.4 respectively. Compare it with clinical isolate which was completely inhibited by C5, C6, C7, C8, and C1 in terms of zone of inhibition 2.9, 2.5, 2.7, 3.0, 2.8 respectively.

Result of DHPM D series showed in figure 4. While analyzing the data it was found that compound DHPM D1 showed very good activity against *E. coli* and *B. spinzienii*. It showed 2.6cm zone of inhibition against *B. spinzienii*. Compound D2 showed good activity against *E. coli, B. spinzizeii* and *P. aurogenosa*. It was found that this compound almost equally inhibited both the ATCC and Clinical isolate of *P. aurogenosa*. Compound D3 showed significant inhibition toward *B.spinzizenii* in terms of 2.3 cm of zone diameter. It was inhibit the growth of *P. auroginosa* ATCC strain and *P. auroginosa* clinical isolate.

Figure 5 represents the data of DHPM F series. Data indicate that F1 showed very good activity against the entire organism except *B. spinzizenii*. It showed highest activity against *E. coli* and *S. aureus* ATCC strain. F2 showed highest activity against *E. coli, E. aerogens* and *B. spinzizenii*. Compound F3 showed very good activity against *E. aerogens* in terms of

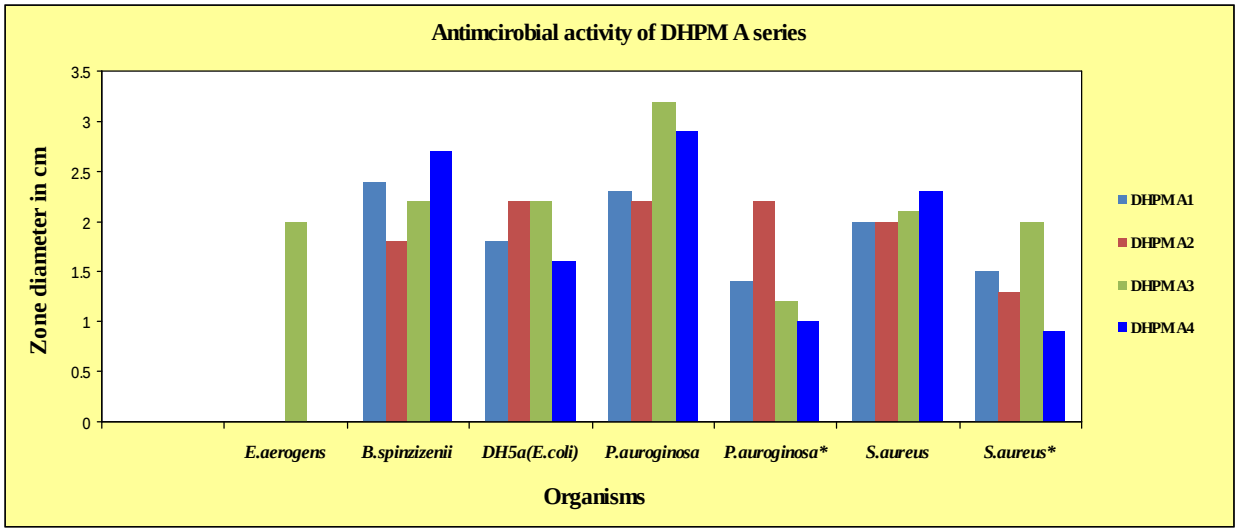


Fig.1: represents antimicrobial activity of DHPM A series of compounds in zone diameter in cm

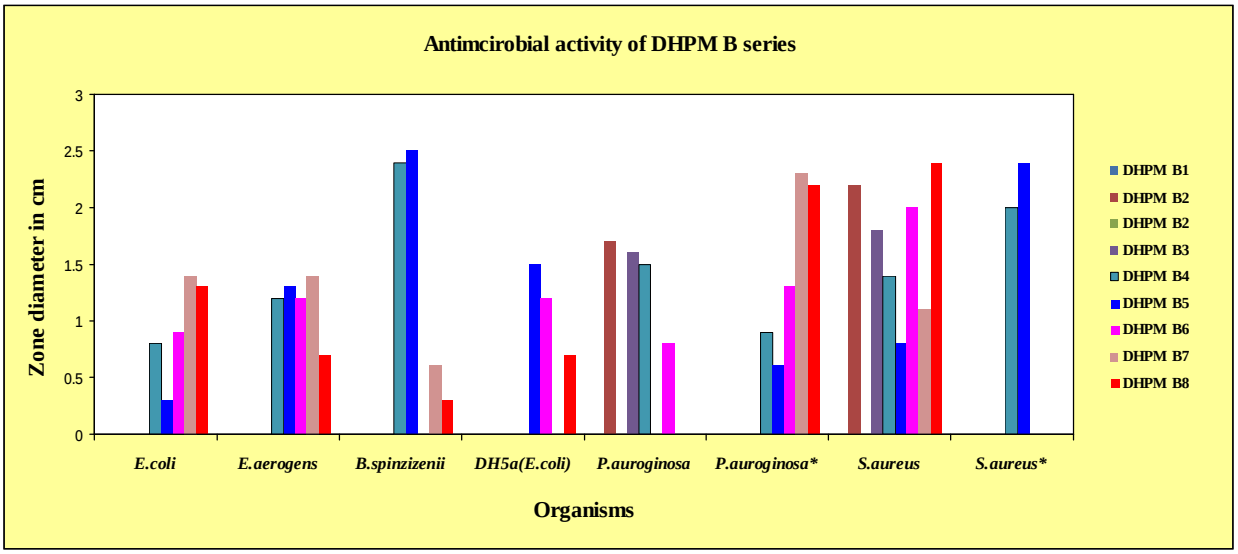


Fig. 2: represents antimicrobial activity of DHPM B series of compounds in zone diameter in cm

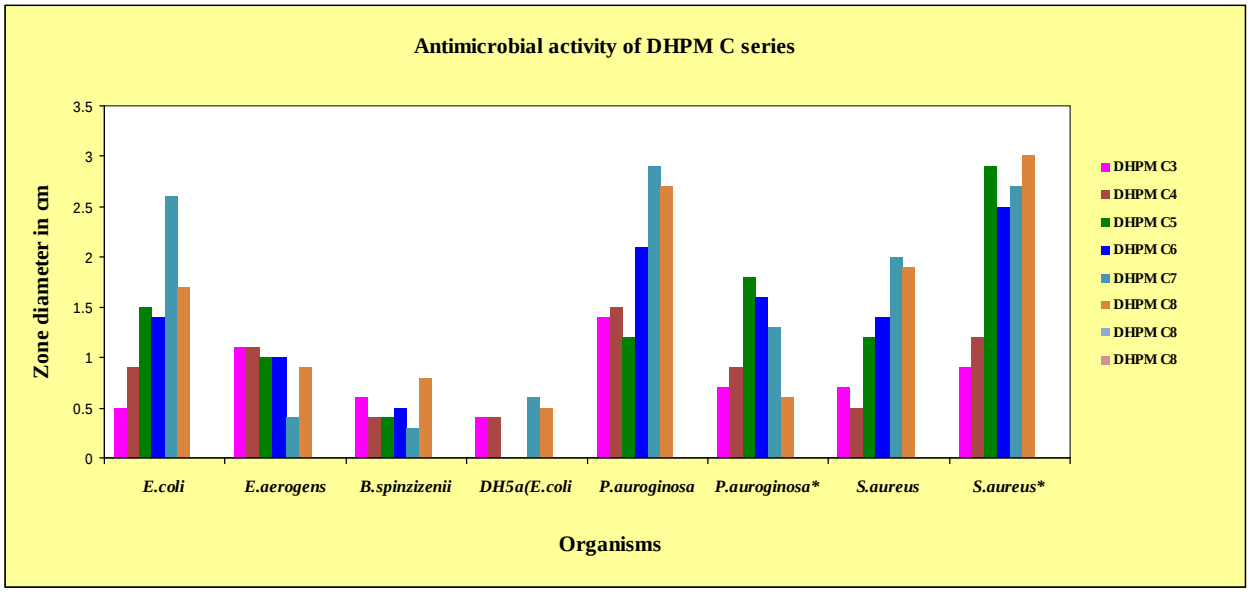


Fig. 3: represents antimicrobial activity of DHPM C series of compounds in zone diameter in cm

2.4 cm zone of inhibition. It showed good activity against *E. coli* and *S. aureus*. It was not active against organisms *B. spinzizenii, DH5α* and *P. auroginosa* ATCC strain. Compound F4 higly effective against ATCC strain of *P. auroginosa* and *S.aureus* in the range of 2.4 and 2.0cm zone diameter. It was also found to be active against *E. coli* at the concentration of 100µg/ml. Compound F5 was very active against *B. spinzizenii* but it showed no activity against *E.coli* and clinical isolate *P. auroginosa*. Its activity against clinical isolate *S. aureus, S. aureus* ATCC strain, *P. auroginosa* and *DH5α* were not tested. Compound F6 was very active against *B.spinzizenii* but it showed moderate activity against *E.coli* and *E.aerogens*.

Compound F7 showed good activity against *E. coli* and clinical isolate *P.auroginosa*. But it was found to be inactive against remaining organisms. Compound F8 showed highest activity against *E. coli* and *DH5α*. It showed moderate activity aginst clinical isolates *S. aureus* and *P. auroginosa*. It showed no activity against *E. aerogens, DH5α* and *P. auroginosa*. Compound F9 showed highest activity against *E. coli* and *DH5α*. It showed moderate activity aginst *E. aerogens* and *B. spinzizenii*. It showed no activity against *DH5α* and *P.auroginosa*. Compound F10 showed highest activity against *E. aerogens*, clinical isolate *P. auroginosa, S. aureus* ATCC strain. It showed no activity aginst *E. coli B. spinzizenii, DH5α, P. auroginosa*. Compound F11 and F12 were only slightly affecting the organism *E. aerogens*, clinical isolate *P. auroginosa* and *S. aureus*.

Compound F13 showed very good activity against *E.coli*. It also inhibits the growth of *S. aureus* ATCC as well as Clinical isolate. It showed moderate activity against *E. aerogens, DH5α and P. auroginosa*. Compound F14 showed very good activity against *E. coli, B. spinzizenii* and clinical isolate *S.aureus*. It showed no activity against *P. auroginosa* ATCC strain. Compound F15 showed significant activity against clinical isolate *S. aureus*. It showed moderate activity against *E. coli, DH5α* and *S. aureus* it showed no activity against *P. auroginosa* ATCC strain. Compound F16 showed very good activity against *DH5α* and *S. aureus*. It showed moderate activity against *E. aerogen*, clinical isolate *S. aureus, P. auroginosa* ATCC strain. Compound F18 showed very good activity against *B.spinzizenii*. It showed no activity against *E. coli*. Compound F19 showed good activity against *DH5α*. It showed no activity *B. spinzizenii*, clinical isolate *S. aureus*, ATCC strain *P. auroginosa*, ATCC strain *S. aureus*, *E. coli*. It showed moderate activity against *E. aerogns* and *E. coli*. Compound F20 showed good activity against *E. coli, B. spinzizenii* and *DH5α*. It showed no activity against *P. auroginosa* and *E. aerogns*. It showed moderate activity against ATCC strain *S. aureus*. Compound F21 showed good activity against, *E. coli, E. aerogens, B. spinzizeni*, clinical isolate as well as ATCC strain *S.aureus*. It showed no activity against *P. auroginosa* and *DH5α*. Compound F22 showed good activity against *E. aerogens* and *S. aureus*.It showed less activity against clinical isolateS.aureus and *DH5α* it showed no activity *E. coli, B. spinzizenii* and *P. auroginosa* ATCC strain.

DISCUSSION

Pyrimidine is the most important member of all the diazines as this ring system occurs widely in living organisms. Purines, uric acid, barbituric acid, anti-malarial and anti-bacterial agents also contain the pyrimidine ring. Despite the importance of dihydroazines (particularly those containing the 1,4-dihydropyrimidine and dihydropyridine moiety1) for clarifying a wide range of theoretical, medicinal and biological problems. Dihydroazines compounds are important because of presence of this group at the active site of the *hydrogen transferring coenzyme* (nicotinamide adenine dinucleotied hydrogenase-NADH or reduced nicotinamide adenine dinucleotide). This nucleotide, a central participant in metabolic processes in living organisms, participates in the reduction of various unsaturated functionalities. Additionally, dihydropyridines have been found to actively transport medication across biological membranes [19]. Literature survey revealed that dihydropyrimidines are having very good pharmacological profile. Many DHP analogs have now been synthesized and numerous second-generation commercial products have appeared on the market (e.g.nitrendipine, nicardipine and amlodipine) [20].

Because of the importance of dihydroazines in antimicrobial activity [21-25], in present investigation forty compounds of dihydropyrimidines were synthesized in our laboratory of chemistry department and analyzed for antimicrobial activity against several pathogenic strains isolated and obtained from the local pathology laboratory as well as the ATCC strains

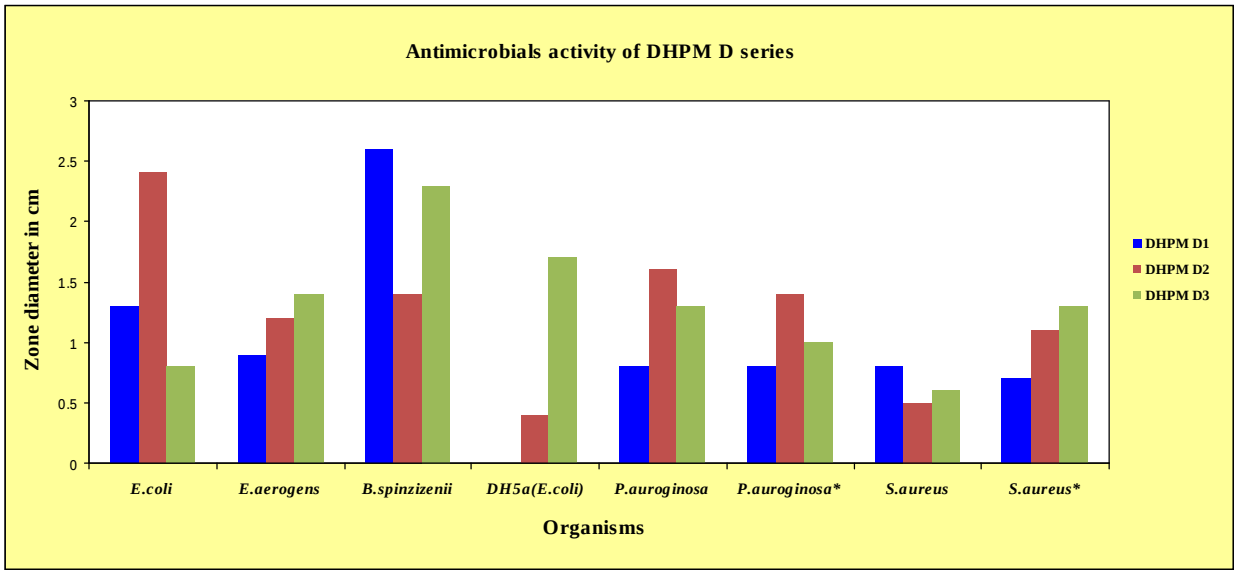


Fig. 4: represents antimicrobial activity of DHPM D series of compounds in zone diameter in cm

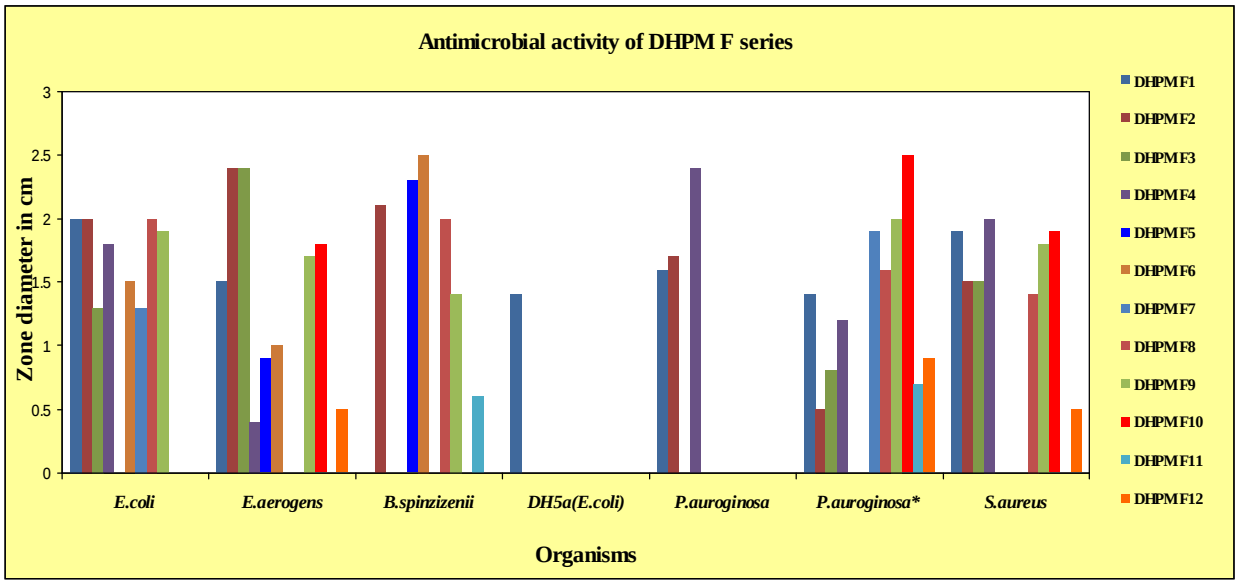


Fig. 5: represents antimicrobial activity of DHPM F series of compounds in zone diameter in cm

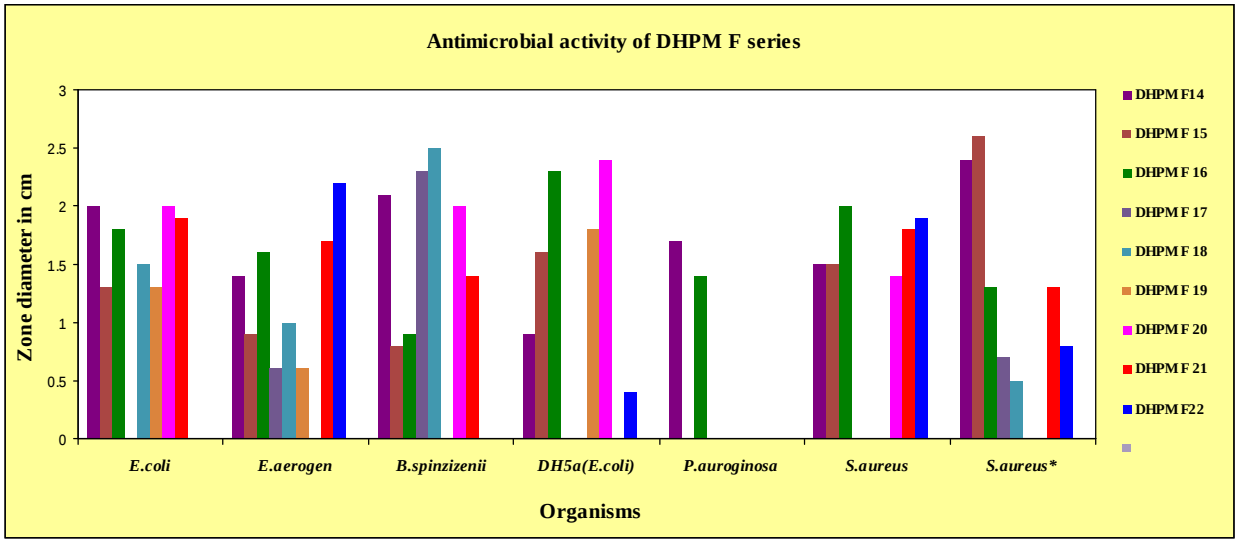


Fig. 6: represents antimicrobial activity of DHPM F series of compounds in zone diameter in cm

employing Agar Diffusion Method.

In all the set, controls were kept which showed moderate activity. So the control zone diameter was subtracted from the diameter of test compounds. While analyzing the data it is revealed that all the compounds are showing moderate activity against the bacterial strains.

CONCLUSIONS

All the DHPM A series of compound showed significant zone of inhibition against most of the bacterium compare to rest of the compounds. Amongst 40 screened compounds a few of them showed very good zone diameter in the range of 2.0 to 3.2 cm. Among these, at least 12 compounds are promising and come in the said range (A3,A4,B2,B4,B5,C7,C8,D1,D2,F4,F6,F18). However, a detail separate study is required for further clarification again each and every pathogen. Studies in this respect are in progress.

ACKNOWLEDGMENTS

Authors are highly thankful to the Head, Department of Chemistry and Biochemistry for providing laboratory facilities. We also convey thanks to Dr. S.J.Pathak and Dr. Rajesh Patel for their valuable guidance.

REFERENCES

[1] Rovnyak, G.G., Kimball, S.D., Beyer, B., Cucinotta, G., DiMacro, J.D., Gougoutas, J., Hedber, A., Malley, M., McCarthy, J.P., Zhang, R. and Moreland, S.: J. Med. Chem., 38: 119-129 (1995).

[2] Brown, D.J.: In: *Comprehensive Heterocylic Chemistry* (Katrizky, A.R. and C W Rees, C.W. ed), Pergamon Press, Oxford (1984).

[3] Petrie, C.R., Cottam, H.B. Mickernan, P.A., Robins, R.K. and Revankar, G.R.: J. Med. Chem., (28): 1010-1016 (1985).

[4] Roth, B. and Cheng, C.: *In Progress in Medicinal Chemistry* (Ellis, G.P. and West, G.B. ed), Elsevier Biomedical Press, New York (1982).

[5] Noll, S., Kralj, M., Suman, L., Stephan, H. and Piantanida, I.: Euro. J. Med. Chem., (44): 1172-1179 (2009).

[6] Ali, H.I., Ashida, N. and Nagamatsu, T.: Bioorg. Med. Chem., (15): 6336-6352 (2007).

[7] Baraldi, P.G., Pavani, M.G., Nunez, M., Brigidi, P., Vitali, B., Gambar, I.R. and Romagnoli, R.: Bioorg. Med. Chem., 10: 449-456 (2002).

[8] Sondhi, S.M., Johar, M., Rajvanshi, S., Dastidar, S.G., Shukla, R., Raghubir, R. and Lown, J.W.: Aust. J. Chem., (54): 69-74 (2001).

[9] Narayana, L.B., Rao, R.R. and Rao, S.P.: Eur. J. Med. Chem., (44): 1369-1374 (2009).

[10] Mangalagiu., G., Ungureanu, M., Grosu, G., Mangalagiu, I. and Petrovanu, M.: Ann. Pharm. Fr., 59(2): 139-140 (2001).

[11] Chhabria, M.T. and Jani, M.H.: Eur. J. Med. Chem., 44(10): 3837-3844 (2009).

[12] Beck, G. and Heitzer, H.: US Patent 4, 7482, 43 (1988).

[13] Jackson, A., Heyes, V. and Grayson, J.I.: US Patent 5, 180, 833 (1998).

[14] Zhang, Q., Haemers, A., VadenBerghe, D., Pattyn, S.R. and Bolaert, W.: J. Heter. Chem, 28(3): 673-683 (1991).

[15] Akbari, J.D.: *Studies on bioactive heterocyclic compounds*, Ph.D. Thesis submitted to Saurashtra University, Rajkot (2008).

[16] Wayne, P.A.: In: Methods for dilution Antimicrobial Susceptibility Tests for Bacteria that grow aerobically, 5th edi. Approved standard M7-A5,NCCLS (2000).

[17] Fernandes, C.J., Steven, D.A., Groot Obbink, D.J. and Ackerman, V.P.: J. Antimicrob. Chemother., (15) : 53-60 (1985).

[18] Woods, G.L. In: *Manual of Clinical Microbiology*. ASM Press, Washington DC (1995).

[19] Roche, E.B., Bossert, F. and Vater, W.: Med. Res. Rev., 9: 291-324 (1989).

[20] Venkatesan, J., Pandeya, S.N. and Selvakumar, D.: Elec. J. Chem., 69(4): 586-589 (2007).

[21] Venkatesan, J., Pandeya, S.N. and Selvakumar, D.: Indian J. Chem., 48B: 1732-1737 (2009).

[22] Heda, C., Sharma, R., Pareek, C. and Chaudhari, P.B.: Elec. J. Chem., 6(3), 770-774 (2009).

[23] Baluja, S., Gajera, R., Kulshrestha, A., Patel, A., Chanda, S., Vaghasiya, Y. and Barvaliya, Y.: Arch. Appl. Sci. Res., 2(2): 72-78 (2010).

[24] Wetzel, J.M., Miao, S.W., Borden, L.A., Branhek, T.A. and Gluchowski, C.: J. Med. Chem., 38: 1579-1581 (1995).

[25] Sweet, F. and Fissekis, J.D.: J. Am. Chem. Soc., 95: 8741- 8749 (1973).

.