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Synthesis and *in vitro* antibacterial activity of 3-(5-amino-6(2,3-dichlorophenyl)-1,2,4-triazin-3-yl)-2-*aryl*-quinazoline-4(3H)-ones

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Summary. In the present study some new 3-(5-amino-6(2,3-dichlorophenyl)-1,2,4-triazin-3-yl)-2-*aryl*-quinazoline-4(3*H*)-ones (**3a-3f**) was synthesized. The newly synthesized compounds were characterized on the basis of elemental analysis, IR and ^{1}H-NMR spectra. All the synthesized compounds were tested for their antibacterial activity against 20 strains of Gram positive and Gram negative bacteria. Among the compounds tested, the compounds **3a** & **3f** showed good antimicrobial activity in comparison to standard sulphamethoxazole. Compound **3f** was found to be most active in the series against *H.pylori* with MIC 25 μg/mL.

Key words: quinazolinones, *in vitro* antibacterial activity, MIC value.

Introduction. 4(3*H*)-quinazolinone derivatives were reported to posses analgesic [1], anti-inflammatory [2], anti-bacterial, anti-fungal [3-7], anti-HIV [8, 9], antihelminthic [10], antiallergic [11], antitomour [12], anticancer [13], MOA inhibitory [14] and central nervous system activities [15]. 4(3*H*)-quinazolinones its 3 substitution has been reported to be associated with antimicrobial properties [16-18]. The 3 substitution which were reported are various substituted phenyl ring moieties [19], bridged phenyl rings [20], heterocyclic rings [21-23] and aliphatic systems [24]. 2,3-substituted-4(3*H*)-quinazolinone [25] were reported to possess antimicrobial properties. The antimicrobial, antifungal and anti HIV screening of semi-derivatized conventional antibacterial agents namely trimethoprim [26, 27], sulphadoxime [28], norfloxacin, ciprofloxacin and lomefloxacin have been reported.

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These observation lead to the conception that a new series of 2-phenyl-4(3*H*)-quinazolinones with 3,5 diaminotriazine (lamotrizine) substituent in 3rd position would exhibit potential antibacterial activity. Trizines selected for the study posses dihydrofolate reductase inhibitory property. In continuation of earlier work 4(3*H*) quinazolinones the present deals with synthesis of a series of 2,3-disubstituted-4(3*H*)-quinozolinones by condensation of 2-substituted benzoxazin-4-one with lamotrigine. The chemical structure of the synthesized compounds was conformed by means of IR, ^{1}H-NMR, mass spectral and elemental analysis.

Chemistry. The melting points were taken in open capillary tube on a Thomas hoover melting point apparatus and are uncorrected. The IR spectra of the compounds were recorded on Bruker vector-22 FT-IR with KBr pellets. ^{1}H-NMR spectra recorded on 500 MHz Bruker AMX 500 using DMSO-d_6 as solvent. The chemical shifts are reported as parts per million downfield from tetramethylsilane (Me_4Si). Mass

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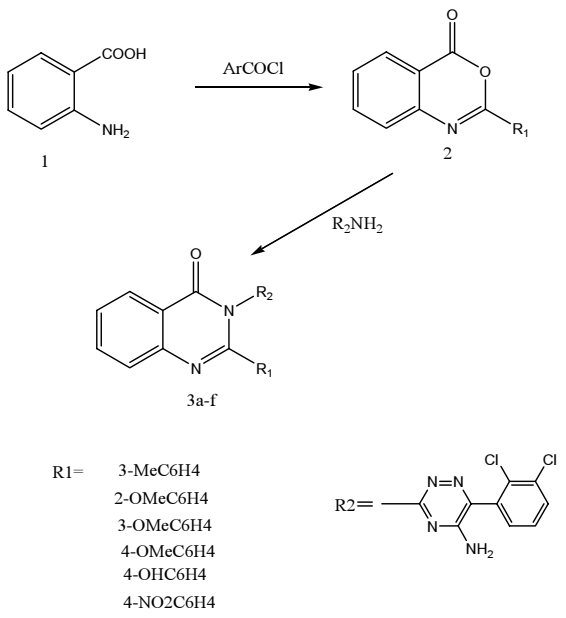

spectra were recorded on varian atlas CH-7. Microanalysis for C, H, N were performed in Heraes CHN Rapid analyzer. All the compounds gave satisfactory chemical analyses (±0.4 %). The purity of the compounds were checked by TLC on SiO_2 gel (HF_{254}, 200 mesh) coated glass plates using (4:1) CH_3OH:$CHCl_3$ as mobile phase and visualized by iodine vapours.

Biological evaluation.

In vitro antibacterial activity. Synthesized compounds were evaluated for their *in vitro* antibacterial activity against pathogenic bacteria by the agar dilution method. MIC values (Table 1) were considered to be the lowest concentration that was completely inhibited growth on agar plates. Sulphamethoxazole was used as the standard in all antibacterial screening studies.

Synthesis of 2-aryl-3,1-benzoxazine-4-one. To a solution of anthranillic acid (0.1 mol) in 50 ml of dry pyridine, benzoyl chloride (0.2 mol) as added drop wise with constant stirring at 15 ˚C. The reaction mixture was cooled to 5 ˚C and aqueous sodium carbonate (15 ml, 10 % w/v) was added. The product formed was filtered, vacuum dried and recrystallized using absolute ethanol.

General procedure for 2,3-disubstituted-4(3H)-quinazolinones.

Equimolar quantities (0.01 mol) of 2-substituted-3,1-benzaxocin-one and the primary amine (lamotrigine) in glacial acetic acid (10 ml) was refluxed for 6 h. The reaction content as cooled o room temperature and poured into crushed ice. The product formed was filtered, vacuum dried and recrystallized using absolute ethanol.

*3-(5-amino-6(2,3-dichlorophenyl)-1,2,4-triazin-3-yl)-2-p-tolylquinazoline-4(3H)-one (**3a**):* yield=86 %, mp 210-211 ˚C. ^{1}H-NMR (DMSO-d_6) δ; 8.52 (s, 1H; 5-H), 8.14 (d, *J*=6 Hz, 1H; 8H), 7.71 (d, *J*=6 Hz, 1H; 7-H), 7.09-7.48 (m, 3H phenyl), 3.56 (s, 2H; NH_2) 2.15 (s, 3H; CH_3). IR (KBr) cm^{-1}; 3455 (NH_2), 1681 (C=O), 1645 (C=N), 1438 (N=N), 830, 792, 737 (Ar-H). EI-MS *m/z*: 474.0763 (calcd for $C_{24}H_{16}Cl_2N_6O$:475.32). *Anal* Calcd for $C_{24}H_{16}Cl_2N_6O$: C, 60.64; H, 3.39; N, 17.68. Found: C, 60.46; H, 3.04; N, 17.74.

*3-(5-amino-6(2,3-dichlorophenyl)-1,2,4-triazin-3-yl)-2-(2-methoxyphenyl)quinazoline-4(3H)-one (**3b**):* yield=82 %, mp 240-241 ˚C. ^{1}H-NMR (DMSO-d_6) δ; 8.82 (s, 1H; 5-H), 8.36 (d, *J*=7.1 Hz, 1H; 8-H), 8.08 (d, *J*=7.1 Hz, 1H; 7-H), 6.85-7.51 (m, H-aromatic), 3.42 (s, 2H; NH_2), 3.68 (s, 3H; CH_3O). IR (KBr) cm^{-1}; 3393 (NH_2), 1679 (C=O), 1611 (C=N), 1435 (N=N), 827, 799, 781 (Ar-H). EI-MS *m/z*: 490.07 (calcd for $C_{24}H_{16}Cl_2N_6O_2$: 491.32). *Anal* Calcd for $C_{24}H_{16}Cl_2N_6O_2$: C, 58.67; H, 3.28; N, 17.10. Found: C, 58.45; H, 3.24; N, 17.14.

*3-(5-amino-6(2,3-dichlorophenyl)-1,2,4-triazin-3-yl)-2-(3-methoxyphenyl)quinazoline-4(3H)-one (**3c**):* yield=83 %, mp 270-271 ˚C. ^{1}H-NMR (DMSO-d_6) δ; 8.42 (s, 1H; 5-H), 8.04 (d, *J*=6.8 Hz, 1H; 8-H), 7.78 (d, *J*=6.8 Hz, 1H; 7-H), 6.74-7.26 (m, H-aromatic), 3.83 (s, 2H; NH_2) 3.73 (s, 3H; CH_3O). IR (KBr) cm^{-1}; 3363 (NH_2), 1657 (C=O), 1605 (C=N), 1425 (N=N), 816, 785, 772 (Ar-H). EI-MS *m/z*: 490.07 (calcd for $C_{24}H_{16}Cl_2N_6O_2$: 491.32). *Anal* Calcd for $C_{24}H_{16}Cl_2N_6O_2$: C, 58.67; H, 3.28; N, 17.10. Found: C, 58.71; H, 3.30; N, 17.12.

*3-(5-amino-6(2,3-dichlorophenyl)-1,2,4-triazin-3-yl)-2-(4-methoxyphenyl)quinazoline-4(3H)-one (**3d**):* yield=79 %, mp 192-193 ˚C. ^{1}H-NMR (DMSO-d_6) δ; 8.32 (s, 1H; 5-H), 7.94 (d, J=7.0 Hz, 1H; 8-H), 8.06 (d, *J*=7.0 Hz, 1H; 7-H), 6.96-7.69 (m, H-aromatic), 3.9 (s, 2H; NH_2) 3.63 (s, 3H; CH_3O). IR (KBr) cm^{-1}; 3343 (NH_2), 1634 (C=O), 1623 (C=N), 1417 (N=N), 811, 783, 769 (Ar-H). EI-MS *m/z*: 490.07 (calcd for $C_{24}H_{16}Cl_2N_6O_2$: 491.32). *Anal* Calcd for $C_{24}H_{16}Cl_2N_6O_2$: C, 58.67; H, 3.28; N, 17.10. Found: C, 58.65; H, 3.25; N, 17.02.

3-(5-amino-6(2,3-dichlorophenyl)-1,2,4-triazin-3-yl)-2-(4-nitrophenyl)quinazoline-4(3H)-

Table 1

*In vitro antibacterial activities of compounds **3a-f** against selected strains (MICs in μg/mL)*

S.No.	Name of bacteria	3a	3b	3c	3d	3e	3f	Sulph.
1	*Staphylococcus aureus*	300	–	200	300	200	400	5000
2	*E.coli*	50	400	300	300	150	400	1250
3	*E.coli* ATCC35218	–	500	200	–	400	–	1250
4	*E.faecalis*	50	500	400	200	300	250	5000
5	*H. pylori*	50	–	100	400	–	25	2500
6	*Klebsiella oxytoca*	150	–	–	–	–	–	5000
7	*Kleb. Pneumoniae*	–	–	–	–	–	–	2500
8	*Morganella morganii*	300	500	–	300	–	500	2500
9	*Proteus vulgaris*	–	–	–	–	–	–	2500
10	*Pseudo.aeruginosa* ATCC27853	–	–	200	500	–	250	5000
11	*Providencia rettgeri*	–	–	300	400	250	–	2500
12	*Proteus mirabilis*	–	–	–	300	400	–	2500
13	*Shigella sonnei*	–	500	500	400	400	200	2500
14	*Shigella boyedli*	–	–	–	–	–	–	2500
15	*Salmonella paratyphi*	–	500	400	500	300	200	2500
16	*Shigella flexinerii*	50	–	250	300	200	400	2500
17	*Salmonella enteritidis*	–	–	–	–	–	–	2500
18	*Salmonella typhi* MTCC 3216	–	–	–	–	–	–	2500
19	*Salmonella typhi*	–	–	300	–	300	500	2500
20	*Vibrio cholera*	–	–	–	300	–	250	5000

Sulphamethoxazole as standard drug; – =Insensitive (inactive).

*one (**3e**):* yield=84 %, mp 235-236 °C. ^{1}H-NMR (DMSO-d_6) δ; 8.72 (s, 1H; 5-H), 8.34 (d, *J*=6.9 Hz, 1H; 8-H), 7.89 (d, J=7.1 Hz, 1H; 7-H), 7.14-8.26 (m, H-aromatic), 3.39 (s, 2H; NH_2). IR (KBr) cm^{-1}; 3385 (NH_2), 1668 (C=O), 1623 (C=N), 1440 (N=N), 831, 785, 779 (Ar-H). EI-MS *m/z*: 505.04 (calcd for $C_{23}H_{13}Cl_2N_7O_3$: 506.30). *Anal* Calcd for $C_{23}H_{13}Cl_2N_7O_3$: C, 54.56; H, 2.59; N, 19.37. Found: C, 54.48; H, 2.43; N, 19.41.

*3-(5-amino-6(2,3-dichlorophenyl)-1,2,4-triazin-3-yl)-2-(4-hydroxyphenyl)quinazoline-4(3H)-one (**3f**):* yield=82 %, mp 296-297 °C. ^{1}H-NMR (DMSO-d_6) δ; 8.72 (s, 1H; 5-H), 7.54 (d, *J*=5.9 Hz, 1H; 8-H), 7.76 (d, *J*=5.9 Hz, 1H; 7-H), 6.76-7.45 (m, H-aromatic), 3.89 (s, 2H; NH_2), 4.9 (aromatic OH), IR (KBr) cm^{-1}; 3378 (NH_2), 1672 (C=O), 1635 (C=N), 1420 (N=N), 825, 743, 765 (Ar-H). EI-MS m/z: 476.05 (calcd for $C_{23}H_{14}Cl_2N_6O_2$:477.30). *Anal* Calcd for $C_{23}H_{14}Cl_2N_6O_2$: C, 57.88; H, 2.96; N, 17.61. Found: C, 57.57; H, 2.74; N, 17.53.

Results and discussion. Quinazolinones heterocyclic entity are very interesting components in terms of their biological properties, such as anti fungal, antibacterial and herbicidal. Synthesized compounds **3a-3f** were tested against a panel of microorganisms including Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli, Escherichia coli ATCC-35218, Morganella morganii, Prot. mirabilis, Providencia rettgeri, Salmonella paratyphi, Shigella sonnei, Shigella boyelli, Vibrio cholerae, Prot.vulgaris, E.faecalis, Pseudo.aeruginosa ATCC-27853, Shigella flexneri, Klebsiella oxytoca, Kleb. neumoniae, Salmonella enteritidis, Salmonella typhi MTCC 2316, H.pylori, Salmonella typhi*) was using conventional agar-dilution method. He MIC values for these compounds (**3a-3f**) were determined by comparison to sulphamethoxazole as reference drug.

Sensitivity testing was performed for all compounds. This showed that synthetic compounds were sensitive against all bacteria.

Compounds **3a-3f** were not active against *Shigella boyedli, Proteus vulgaris, Salmonella*

typhi MTCC 3216, Salmonella enteritidis, Kleb. pneumoniae. Further some compounds were not active against specific bacteria like **3a** (*Shigella sonnei*), **3a**, **3b** and **3f** (*Providencia rettgeri*), **3a**, **3d** and **3f** (*E.coli ATCC35218*), **3a**, **3b**, **3c** and **3f** (*Proteus mirabilis*), **3c** and **3e** (*Morganella morganii*), **3a** (*Salmonella paratyphi*), **3a**, **3b**, **3c** and **3e** (*Vibrio cholerae*), **3a**, **3b** and **3e** (*Pseudo. aeruginosa ATCC27853*), **3b** (*Shigella flexinerii*), **3b**, **3c**, **3d**, **3e** and **3f** (*Klebsiella oxytoca*), **3b** and **3e** (*H. Pylori*), **3a**, **3b** and **3d** (*Salmonella typhi*), **3a** (*Staphylococcus aureus*).

The MIC values of synthesized compounds were tested against organism displayed a significant activity with wide degree of variation (Table 1). On gram positive bacteria, Compounds **3a-3f** was found to be 12.5 to 25 times more active than standard drug. On gram negative bacteria, compounds **3a-3f** were found to be 2.5 to 100 times more active than standard drug.

Compound **3a** exhibited significant activity against *E.coli*, *H.pylori* and *Shigella flexinerii* with MIC value 50 μg/mL. Other then these bacteria, compound **3a** showed moderate activity. Compounds **3f** showed greater activity against *H.pylori* with MIC value 25 μg/mL. Other then this bacteria, this compound was showed moderate activity. Synthesized compounds **3a**, **3b**, **3c**, **3d**, **3e** and **3f** exhibited moderate activity against all bacteria.

Nitro group containing compounds (NO_2) (**3f**) was found to be active against some bacteria than other group containing compounds. It was found to be 5 to 12.5 times more active than standard drug.

On the basis of MIC values, synthesized compounds were divided into three parts 1-Weak active 300-500) — **3c**.

2-Moderate active (50-300 μg/mL) — **3d** & **3e**.

3-Most active (12.5-50 μg/mL) — **3a** & **3f**.

Antibacterial screening revealed that synthetic compounds exhibited moderate activity as compared to standard.

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Синтез та антибактеріальна активність *in vitro* 3-(5-аміно-6(2,3-дихлорофеніл)-1,2,4-триазин-3-іл)-2-*арил*-хіназолін-4(3*H*)-онів

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Резюме. Синтезовано деякі нові похідні 3-(5-аміно-6(2,3-дихлорофеніл)-1,2,4-триазин-3-іл)-2-*арил*-хіназолін-4(3*H*)-онів. Новизну ситезованих сполук доведено на основі елементного аналізу, ІЧ- та ^{1}Н-ЯМР-спектрами. Одержані сполуки перевірено на їхню антибактеріальну активність проти 20 штамів грампозитивних і грамнегативних бактерій. Визначено, що серед усіх протестованих сполук тільки сполуки **3a** і **3f** показали високу антибактеріальну активність у порівнянні зі стандартом сульфаметоксазола. Також встановлено, що сполука **3f** виявляє найвищу активність у серії проти *H.pylori* з MIC 25 μg/mL.

Ключові слова: хіназоліни, антибактеріальна активність *in vitro*, MIC value.

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