



## Research Article

## SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF NOVEL SERIES OF PYRAZOLINE DERIVATIVES BEARING 1,4- BENZOXAZINONE

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## ABSTRACT

The starting compound 2*H*-benzo[1,4]oxazin-3(4*H*)-one (**1**) was prepared from *o*-aminophenol (**A**), chloroacetyl chloride (**B**), benzyltriethylammoniumchloride, chloroform, and sodium hydrogen carbonate upon refluxing for 12 h at 55°C. **1** was refluxed for 12 h with ethyl bromo acetate, acetonitrile, potassium carbonate, TBAI to furnish ethyl 2-(3-oxo-2*H*-benzo[1,4]oxazin-4(3*H*)-yl)acetate (**2**) and **2** was stirred for 12 h with lithium hydroxide and THF to furnish 2-(3-oxo-2*H*-benzo[1,4]oxazin-4(3*H*)-yl) acetic acid (**3**). The different Pyrazole compounds (**4a-e**) and secondary amines (**4f-l**) derivatives were synthesized by refluxing with hydroxy benzotriazole (HOBT), EDC HCl, DCM and triethylamine with **3** for 14 h. The synthesized compounds were characterized by their physical and spectral data and subjected to antimicrobial evaluation.

**Keywords:** Benzoxazinone, Chloroacetylchloride, Benzyltriethylammoniumchloride, Bromoethylacetate, Lithiumhydroxide and antimicrobial activity.

## INTRODUCTION

The 2*H*-1,4-benzoxazin-3-(4*H*)-one scaffold has been studied intensively as important heterocyclic systems for building natural <sup>1</sup> and designed synthetic compounds. The 2*H*-1,4-benzoxazin-3-(4*H*)-ones and 3,4-dihydro-2*H*-1,4-benzoxazines have been frequently utilized as suitable skeletons for the design of biologically active compounds, ranging from herbicides and fungicides to therapeutically usable drugs. A literature provided/identified several 1,4-benzoxazinone based compounds in the development phase as potential new drugs. Novel antibacterial agents like 1-(4-(2-(4-(2-chlorobenzyl)-3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]oxazin-2-yl)ethoxy)benzyl)guanidine and 1-(2-(4-(3-chlorobenzyl)-3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]oxazin-2-yl)ethyl)guanidine are inhibitors of bacterial histidine protein kinase<sup>2,3</sup>. The (E)-mesityl 3-oxo-2-(2-oxo-2*H*-benzo[b][1,4]oxazin-3(4*H*)-ylidene)-3-phenylpropanoate is potentially useful for treating infections caused by *Mycobacterium* species<sup>4</sup>.

The N-(diaminomethylene)-2-ethyl-4-isopropyl-3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]oxazine-7-carboxamide is a potential drug for treating heart disease, myocardial necrosis or arrhythmia<sup>5</sup> and 3-(4-(4-(2*H*-benzo[b][1,4]oxazin-4(3*H*)-yl)butyl)phenyl)-2-ethoxypropanoic acid possesses peroxisome proliferator activated receptor a (PPAR  $\alpha$ ) and (PPAR  $\gamma$ ) agonist activity and could be used in treating diabetes, hyperlipidemia and other diabetic complications<sup>6</sup>. French investigators recently introduced new 8-aryl alkyl amino-1,4-benzoxazine neuro protectants<sup>7</sup> and Schering has disclosed 1,4-benzoxazines like N-(4-(((3-amino-2-methyl-2*H*-benzo[b][1,4]oxazin-6-yl)methyl)amino)butyl)-2,2,2-trifluoroacetamide and tert-butyl 3-(3-amino-2-methyl-2*H*-benzo[b][1,4]oxazin-6-yl)-2-(3-(((tert-butoxycarbonyl)amino)methyl)benzyl)propanoate as inhibitors

of nitric oxide synthase (NOS) which are potential drugs for treating neurodegenerative, inflammatory, auto immune and cardiovascular disorders<sup>8,9</sup>. 3-(4-(5-((2*S*,6*R*)-2,6-dimethylpiperidin-1-yl)pentyl)-3,4-dihydro-2*H*-benzo[b][1,4]oxazin-2-yl)-4-hydroxybenzimidamide inhibits the coagulation serine proteases factor Xa, thrombin and factor VIIa (Berryman *et al*, 1999), and 6-(2-(4-(6-methylnaphthalen-1-yl)piperazin-1-yl)ethyl)-2*H*-benzo[b][1,4]oxazin-3(4*H*)-one is a potential agent for treating anxiety and depression<sup>11</sup>.

The 6-((4-(4-methoxyphenyl)piperazin-1-yl)methyl)-2*H*-benzo[b][1,4]oxazin-3(4*H*)-one possesses D<sub>2</sub> receptor antagonistic activity and is a potential antipsychotic agent<sup>12</sup>. The 2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylic acid derivative was found to be a potent immune stimulant<sup>13</sup>. The 2*H*-1,4-benzoxazin-3(4*H*)-ones bearing a carboxylate and a benzamide side chain are fibrinogen receptor antagonists<sup>14</sup> and 5-(2-((4-carbamimidoylbenzyl)oxy)-3,4-dihydro-2*H*-benzo[b][1,4]oxazin-7-yl)-5-oxo-3-phenylpentanoic acid possesses a dual antithrombotic action, exhibiting both thrombin inhibitory and fibrinogen receptor antagonistic activities<sup>15</sup>.

Substituted benzoxazinones have received considerable attention during last two decades as they are endowed with variety of biological activities and have wide range of therapeutic properties. Similarly several heterocyclic compounds like pyrazoles and other nitrogen heterocycles are also endowed with different pharmacological and biological activities. A literature survey indicates that benzoxazinones derivatives possess different pharmacological and biological activities, of which the most potent is anti-microbial, antifungal, antiulcer and anti-inflammatory activities<sup>16</sup>. In view of above literature survey, we thought to synthesize some new substituted benzoxazinones

derivatives containing pyrazoles and other nitrogen heterocyclic moieties as partner and determine their anti-microbial activities.

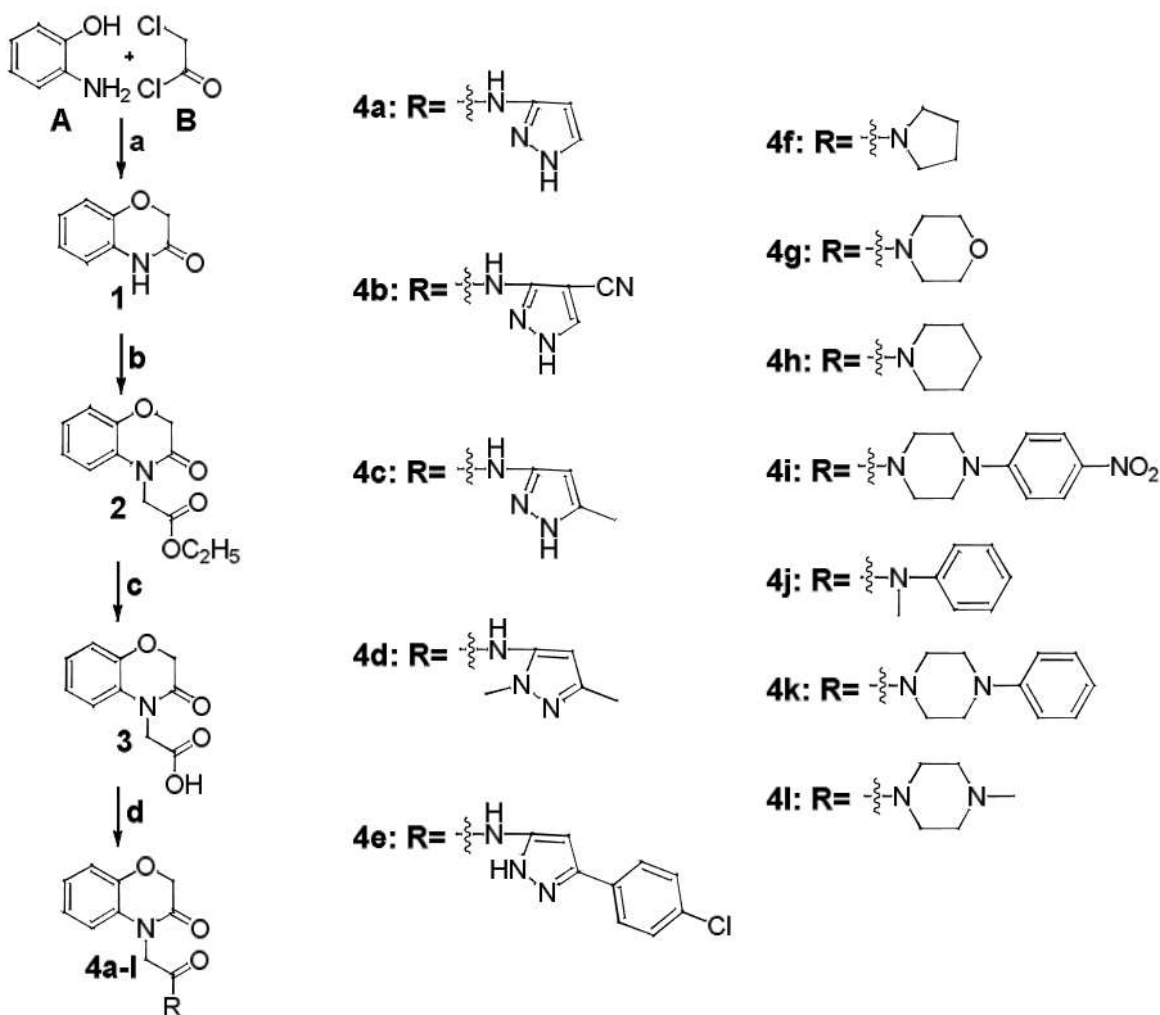
## MATERIAL AND METHODS

### Chemicals and Instrumentations

Melting points were determined by using Toshniwal apparatus in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel G plates using chloroform: ethyl acetate (7:3) as solvent system and U.V lamp used as a visualizing agent. IR spectra were recorded using KBr pellets on a Shimadzu 8000 series and Jasco FT/IR 5300 Series spectrophotometer.  $^1\text{H}$ NMR and  $^{13}\text{C}$ NMR spectra on a Varian EM-200, Avance 200MHz spectrophotometer using  $\text{DMSO}-d_6$  and  $\text{CDCl}_3$  as solvent and TMS as internal standard (chemical shift values expressed in ppm). Mass spectra were recorded on a Shimadzu 2010A series spectrophotometer by LC-MS method.

### Methods for development of analogues

The starting compound 2*H*-benzo[1,4]oxazin-3(4*H*)-one (1) was prepared from *o*-aminophenol, chloroacetyl chloride, benzyltriethylammoniumchloride, chloroform, and sodium hydrogen carbonate upon refluxing for 12 h at 55 °C in single step respectively. The 2*H*-benzo[1,4]oxazin-3(4*H*)-one (1) was refluxed for 12 h with ethyl bromo acetate, acetonitrile, potassium carbonate, TBAI to furnish ethyl 2-(3-oxo-2*H*-benzo[1,4]oxazin-4(3*H*)-yl)acetate (2) respectively. Then ethyl 2-(3-oxo-2*H*-benzo[1,4]oxazin-4(3*H*)-yl)acetate (2) was stirred for 12 h with lithium hydroxide and THF to furnish 2-(3-oxo-2*H*-benzo[1,4]oxazin-4(3*H*)-yl) acetic acid (3). The different Pyrazole compounds and different sec amines (4a-l) were synthesized by refluxing with hydroxy benzotriazole (HOBT), EDC HCl, DCM and triethylamine with 2-(3-oxo-2*H*-benzo[1,4]oxazin-4(3*H*)-yl) acetic acid (3) for 14 h. The synthesized compounds were characterized by their physical and spectral data.



Scheme 1: a) TEBA,  $\text{CHCl}_3$ ,  $\text{NaHCO}_3$ , 0-5°C for 1 h and 55°C for 12 h, b)  $\text{K}_2\text{CO}_3$  in acetonitrile for 30 mins, TBAI for 12 h, c) LiOH, THF for 12 h, d) HOBT, EDC. HCl, DCM for 30 mins, trimethylamine for 12 h.

### Anti-Microbial Activity

Anti-bacterial activity was carried out by cup and plate method using *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* organisms for antibacterial activity. The potency of synthesized compounds was determined against standard drug Penicillin and Streptomycin by measuring the minimum inhibitory concentration.

Preparation of test solution: 20 mg of the test compound was dissolved in 20 ml of DMF, from this stock solution, 1 ml of solution was taken and further diluted to required concentration with DMF. These sample solution were made in suitably labeled sterilized test tubes.

Preparation of standard solution: The standard drug used for the comparison are Penicillin and Streptomycin, the solutions were prepared from sterile water soluble.

Method of testing: The prepared nutrient agar media sterilized using autoclave and is cooled to 45°C with gentle shaking to bring about uniform cooling. To this 0.5–0.6 mL of 18-24 h old culture was injected aseptically and mixed well by gentle shaking. This was poured onto the petri dishes and was allowed to set for 1 h. Thereafter the cups were made by punching into the set agar with a sterile cork borer and scooping out the punched part of the agar. The diameter of each cup was 6 mm. To these cups 50 µl of the test compound was put, which was prepared in DMF. After adding the drug solution, it was allowed to diffuse for about 45 minutes, at room temperature. Then the plates were incubated at 37 °C for 24 h in an incubator. The minimum inhibitory concentration (MIC) is taken as a parameter of antibacterial activity, results were tabulated (Table 1)

Anti-fungal activity was carried out by cup and plate method using *Rhizopus oryzae*, *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Saccharomyces cerevisiae* on potato dextrose agar media. Amphotericin-B 100 µg/ml is used as standard.

Preparation of test solution: 10 mg of the compound was dissolved in 10 ml of DMF, from this stock solution further required concentration solutions were prepared by dilution using DMF.

Preparation of standard anti-fungal solution: Amphotericin-B was used as standard anti-fungal for comparison and solution were prepared by using sterile water, so that the concentrations of the solution were 100 µg/ml.

Method of testing: The method of testing for fungicidal activity is the same as that of antibacterial testing. DMF was used as a solvent control, zone of inhibition is taken as a parameter of antifungal activity and results were tabulated.

### RESULTS

Procedure for preparation of 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (1). To a stirred solution of *o*-amino phenol (5g, 0.045 mol) and TEBA (benzyl triethyl ammonium chloride) (10.44g, 0.045 mol) in chloroform (25 mL) was added finely powdered sodium hydrogen carbonate (15.3g, 0.18 mol). The resultant mixture was cooled in an ice bath and then a solution of chloro acetyl chloride (6.1g, 0.054 mol) in chloroform was added drop wise over a period of 20 min. After the addition was completed the mixture was stirred at 0-5°C for 1 h and then heated at 55°C for 12 h. The solvent was removed and water (40 mL) was added. The crude product was collected by filtration and washed with water and

then recrystallised from ethanol. The melting point was 232-34°C and percentage yield was 86%. IR (KBr, cm<sup>-1</sup>): 1704, 2902, 2982 and 3134. <sup>1</sup>H NMR (200MHz, DMSO-*d*<sub>6</sub>, 25°C): δ= 4.4 (s, 2H, CH<sub>2</sub>), 6.8 (m, 4H, Ar-H), 10.66 (s, 1H, NH). <sup>13</sup>C NMR (200MHz, DMSO-*d*<sub>6</sub>, 25°C): δ= 69.9, 112.8, 120.3, 121.2, 126.8, 128.2, 148.2 and 168.2 ppm. HRMS: found for C<sub>8</sub>H<sub>7</sub>O<sub>2</sub>N[M+H]<sup>+</sup> is 150.

Ethyl 2-(3-oxo-2*H*-benzo[*b*][1,4]oxazin-4(3*H*)-yl) acetate (2). A mixture of compound (1) (1.5g, 0.006 mol) and anhydrous potassium carbonate (3g, 0.0018 mol) in acetonitrile (25mL) was stirred for 30 min. To this reaction mixture, added ethyl bromoacetate (0.61mL, 0.006 mol) and catalytic amount of TBAI (tetra butyl ammonium iodide) was added and then refluxed for 12 h. The solvent was removed under reduced pressure and the separated solid was recrystallised from ethanol. Melting point 176-78°C and percentage yield 80%. IR (KBr, cm<sup>-1</sup>): 1738, 2915 and 2983. <sup>1</sup>H NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 1.2-1.4 (t, 3H, CH<sub>3</sub>), 4.2 (q, 2H, CH<sub>2</sub> of C<sub>2</sub>H<sub>5</sub>), 4.6-4.7 (s, 4H, CH<sub>2</sub>), 6.8-7.1 (m, 4H, Ar-H). <sup>13</sup>C NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 14.1, 54.3, 61.0, 67.4, 112.8, 117.1, 121.2, 128.2, 128.6, 145.1, 164.8 and 167.6 ppm. HRMS: found for C<sub>12</sub>H<sub>13</sub>O<sub>4</sub>N[M+H]<sup>+</sup> is 236.

2-(3-oxo-2*H*-benzo[*b*][1,4]oxazin-4(3*H*)-yl) acetic acid (3). To the compound 2 (0.25g, 0.001 mol) added lithium hydroxide (10mL) and to this mixture added tetrahydrofuran (THF) (3mL) and stirred the reaction mixture for 12 h. The solvent was removed under reduced pressure and the separated solid was recrystallised from ethanol. Melting point 162-64°C and percentage yield 74%. IR (KBr, cm<sup>-1</sup>): 1638, 1921, 2964 and 3153. <sup>1</sup>H NMR (200MHz, DMSO-*d*<sub>6</sub>, 25°C): δ= 4.6-4.8 (s, 4H, CH<sub>2</sub>), 6.7-7.1 (m, 4H, Ar-H), 12.9-13.0 (s, 1H, OH). <sup>13</sup>C NMR (200MHz, DMSO-*d*<sub>6</sub>, 25°C): δ= 53.8, 67.4, 112.8, 117.1, 121.2, 128.2, 128.6, 145.1, 164.8 and 171.0 ppm. HRMS: found for C<sub>10</sub>H<sub>9</sub>O<sub>4</sub>N[M+H]<sup>+</sup> is 208.

2-(3-oxo-2*H*-benzo[*b*][1,4]oxazin-4(3*H*)-yl)-N-(1*H*-pyrazol-3-yl)acetamide (4a). A mixture of (3) (1g, 0.005 mol) and HOBt (hydroxy benzotriazole) (1.2g, 0.006 mol) and EDC HCL [1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride] (1.1g, 0.005 mol) and suitable amount of dichloro methane (DCM) solvent is added and the reaction is stirred for 30 min. Then add 1*H*-pyrazol-3-amine and then triethylamine (2.5mL, 0.012 mol) to the reaction mixture and stir the reaction mixture for 14 h. The solvent was removed and then wash the reaction mixture with sodium bicarbonate 3-4 times and then concentrate the reaction mixture and then the solid separates. The obtained solid was recrystallized from ethanol. Melting point 232-234°C and percentage yield 66%. <sup>1</sup>H NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 4.6-4.7 (s, 2H, N-CH<sub>2</sub> and s, 2H, O-CH<sub>2</sub>), 6.2-6.3 (m, 1H, CH of pyrazole), 6.7-7.5 (m, 4H, Ar-H), 7.5-7.6 (s, 1H, CH of pyrazole), 10.5-10.6 (s, 1H, NH of CONH) and 12.5-12.6 (s, 1H, NH of pyrazole). <sup>13</sup>C NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 56.0, 67.0, 91.5, 112.8, 117.1, 121.2, 128.2, 128.6, 132.0, 135.5, 145.1, 164.8 and 168.5 ppm. HRMS: found for C<sub>13</sub>H<sub>12</sub>O<sub>3</sub>N<sub>4</sub>[M+H]<sup>+</sup> is 273.

N-(4-cyano-1*H*-pyrazol-3-yl)-2-(3-oxo-2*H*-benzo[*b*][1,4]oxazin-4(3*H*)-yl)acetamide (4b). This compound was prepared in a yield of 58%, according to the procedure for the synthesis of compound 4a, using 3-amino-1*H*-pyrazole-4-carbonitrile. Mp: 278-280°C. <sup>1</sup>H NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 4.6-4.7 (s, 2H, N-CH<sub>2</sub> and s, 2H, O-CH<sub>2</sub>), 6.8-7.1 (m, 4H, Ar-H), 7.5-7.6 (s, 1H, CH of pyrazole), 10.5-10.6 (s, 1H, NH of CONH) and 12.5-12.6 (s, 1H, NH of pyrazole). <sup>13</sup>C NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 56.0, 67.0, 76.7, 112.8, 117.1, 121.2, 128.2, 128.6, 137.4, 145.1, 146.6, 164.8 and 168.5 ppm. HRMS: found for C<sub>14</sub>H<sub>11</sub>O<sub>3</sub>N<sub>5</sub>[M+H]<sup>+</sup> is 298.

N-(5-methyl-1H-pyrazol-3-yl)-2-(3-oxo-2H-benzo[b][1,4]oxazin-4(3H)-yl)acetamide (4c). This compound was prepared in a yield of 64%, according to the procedure for the synthesis of compound 4a, using 5-methyl-1H-pyrazol-3-amine. Mp: 266-268°C. <sup>1</sup>H NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 2.3-2.5 (s, 3H, CH<sub>3</sub>), 4.6-4.8 (s, 2H, N-CH<sub>2</sub> and s, 2H, O-CH<sub>2</sub>), 6.1-6.2 (s, 1H, CH of pyrazole), 6.8-7.2 (m, 4H, Ar-H), 10.5-10.6 (s, 1H, NH of CONH) and 12.1-12.2 (s, 1H, NH of pyrazole). <sup>13</sup>C NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 13.1, 56.0, 67.0, 98.3, 112.8, 117.1, 121.2, 128.2, 128.6, 138.0, 145.1, 148.8, 164.8 and 168.5 ppm. HRMS: found for C<sub>14</sub>H<sub>14</sub>O<sub>3</sub>N<sub>4</sub>[M+H]<sup>+</sup> is 287.

N-(1,3-dimethyl-1H-pyrazol-5-yl)-2-(3-oxo-2H-benzo[b][1,4]oxazin-4(3H)-yl)acetamide (4d). This compound was prepared in a yield of 68%, according to the procedure for the synthesis of compound 4a, using 1,3-dimethyl-1H-pyrazol-5-amine. Mp: 274-276°C. <sup>1</sup>H NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 2.6 (s, 3H, CH<sub>3</sub>), 3.4 (s, 2H, N-CH<sub>3</sub>), 4.7-4.8 (s, 4H, N-CH<sub>2</sub>, O-CH<sub>2</sub>), 6.7-7.1 (m, 4H, Ar-H) and 10.5-10.6 (s, 1H, NH of CONH). <sup>13</sup>C NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 13.2, 35.4, 56.0, 67.0, 88.9, 112.8, 117.1, 121.2, 128.2, 128.6, 139.1, 145.1, 148.8, 164.8 and 168.5 ppm. HRMS: found for C<sub>15</sub>H<sub>16</sub>O<sub>3</sub>N<sub>4</sub>[M+H]<sup>+</sup> is 301.

N-(3-(4-chlorophenyl)-1H-pyrazol-5-yl)-2-(3-oxo-2H-benzo[b][1,4]oxazin-4(3H)-yl)acetamide (4e) This compound was prepared in a yield of 62%, according to the procedure for the synthesis of compound 4a, using 3-(4-chlorophenyl)-1H-pyrazol. Mp: 312-314°C. <sup>1</sup>H NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 4.6-4.8 (s, 2H, N-CH<sub>2</sub> and s, 2H, O-CH<sub>2</sub>), 6.4-6.6 (s, 1H, CH of pyrazole), 6.8-8.0 (m, 8H, Ar-H), 10.8-10.9 (s, 1H, NH of CONH) and 12.6-12.7 (s, 1H, NH of pyrazole). <sup>13</sup>C NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 56.0, 67.0, 87.8, 112.8, 117.1, 121.2, 128.2, 128.9 (3), 131.1, 134.3, 136.6, 145.1, 153.2, 164.8 and 168.5 ppm. HRMS: found for C<sub>19</sub>H<sub>15</sub>O<sub>3</sub>N<sub>4</sub>Cl[M+H]<sup>+</sup> is 383.

4-(2-oxo-2-(pyrrolidin-1-yl)ethyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (4f). A mixture of (3) (1 gm, 0.005 mol) and HOBT (hydroxy benzotriazole) (1.2 gm, 0.006 mol) and EDC HCL [1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride] (1.1 gm, 0.005 mol) and suitable amount of dichloro methane (DCM) solvent is added and the reaction is stirred for 30 min. Then add pyrrolidine and then triethylamine (2.5 ml, 0.012 mol) to the reaction mixture and stir the reaction mixture for 14 h. The solvent was removed and then wash the reaction mixture with sodium bicarbonate 3-4 times and then concentrate the reaction mixture and then the solid separates. The obtained solid was recrystallized from ethanol. Melting point 256-258°C and percentage yield 76%. IR (KBr, cm<sup>-1</sup>): 1693, 2990 and 2960. <sup>1</sup>H NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 1.8-2.2 (d, 4H, CH<sub>2</sub> of pyrrolidine), 3.5-3.7 (d, 4H, CH<sub>2</sub> of pyrrolidine), 4.6 (s, 2H, O-CH<sub>2</sub>), 4.8 (s, 2H, N-CH<sub>2</sub>), 6.8-7.1 (m, 4H, Ar-H). <sup>13</sup>C NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 25.4 (2), 48.7 (2), 54.2, 67.0, 112.8, 117.1, 121.2, 128.2, 128.6, 145.1, 164.8 and 167.8 ppm. HRMS: found for C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub>[M+H]<sup>+</sup> is 261.

4-(2-morpholino-2-oxoethyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (4g). This compound was prepared in a yield of 74%, according to the procedure for the synthesis of compound 4f, using morpholine. Mp: 224-226°C. IR (KBr, cm<sup>-1</sup>): 1772, 2984 and 2910. <sup>1</sup>H NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 3.4-3.8 (d, 4H, CH<sub>2</sub> of morpholine), 4.0-4.2 (d, 4H, morpholine), 4.6-4.8 (s, 2H, N-CH<sub>2</sub> and s, 2H, O-CH<sub>2</sub>), 6.8-7.1 (m, 4H, Ar-H). <sup>13</sup>C NMR

(200MHz, CDCl<sub>3</sub>, 25°C): δ= 47.7 (2), 54.2, 66.2 (2), 67.0, 112.8, 117.1, 121.2, 128.2, 128.6, 145.1, 164.2 and 167.8 ppm. HRMS: found for C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>O<sub>4</sub>[M+H]<sup>+</sup> is 277.

4-(2-oxo-2-(piperidin-1-yl)ethyl)-2Hbenzo[b][1,4]oxazin-3(4H)-one(4h). This compound was prepared in a yield of 70%, according to the procedure for the synthesis of compound 4f, using piperidine. Mp: 234-236°C. IR (KBr, cm<sup>-1</sup>): 1750, 2982 and 2919. <sup>1</sup>H NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 1.5-1.8 (t, 6H, CH<sub>2</sub> of piperidine), 3.4-3.6 (d, 4H, CH<sub>2</sub> of piperidine), 4.6 (s, 2H, O-CH<sub>2</sub>), 4.8 (s, 2H, N-CH<sub>2</sub>), 6.8-7.1 (m, 4H, Ar-H). <sup>13</sup>C NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 24.2, 25.4 (2), 48.6 (2), 54.2, 67.0, 112.8, 117.1, 121.2, 128.2, 128.6, 145.1, 164.8 and 167.8 ppm. HRMS: found for C<sub>15</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub>[M+H]<sup>+</sup> is 275.

4-(2-(4-(4-nitrophenyl)piperazin-1-yl)-2-oxoethyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (4i). This compound was prepared in a yield of 70%, according to the procedure for the synthesis of compound 4f, using N-nitro phenyl piperazine. Mp: 234-236°C. IR (KBr, cm<sup>-1</sup>): 1597, 1732 and 2911. <sup>1</sup>H NMR (200MHz, DMSO-*d*<sub>6</sub>, 25°C): δ= 3.0-3.2 (d, 4H, CH<sub>2</sub> of CH<sub>2</sub>), 3.4-3.6 (d, 4H, CH<sub>2</sub> of CH<sub>2</sub>), 4.6-4.8 (s, 2H, N-CH<sub>2</sub> and s, 2H, O-CH<sub>2</sub>), 6.8-7.8 (m, 8H, Ar-H). <sup>13</sup>C NMR (200MHz, DMSO-*d*<sub>6</sub>, 25°C): δ= 46.2 (2), 53.2 (2), 54.2, 67.0, 112.3 (2), 112.8, 117.1, 121.2, 124.8 (2), 128.2, 128.6, 137.4, 145.1, 155.7, 164.2 and 167.8 ppm. HRMS: found for C<sub>20</sub>H<sub>20</sub>N<sub>4</sub>O<sub>5</sub>[M+H]<sup>+</sup> is 397.

N-methyl-2-(3-oxo-2H-benzo[b][1,4]oxazin-4(3H)-yl)-N-phenylacetamide(4j). This compound was prepared in a yield of 58%, according to the procedure for the synthesis of compound 4f, using N-methyl aniline. Mp: 204-206°C. IR (KBr, cm<sup>-1</sup>): 1604, 1720, 2960 and 2923. <sup>1</sup>H NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 2.8-2.9 (s, 3H, CH<sub>3</sub>), 4.7 (s, 2H, O-CH<sub>2</sub>), 4.8 (s, 2H, N-CH<sub>2</sub>), 6.6-7.3 (m, 9H, Ar-H). <sup>13</sup>C NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 35.8, 53.5, 67.0, 112.8, 117.1, 121.2, 127.5 (2), 128.0, 128.9 (2), 144.4, 145.1, 164.8 and 166.7 ppm. HRMS: found for C<sub>17</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub>[M+H]<sup>+</sup> is 297.

4-(2-oxo-2-(4-phenylpiperazin-1-yl)ethyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (4k). This compound was prepared in a yield of 60%, according to the procedure for the synthesis of compound 4f, using N-phenyl piperazine. Mp: 268-270°C. IR (KBr, cm<sup>-1</sup>): 1664, 1690, 2980 and 2927. <sup>1</sup>H NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 3.1-3.3 (d, 4H, CH<sub>2</sub>), 3.8-4.0 (d, 4H, CH<sub>2</sub>), 4.6 (s, 2H, O-CH<sub>2</sub>), 4.8 (s, 2H, N-CH<sub>2</sub>), 6.8-7.4 (m, 9H, Ar-H). <sup>13</sup>C NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 46.2 (2), 53.3 (2), 54.2, 67.0, 112.8, 114.3 (2), 117.1, 121.2, 121.9, 128.2, 129.6 (2), 145.1, 149.6, 164.2 and 166.7 ppm. HRMS: found for C<sub>20</sub>H<sub>21</sub>N<sub>3</sub>O<sub>3</sub>[M+H]<sup>+</sup> is 352.

4-(2-(4-methylpiperazin-1-yl)-2-oxoethyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (4l). This compound was prepared in a yield of 64%, according to the procedure for the synthesis of compound 4f, using N-methyl piperazine. Mp: 268-270°C. IR (KBr, cm<sup>-1</sup>): 1621, 1691, 1650, 2964 and 2923. <sup>1</sup>H NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 2.3 (s, 3H, CH<sub>3</sub>), 2.4-2.6 (d, 4H, 4.7 CH<sub>2</sub>), 3.5-3.7 (d, 4H, CH<sub>2</sub>), 4.7-4.8 (s, 2H, N-CH<sub>2</sub> and s, 2H, O-CH<sub>2</sub>), 6.8-7.0 (m, 4H, Ar-H). <sup>13</sup>C NMR (200MHz, CDCl<sub>3</sub>, 25°C): δ= 46.6, 49.3 (2), 51.5 (2), 54.2, 67.0, 112.8, 117.1, 121.2, 127.5 (2), 128.2, 128.9, 145.1, 164.8 and 166.7 ppm. HRMS: found for C<sub>15</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>[M+H]<sup>+</sup> is 290.

Table 1: Antibacterial activity of synthesized compounds 4a-1

Compound Code	Minimum Inhibitory Concentration ( $\mu\text{g/ml}$ )					
	Gram Positive Organism			Gram Negative Organism		
	<i>B.Subtilis</i>	<i>S.aureus</i>	<i>S.epidermis</i>	<i>E.coli</i>	<i>P.aeruginosa</i>	<i>K.pneumoniae</i>
4a	23.15	150	17.10	75	150	18.0
4b	18.60	75	22.50	14.55	13.0	75
4c	75	30.15	75	150	150	75
4d	24.50	10.25	12.16	75	75	29.35
4e	24.75	4.65	75	75	17.75	16.75
4f	21.60	75	85	13.50	75	150
4g	18.65	75	150	150	150	18.66
4h	15.65	75	75	150	150	75
4i	26.15	18.65	16.75	12.25	16.0	75
4j	75	18.68	22.15	23.25	28.75	150
4k	16.15	18.75	28.0	75	75	23.75
4l	18.68	22.65	18.68	75	75	150
Penicillin	1.526	6.25	3.125	7.81	12.50	6.25
Streptomycin	6.25	1.56	1.56	3.125	3.125	3.125

Note: 20  $\mu\text{g/ml}$  and above poor activity, 14-20  $\mu\text{g/ml}$  moderate activity and 4-13  $\mu\text{g/ml}$  significant activity.

Table 2: Antifungal activity of synthesized compounds 4a-1

Zone of Inhibition(mm)						
Compound (100 $\mu\text{g/ml}$ )	<i>R. oryzae</i>	<i>A.niger</i>	<i>A.flavis</i>	<i>C.albicans</i>	<i>S.cerevisiae</i>	
4a	16	16	-	-	-	
4b	18	14	12	12	10	
4c	12	14	16	-	18	
4d	24	18	-	-	16	
4e	18	16	12	14	22	
4f	16	12	14	-	-	
4g	14	10	12	14	16	
4h	-	-	14	16	18	
4i	16	14	18	12	12	
4j	28	16	14	-	-	
4k	-	-	14	12	-	
4l	14	-	21	18	16	
Amphotericin-B	24	25	24	23.5	22	

Note: '-' denotes no activity, 10-14 mm poor activity, 15-17 mm moderate activity, 18-25 mm significant activity.

## DISCUSSION

The data in the Table 1 indicate that compound 4e show significant activity and compound 4d shows moderate activity against *staphylococcus aureus*, and compound 4h shows moderate activity against *Bacillus subtilis* and compound 4d shows moderate activity against *staphylococcus epidermidis* and compound 4i shows significant activity and compound 4b and 4f shows moderate activity against *Escherichia coli* and compound 4b shows significant activity and compounds 4e and 4i shows moderate activity against *Pseudomonas aeruginosa* and compound 4e shows moderate activity against *Klebsiella pneumoniae* and rest of the compounds were found to exhibit poor activity when compared to the standard Penicillin and Streptomycin.

The data in Table 2 indicates that compounds 4d and 4j show significant activity and compounds 4b and 4e show moderate activity against *Rhizopus oryzae* and compound 4d show moderate activity against *Aspergillus niger* and compound 3b7 show significant activity and compound 4i show moderate activity against *Aspergillus flavus* and compound 4l show moderate activity against *Candida albicans* and compound 4e show significant activity and compounds 4c and 4h show moderate activity against *Saccharomyces cerevisiae* and rest of the compounds were found to exhibit poor activity when compared to the standard Amphotericin-B.

## CONCLUSION

From the data of the antibacterial and antifungal activity, it is clearly concluded that the synthesized benzoxazinone derivatives were found to be moderate to weak antibacterial agents. When the two moieties are fused or combined and screened for antibacterial studies they showed moderate to weak antibacterial activity against Gram (+ve) and Gram (-ve) bacteria. Further the detail structure activity relationship studies are required along with the molecular manipulation i.e. molecular modeling may give better drugs and further toxicological study is needed. Molecules prepared for the biological testing do not always turn out as potential new molecules, but may be intended to serve as models for evaluation of the hypothesis. Since the synthesized compounds were reported to possess several other pharmacological activities further molecular manipulation of active compound may result in to a better pharmacological active agent.

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