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ANTIBACTERIAL, ANTIFUNGAL AND ANTIFEEDANT ACTIVITY OF QUINAZOLINONYL-β-LACTAMS/QUINAZOLINONES AND BIS (QUINAZOLINONYL-β-LACTAMS)

P. S. N. Reddy[1,*], Vasantha Mittapelli[2] and V. D. Reddy[3]
[1]Department of Chemistry, Osmania University, Hyderabad-500 007, India.
[2]Matrix Laboratories Limited, Plot No 34 A, Anrich Industrial Estate, Bollaram, Jinnaram (Mandal), Medak Dist- 502 325, India.
[3]Centre for Plant Molecular Biology Osmania University, Hyderabad-500 007
*E-mail: psnreddyou@yahoo.com

ABSTRACT

Quinazolinonyl-β-lactams/quinazolinones, bis(quinazolinonyl-β-lactams) earlier synthesised by us are screened for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, antifungal activity against *Fusarium oxisporium*, *Macrophomina sorgina* on PDA plates and antifeedant activity against agriculture pest *Achoea janata* were studied. All the compounds proved to be ineffective in antibacterial activity. The compounds 1, 2, 4, 9, 10 and 11 proved to be effective antifeedants. Compound 1 with chlorine substituent showed both antifungal & antifeedant activities.

Keywords: Quinazolinones, β-lactams, bis-β-lactams, bisquinazolinones, antimicrobial, antifungal, antifeedant activity.

INTRODUCTION

2-Heteryl and heteroalkyl-4(3*H*)-quinazolinones exhibited a wide range of pharmacological properties such as CNS depressant, antimicrobial, antibacterial, analgesic, antifungal, antiinflammatory, antiulcer, anticonvulsant, antihypertensive, sedative, anaesthetic, tranquilising and muscle relaxant, body temperature lowering, spore germination inhibition in *Drechslera rostrata* and *Fusarium oxysporum*, CNS active, hypnotic, antidepressant, antihelmentic, inhibition of AMPA receptor activation, antihistamine, virucidal, hypoglycemic, MAO inhibition, insecticidal, radioprotective, spasmolytic, contraceptive, antitubercular, antimonomine oxidase, H_2-antagonist and antisecretion activity.[1] They are also useful in the treatment of gastrointestinal and appetite disorders as cholecystokinin β-receptor and cholecystokinin gastric receptors. They also find application as heat stable epoxy resins, fiber reactive dyes and polymers. In view of these findings, we synthesised quinazolinonyl-β-lactams/quinazolinones, bis(quinazolinonyl-β-lactams) and tested for possible antibacterial, antifungal and antifeedant activities.

The following compounds were synthesised in our laboratory and the detailed procedures were reported. 2-Chloromethyl-3-methyl-4 (3*H*)-quinazolinone (**1**)[2], 2- (4-ethylphenyl)aminomethyl-3-methyl-4(3*H*)-quinazolinone (**2**)[2], 2-N-chloroacetyl 2 (4-methoxyphenyl)aminomethyl-3-methyl-4(3*H*)-quinazolinone (**3**)[2], 4-(3-methyl-4(3*H*)-quinazolinone-2-yl)-1-(4-methylphenyl)azetidin-2-one (**4**)[2], 4-(3-methyl-4(3*H*)-quinazolinone-2-yl)-1-(4-ethoxyphenyl)azetidin-2-one (**5**)[2], 4-(3-methyl-4(3*H*)-quinazolinone-2-yl)-1-(4-ethylphenyl)azetidin-2-one (**6**)[2], 3,3'-dimethyl-4,4'-[*N*,*N*'-bis(4-(3-methyl-4(3*H*)-quinazolinone-2-yl)methyl)]diaminobiphenyl (**7**)[3], 3,3'-dimethyl-4,4'-[*N*,*N*'-dichloroacetyl-*N*,*N*'-bis((3-methyl-4(3*H*)-quinazolin-one-2-yl)methyl)]diaminobiphenyl (**8**)[3], 3,3'-dimethyl-4,4'-[bis(4-(3-methyl-4(3*H*)-quinazolinone-2-yl)azetidin-2-one-1-yl)]biphenyl (**9**)[3], 2-*N*-azidoacetylphenyl- aminomethyl-3-methyl-4(3*H*)-quinazolinone (**10**)[4], 2-*N*-azidoacetyl(4-ethylphenyl) aminomethyl-3-methyl-4(3*H*)-quinazolinone (**11**)[4], 3'-acetamido-6'-chloro-3-methyl-2,2'-bis-4(3*H*)-quinazolinone (**12**)[5], 3'-acetamido-6'-bromo-3-

methyl-2,2'-bis-4(*3H*)-quinazolinone (**13**)[5], 3'-acetamido-6',8'-dichloro-3-methyl-2,2'-bis-4(3*H*)-quinazolinone (**14**)[5] were screened for their antibacterial, antifungal and antifeedant activities.

EXPERIMENTAL

Evaluation of antifungal activity

Organisms and growth conditions

Fusarium oxisporium and *Macrophomina sorgina* fungi were grown in medium (pH 5.0), comprising of 1.5% potato dextrose agar (PDA), 0.09% KH_2PO_4, 0.02% $MgSO_4.7H_2O$, 0.02% KCl, 0.1% NH_4NO_3, 0.0002% $FeSO_4$, 0.0002% $ZnSO_4$ and 2% agar. All the constituents were mixed in double distilled water and autoclaved at 121^0C (at 15lb pressure) for 15 minutes. The medium when cooled to about 50 to 45^0C temperature mixed with fungal strains separately and poured into sterile disposable petri plates for solidification of the medium. The PDA plates thus prepared were then kept for antifungal testing.

Antifungal testing[6]

The above mentioned PDA plates inoculated with two different fungal strains were taken and labeled separately. Sterile filter paper discs (6 mm diameter) prepared from standard Whatman No.1 filter paper was applied with test solution of different concentrations. After drying the discs were introduced on to the above inoculated PDA plates containing fungal strains. The plates with test compound discs were incubated for 3 days at 30^0C.

After 3 days of the treatment, petri dishes were checked for growth inhibition zone. The presence or absence of growth inhibition zone around each disc was recorded by comparing with control (acetone). Presence of clear zone around the disc indicated the inhibition of fungal growth. The compound was then considered to be active against the fungi and the area of inhibition zone was measured. If no clear zone was observed around the disc, it indicated the inactivity of the test sample. The inhibition zone was calculated and compared with the control (untreated check).

Evaluation of Insect-antifeedant activity:

Organism

The test insect *Achoea janata* was maintained under laboratory conditions of 27 ± 1 ^{0}C and 70 ± 5% RH. The test larvae (IV instar) were fed on natural food - castor leaves. The antifeedant activity was assessed by using leaf discs in a "non-choice test method of Ascher and Rones.[7]

Antifeedant Test

Circular leaf discs of 9 cm diameter were cut from fresh castor leaves and treated with the solution of test compound from 100 to 3.125µg/cm2 by using serial dilutions. These leaf discs were air dried for 2-5 sec in acetone and were kept in petri dishes. Control discs were treated with acetone and Carbindazime and kept in separate petri dishes. Preserved IV instar larvae of *Achoea janata* were released simultaneously into petri dishes.

The consumption of leaf by the insect was measured after 48h. The leaf area consumed by the insect (in both control and treated) was measured by planimeter and the percentage of protection (antifeedant activity) was assessed by Singh and Panth formula.[8]

$$\% \text{ of antifeedant} = \frac{\left(\begin{matrix}\% \text{ of protection in} \\ \text{treated}\end{matrix}\right) - \left(\begin{matrix}\% \text{ of protection in} \\ \text{control}\end{matrix}\right)}{100 - \left(\% \text{ of protection in control}\right)} \times 100$$

Evaluation of antibacterial activity

Organisms

The bacterial strains used in the experiments were *E. coli* of MTCC 119 (Gram -ve) and *S. aureus* of MTCC 96 (Gram +ve) and were procured from IMTECH, Chandigarh, India.

Preparation of sample solution

The test compounds were dissolved in acetone (AR grade) and prepared into different concentrations by successive dilutions. Whatman No.1 filter paper discs were dipped in different sample solution and kept for air drying for further use in testing for bacteriological activity. The concentrations of test compounds ranged from 50 to 1.625μg/disc.

Preparation of medium

The medium was prepared by adding agar powder (Thomas Baker) to nutrient broth, which was prepared with the beef extract known Lablemco (10 g), sodium chloride (5 g) and peptone (10 g) in water (1 L) and pH was adjusted to 7.2 – 7.4 and sterilised in autoclave at 121^0C (at 15 lb pressure) for 15 minutes. The sterile nutrient agar medium was cooled down to 45-50^0C and poured into sterile petri plates of 6" diameter with a volume of 20 mL per plate and kept for solidification. The sterile agar plates were used for inoculating the bacterial cultures.

Bacteriological testing

Actively growing agar slant culture suspension of bacteria swab was inoculated separately on these solidified agar plates. Sterile filter papers were dipped in the test solution of different concentrations in acetone solvent (0.01 mL/disc). After drying the discs, they were introduced on to the above inoculated agar plates containing bacterial strains. The plates with test compound discs were incubated for 24h at 37^0C. After 24h, the petri dishes were checked for growth inhibition zone. The presence or absence of growth inhibition zone around each disc was recorded by comparing with inhibition zone of standard disc (Streptomycin, 10 μg/disc). Presence of clear zone around the disc indicated the inhibition of organism's growth and measured the area of inhibition zone. The compound was then considered to be active against the organism. If no clear zone was observed around the disc, it indicated the inactivity of the test sample.

RESULTS AND DISCUSSION

Antifungal activity

Fusarium oxisporium and *Macrophomina sorgina* fungi were used to evaluate the antifungal activity of the test compounds. The experimental results and assessment of antifungal activity of the compounds are given in the Table-1.

Antifeedant activity

After 48h, antifeedant activity of compounds against *Achoea janata* was measured by using the planimeter and protected area (antifeedant activity) was calculated by using Singh and Panth formula. Likewise, all the experiments were carried out in triplicate and the average is reported in table **–** II.

Antibacterial activity

The above mentioned compounds were tested for antibacterial activity by using inhibition zone method on agar plates[9] against *Escherichia coli* (Gram -ve) and *Staphylococcus aureus* (Gram +ve). None of the compounds showed antibacterial activity.

CONCLUSION

Antifungal activity

Nine out of fourteen compounds showed most promising antifungal activity against *Fusarium oxisporiu*m at 100μg/disc concentration and four compounds were fairly active against *Macrophomina sorgina* at 12.5μg/disc concentration. In general compound **1** has exhibited ~83.00% antifungal activity against both fungal strains at 50 μg/disc concentration. Compound **12** has exhibited 70.25% antifungal activity against *Macrophomina sorgina* at 25μg/disc.

Antifeedant activity

All the compounds tested (**1** to **14**) exhibited moderate to good antifeedant activity against *Achoea janata* at 200 to 6.25μg/cm^2 concentrations. The activity persisted even after 48h, though it generally recedes after 24h. Of the fourteen compounds tested, 3,3'-dimethyl-4,4'-[*N*,*N*'-dichloroacetyl-*N*,*N*'-bis((3-methyl-4(3*H*)-quinazo- linone-2-yl)methyl)]diaminobiphenyl (**8**) is the most promising with 66.26% antifeedant activity even at 6.25μg/cm^2 concentration, and can be considered as a potent antifeedant. SAR studies of

8 are preserved for future studies. Compounds **1, 2, 4, 8, 9** and **10** showed more than 50% leaf protection at 6.25µg/cm^2.

Antibacterial activity

None of the compounds showed antibacterial activity.

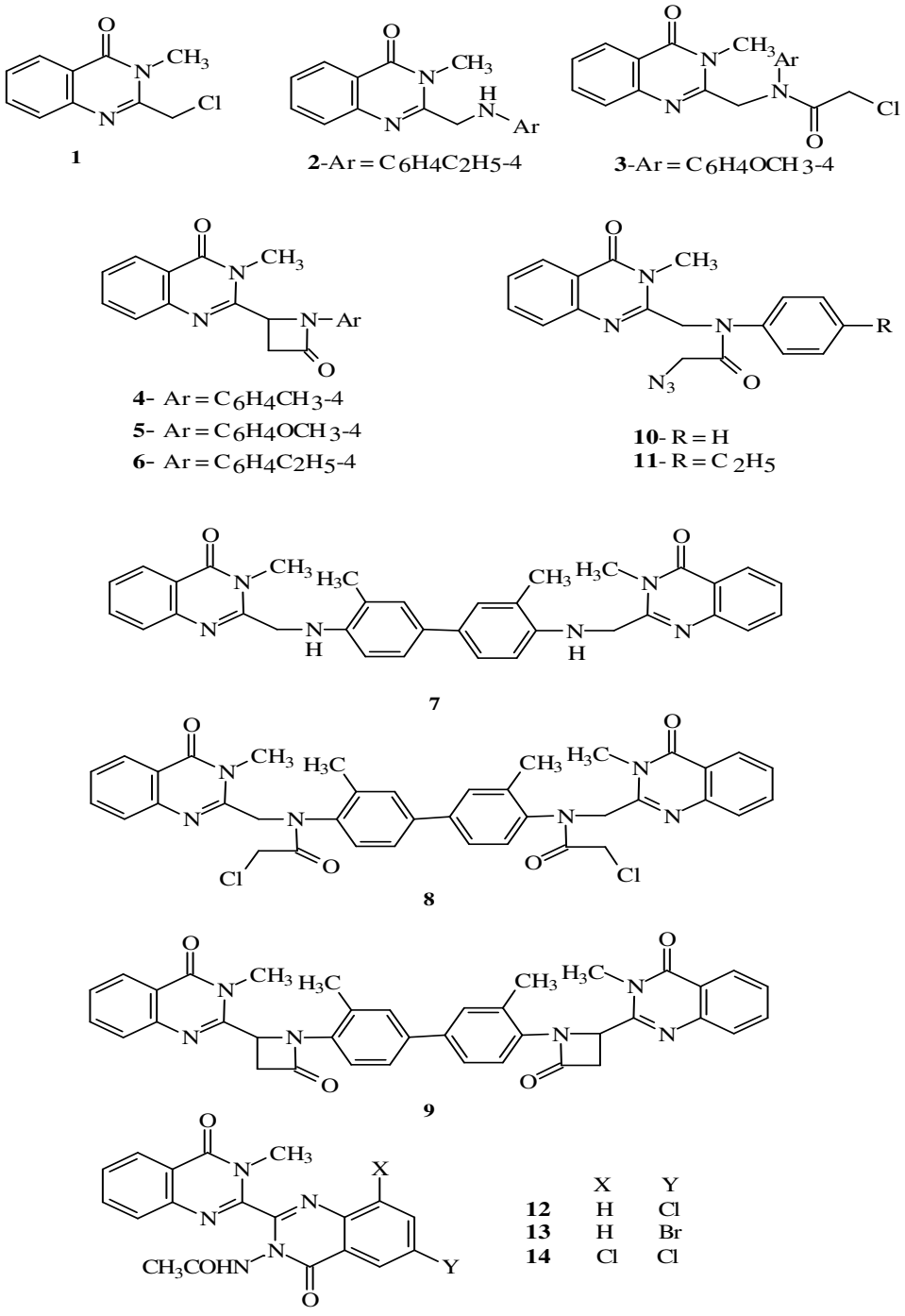

Fig.-1: Structures of Different compounds

Table-1: Antifungal activity of compounds

Com	*Fusarium oxisporium*						*Macrophomina sorgina*					
	Concentration*						Concentration*					
	100	50	25	12.5	6.25	3.125	100	50	25	12.5	6.25	3.125
1	86.80	73.34	39.65	12.88	---	---	89.66	72.23	69.38	35.23	28.66	10.78
2	54.76	53.86	---	---	---	---	---	---	---	---	---	---
3	---	---	---	---	---	---	40.77	35.45	30.67	10.26	---	---
4	---	---	---	---	---	---	---	---	---	---	---	---
5	21.26	---	---	---	---	---	---	---	---	---	---	---
6	28.33	23.86	---	---	---	---	---	---	---	---	---	---
7	---	---	---	---	---	---	---	---	---	---	---	---
8	---	---	---	---	---	---	---	---	---	---	---	---
9	32.86	21.66	---	---	---	---	39.66	22.86	---	---	---	---
10	26.54	9.34	---	---	---	---	---	---	---	---	---	---
11	13.54	---	---	---	---	---	---	---	---	---	---	---
12	28.96	19.88	---	---	---	---	82.3	70.28	70.25	52.86	22.39	---
13	---	---	---	---	---	---	61.86	55.58	48.33	25.62	7.23	---
14	16.77	---	---	---	---	---	61.87	49.26	27.60	8.25	---	---
TC	96.25	92.33	78.86	---	---	---	98.87	96.33	89.89	76.38	64.88	58.66
UTC	---	---	---	---	---	---	---	---	---	---	---	---

* Concentration expressed in μg/cm^2 dissolved in Acetone
(-) No inhibition of the fungal growth (i.e., compound is not antifungal)
UTC Untreated check (acetone)
TC Treated check (carbindazime)

Table-2: Antifeedant activity of compounds against *Achoea janata* after 48 hrs of treatment

Compound No.	% of Antifeedant activity after 48 hrs of treatment					
	Concentration in μg/cm^2					
	200	100	50	25	12.5	6.25
1.	82.70±8.62 (65.82)	81.36±5.00 (64.55)	60.39±2.90 (51.00)	58.13±2.86 (49.68)	53.72±4.31 (47.14)	55.96±2.75 (48.43)
2.	71.76±0.64 (57.90)	67.88±3.06 (55.49)	60.46±3.06 (51.05)	53.94±3.86 (47.27)	52.67±4.05 (46.54)	50.73±3.34 (45.42)
3.	68.11±1.29 (55.62)	45.65±3.08 (42.50)	26.20±4.48 (30.73)	21.40±2.53 (27.53)	15.21±0.55 (22.96)	11.28±1.70 (19.59)
4.	68.41±1.01 (55.80)	65.32±4.60 (53.94)	63.75±1.77 (52.98)	57.43±3.22 (49.28)	53.66±3.84 (47.11)	51.29±3.21 (45.74)
5.	72.98±7.05 (58.80)	70.70±2.20 (57.28)	60.94±3.67 (51.33)	49.26±1.40 (44.55)	36.97±4.46 (37.43)	37.76±2.39 (37.91)
6.	62.90±2.51 (52.48)	58.74±4.71 (50.05)	60.50±2.78 (51.06)	54.44±3.02 (47.55)	45.04±4.75 (42.15)	43.25±6.08 (41.10)
7.	61.96±5.45 (51.95)	60.73±2.65 (51.20)	60.33±1.16 (50.96)	48.01±3.77 (57.72)	44.11±1.71 (41.62)	38.33±2.64 (38.24)
8.	88.99±2.40 (66.45)	84.64±2.46 (63.93)	82.51±4.14 (65.37)	78.37±1.80 (62.29)	70.67±3.26 (57.23)	66.26±0.83 (54.490)

9.	73.34±1.87 (58.92)	68.42±4.71 (55.84)	66.71±3.20 (54.77)	62.92±3.24 (52.50)	57.30±1.03 (49.20)	53.12±0.64 (46.79)
10.	91.70±1.88 (73.32)	91.52±1.56 (73.11)	65.39±0.98 (53.96)	61.01±3.22 (51.36)	59.55±2.54 (50.51)	53.18±4.14 (46.83)
11.	90.73±2.54 (72.39)	74.25±3.50 (59.54)	17.44±2.44 (24.65)	10.00±1.77 (18.79)	8.02±2.01 (16.37)	5.60±0.84 (13.66)
12.	89.88±4.52 (71.77)	63.62±4.94 (53.93)	63.27±2.56 (52.70)	54.17±5.39 (47.41)	46.67±3.12 (43.09)	39.80±1.80 (39.11)
13.	86.18±2.92 (68.25)	81.05±1.93 (64.21)	55.21±4.50 (48.00)	43.25±2.39 (41.12)	38.79±1.50 (38.52)	29.23±1.84 (32.72)
14.	59.44±2.69 (50.45)	60.92±2.16 (51.31)	47.27±0.74 (43.43)	44.24±3.07 (41.69)	44.82±4.46 (42.02)	42.36±1.77 (40.60)
UTC.	0.00 (4.05±0.00)	0.00 (4.05±0.00)	0.00 (4.05±0.00)	0.00 (4.05±0.00)	0.00 (4.05±0.00)	0.00 (4.05±0.00)

UTC : untreated control
* Values in the parenthesis are transformed values (Arc sine)
Significance at $p<0.01$.
SEM 9.47
CD at 0.05%; CV(%) 5.56

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