SYNTHESIS AND EVALUATION OF NOVEL-3-AMINO-2-ARYL [1,2,4] TRIAZOLO [5,1-b] QUINOZOLIN-9 (3H)-ONES OF BIOLOGICAL INTEREST

Abdul Kadar Mujahid1*, Kalyane N V2, Shivkumar B2, Hemasundar Naidu M1, Pramod N3 and Rizwan M4

1 Semler Research Centre, Bangalore.
2 Department of pharmaceutical chemistry, B.L.D.E’s College of Pharmacy, Bijapur, Karnataka, India.
3 Department of pharmaceutical chemistry, A.M. Reddy Memorial College of Pharmacy, Narasaraopet, Guntur dist. A.P, India.
4 Department of pharmacology, SETs College of Pharmacy, Dharwad, Karnataka, India.

ABSTRACT

The synthesis of ten novel fused triazoloquinazolinones by making use of 3-amino-2-hydrazino-4 (3H)-quinazolinones as synthon with view to evaluation them for their possible biological and pharmacological properties. For this purpose, the required 3-amino-2-hydrazino-4 (3H)-quinazolinones prepared from the 3-amino-2-mercapto-4 (3H)-quinazolinone by direct hydrazinolysis/alkylation followed by hydrazinolysis of 3-amino-2-mercapto-4 (3H) quinazolinone. 3-amino-2-mercapto-4 (3H) quinazolinones are prepared from respective 2-amino benzoic acid. This on condensation with a various benzoylchlorides to get 3-amino-2-aryl [1,2,4] triazolo [5,1-b] quinazolin-9 (3H)-ones, further the free amino group of triazole is condensed with aromatic aldehydes to get Schiff bases. The newly synthesized compounds have been characterized by their analytical and spectral (IR, 1HNMR and Mass) properties. Further, they have been screened for their antibacterial, antifungal, anti-inflammatory (in vitro) and by standard method. Results of the activities reveals that, compounds exhibited moderate to good antibacterial and antifungal activities.

Key words: Quinazolinones, Triazoloquinazolinones, Schiff bases, Anti-microbial and Anti-fungal.

INTRODUCTION

Quinazoline (1) is a bicyclic compound earlier known as benzo-1,3-diazine was first prepared in the laboratory by Gabriel in 1903 although one of its derivatives was known much earlier.

The name quinazolinone (German: Chinazolin) was first proposed for its compound by Weddige, on observing that this was isomeric with the then compounds cinoiline (2) and quinaoxaline (3). The numbering of the quinazoline ring system, which is currently used, was suggested by Paal and Busch. The other less commonly used names for this ring system are ‘phenmiazine’ and 5,6-benzopyrimidine. However, the name ‘quinazoline’ is now universally accepted. The ‘oxo’ derivative is suffixed by ‘one’, that is ‘quinazolinone’.

Quinazolines and their derivatives have been reported to possess varied pharmacological properties like sedatives, hypnotics, anticonvulsants [1] antibacterials [2], anti-inflammatory [3], diuretics [4], antihistaminics agents [5], analgesics [6], antitubercular [7] antivirals, cytotoxicity [8] etc. The prominence enthused several chemists and medicinal chemists to prepare newer and newer quinazolinones by different synthetic routes while incorporating a variety of known pharmacophores into their molecular systems and evaluating them for their possible biological and pharmacological properties.

The present work made an attempt to bring out the various routes that are intended to achieve the synthesis of quinazolinones and their derivatives of specific biological and pharmacological importance.
MATERIALS AND METHOD
All chemicals used were of analytical grade from, SD Fine. Melting points of all the synthesized compounds were determined by open capillary tube method. These are uncorrected. The purity of all compounds was checked by TLC was run on Silica Gel G plates using Chloroform and Methanol (9:1). Spots were visualized using iodine vapour chamber. IR spectra were recorded on Shimadzu IR spectrophotometer by using KBr pellets technique.\(^1\)\(^H\)-NMR was recorded on Bruker AMX 60 MHz spectrophotometer by using DMSO as solvent.

EXPERIMENTAL
SYNTHESIS OF 3-AMINO-2-MERCAPTOQUINAZOLIN-4(3H)-ONES (II)

General Procedure:
The compound 3-amino-2-mercaptoquinazolin-4(3H)-ones (II) was synthesized by adding carbon disulphide (1.6 ml, 0.026 mol) and aqueous sodium hydroxide (1.2 ml, 20M) dropwise to a vigorously stirred solution of anthranilic acid (2.74g, 0.02 mol) in dimethylsulfoxide (10 ml) at room temperature. After thirty min dimethylsulphate (1.89 ml, 0.02 mol) was added dropwise under cooling with an ice bath. Stirring was continued for 3hrs, the reaction mixture was then poured into ice water and extracted with chloroform. The solvent was removed by distillation under reduced pressure. Thus the obtained methyl-N-(2-carboxyphenyl) dithiocarbamate was used for further reaction without purification. Hydrazine hydrate (9.80 ml, 0.2 mol, 80%) was added dropwise with stirring to methyl-N-(2-carboxyphenyl) dithiocarbamate in cold condition. After complete addition, stirring was continued for 1½ hrs at 50\(^\circ\)C and then it was poured into ice water, the solid obtained was filtered, washed with water dried and recrystallized from dimethylformamide-ethanol mixture to yield (II) as a white crystalline product.

SYNTHESIS OF 3-AMINO-2-(METHYLTHIO)QUINAZOLIN-4(3H)-ONE (III).
To a solution of 3-amino-2-mercapto-3H-quinazolin-4-one (3) (0.01 mol) in sodium hydroxide (10 ml, 10\%solution), dimethyl sulfate (0.01 mol) was added drop- wise under constant stirring. The solution was stirred further at room temperature for 12 h. The solid obtained was filtered, washed with cold water, dried and recrystallized from chloroform/ethanol (50:50), yield = 91\%, mp 155–159 \(^\circ\)C.

SYNTHESIS OF 3-AMINO-2-HYDRAZINOQUINAZOLIN-4(3H)-ONES (IVa-j).
The 3-amino-2-mercaptoquinazolin-4(3H)-ones (II) 1.93g (0.01mol) OR 3-amino-2-(methylthio)quinazolin-4(3H)-one (III) 2.07g (0.01 mol) was dissolved in ethanol (25 ml). To this hydrazine hydrate (99\%) 5 g (0.1 mol) was added and refluxed for 15 hrs. The reaction mixture was cooled and poured into ice-water. The solid so obtained was filtered washed with water, dried and recrystallized from chloroform–benzene (25 : 75) mixture.

BIOLOGICAL ACTIVITY
Antibacterial activity
Agar diffusion method [9-11]
The antibacterial activity of the synthesized compounds were studied, systematically against four different strains of bacteria (gram-positive and gram-negative) by the agar diffusion method. Generally, the antibacterial activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria in nutrient broth or agar, the bacterial inhibition can be measured by two methods: one is the serial dilution method and the other is diffusion method. The serial dilution method is very much useful for the determination of the antibacterial activity. But it is not much useful for the qualitative detection tests and also for the evaluation of a large number of compounds. Therefore, in this investigation the latter is employed. Further, the contemplated the agar diffusion method is of three types :

(i) cup-plate method (disc method),
(ii) filter paper strip method, and
(iii) gradient plate method. The specific method adopted in the present investigation was cup-plate method involving cups of standard diameter, the nutrient agar medium and containing standard bacteria inoculum.

All the test compounds were evaluated for antibacterial activity against Staphylococcus aureus (gram-positive), Eschrichia colli (gram-negative) and streptococci (gram-negative), Pseudomonas typhii (gram-negative), following the agar diffusion method of assay using Sparfloxacin, Cetazolin sodium, Procaine Penicillin and Streptomycin as a standard drugs. The organisms were sub-cultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at 37 \(\pm\) 1 \(^\circ\)C for 24 hrs. they were stored in a refrigerator. Thus stock cultures were maintained. Bacterial inoculum was prepared by transferring a loopful of stock culture to nutrient broth (100 ml) in a clean and sterilized conical flask (250 ml). The flasks were incubated at 37 \(\pm\) 1 \(^\circ\)C for 18 hrs. before the experimentation. Solutions of the test compounds were prepared by dissolving 5 mg and 10 mg of each in dimethylforamide (10 ml AR). A reference standard for gram-positive and gram-negative bacteria were made by dissolving accurately weighed quantity of Procaine Penicillin, Sparfloxacin and Cetazolin sodium, Streptomycin respectively in dimethylformamide solution, separately. The nutrient agar medium was sterilized by autoclaving at 121\(^\circ\)C for 15 min (15 lb/sq.inch). The petriplates, tubes and flasks plugged with cotton were sterilized in hot air-oven at 160\(^\circ\)C, for an hour. Into each sterilized
petri-plate (10 cm diameter), about 30 ml each of molten nutrient bacteria (6 ml of inoculum to 300 ml of nutrient agar medium) was transferred, aseptically. The plates were kept at room temperature to allow the solidification. In each plate, four cups of 8 mm diameter were made with a sterile borer. Then, 0.1 ml of the test solution was added to the cups, aseptically and labeled, accordingly. The plates were kept undisturbed for at least 2 hrs at room temperature to allow diffusion of the solution properly, into nutrient agar medium. After incubation of the plates at 37 ± 1°C for 24 hrs the diameter of the zone of inhibition surrounding each of the cups was measured with the help of an ‘antibiotic zone reader’. All the experiments were carried out in triplicate. Simultaneously, controls were maintained employing 0.1 ml of dimethylformamide to observe the solvent effects. The results are tabulated in Table No.2.

ANTIFUNGAL ACTIVITY

All those compounds screened for antibacterial activity were also tested for their antifungal activity [12-15]. The fungi employed for screening were: *Aspergillus flavus* and *Candida albicans*. The test organisms were sub-cultured using potato dextrose agar medium. The tubes containing sterilized medium were inoculated with test fungi and after incubation at 25°C for 48 hrs they were stored 4°C in a refrigerator. The inoculums was prepared by taking a loopful of stock culture to about 100 ml of nutrient broth, in 250 ml cleaned sterilized conical flasks. The flasks were incubated at 25°C for 24 hrs before use. The solutions of test substances were prepared by a similar procedure described under the antibacterial activity. A reference standard (0.5 mg and 1 mg/ml conc.) were prepared by dissolving 5 mg and 10 mg of Griseofulvin in 10 ml of dimethylformamide to obtain a solutions of 50 µg/ml and 100 µg/ml concentration. The potato-dextrose-agar medium was sterilized by autoclaving at 121°C for 15 min (15 lb/sq. inch). The petri-plates, tubes and flasks plugged with cotton plugs were sterilized in hot air-oven at 150°C, for an hour. Into each sterilized petri-plate (10 cm diameter), about 30 ml each of molten potato dextrose-agar medium inoculated with respective fungus (6 ml of inoculum to 300 ml of potato-dextrose-agar medium) was transferred, aseptically. After solidification of the medium at room temperature four cups of 8 mm diameter were made in each plate with a sterile borer. Accurately 0.1 ml (100 µg/ml conc.) of test solution was transferred to the cups, aseptically and labeled, accordingly. The reference standard 0.1 ml (50 µg/ml conc., 100 µg/ml conc.) were also added to the cups in each plate. The plates were kept undisturbed for at least two hours at room temperature to allow diffusion of the solution properly, into potato-dextrose-agar medium. Then the plates were incubated at 25°C for 48 hrs. The diameter of the zone of inhibition was read with help of an ‘antibiotic zone reader’. The experiments were performed in triplicate in order to minimize the errors. The results are tabulated in Table No.3.

SPECTRAL DATA

**IVa**: IR (KBr) cm⁻¹: 3054 & 2930 (-CH), 1684 (C=O), 1550 (C=C), 1475 (C=N). ¹H NMR (DMSO) δ ppm: 7.20-8.24 (m,12H,Ar-H),8.40-8.42(s,1H,-N=CH). FABMS (m/z): 355 (M⁺).

**IVb**: IR (KBr) cm⁻¹: 3075 & 2853 (-CH), 1680 (C=O), 1560 (C=C), 1483 (C=N). ¹H NMR (DMSO) δ ppm: 6.97-7.86 (m,13H, Ar-H), 9.89 (s,1H, -N=CH),11.00(s,1H,Ar-OH). FABMS (m/z): 381 (M⁺).

**IVc**: IR (KBr) cm⁻¹: 3080 & 2910 (-CH), 1682 (C=O), 1520 (C=C), 1441 (C=N). ¹H NMR (DMSO) δ ppm: 2.84-3.03 (s,6H,-N(CH3)2),6.49-8.54 (m,13H,Ar-H),9.69 (s,1H, -N=CH). FABMS (m/z): 382 (M⁺).

RESULTS

Table 1. Physical and analytical data of 3-[arylidine amino]-2-phenyl [1,2,4] triazolo[5,1-b]quinazolin-9(3H)-ones (Schiff bases).
Table 2. Results of Antibacterial activity by cup-plate method.

<table>
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<tr>
<th>Sl. No.</th>
<th>Compound Code</th>
<th>Mean zone of inhibition in (mm)</th>
<th>streptococci (G+ve)</th>
<th>Pseudomonas (G-ve)</th>
<th>Staphylococcus aureus (G+ve)</th>
<th>Escherichia coli (G-ve)</th>
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<td></td>
<td></td>
<td>50 µg/ml</td>
<td>100 µg/ml</td>
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<td>100 µg/ml</td>
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<td>50 µg/ml</td>
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<td>Sparfloxa</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>02</td>
<td>Cetazolin Sodium</td>
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<td>-</td>
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<tr>
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<td>Procaine Penicillin</td>
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<td>Streptomycin</td>
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<tr>
<td>05</td>
<td>Iva</td>
<td>15 (0.62)</td>
<td>18 (0.62)</td>
<td>14 (0.64)</td>
<td>17 (0.71)</td>
<td>14 (0.70)</td>
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<tr>
<td>06</td>
<td>IVb</td>
<td>11 (0.46)</td>
<td>14 (0.48)</td>
<td>12 (0.55)</td>
<td>16 (0.67)</td>
<td>14 (0.70)</td>
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<td>07</td>
<td>IVc</td>
<td>11 (0.46)</td>
<td>13 (0.45)</td>
<td>11 (0.50)</td>
<td>15 (0.63)</td>
<td>14 (0.70)</td>
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<tr>
<td>08</td>
<td>IVd</td>
<td>08 (0.33)</td>
<td>10 (0.34)</td>
<td>12 (0.55)</td>
<td>16 (0.67)</td>
<td>16 (0.75)</td>
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<tr>
<td>09</td>
<td>IVe</td>
<td>11 (0.46)</td>
<td>14 (0.48)</td>
<td>15 (0.68)</td>
<td>20 (0.83)</td>
<td>15 (0.71)</td>
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<tr>
<td>10</td>
<td>IVf</td>
<td>11 (0.46)</td>
<td>12 (0.41)</td>
<td>17 (0.77)</td>
<td>19 (0.79)</td>
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<td>11</td>
<td>IVg</td>
<td>14 (0.58)</td>
<td>17 (0.59)</td>
<td>11 (0.50)</td>
<td>14 (0.58)</td>
<td>18 (0.90)</td>
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<tr>
<td>12</td>
<td>IVh</td>
<td>14 (0.58)</td>
<td>16 (0.55)</td>
<td>17 (0.77)</td>
<td>20 (0.83)</td>
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<td>13</td>
<td>IVi</td>
<td>10 (0.42)</td>
<td>11 (0.38)</td>
<td>15 (0.68)</td>
<td>17 (0.71)</td>
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<td>14</td>
<td>IVj</td>
<td>12 (0.50)</td>
<td>15 (0.52)</td>
<td>16 (0.73)</td>
<td>20 (0.83)</td>
<td>13 (0.65)</td>
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</table>
Table 3. Results of Antifungal activity

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Compound code</th>
<th>Candida albicans</th>
<th>Aspergillus flavus</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>50μg/ml</td>
<td>100μg/ml</td>
</tr>
<tr>
<td>01</td>
<td>Griseofulvin</td>
<td>18</td>
<td>23</td>
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<tr>
<td>02</td>
<td>IVa</td>
<td>15(0.83)</td>
<td>13(0.56)</td>
</tr>
<tr>
<td>03</td>
<td>IVb</td>
<td>16(0.88)</td>
<td>19(0.83)</td>
</tr>
<tr>
<td>04</td>
<td>IVc</td>
<td>13(0.72)</td>
<td>16(0.69)</td>
</tr>
<tr>
<td>05</td>
<td>IVd</td>
<td>17(0.94)</td>
<td>17(0.73)</td>
</tr>
<tr>
<td>06</td>
<td>IVe</td>
<td>14(0.77)</td>
<td>19(0.83)</td>
</tr>
<tr>
<td>07</td>
<td>IVf</td>
<td>10(0.55)</td>
<td>17(0.73)</td>
</tr>
<tr>
<td>08</td>
<td>IVg</td>
<td>9(0.50)</td>
<td>18(0.78)</td>
</tr>
<tr>
<td>09</td>
<td>IVh</td>
<td>16(0.88)</td>
<td>20(0.87)</td>
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<tr>
<td>10</td>
<td>IVi</td>
<td>14(0.77)</td>
<td>12(0.52)</td>
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<tr>
<td>11</td>
<td>IVj</td>
<td>17(0.94)</td>
<td>14(0.61)</td>
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Fig 1. IR Spectra of compound [IVa]:

![IR Spectra of compound IVa](image-url)
Fig 2. I.R. Spectra of compound

Fig 3. I.R. Spectra of compound [IVb]
Scheme-I.

I.  

\[
\text{C}_6\text{H}_5\text{COCl} \quad \text{POCl}_3 \quad C_6\text{H}_5\text{COCl}
\]

II.  

III.  

IV.  

Alkylation

V.  

VI(a-j)
DISCUSSION

Antibacterial activity by cup-plate method:

Synthesized compounds have been evaluated for their antibacterial activity at concentration of 50 µg/ml and 100 µg/ml by standard method, against following bacteria's:

a) (i) *Streptococci* (ii) *Staphylococcus aureus* gram (+ve).
b) (i) *Pseudomonas* (ii) *Escherichia coli* gram (-ve).

From the results revealed that all the compounds have been shown to exhibit a moderate broad spectrum of activity. The test compounds have been found to active against both gram (+ve) and gram (-ve) organisms. The test compounds IVa, IVh, and IVj showed potent antibacterial activity at lower (50 µg/ml), compounds IVb, IVe, IVg and IVi at higher (100 µg/ml) concentration against *Streptococci* gram (+ve), compare to standard drug Sparfloxacine.

Compounds IVe, IVf, IVh, IVi and IVj at (50 µg/ml) and IVa, IVb, IVc, IVd, IVf IVh, IVi , IVj (100 µg/ml) showed promising antibacterial activity against *Pseudomonas* gram (-ve) bacteria compare to standard drug Cetazolin sodium. Compounds IVe, IVf, IVg, IVh, IVi at (50 µg/ml) and IVc, IVd at (100 µg/ml) showed moderate activity against *Staphylococcus aureus* gram (+ve) bacteria compare to standard drug procaine penicillin. The compounds IVa, IVb, IVc, IVd, IVg, IVh, IVi at (50 µg/ml) and IVb, IVe (100 µg/ml) being same has been found exhibit relatively by more in their inhibitory action against *Escherichia coli* gram (-ve) bacteria, compare to standard drug streptomycine.

Antifungal activity:

All the compounds were subjected to antifungal activity. For these activities *Candida albicans* and *Aspergillus flavus* fungal organisms were used. The Griseofulvin was used as standard. Standard and synthesized compounds were tested at two conc. Viz., 50 µg/ml and 100µg/ml. The experimental results are presented in Table No. 5. The compounds have shown activity, but none of them have shown better activity than standard. Some of compounds like IVa, IVd, IVe, IVh IVi have shown activity near to standard against *Candida albicans* at 50 µg/ml conc. And same compounds have shown activity almost equipotent antifungal activity to that of standard standard at 100 µg/ml conc. and the compounds IVb, IVe, IVh IVi have shown activity same to standard against *Aspergillus flavus* organism at 50 µg/ml conc. and all compounds IV a-j have shown near to standard at 100 µg/ml conc.

CONCLUSION

Ten different novel compounds of 3-amino-2-aryl(1,2,4)triazolo(5,1-b) quinazolin-9 (3H)-were synthesized, by reaction with ten different aromatic aldehydes. All the synthesized compounds were characterized by IR, 1H NMR and Mass spectral properties. The synthesized compounds were screened for antibacterial, antifungal and anti-inflammatory activity. Tested compounds exhibited moderate to good antibacterial activity against both gram (+ve) and gram (-ve) bacteria. A few of compounds exhibited antifungal activity near to standard drug.

ACKNOWLEDGEMENT

The authors are thankful to Secretary of B.L.D.E’s College of Pharmacy, Bijapur, Karnataka, through Principal, for providing necessary facilities to carry out this research work.

REFERENCES