ANTI-INFLAMMATORY AND ANTIMICROBIAL POTENTIAL OF SOME NOVEL FUSED BENZOPYRIMIDINE DERIVATIVES

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Abbreviated title: vachala *et al*.,

Summary

In the present study, a series of novel 2-phenyl-3, 6, 7-trisubstituted quinazolin-4(3H)-ones (ABM 1-8) were synthesized by microwave irradiation method using 2-benzamido benzoic acid and different primary amines. The structure of these compounds was confirmed by IR, ^{1}H-NMR and mass spectral analysis. All the synthesized compounds were tested for their anti-inflammatory activity; *in vitro* antimicrobial and time kill studies. The anti-inflammatory activity was performed at two different concentrations (50 and 100mg/kg body mass) by rat paw oedema model. Ibuprofen (100mg/kg) was used as standard. Compounds ABM-5 and ABM-7 showed a very good anti-inflammatory activity at 50 and 100mg/kg body weight respectively. Among the ABM series, compound ABM-4 showed microbial growth inhibition at very low concentrations. The minimum inhibitory concentration of ABM-4 against *P. aeroginosa*, *S. aureus*, *E. coli*, *C. albicans* and *A. flavus* was found to be 0.78, 0.39, 0.39, 1.56 and 6.25 µg/ml respectively. The most potent compound ABM-4 was then considered for time kill study for 0-24h. It showed 100% growth inhibition at the 12th h against *P. aeroginosa.* These data revealed that the compounds containing 4-chlorophenyl, N,N-dimethyl ethyl groups and 4-fluorophenyl substitutions at 3rd position in the quinazolinone nucleus played an important role in anti-inflammatory and antimicrobial activities.

Key words: 4(3*H*)-Quinazolinone; anti-inflammatory; antimicrobial activity; minimum inhibitory concentration; time kill study

Fused Pyrimidines have been the subject of substantial attention by synthetic and medicinal chemists because of the role of this heteroaromatic ring in many biological systems. 4(3*H*)-Quinazolinone is a versatile lead molecule and the synthesis of derivatives of 4(3*H*)-Quinazolinone has been the focus of great interest recently. Literature survey indicates that compounds having pyrimidine nucleus have wide range of therapeutic uses which includes, anticonvulsant [1], anti-inflammatory [2-4], antitumor [5-6], antihelminthic [7], antiallergic [8], antifungal [9], antibacterial [10], MAO inhibitory activities [11] and antiproliferative activities as well as inhibitory effects for thymidylate synthase and poly-(*ADP*-ribose) polymerase (*PARP*) activities [12]. Several 2, 3-disubstituted benzopyrimidine derivatives were synthesized and tested for different biological activities. The reports showed that aryl substitution at 3rd position enhances the biological activities.

Several methods have been reported for the synthesis of 4(3*H*)-Quinazolinone derivatives [13-14]. However, these methods suffer from drawbacks, such as longer reaction time, complicated workup, and use of expensive and hazardous chemicals with low yield [15-16]. The use of microwaves in organic synthesis has increased dramatically in the last years, receiving widespread acceptance and had become an indispensable tool [17]. Since by employing this technique it is generally possible to prepare organic compounds very fast, with high purity and better yields compared to other more conventional methods. This methodology also avoids the use of solvents and harmful acids or bases, which are generally used for the catalysis of the reactions.

The simultaneous use of several drugs to treat inflammatory conditions, associated with some microbial infections may cause health problems especially in patients with impaired liver or kidney functions. Also, from the pharmaco-economic point of view, and for better patient compliance, an anti-inflammatory antimicrobial agent with minimum adverse effects and high safety margin is highly desirable. In view of the fact that several 4-oxoquinazoline derivatives possess useful anti-inflammatory as well as antimicrobial properties, we have designed and synthesized some novel 4(3*H*)-Quinazolinone analogs having the general structure of figure 1 as potential antimicrobial and anti-inflammatory agents.

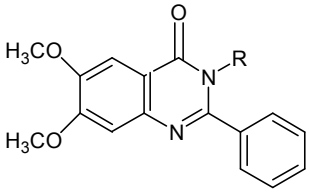


Figure 1

Methods

Experimental

The melting points of the synthesized compounds were taken in open capillary tubes and are uncorrected. The IR spectra of the compounds were recorded in the 500-4000 cm^{-1} range on ABB Bomen MB 104 series using KBr pellets. The ^{1}H-NMR spectra were recorded on Joel, model GSV-400 MHz spectrometer in $CDCl_3$/DMSO-d_6 as solvent. The chemical shifts were reported as parts per million downfield from tetramethylsilane (Me_4Si). Mass spectra were recorded on the Finningan MAT 8230. The purity of the compounds were checked by TLC on SiO_2 gel (HF_{254}, 200 mesh) coated glass plates.

Synthesis of quinazolin-4(3H)-one derivatives

4,5-dimethoxy anthranilic acid (0.5g) was reacted with benzoyl chlorides (1ml) in presence of sodium hydroxide to give 2-(benzamido)-4,5-dimethoxybenzoic acid. Then it was condensed with different primary amines by microwave irradiation technique to produce fused benzopyrimidine (Quinazolin-4(3H)-ones) analogues.

Anti-inflammatory activity by Carrageenean induced hind paw edema in rats

The method adopted resembles essentially that described by Winter *et al*. [18]. Carboxymethylcellulose (0.5% w/v) was selected as vehicle to suspend the standard drug and test compounds. The rats were starved for 18 h prior to the experiment. The animals were weighed, marked for identification and divided into 18 groups, each containing of six animals. Edema was induced in the left hind paw of all rats by subcutaneous injection of 0.1 mL of 1% (w/v) carrageenean in 0.9% saline into their footpads. The 1st group was kept as control and was given 0.5% CMC. The 2nd to 9th groups were given an aqueous suspension of

the synthesized compounds at 50 mg kg^{-1} body mass. And 10^{th} to 17^{th} groups were received an aqueous suspension of the synthesized compounds at100 mg kg^{-1} body mass. The last group (18^{th}; standard) was administered Ibuprofen (100 mg kg^{-1} body mass, suspension in 0.5% CMC). All the test compounds and the standard drug were administered orally, 1 hour before the carrageenean injection. The paw volume of each rat was measured using a digital plethysmometer (UGO Basil, Italy), just before the carrageenean injection (0 h) and then hourly for 5 hours post administration of the carrageenan injection. The percentage inhibition of paw volume for each test group was calculated using the following equation.

Percentage of inhibition (%) = 100(1- (a-x/b-y))

Where a = mean paw volume of treated animals after carrageenan injection

x = mean paw volume of treated animals before carrageenan injection

b = mean paw volume of control animals after carrageenan injection

y = mean paw volume of control animals before carrageenan injection

Anti-microbial activity

Microbial strains

Transfer culture of bacterial strains; *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) *and fungal strains Candida albicans* (ATCC 24433) and *Aspergillus flavus* (ATCC 15517), from the stock collection were carried out on to Muller Hinton Agar (MHA) and Sabourauds Dextrose Agar (SDA) plates respectively. The cultures were passaged aseptically thrice to ensure purity.

Minimum inhibitory concentration-Microdilution Assay

The broth micro dilution test was performed using sterile, disposable, multiwell microdilution plates (96 U-shaped wells) in 12X8 format [19]. Column 1 was the media control, containing 200µL of the medium to determine the sterility of the medium. Column 11 was the test compound growth control with 100 µL of sterile medium and 100µL of the test compound. It determines the purity of the compound tested. Column 12 was the growth control which contains 100µL of medium and 100µL of diluted inoculum suspension. Initially, 100 µl of medium (MHB for bacteria and RPMI for fungi) were added to all the wells of the plate except the media control, where 200 µl was added. Next, 100 µl of the test compounds were added respectively to their wells in column 2. Column 2 contains 100µL of the highest concentration of the drugs and 100µL of the medium. By performing a serial dilution from column 2 to column 10, varying concentrations of the test compounds ranging from 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 and 0.39 µg/ml were achieved. Column 10 had 100µL of the lowest concentration of the drugs and 100µL of the medium. Finally, 100 µl of the diluted inoculum suspension was added to wells from column 10 to column 2. The micro dilution plates were incubated at 37^0C and observed for interpretation of bacterial and fungal MIC values after 24 and 48 h respectively. The last well in the dilution series that *does not* demonstrate growth indicated by turbidity and/or a pellet of microorganisms in the bottom of the vessel corresponds with the minimum inhibitory concentration (MIC) of the antimicrobial agent.

Time kill study

The compound which showed good antimicrobial activity at low concentration was considered for time kill study. Test tubes with 5 ml Mueller-Hinton broth (Difco) for *S. aureus, P. aeruginosa* and *E. coli* (Difco) and RPMI broth (Difco) *C. albicans, A. flavus* were used. Test drug (ABM-4) concentrations were chosen to comprise dilutions at the MIC range 1mg/ml. A sensitivity threshold of 5 X 10^6 CFU/ml was used to determine the 99.9% killing.

The inoculum for *P. aeroginosa*, *S. aureus* and *E. coli* and *C. albicans* was prepared by diluting a 16h broth culture to obtain the correct inoculum 5 X 10^6 CFU/ml. In case of *A. flavus* a spore suspension was adjusted to 5 X 10^6 CFU/ml by counting using a cell counter. After vortexing briefly 1 ml of each diluted inoculum was delivered by pipette and plated for viability counts (0 h). Only tubes with an initial inoculum within the range 5 X 10^6 CFU/ml were acceptable for the study.

The viability count of test drug (ABM-4) suspension was performed at 2, 4, 6, 12, and 24 h by plating 0.1ml aliquots of inoculum of each test microorganisms onto Trypticase soy agar (TSA) for bacteria and Sabourauds dextrose agar (SDA) for fungi. The plates were incubated for up to 48 h and colony counts were noted for the recovery, yielding 30 to 300 colonies. The lower limit of sensitivity of colony counts was 250 CFU/ml.

Time-kill assay results were analyzed by determining the numbers of strains which yielded $\log_{10}^{-1}$ CFU/ml in comparison with counts at 0 h, for test drugs at different time intervals [20]. Test drugs were considered microbicidal at the MIC concentration that reduced the original inoculum by $\geq$3 $\log_{10}^{-1}$ CFU/ml (99.9%) and microbistatic if inoculum was reduced by < 3 $\log_{10}^{-1}$ CFU/ml.

Results and conclusion

The syntheses of hitherto unreported title compounds were prepared from 4, 5-disubstituted anthranillic acid. 2-phenyl-3, 6, 7-trisubstituted quinazolin-4(*3H*)-ones (ABM 1-8) were synthesized by the condensation of 2-benzamido benzoic acid with different amines by MWI technique and their physico-chemical data were given Table-1. The structure of the newly synthesized compounds had been established on the basis of spectral data.

Table 1: Physical data of 6, 7-dimethoxy-2-phenyl-3-substituted quinazolin-4(3H)-ones.

Cpd code	R	Molecular formula	Yield %	mp (ºC)	R_f value	Time in minutes
ABM-1	4′-fluoro-3′-chloro phenyl	$C_{22}H_{16}ClFN_2O_3$	65	200	0.71	7
ABM-2	4′-fluorobenzyl	$C_{23}H_{19}FN_2O_3$	75	216	0.70	5
ABM-3	4′-chlorobenzyl	$C_{23}H_{19}ClN_2O_3$	77	126	0.80	6
ABM-4	4′-fluorophenyl	$C_{22}H_{17}FN_2O_3$	73	220	0.61	6.5
ABM-5	4′-chlorophenyl	$C_{22}H_{17}ClN_2O_3$	71	224	0.71	7
ABM-6	3′-chlorophenyl	$C_{22}H_{17}ClN_2O_3$	74	184	0.70	7
ABM-7	N,N-dimethyl ethyl	$C_{20}H_{23}N_3O_3$	68	176	0.80	5
ABM-8	4′-methoxyphenyl	$C_{24}H_{21}N_2O_4$	80	182	0.90	7

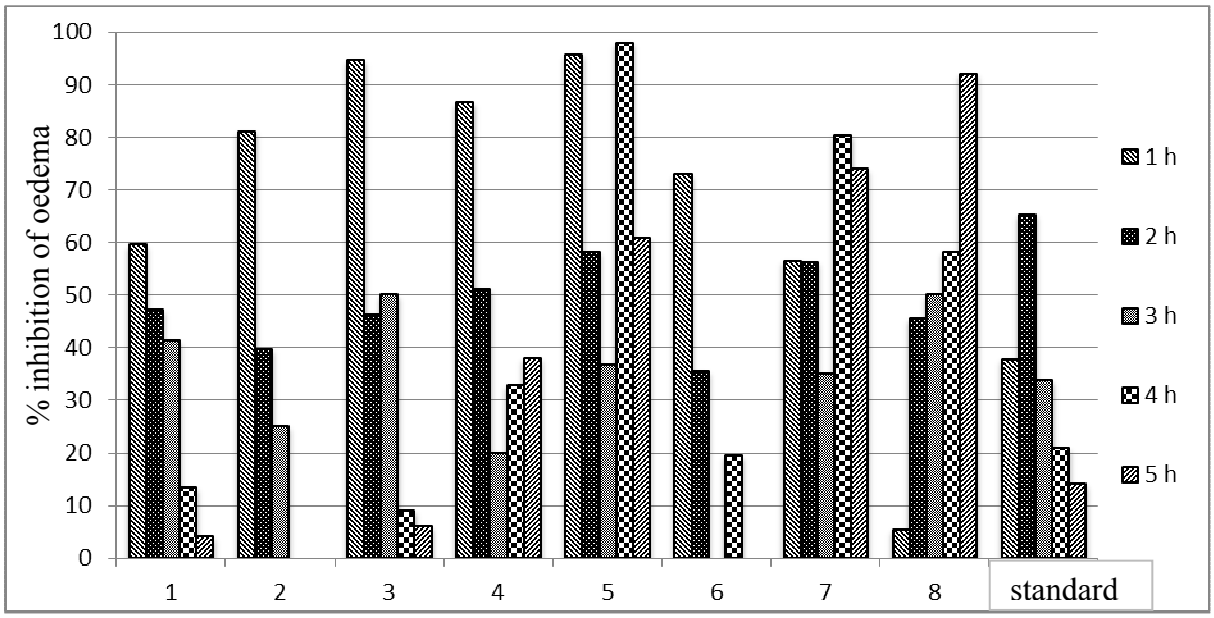
The anti-inflammatory activity was performed at 50 and 100mg/kg body mass by rat paw oedema model. Ibuprofen (100mg/kg) was used as standard. The results were given in table-2.

Table 2: Anti-inflammatory activity of the synthesized compounds at 50 and 100mg/kg body weight

Cpd Code	Dose (mg/ kg)	Paw volume ± SEM					
		0h	1h	2h	3h	4h	5h
Ctrl	-	1.1150±0.0437	1.2075±0.0405	1.4175±0.1931	1.225±0.0484	1.2825±0.0862	1.2400±0.0754
ABM-1	50	1.0825±0.0922	1.0450±0.1515	1.2425±0.2035	1.2600±0.2754	1.2275±0.2198	1.2025±0.2198
	100	1.0212±0.0923	1.0832±0.0312	1.0952±0.0321	1.0981±0.0382	1.0975±0.0823	1.0785±0.0321
ABM-2	50	1.0750±0.2624	1.0925±0.0789	1.2575±0.0768	1.225±0.0818	1.2625±0.0932	1.2075±0.0869
	100	1.0650±0.0233	1.0950±0.0171	1.3000±0.0828	1.2725±0.0995	1.1575±0.0666	1.1075±0.0581
ABM-3	50	1.2400±0.0810	1.1525±0.1417	1.4025±0.1406	1.3400±0.1650	1.3925±0.1650	1.3575±0.1935
	100	1.0150±0.0409	1.0650±0.0457	1.1000±0.0672	1.0475±0.0531	1.0175±0.0409	1.2350±0.2417
ABM-4	50	1.0650±0.1193	1.0775±0.0793	1.2125±0.1391	1.2250±0.1891	1.1775±0.2251	1.1425±0.2133
	100	0.9875±0.2193	1.0700±0.0837	1.2000±0.0698	1.0725±0.0357	1.0600±0.0387	0.9875±0.0382
ABM-5	50	1.0010±0.0424	1.0050±0.0995	1.1275±0.0963	1.1275±0.0964	1.0872±0.0921	1.0500±0.0559
	100	1.0425±0.0309	1.0975±0.0509	1.1950±0.0487	1.1640±0.0158	1.1010±0.0227	1.0970±0.0196
ABM-6	50	1.1825±0.3335	1.2075±0.0943	1.3775±0.1204	1.4000±0.1246	1.3175±0.1109	1.3150±0.1066
	100	0.9025±0.0259	1.0350±0.0240	1.0950±0.0477	1.0375±0.0442	0.9650±0.0574	0.9075±0.0340
ABM-7	50	1.2800±0.0178	1.3202±0.0182	1.4123±0.0283	1.4100±0.0268	1.3118±0.0318	1.3126±0.0138
	100	1.1678±0.0132	1.2268±0.0172	1.2891±0.0138	1.2022±0.0173	1.1856±0.0752	1.1692±0.0532
ABM-8	50	1.2400±0.0739	1.3275±0.0568	1.4050±0.0351	1.3400±0.0497	1.3100±0.0891	1.2500±0.1023
	100	0.8750±0.0413	1.1150±0.1635	1.0150±0.0388	0.9525±0.0541	0.9450±0.0511	0.8780±0.0507
Ibuprofen	100	1.0925±0.0437	1.0350±0.0456	0.9875±0.0411	0.9600±0.0474	0.9500±0.0397	0.9600±0.0543

All the compounds were showing anti-inflammatory activity at 50mg/kg body mass. The percentage inhibition of oedema was presented in figure-2.

Figure 2: The percentage inhibition of oedema of all the synthesized compounds at 50mg/kg body weight.

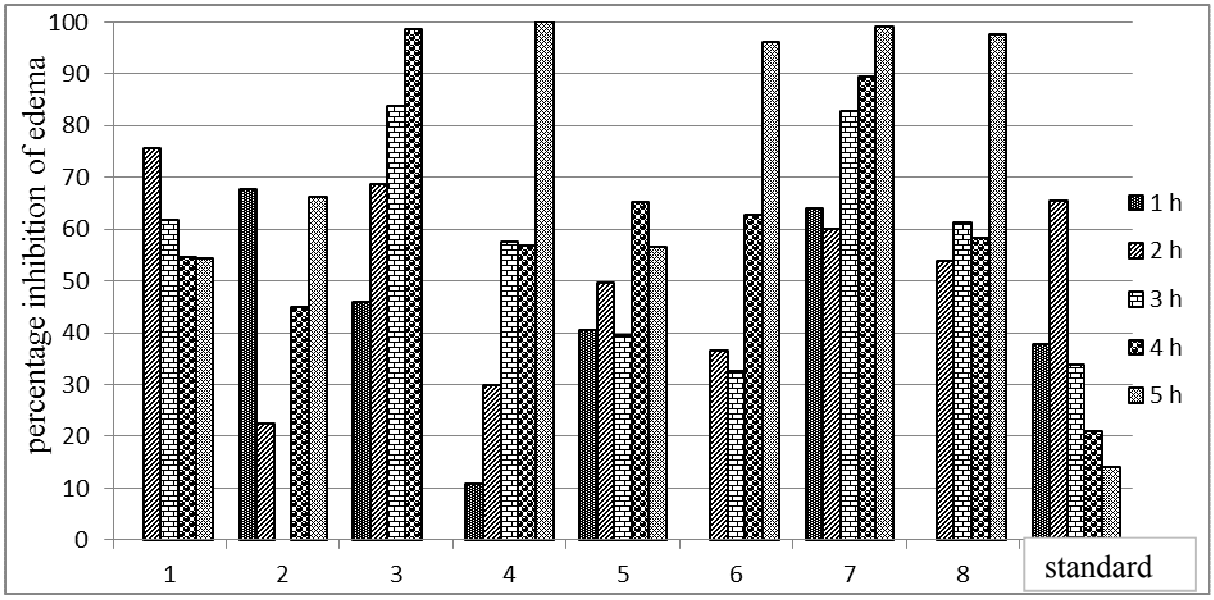


At 1st h compounds ABM-5, 3 and 4 showed good inhibition of inflammation (1st h) when compared with other compounds. At 2nd h, compounds ABM-5 and ABM-7 showed good

percentage inhibition of paw oedema when compared to all the other compounds (58.2 and 56.3). The standard drug showed 65.3% inhibition of inflammation at 2nd h and compound ABM-2 was not reducing the inflammation after 2nd hour. Compound ABM-1, 4, 5, 6, 7 and 8 were not showing any inhibitory activity at 3rd hour. At 5th h, compounds ABM-8 and ABM-7 showed good percentage inhibition of inflammation (92 and 73.9%). These results revealed that the compounds containing 4-chloro phenyl, 4-methoxy phenyl and N, N-dimethyl ethyl substitutions at 3rd position in the quinazolinone nucleus enhances their anti-inflammatory activity.

All the compounds were tested at 100mg/kg body mass. The percentage inhibition of inflammation was given in figure-3.

Figure 3: The percentage inhibition of oedema of all the synthesized compounds at 100mg/kg body weight.



At 100mg/kg, compounds ABM-1, 6 and 8 were not showing any anti-inflammatory activity at 1st h. But compound ABM-1 was showing very good percentage inhibition of inflammation at 2nd h (75.5) when compared with other compounds. At 3rd h, compound ABM-2 did not show any activity. But compounds ABM-3 and 7 were showing very good anti-inflammatory activity (83.8 and 82.8) at 3rd h. At 4th h, compound ABM-3 showed 98.5 % inhibition of paw oedema. Compound ABM-4 showed 100% inhibition of inflammation at 5th h. And compounds ABM-7, 8 and 6 also showed very good anti-inflammatory activity (98.9, 97.6 and 96%). When comparing the anti-inflammatory activity of the test compounds at 100mg/kg body mass, compound ABM-7 showed very good percentage inhibition of oedema throughout the study time. These data revealed that the compound containing N, N-dimethyl ethyl substitution at 3rd position in the quinazolinone nucleus played an important role in inhibition of inflammation.

All the synthesized compounds were tested for their *in vitro* antimicrobial activity against five microorganisms belonging to bacteria and fungi classes. The minimum inhibitory concentration of all the synthesized compounds was studied by microdilution assay method. The MIC data were given in table-3.

Table 3: Minimum inhibitory concentration of 6, 7-dimethoxy-2-phenyl-3-substituted quinazolin-4(3H)-ones.

Cpd code	Minimum inhibitory concentration (µg/ml)				
	P. aeroginosa	*S. aureus*	*E. coli*	*C. albicans*	*A. flavus*
ABM-1	3.12	0.78	1.56	12.5	25
ABM-2	>100	>100	>100	>100	>100
ABM-3	>100	>100	>100	>100	>100
ABM-4	0.78	0.39	0.39	1.56	6.25
ABM-5	25	3.12	6.25	>100	>100
ABM-6	6.25	1.56	3.12	50	100
ABM-7	3.12	0.78	1.56	12.5	25
ABM-8	>100	>100	>100	>100	>100

Among the ABM series, compound ABM-1 and ABM-7 showed similar growth inhibition against all the organisms tested. The MIC value of compounds ABM-2, ABM-3 and ABM-8 were found to be more than 100 µg/ml. Compound ABM-6 showed minimum growth inhibition at moderate concentrations. Compound ABM-4 showed good inhibitory activity at very low concentrations. The MIC value of ABM-4 against *P. aeroginosa*, *S. aureus*, *E. coli*, *C. albicans* and *A. flavus* was found to be 0.78, 0.39, 0.39, 1.56 and 6.25 µg/ml respectively. The most potent compound ABM-4 was selected for time kill study and the result was given in table-4.

Table 4: Time kill study of the compound ABM-4.

Test strains	No (%) of strains with the indicated $\log_{10}^{-1}$ CFU/ml at time intervals				
	2h	4h	6h	12h	24h
P. aeroginosa (ATCC 27853)	7 (77.8)	9 (100)	9 (100)	0	0
S. aureus (ATCC 25023)	4 (44.4)	8 (88.9)	9 (100)	9 (100)	0
E. coli (ATCC 25922)	5 (55.6)	8 (88.9)	8 (88.9)	8 (88.9)	0
C. albicans (ATCC 24433)	7 (77.8)	6 (66.7)	9 (100)	9 (100)	9(100)
A.Flavus (ATCC 15517)	2(22.2)	6 (66.7)	7 (77.8)	8 (88.9)	9 (100)

The time kill study report showed that the compound ABM-4 inhibited the growth of *P. aeroginosa*, *S. aureus* and *E. coli* completely at 12 and 24 h incubation. But it required more than 24 h incubation period to prevent the growth of *C. albicans* and *A.Flavus*. Compounds containing 3-chloro-4-fluro phenyl and N, N-dimethyl ethyl substitution at 3rd position in the quinazolinone showed the microbial growth inhibition at moderate levels. These results revealed that the compound containing 4-fluorophenyl substitution in the quinazolinone nucleus showed very good antimicrobial activity at low concentrations.

Finally on the basis of these observations, it can be concluded that the presence of 4-chlorobenzyl, 4-fluoro phenyl, 3-chloro-4-fluro phenyl and N, N-dimethyl ethyl substitutions at 3rd position in the quinazolin-4(*3H*)-one nucleus enhances their pharmacological activities.

Acknowledgement

The authors are thankful to AICTE, New Delhi for providing the research grant and also we express our gratitude to Prof. Dr. N.Udupa, The Principal, Manipal College of Pharmaceutical Sciences; Prof. Dr. Indira Bairy, The HOD, Department of microbiology, KMC and the management of Manipal University, Manipal for providing all the facilities to carry out this work.

References

1. Wolf JF, Ratham TL, Sleevi MC, Campbell JA, Greenwood TD. Synthesis and anticonvulsant activity of some new 2-substituted-3-aryl-4(3H) quinazolinones. J Med Chem 1990;33:161-166
2. Verma M, Sinha JN, Gujrati VR, Bhalla TN, Bhargava KP, Shanker K. A new potent anti-inflammatory quinazolone. Pharmacol Res Commun 1981;13:967-979
3. Kumar A, Verma RS, Jaju BP, Sinha JN. Solvent free synthesis of lactones. J Ind Chem Soc 1990;67:920-928
4. Sarvanan J, Mohan S, Manjunatha KS. Cyclisation of unsaturated ketones. Ind J Heterocycl Chem 1998;8:55-61
5. Nair MG, Nanavati NT, Nair IG, Kusliuk RL, Gaumont Y, HsiaoM C, Kalman TI. Isolation and characterization of black cumin Compounds. J Med Chem 1986;29:1754-1762
6. Sikora E, Jackman AL, Newell DR, Calvert AH. Formation and retention and biological activity of N10-propargyl-5, 8-dideazafolic acid (CB3717) polyglutamates in L1210 cells in vitro. Biochem Pharmacol 1998;37:4047-4054
7. Rastogi R and Sharma S. Synthesis of 2-substituted quinazolines and quinazolones as potential anthelmintics. Indian J Chem 1982;21B:744-746
8. Doria G, Romeo C, Giraldi P, Lauria F, Corno ML, Sberza P, Tibolla M, Ger patent No.2654215, 1977
9. Gamal El-Hiti A, Mohamed Abdel-Megeed F, Yehia Mahmoud AG. Biological screening of anthraquinone derivatives. Ind J Chem 2000;39B:368-376
10. Ghorab MM, Abdel-Hamide SG, El-Hakim AE. Synthesis of Some New Biologically Active 2-Phenyl-6-iodo-3-substituted-4(3H) Quinazolinones. Ind J Heterocycl Chem 1995;5:115-120
11. Srivastava VK, Satsangi RK, Kumar P, Kishore K. Monoamine oxidase inhibitory activity of -2-aryl-(5′-chlorobenzophenon-2′-yl)quinazolin-4-(3H)ones. Ind J Physiol Pharmacol 1980;24:361-363
12. Griffin J, Srinivasan S, Bowman K, Kalvert HA, Curtin NJ, Newel DR, Pemberton LC, Golding BT. Synthesis and biological properties of quinazolinone inhibitors of the DNA repair enzyme poly(ADP-ribose) polymerase (PARP). J Med Chem 1998;41:5247-5256
13. McNamara DJ, Berman EM, Fry DW, Werbel LM. Potent inhibition of thymidylate synthase by two series of nonclassical quinazolines. J Med Chem 1990;33:2045-2051
14. Archana PR, Srivastava VK, Kumar A. Microwave synthesis of pyrrolopyrimidines. Ind J Chem 2002;41B:2642-2649
15. Caddick S. Microwave assisted synthesis of heterocycles. Tetrahed 1995;51:10403-10409
16. Varma RS, Clark DE, Sutton WH, Lewis DA. Microwave-Assisted Reactions under Solvent-Free 'Dry' Conditions" in Microwaves: Theory and Application in Material Processing. Am Cer Soc Cer Trans Ohio 1997;80:357-365

17. Thierney JP, Lidström P. Microwave Assisted Organic Synthesis. Eds.; Blackwell Publishing Ltd, 96-97
18. Winter CA, Risley EA, Nuss GW. Carrageenan-induced oedema in hind paw of the rat as an assay for anti-inflammatory. Proc Soc Exp Biol Med 1962;3:111:544-7
19. Venugopal D, Kumar S, Isa M, Bose M (2007) Drug resistance profile of human Mycobacterium avium complex strains from India. Ind J Med Microb. Vol. 25, No. 2: 115-120
20. Pankuch GA, Jacobs MR, Appelbaum PC (1994) Study of comparative antipneumococcal activities of penicillin G, RP 59500, erythromycin, sparfloxacin, ciprofloxacin, and vancomycin by using time-kill methodology. Antimicrob Agents Chemother 38:2065-2072