INTRODUCTION
During the ancient era the isolation of various compounds was done by the process of extraction. But this process was time consuming as well as laborious. Moreover the yield was very low and the process of isolation required large amount of the starting material. Today the process of isolation has been replaced by the synthetic routes. A large number of compounds can be synthesized by using small amount of chemicals. More over the Synthetic routes take less amounts of time and can easily be carried out. Quinazoline derivatives hold a place of significant in todays world for their important application in chemical, clinical and biological spheres. Medicinally quinazoline has been used in various areas especially as an analgesic1,2, anti-oxidant3-5, anti-inflammatory1,12, anti-convulsant13, anti-bacterial14, anti-fungal15 and anti-mycobacterial agents16, 17. It has also been found in the treatment of malaria18, 19. Considering the vast potential of quinazoline, it was thought appropriate to synthesized, characterized quinazoline analogues and investigates their biological activity. In this investigation, we have prepared quinazolinederivatives and characterized them using spectral data. Biological screenings of these compounds were also reported here.

MATERIALS AND METHODS
Experimental Instrumentation
All melting points are uncorrected and measured by use of an electrothermal capillary melting point apparatus. Infrared spectra were acquired with a Jasco FT/ IR-6100 using KBr discs. 1H NMR spectra were acquired with Jeol 270 MHz andjeolsx 500 MHz spectrometers, using TMS as internal standard. Mass spectra were acquired with a JeolJMS-AX 500. All reactions were followed and checked by TLC (aluminum-backed plates) with chloroform-methanol (9:1 V/V) as mobile phase. For detection the plates were sprayed with iodine.

Synthesis
5-Chloro-2-((isonicotinamido)benzoic acid (1)
A mixture of 5-chloroanthranilic acid (0.01 mol) and isonicotinyl chloride (0.27 g, 0.001 mol) in dry pyridine (30 mL) was stirred at room temperature for 12h. The reaction mixture was poured onto ice/water then, acidified with diluted HCl, the formed precipitated solid was then filtered off and recrystallized from acetic acid to give 1.
Crystallized from acetic acid, yellow crystal, m.p. 205-209 °C, yield 82 %. Analysis: for C13H9ClN2O3,M.Wt. 276.5, calcd: C, 48.62; H, 2.82; N, 8.72. Found: C, 48.60; H, 2.83; N,
8.73. IR (KBr, cm⁻¹): 3310-3558 (OH), 3243 (NH), 1680 (C=O of COOH), 1652 (C=O of CONH), 1612 (C=N).

¹H NMR (DMSO- d₆, δ ppm): 6.57-7.96 (7H, m, H aromatic), 8.63 (1H, s, NH exchangeable with D₂O), 11.32 (1H, s, OH exchangeable with D₂O). [M⁺] m/z =276(58.16%) and [M+2]⁺ m/z =278(60%).

6-Chloro-2-(pyridin-4-yl)-4H-benzo[d][1,3]oxazin-4-one (2)

A mixture of compound 1 (0.27 g, 0.001 mol) and acetic anhydride (5 mL) was heated together upon fusion at 150 °C on sand bath for 2h. After cooling, the crude mass was recrystallized from ethanol to give dark brown crystals of compound 2.

Crystallized from ethanol, dark brown, m.p. 195-197°C, yield 82%. Analysis: for C₁₃₃H₂₇ClN₂O₂, M.Wt: 258.5, calcd: C, 51.51; H, 2.33; N, 9.24. Found: C, 51.49; H, 2.31; N, 9.22. IR (KBr, cm⁻¹): 3035 (CH-aromatic), 1675 (C=O), 1626 (C=N).¹H NMR (DMSO- d₆, δ ppm): 7.72-8.53 (7H, m, H aromatic). MS (m/z) 258(23.83%) and [M+2]⁺ m/z 260(29.99%).

6-Chloro-2-(pyridin-4-yl)quinazolin-4(3H)-one (3)

Procedure A

A mixture of compound 1 (2.7 g, 0.01 mol), ammonium acetate (0.77 g, 0.01 mol), ammonium hydroxide (2 mL) and 10% sodium hydroxide (5 mL) in pyridine (15 mL), was heated under reflux for 2h, then left to cool. The reaction mixture was then titrated with cold water (50 mL) and neutralized with 1N HCl (5 mL) the resulting precipitated solid was collected by filtration, washed with water, dried and recrystallized from ethanol to give compound 3.

Procedure B

A mixture of compound 2 (2.58 g, 0.01 mol) and ammonium acetate (0.01 mol) in ethanol (50 mL), was refluxed for 5h. The mixture then cooled and the separated solid was filtered off and recrystallized to give compound 3, m.p. and mixed m.p. determined with authentic sample gave no depression.

Crystallized from ethanol, yellow crystal, m.p. 185-187°C, yield 78%. Analysis: for C₁₃₃H₂₇ClN₂O₂, M.Wt: 257.5, calcd: C, 51.68; H, 2.67; N, 13.91. Found: C, 51.66; H, 2.65; N, 13.89. IR (KBr, cm⁻¹): 3421 (NH), 3074 (CH aromatic), 1751 (C=O), 1595 (C=N).¹H NMR (DMSO-d₆, δ ppm): 6.90-7.90 (7H, m, H aromatic), 9.0 (1H, s, NH, exchangeable with D₂O). MS [M⁺] m/z 257(38.86%) and [M+2]⁺ m/z 259.

6-chloro-4-chloro-2-(pyridin-4-yl)quinazoline (4)

A mixture of compound 3 (2.5 g, 0.01 mol) and phosphorus pentachloride (0.015 mol) in phosphorus oxychloride (20 mL) was heated on a water bath for 8 h. and the reaction mixture poured gradually onto crushed ice. The separated solid was filtered off, dried then recrystallized from acetic acid to give compound 4.

Crystallized from acetic acid, yellow crystal, m.p. 130-135°C, yield 82%. Analysis: for C₁₃₃H₂₇ClN₂O₂, M.Wt: 276, calcd: C, 48.71; H, 2.20; N, 13.11. Found: C, 48.69; H, 2.18; N, 13.09. IR (KBr, cm⁻¹): 3020 (CH aromatic), 1620 (C=N).¹H NMR (DMSO- d₆, δ ppm): 6.70-8.20 (7H, m, H aromatic). MS: (m/z) [M+1]⁺ m/z 276(12.14%).

1-(4-(6-chloro-2-(pyridin-4-yl)quinazolin-4-ylamino)phenyl)ethanone (5)

A mixture of compound 4 (2.75 g, 0.01 mol) and p-aminoacetophenone (0.01 mol) in pyridine (30 mL) was refluxed for 6h. and the reaction mixture poured gradually on water and then neutralized till acidification. The precipitate was filtered off, dried and recrystallized.

Crystallized from methanol, yellow crystal, m.p. 120-123°C, yield 65%. Analysis for C₂₁H₁₉ClN₄O, M.Wt: 374.5, calcd: C, 60.16; H, 3.61; N, 13.36. Found: C, 60.10; H, 3.56; N, 13.40. IR (KBr, cm⁻¹): 3421 (NH), 3099 (CH aromatic), 2937 (CH aliphatic), 1772 (C=O), 1612 (C=N).¹H NMR (DMSO-d₆, δ ppm): 2.10 (3H, s, CH₃), 6.20 (1H, s, NH exchangeable with D₂O) 7.51-8.90 (11H, m, H aromatic). MS [M⁺] m/z 374(30.30%) and [M+2]⁺ m/z =376.

General procedure for preparation of compounds (6a-d, 7a-d)

A mixture of compound 5 (1.12 g, 0.003 mol) and the appropriate aromatic aldehydes, namely, benzaldehyde, 4-methoxybenzaldehyde, 4-chlorobenzaldehyde and/or 4-nitrobenzaldehyde (0.003mol), malononitrile and/or ethylcyanoacetate (0.021 mol) and excess ammonium acetate in n-butanol (50 mL) was refluxed for 10 hrs. The reaction mixture was concentrated till its half volume, then cooled and left overnight. The precipitate was filtered off, dried under vacuum then recrystallized from DMF/water.

2-amino-6-(4-(6-chloro-2-(pyridine-4-yl)quinazoline-4-ylamino)phenyl)-4-phenyl nicotinonitrile (6a)

Crystallized from Di methyl formamide, white crystals, m.p. 162-164°C, yield 57%. Analysis for C₂₁H₂₀ClN₄, M.Wt 525.5, calcd: C, 65.27;
H, 3.53; N, 17.19. Found: C, 65.25; H, 3.51; N, 17.17. IR (KBr, cm⁻¹): 3366, 3205 (NH₃), 3037 (CH aromatic), 2191 (C=O), 1615 (C≡N). ¹H NMR (DMSO- d₆, δ ppm): 5.12.9.14 (3H.2s, NH, NH exchangeable with D₂O). 7.27-8.08 (17H, m, H aromatic). MS: (m/z) [M]+ m/z525(95.15)% and [M+2]+ m/z 527(68.93%).

6-[(4-(B-chloro-2-(pyridine-4-yl)quinazoline-4-ylamino)phenyl)-2-amino-(4-methoxy-phenyl)]-pyridine-3-carbonitrile (6b)

Crystallized from DI methyl formamide, yellow crystal, m.p. 99-102°C, yield 55%. Analysis for C₈₇H₈₂ClN₄O₉. M.Wt. 555.5, calcd: C, 64.01; H, 3.69; N, 16.33. Found: C, 63.98; H, 3.66; N, 16.31. IR (KBr, cm⁻¹): 3365, 3204 (NH₃), 3100 (CH aromatic), 2991 (CH aliphatic), 2191 (C≡N), 1660 (C=O), 1619 (C≡N). ¹H NMR (DMSO- d₆, δ ppm): 3.79 (3H, s, OCH₃), 6.90-8.20 (16H, m, H aromatic) 6.17-9.18 (2H, s, NH, NH exchangeable with D₂O). MS: (m/z) [M]+ m/z556(68.24)% and [M+2]+ m/z557(48%).

2-amino-6-[(4-(6-chloro-2-(pyridine-4-yl)quinazoline-4-ylamino)phenyl)-4-(4-chlorophenyl)]-nicotinonitrile (6c)

Crystallized from DI methyl formamide, white crystal. m.p. 130-131°C, yield 80%. Analysis for C₈₇H₇₉ClN₄O₇. M.Wt. 560, calcd: C, 61.55; H, 3.17; N, 16.21. Found: C, 61.60; H, 3.05; N, 16.08. IR (KBr, cm⁻¹): 3364, 3203 (NH₃), 3022 (CH aromatic) 2191 (C≡N), 1612 (C=N). ¹H NMR (DMSO- d₆, δ ppm): 7.70-8.50 (16H, m, H aromatic) 10.10-11.20 (3H, s, NH, NH exchangeable with D₂O). MS: [M]+ m/z560(23.50) and [M+2]+ m/z 562(22%).

2-amino-6-[(4-(6-chloro-2-(pyridine-4-yl)quinazoline-4-ylamino)phenyl)-4-(4-nitrophenyl)]-nicotinonitrile (6d)

Crystallized from DI methyl formamide, dark crystal, m.p. 200-203°C, yield 67%. Analysis for C₈₇H₇₉ClN₄O₇. M.Wt. 570.5, calcd: C, 60.50; H, 3.11; N, 18.21. Found: C, 60.48; H, 3.09; N, 18.19. IR (KBr, cm⁻¹): 3364, 3203 (NH₃), 3022 (CH aromatic) 2191 (C≡N), 1623 (C=N). ¹H NMR (DMSO- d₆, δ ppm): 7.67-8.50 (16H, m, H aromatic) 6.12-9.14 (3H, s, NH, NH exchangeable with D₂O). MS: (m/z) [M]+ m/z570(77.78)% and [M+2]+ m/z 572.

6-[(4-(6-chloro-2-(pyridin-4-yl)quinazoline-4-ylamino)phenyl)-2-oxo-4-phenyl-1,2-dihydroppyridine-3-carbonitrile (7a)

Crystallized from methanol, white crystal, m.p. 290-293°C, yield 68%. Analysis for C₈₇H₇₉ClN₄O. M.Wt. 526.5, calcd: C, 65.16; H, 3.35; N, 14.71. Found: C, 65.14; H, 3.33; N, 14.69. IR (KBr, cm⁻¹): 3421, 3365 (2NH), 3026 (CH aromatic), 2221 (C≡N), 1654 (C=O), 1628 (C≡N). ¹H NMR (DMSO- d₆, δ ppm): 5.03-9.77(2H, 2s, 2NH exchangeable with D₂O) 6.82-8.05(17H, m, H aromatic and CH of pyridine ring). MS: (m/z) [M]+ m/z526(66.13)% and [M+2]+ m/z 528.

6-[(4-(6-chloro-2-(pyridin-4-yl)quinazoline-4-ylamino)phenyl)-4-(4-methoxyphenyl)]-2-oxo-1,2-dihydroppyridine-3-carbonitrile (7b)

Crystallized from ethanol. Yellow crystal, m.p. >300°C, yield 68%. Analysis for C₈₇H₇₉ClN₄O₂. M.Wt. 556.5, calcd: C, 63.90; H, 3.52; N, 13.97. Found: C, 63.43; H, 3.50; N, 13.95. IR (KBr, cm⁻¹): 3212, 3198 (2NH), 3010 (CH aromatic), 2207 (C≡N), 1711 (C=O), 1623 (C≡N). ¹H NMR (DMSO- d₆, δ ppm): 3.76 (3H, s, OCH₃), 6.82-8.05(16H, m, H aromatic and CH of pyridine ring), 5.63-9.77(2H, 2s, 2NH exchangeable with D₂O). MS: (m/z) [M]+ m/z556(10.0)% and [M+2]+ m/z 558(0.48%).

6-[(4-(6-chloro-2-(pyridin-4-yl)quinazoline-4-ylamino)phenyl)-4-(4-chlorophenyl)]-2-oxo-1,2-dihydroppyridine-3-carbonitrile (7c)

Crystallized from Dioxane, white crystal, m.p. >300°C, yield 76%. Analysis for C₈₇H₇₉ClN₂O₂. M.Wt. 561, calcd: C, 61.45; H, 2.99; N, 13.87. Found: C, 61.43; H, 2.97; N, 13.85. IR (KBr, cm⁻¹): 3125, 3110 (2NH), 3030 (CH aromatic), 2216 (C≡N), 1666 (C=O), 1627 (C≡N). ¹H NMR (DMSO- d₆, δ ppm): 6.82-8.05(16H, m, H aromatic and CH of pyridine ring), 5.63-9.77(2H, 2s, 2NH exchangeable with D₂O). MS: (m/z) [M]+ m/z561(51.16)% and [M+2]+ m/z 563(50.39%).

6-[(4-(6-chloro-2-(pyridin-4-yl)quinazoline-4-y lamino)phenyl)-4-(4-nitrophenyl)]-2-oxo-1,2-dihydroppyridine-3-carbonitrile (7d)

Crystallized from Acetic acid, dark crystals, m.p. 285-286°C, yield 80%. Analysis for C₃₁H₁₉ClN₄O₂. M.Wt. 571.5, calcd: C, 60.40; H, 2.94; N, 15.91. Found: C, 60.30; H, 2.86; N, 15.73. IR (KBr, cm⁻¹): 3219, 3125 (2NH), 3050 (CH aromatic), 2215 (C≡N), 1680 (C=O), 1629 (C≡N). ¹H NMR (DMSO- d₆, δ ppm): 6.82-8.05(16H, m, H aromatic and CH of pyridine ring), 5.63-9.77(2H, 2s, 2NH exchangeable with D₂O). MS: (m/z) [M]+ m/z571(13.31)% and [M+2]+ m/z 573(13.60%).
General procedure for preparation of compounds (8a-d)
A mixture of 11 (1.12 g, 0.003 mol) and (0.003mol) of the appropriate aromatic aldehyde namely benzaldehyde, 4-methoxy benzaldehyde, 4-chloro benzaldehyde and 4-nitro benzaldehyde in 10 % ethanolic sodium hydroxide solution ( 50 ml) was shaken at room temperature for 48 hrs, then refluxed for 1 hrs and then poured onto ice-cold water. The precipitate that appeared after neutralization with dilute HCL was filtered off and recrystallized from acetic acid to give compounds 8a-d.

2-amino-6-(4-(6-chloro-2-(pyridin-4-y1)quinazolin-4-ylamino)phenyl)-4-phenylnicotinonitrile (8a)
Crystallized from acetic acid, white crystal, m.p. 266-268°C, yield 57%. Analysis for C29H18ClN4O2. M.Wt. 492.5, calcd: C, 64.28; H, 3.77; N, 11.04. Found: C, 66.26; H, 3.75; N, 11.02. IR (KBr, cm⁻¹): 3425 (NH), 3075 (CH aromatic), 2942(CH aliphatic), 1660(C=O) , 1595 (C=N). ¹H NMR (DMSO- d₆ ppm): 6.52-6.60 (2H, dd, HC=CH), 7.20-8.80 (16H, m,H aromatic), 11.2 (1H, s,NH, exchangeable with D₂O).

RESULTS AND DISCUSSION
The starting material 5-chloro-2-(isonicotinamido)benzoic acid (1) was prepared by reaction of 5-chloroaanthranilic acid with isonicotinyl chloride in dry pyridine. Cyclocondensation of compound 1 with acetic anhydride afforded 6-chloro-2-(pyridin-4-yl)-4H-benzo[d][1,3]oxazin-4-one. Interaction of 5-chloro-2-(isonicotinamido)benzoic acid 1 with ammonium acetate in the presence of ammonium hydroxide in sand bath afforded easily separated and highly yield product 6-chloro-2-(pyridin-4-yl)quinazolin-4(3H)-one 3. Further confirmation of the compound 3 was obtained through its synthesis via another reaction route. Thus, the reaction of compound 2 with ammonium acetate afforded a product which was found to be identical in all respects (m.p., mixed m.p., and IR spectrum) with compound 3. The stepwise synthesis of 4 was prepared via the chlorination of compound 3 with phosphorus oxychloride in the presence of phosphoruspenta chloride to give 6-chloro-4-chloro-2-(pyridin-4-yl)quinazoline (4).

Interaction of compound 4 with p-aminoacetophenone gave 1-(4-(6-chloro-2-(pyridin-4-yl)quinazolin-4-ylamino)phenyl)ethanone 5. Compound 5 was reacted with appropriate aldehydes, namely benzaldehyde, 4-methoxybenzaldehyde, 4-chlorobenzaldehyde and 4-nitrobenzaldehyde, malononitride and/or ethyl cyano acetate, excess ammonium acetate in n-butanol to give compounds 6a-d and 7a-d respectively. Compound 5 was reacted with appropriate aldehydes, namely benzaldehyde, 4-methoxybenzaldehyde, 4-chlorobenzaldehyde and 4-nitrobenzaldehyde, in the presence of ethanolic sodium hydroxide solution afforded the derivatives 8a-d, respectively.
Biological activity

This study comprises the biological evaluation of some novel selected compounds of quinazoline derivatives which may possessing a broader spectrum of antitumor activity and fewer toxic side effects than Doxorubicin (DOX). Certain new quinazoline derivatives (3,4,5,6b,6d,8b,8d) were synthesized, characterized, and subjected to a screening system for evaluation of antitumor activity against Liver Cancer (HEPG2) tumor cell line. The antitumor activity results indicated that the selected quinazoline derivatives showed antitumor activity against liver cancer (HEPG2) tumor cell line tested but with varying intensities in comparison to the known antitumor drug: Doxorubicin. Results revealed that compounds (6b,8d) exhibited a strong growth inhibition activity against liver cancer (HEPG2) on the tested tumor panel cell line in comparison to the known antitumor drug: Doxorubicin.

Table 1: Anticancer activity of selected Compounds (3,4,11,12b,14b and 14d)

<table>
<thead>
<tr>
<th>compound</th>
<th>Cytotoxicity(IC50 in µg)</th>
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<tr>
<td></td>
<td>HepG2c</td>
</tr>
<tr>
<td>3</td>
<td>32.41 +/- 6.37</td>
</tr>
<tr>
<td>4</td>
<td>52.51 +/- 8.62</td>
</tr>
<tr>
<td>5</td>
<td>16.32 +/- 2.27</td>
</tr>
<tr>
<td>6b</td>
<td>2.04 +/- 0.63</td>
</tr>
<tr>
<td>8b</td>
<td>18.36 +/- 6.38</td>
</tr>
<tr>
<td>8d</td>
<td>8.23 +/- 1.51</td>
</tr>
<tr>
<td>Doxorubicin</td>
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</tr>
</tbody>
</table>

Fig. 1: Graphical representation of anticancer activity of compounds (3,4,5,6b,8b and 8d)

CONCLUSION

The varied biological activities of the newly synthesized compounds promoted to synthesis some new derivatives of these ring systems and study their antitumor activities their biological activities depended mainly on the nature and the position the substituents. The antitumor activity studies revealed that compounds 6b,8d show strong effects against human hepatocellular liver carcinoma (HepG2). So we can say that synthesis of new derivatives of these compounds is still an active area of research. Where synthesis and study of the anticancer activities of new analogous of these compounds will be helpful for medicinal chemist to focus design of novel chemical entities containing quinazoline derivatives as a part of antitumor drugs.

REFERENCES

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