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ORIGINAL ARTICLE

Design, synthesis and cytotoxic evaluation of novel imidazolone fused quinazolinone derivatives

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Abstract A congeneric series of novel imidazolone fused quinazolinone derivatives were synthesized and characterized by IR, NMR, mass spectra and elemental analyses. All the compounds were evaluated for their in vitro cytotoxic activity against cervical cancer (HeLa), breast cancer (MCF-7), leukemia cells (HL-60) and hepatocellular carcinoma (HepG2) cell lines. The derivative **4e** which bears a methoxy group at *para* position in phenyl ring of imidazolone displayed three fold potent activity against MCF-7 than that of the standard drug Cisplatin. The IC_{50} value of **4e** against HepG2 is twofold lesser than Cisplatin whereas the IC_{50} value against HeLa and HL-60 was equivalent to Cisplatin. The result concludes that these derivatives can be further utilized as a promising anticancer agent.

1. Introduction

Cancer is a leading cause of death worldwide that is accounted for 7.6 million deaths in 2008. More than 70% of all cancer deaths occurred in low and middle income countries. Deaths from cancer worldwide are projected to rise over 11 million in 2030 as per WHO Cancer Fact sheet No 297 February 2011. Chemotherapy constitutes one of the modalities of cancer treatment, either *per se* or in conjunction with other treatment regimens. However, despite much progress in the chemotherapy of cancerous diseases, anticancer drugs in current clinical use generally do not address issues of excessive organ toxicity, lack of cell specificity, short circulation half-life, angiogenesis, metastasis and a pronounced tendency to induce resistance in the target cells. Hence it is imperative to develop a safe and effective drug candidate to save the lives of million people worldwide.

Quinazolinone and its derivatives have drawn much attention because of their pharmacological activities particularly a wide range of antitumor activities (Khalil et al., 2003). Anilinoquinazolines in particular are potent inhibitors of growth

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factor receptor tyrosine kinase (GFRTK) and have found clinical applications in epidermal and vascular endothelial GFR targets (Grunwald and Hidalgo, 2003). Quinazolinone heterocycles possess diverse pharmacological activities including antimycobacterial (Karel et al., 2001) antifungal (Sawhney et al., 1980) antimalarial (Martin et al., 1964), antihypertensive (Dienei et al.,1973), antihistaminic (Alagarsamy et al., 2005, 2006), local anesthetic (Chandrasekhar et al.,1986) anti-Parkinson (Naithani et al., 1989), antiviral (Alagarsamy et al.,2004), and thymidylate synthase inhibitory activities (Hennequin et al.,1996). The simple and condensed quinazolinones are also known to exhibit analgesic (Alagarsamy and Murugesan, 2007), anti-inflammatory (Ravishankar et al., 1984) and anticonvulsant activities (Hori et al.,1990). Interest in quinazolinones as anticancer agents has further increased since the discovery of Raltitrexed and Thymitaq (Fig. 1) and their activity as Thymidylate enzyme inhibitors (Bavetsias et al., 1997). The compounds containing imidazolone chromophore are known to have a wide range of biological activities like anti-cancer, anti-inflammatory, cardioactivity and angiotensin II receptor antagonistic activity (Siamaki et al., 2008). A trisubstituted imidazolone induces a high degree of apoptosis in human leukemia cells and also has prominent cytotoxicity (Fang et al., 2007; Lai et al., 2002). Inspired by these findings; we attempted to synthesize novel quinazolinone derivatives fused with imidazolone and to evaluate anticancer activities against cervical cancer, breast cancer, leukemia and hepatocellular carcinoma cell lines.

2. Experimental

In the present study, fourteen novel congeneric series of quinazolinone derivatives were synthesized as illustrated in Scheme 1. The starting compound 3,5-dibromo anthranilic acid (**1**) was synthesized according to the reported literature procedure (Bogert, 1903) .Compound (**1**) was reacted with acetic anhydride under anhydrous condition for 4 h. The intermediate **(2)** 6,8-dibromo-2-methyl-3,1-benzoxazin-4-one obtained as a solid mass was used immediately for the next step (Misra et al., 1995). Compound (**2**) was subjected to reflux conditions with hydrazine hydrate in the presence of pyridine for 3 h to get the building block 3-amino-6,8-dibromo-2-methyl-quinazolin-4(3*H*)one (**3**) (Raghvendra et al., 2007). Differently substituted oxazolone derivatives were prepared according to the reported literature (Cantello et al., 1994). Compound (**3**) was subjected to reflux with various substituted oxazolone derivatives in pyridine to yield the target compounds (Radadia et al., 2006; Patel et al., 2003).

2.1. Materials

All the chemicals were of synthetic grade and commercially procured from S.D. Fine Chem. Ltd., Mumbai, India. Melting points were recorded on a Buchan capillary melting point apparatus and are uncorrected. IR spectra were recorded on a FT-IR8400S, Fourier Transform (Shimadzu) Infrared spectrophotometer using the KBr disk method. ^{1}H NMR spectra were recorded on a Perkin Elmer NMR Spectrophotometer-300 MHz in DMSO-δ_6 using TMS as an internal standard. Mass spectra were recorded on a Micro mass Q-TOF and Shimadzu LCMS 2010A Mass spectrometer. Elemental analysis was performed using a Perkin Elmer Auto system XL Analyzer.

2.1.1. Synthesis of 6,8-dibromo-2-methyl-3,1-benzoxazin-4-one, 2

A mixture of 3,5-dibromo anthranilic acid **1** (0.05 mol) and acetic anhydride (0.1 mol) was subjected to reflux under anhy-

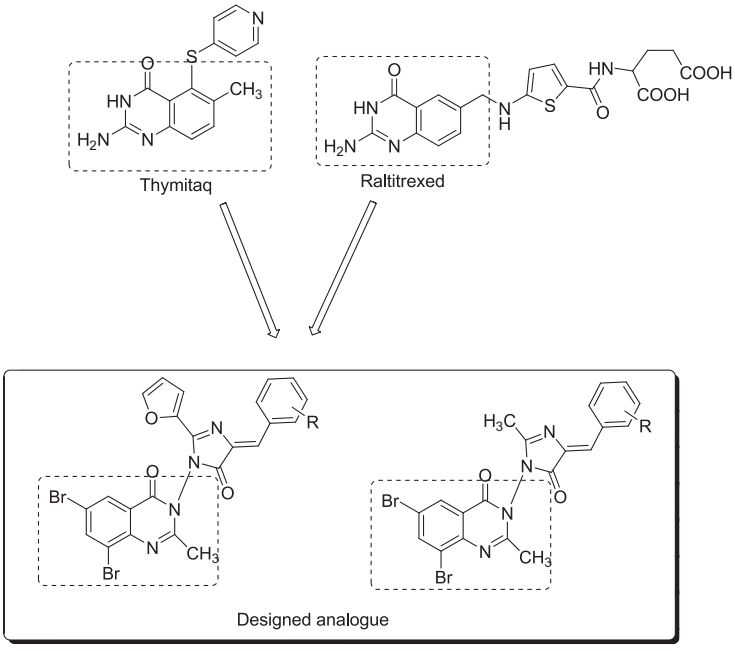
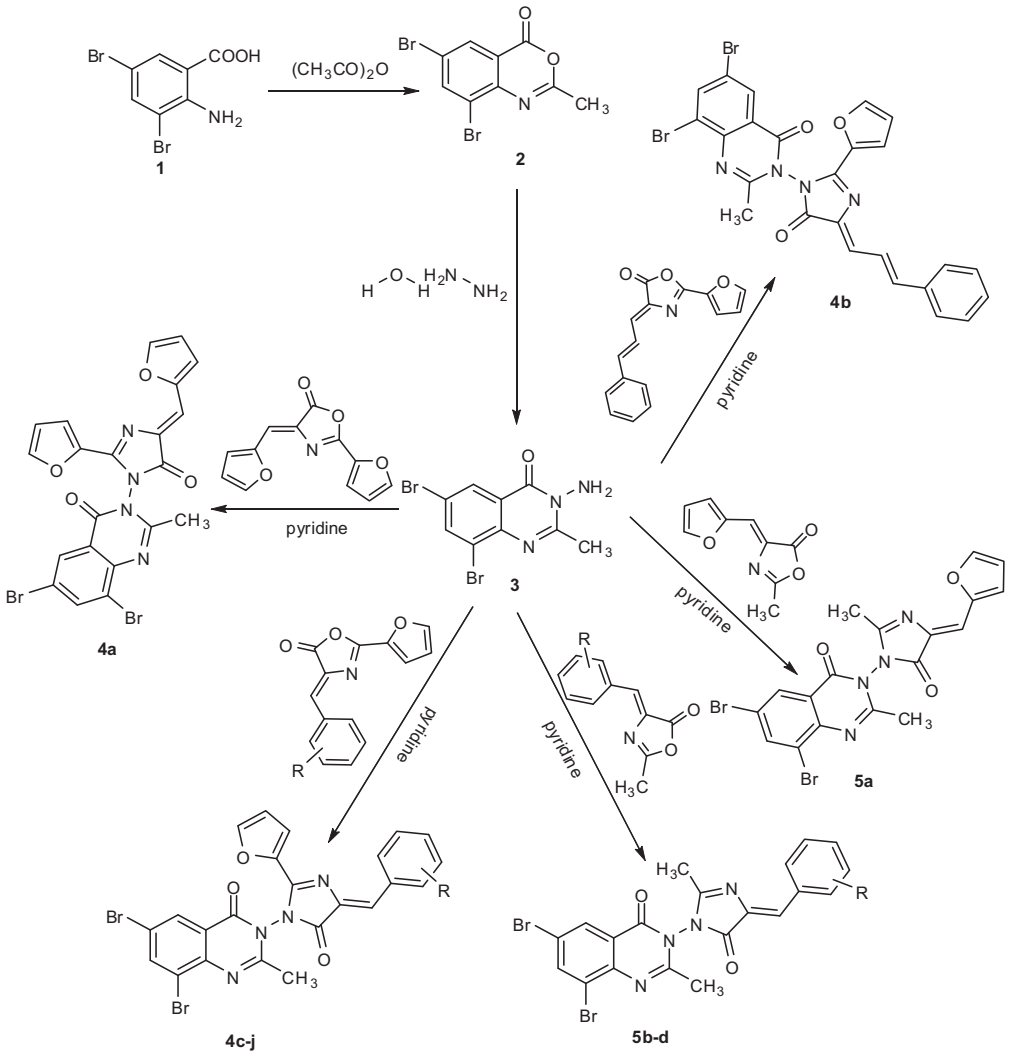


Figure 1 Structural resemblance of Raltitrexed and Thymitaq with designed analogs.



Compd	R	Compd	R	Compd	R
4c	H	**4g**	3-Cl	**5b**	H
4d	2-Cl	**4h**	2-OH	**5c**	2-Cl
4e	4-OMe	**4i**	3-OCH_3	**5d**	3-Cl
4f	4-$N(Me)_2$	**4j**	3-NO_2		

Scheme 1 Synthesis of imidazolone fused quinazolinone derivatives.

drous conditions for 4 h. The excess of acetic anhydride was then distilled off under reduced pressure and reaction mixture was cooled to room temperature. The intermediate benzoxazine-4-one obtained as a solid mass was used immediately for the next step. Yield – 80%, m.p. 173–175 °C.

*2.1.2. Synthesis of 3-amino-6,8-dibromo-2-methylquinazolin-4(3H)one, **3***

Benzoxazin-4-one (**2**) (0.01 mol) was subjected to reflux conditions with hydrazine hydrate (1.5 ml) in the presence of pyridine (15 ml) for 3 h with occasional shaking. On cooling, the crystals formed were filtered, washed with water and dried. The crude product was recrystallized from ethyl acetate. Yield – 81% m.p. 230–232 °C.

*2.1.3. Synthesis of 3-[4-(substituted benzylidene)-4,5-dihydro-5-oxo-2-phenyl-1(H)imidazol-1-yl]-2-phenyl-6,8-disubstituted-4(3H)-oxoquinazoline (**4a**–**5d**)*

A mixture of 3-amino-6,8-dibromo-2-methylquinazolin-4(3*H*) one (**3**, 0.01 mol) and differently substituted oxazolone derivatives (0.01 mol) in pyridine (30 ml) was subjected to reflux for four hours. After cooling, the resultant mixture was poured

over crushed ice and the contents were acidified with dilute hydrochloric acid to remove excess pyridine. The solids were filtered, washed with cold water, dried and recrystallized from ethanol.

*2.1.3.1. 3-(2-(Furan-2-yl)-4-((furan-2-yl)methylene)-4,5-dihydro-5-oxoimidazol-1-yl)-2-methyl-6,8-dibromoquinazolin-4(3H)-one (**4a**).* Yield – 81.33%; m.p. 214–215 °C. IR (KBr cm^{-1}) υ max: 3306 (N—N), 3205 (C═C—H) , 3074 (C—H—Ar), 2889 (C—CH_3) 1666 (C═O imidazoline ring), 1622 (C═O quinazoline ring), 1527 (C═N), 1161 (C—O—Furan), 719 (C—Br); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm: 7.9–7.70 (d, 2H, QNZ-Ar—H), 6.64–7.29, 7.74–8.30 (m, 6H, Furan—H), 6.61 (s, 1H, C═C—H), 2.75 (s, 3H, CH_3); EI-MS (*m*/*z*): 544 $[M]^+$. Anal. Calcd for $C_{21}H_{12}Br_2N_4O_4$: C, 46.35; H, 2.22; N, 10.30; Found: C, 46.05; H, 2.62; N, 10.60.

*2.1.3.2. 3-(2-(Furan-2-yl)-4,5-dihydro-5-oxo-4-phenylallylidene)-imidazol-1-yl)-6,8-dibromo-2-methylquinazolin-4(3H)-one (**4b**).* Yield – 80.21%; m.p. 220–222 °C. IR (KBr cm^{-1}) υ max: 3306 (N—N), 3205 (C═C—H) , 3074 (C—H—Ar), 2889 (C—CH_3), 1666 (C═O imidazoline ring), 1622 (C═O quinazoline ring), 1506 (C═N), 1161 (C—O—Furan), 719 (C—Br); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm: 7.10,7.42 (d, 2H, QNZ—Ar—H), 7.43–8.12 (m, 5H, Ar—H), 6.72 (d, 2H, C═C—H), 6.64–6.65 (m, 3H, Furan—H), 2.75 (s, 3H, CH_3); EI-MS (*m*/*z*): 581 ($M^+ + 1$). Anal. Calcd for $C_{25}H_{16}Br_2N_4O_3$: C, 51.75; H, 2.78; N, 9.66; Found: C, 51.40; H, 2.97; N, 9.22.

*2.1.3.3. 3-(4-Benzylidene-2-(furan-2-yl)-4,5-dihydro-5-oxo-imidazol-1-yl)-6,8-dibromo-2-methyl quinazolin-4(3H)-one (**4c**).* Yield – 80.21%; m.p. 210–213 °C. IR (KBr cm^{-1}) υ max: 3308 (N—N), 2872 (C═C—H) , 3248 (C—H—Ar), 3076 (C—CH_3), 1666 (C═O imidazoline ring), 1624 (C═O quinazoline ring), 1508 (C═N), 1163 (C—O—Furan), 788 (C—Br); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm: 7.29–8.34 (m, 5H, Ar—H), 7.1, 7.26 (d, 2H, QNZ—Ar—H), 7.01 (s, 1H, C═C—H), 6.64–7.26 (m, 3H, Furan—H), 2.7 (s, 3H, CH_3); EI-MS (*m*/*z*): 554 $[M]^+$. Anal. Calcd for $C_{23}H_{14}Br_2N_4O_3$: C, 49.85; H, 2.55; N, 10.11; Found: C, 49.45; H, 2.14; N, 10.50.

*2.1.3.4. 3-(4-(2-Chlorobenzylidene)-2-(furan-2-yl)-4,5-dihydro-5-oxoimidazol-1-yl)-6,8-dibromo-2-methylquinazolin-4(3H)-one (**4d**).* Yield – 86.65%; m.p. 234–236 °C; IR (KBr cm^{-1}) υ max: 3306 (N—N), 3425 (C═C—H) , 3198 (C—H—Ar), 3076 (C—CH_3), 1666 (C═O imidazoline ring), 1624 (C═O quinazoline ring), 1589 (C═N), 1114 (C—O—Furan), 719 (C—Br); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm: 7.74–8.33 (m, 4H, Ar—H), 7.0, 7.29 (d, 2H, QNZ—Ar—H), 6.83 (s, 1H, C═C—H), 6.63–6.97 (m, 3H, Furan—H), 2.75 (s, 3H, CH_3); EI-MS (*m*/*z*): 588 $[M]^+$. Anal. Calcd for $C_{23}H_{13}Br_2ClN_4O_3$: C, 46.93; H, 2.23; N, 9.52; Found: C, 46.50; H, 2.44; N, 9.34.

*2.1.3.5. 3-[4-(4-Methoxybenzylidene)-4,5-dihydro-5-oxo-2phenyl-1(H)imidazol-1-yl]-6,8-dibromo-2-phenyl-4(3H)-oxo-quinazoline (**4e**).* Yield – 83.04%; m.p. 209–211 °C; IR (KBr cm^{-1}) υ max: 3306 (N—N), 3213 (C═C—H) , 3074 (C—H—Ar), 2904 (C—CH_3), 1668 (C═O imidazoline ring), 1622 (C═O quinazoline ring), 1157 (C—O—Furan), 1510 (C═N), 719 (C—Br); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm: 7.29–8.34 (m, 4H, Ar—H), 7.19 (s, 1H, C═C—H), 6.64–7.26 (m, 3H, Furan—H), 6.57–7.28 (d, 2H, QNZ—Ar—H), 3.85 (s, 3H, OCH_3), 2.75 (s, 3H, CH_3); EI-MS (*m*/*z*): 584(M^+). Anal. Calcd for $C_{24}H_{16}Br_2N_4O_4$: C, 49.34; H, 2.76; N, 9.59; Found: C, 49.01; H, 2.47; N, 9.88.

*2.1.3.6. 3-(4-(4-(Dimethylamino)benzylidene)-2-(furan-2-yl)-4,5-dihydro-5-oxoimidazol-1-yl)-6,8-dibromo-2-methylquinazolin-4(3H)-one (**4f**).* Yield – 84.02%; m.p. 217–219 °C; IR (KBr cm^{-1}) υ max: 3433 (N—N), 3360 (C═C—H) , 3261 (C—H—Ar), 3010 (C—CH_3), 1664 (C═O imidazoline ring), 1622 (C═O quinazoline ring), 1587 (C═N), 1161 (C—O—Furan), 719 (C—Br); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm: 8.07–8.13 (m, 4H, Ar—H), 7.38 (s, 1H, C═C—H), 6.74–7.70 (d, 2H, QNZ—Ar—H), 6.61–6.71 (m, 3H, Furan—H), 3.10 (s, 6H, 2×NCH_3), 2.75 (s, 3H, CH_3); EI-MS (*m*/*z*): 597 (M^+). Anal. Calcd for $C_{25}H_{19}Br_2N_5O_3$: C, 50.27; H, 3.21; N, 11.73; Found: C, 50.65; H, 3.53; N, 11.32.

*2.1.3.7. 3-(4-(3-Chlorobenzylidene)-2-(furan-2-yl)-4,5-dihydro-5-oxoimidazol-1-yl)-6,8-dibromo-2-methylquinazolin-4(3H)-one (**4g**).* Yield – 82.0%; m.p. 225–228 °C; IR (KBr cm^{-1}) υ max: 3431 (N—N), 3350 (C═C—H) , 3232 (C—H—Ar), 3076 (C—CH_3), 1666 (C═O imidazoline ring), 1622 (C═O quinazoline ring), 1568 (C═N), 1163 (C—O—Furan), 719 (C—Br); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm: 7.26–8.12 (m, 4H, Ar—H), 6.66–7.16 (d, 2H, QNZ—Ar—H), 6.64–6.65 (m, 3H, Furan—H), 6.33 (s, 1H, C═C—H), 2.75 (s, 3H, CH_3); EI-MS (*m*/*z*): 588 (M^+). Anal Calcd for $C_{23}H_{13}Br_2$ ClN_4O_3: C, 46.93; H, 2.23; N, 9.52; Found: C, 46.62; H, 2.60; N, 9.10.

*2.1.3.8. 3-(4-(2-Hydroxybenzylidene)-2-(furan-2-yl)-4,5-dihydro-5-oxoimidazol-1-yl)-6,8-dibromo-2-methylquinazolin-4(3H)-one (**4h**).* Yield – 85.0%; m.p. 198–200 °C; IR (KBr cm^{-1}) υ max: 3473 (N—N), 3400 (C═C—H), 3344 (C—H—Ar), 3105 (C—CH_3), 1668 (C═O imidazoline ring), 1622 (C═O quinazoline ring), 1568 (C═N), 1163 (C—O—Furan), 719 (C—Br); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm: 8.98 (s, 1H, OH), 7.49–8.98 (m, 4H, Ar—H), 7.48 (s, 1H, C═C—H), 7.26–7.47 (d, 2H, QNZ—Ar—H), 6.67–6.98 (m, 3H, Furan—H), 2.75 (s, 3H, CH_3); EI-MS (*m*/*z*): 571 ($M^+ + 1$). Anal Calcd for $C_{23}H_{14}Br_2N_4O_4$: C, 48.45; H, 2.47; N, 9.83; Found: C, 48.05; H, 2.17; N, 9.43.

*2.1.3.9. 3-(4-(3-Methoxybenzylidene)-2-(furan-2-yl)-4,5-dihydro-5-oxoimidazol-1-yl)-6,8-dibromo-2-methylquinazolin-4(3H)-one (**4i**).* Yield – 87.0%; m.p. 233–235 °C; IR (KBr cm^{-1}) υ max: 3308 (N—N), 3473 (C═C—H) , 3400 (C—H—Ar), 3105 (C—CH_3), 1668 (C═O imidazoline ring), 1624 (C═O quinazoline ring), 1568 (C═N), 1163 (C—O—Furan), 721 (C—Br); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm: 7.35–8.33 (m, 4H, Ar—H), 6.97 (s, 1H, C═C—H), 6.66–7.33 (d, 2H, QNZ—Ar—H), 6.64–6.66 (m, 3H, Furan—H), 3.89 (s, 3H, OCH_3), 2.75 (s, 3H, CH_3); EI-MS (*m*/*z*): 584 (M^+). Anal Calcd for $C_{24}H_{16}Br_2N_4O_4$: C, 49.34; H, 2.76; N, 9.59; Found: C, 49.69; H, 2.30; N, 9.10.

*2.1.3.10. 3-(4-(3-Nitrobenzylidene)-2-(furan-2-yl)-4,5-dihydro-5-oxoimidazol-1-yl)-6,8-dibromo-2-methylquinazolin-4(3H)-one (**4j**).* Yield – 80.0%; m.p. 230–232 °C; IR (KBr cm^{-1}) υ max: 3309 (N—N), 3213 (C═C—H), 3076 (C—H—ArC—H—Ar), 2881 (C—CH_3), 1668 (C═O imidazoline ring), 1622 (C═O quinazoline ring), 1568 (C═N), 1163 (C—O—Furan), 719

(C—Br); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm: 7.70–8.34 (m, 4H, Ar—H), 7.53 (s, 1H, C═C—H), 7.26–7.48 (d, 2H, QNZ—Ar—H), 6.64–7.16 (m, 3H, Furan—H), 2.75 (s, 3H, CH_3); EI-MS (m/z): 599 (M^+). Anal Calcd for $C_{23}H_{13}Br_2N_5O_5$: C, 46.10; H, 2.19; N, 11.69; Found: C, 46.40; H, 2.48; N, 11.88.

*2.1.3.11. 3-(-4-(Furan-2-yl)-4-methylene)-4,5-dihydro-2-methyl-5-oxoimidazol-1-yl)-6,8-dibromo-2-methylquinazolin-4(3H)-one (**5a**)*. Yield – 82.0%; m.p. 246–248 °C; IR (KBr cm^{-1}) υ max: 3448 (N—N), 3203 (C—H—Ar), 3076 (C—CH_3), 1666 (C═O imidazoline ring), 1622 (C═O quinazoline ring), 1585 (C═N), 721 (C—Br); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm: 7.08 (s, 1H, C═C—H), 7.00–8.33 (d, 2H, QNZ—Ar—H), 6.63–6.65 (m, 3H,furan H), 2.75 (s, 3H, CH_3 of QNZ), 2.58 (s, 3H, CH_3 of imidazole); EI-MS (m/z): 492 (M^+). Anal Calcd for $C_{18}H_{12}Br_2N_4O_3$: C, 43.93; H, 2.46; N, 11.38; Found: C, 43.60; H, 2.78; N, 11.04.

*2.1.3.12. 3-(4-Benzylidene-4,5-dihydro-2-methyl-5-oxoimidazol-1-yl)-6,8-dibromo-2-methyl quinazolin-4(3H)-one (**5b**)*. Yield – 83.0%; m.p. 238–240 °C; IR (KBr cm^{-1}) υ max: 3306 (N—N), 3203 (C—H—Ar), 3076 (C—CH_3) 2872 (C═C—H) , 1666 (C═O imidazoline ring), 1622 (C═O quinazoline ring), 1508 (C═N), 788 (C—Br); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm: 7.70–8.34,6.64–7.26 (m, 5H, Ar—H), 7.29–7.48 (d, 2H, QNZ—Ar—H), 7.15 (s, 1H, C═C—H), 2.75 (s, 3H, CH_3 of QNZ), 1.59 (s, 3H, CH_3 of imidazole); EI-MS (m/z): 503 ($M^+ + 1$). Anal Calcd for $C_{20}H_{14}Br_2\ N_4O_2$: C, 47.84; H, 2.81; N, 11.16; Found: C, 47.30; H, 2.81; N, 11.39.

*2.1.3.13. 3-(4-(2-Chlorobenzylidene)-4,5-dihydro-2-methyl-5-oxoimidazol-1-yl)-6,8-dibromo-2-methylquinazolin-4(3H)-one (**5c**)*. Yield – 83.0%; m.p. 240–242 °C; IR (KBr cm^{-1}) υ max: 3331 (N—N), 3230,3246 (C—H—Ar), 3230 (C—CH_3), 1666 (C═O imidazoline ring), 1629 (C═O quinazoline ring), 1543 (C═N), 788 (C—Br), 719,788, 653,596,546 (Aromatic ring vibration); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm: 7.70–8.34,6.64–7.26 (m, 4H, Ar—H), 7.59 (s, 1H, C═C—H), 7.29–7.48 (d, 2H, QNZ—Ar—H), 2.75 (s, 3H, CH_3 of QNZ), 1.55 (s, 3H, CH_3 of imidazole); EI-MS (m/z): 536 (M^+). Anal. Calcd for $C_{20}H_{13}Br_2Cl\ N_4O_2$: C, 44.77; H, 2.44; N, 10.04; Found: C, 44.39; H, 2.80; N, 10.44.

*2.1.3.14. 3-(4-(3-Chlorobenzylidene)-4,5-dihydro-2-methyl-5-oxoimidazol-1-yl)-6,8-dibromo-2-methylquinazolin-4(3H)-one (**5d**)*. Yield – 84.5%; m.p. 244–245 °C; IR (KBr cm^{-1}) υ max: 3431 (N—N), 3306 (C═C—H), 3203 (C—H—Ar), 3076 (C—CH_3), 1666 (C═O imidazoline ring), 1622, 1602 (C═O quinazoline ring), 1543 (C═N), 721 (C—Br); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm: 7.26–8.33 (m, 4H, Ar—H), 6.88 (s, 1H, C═C—H), 6.62–7.21 (d, 2H, QNZ—Ar—H), 2.75 (s, 3H, CH_3 of QNZ), 1.59 (s, 3H, CH_3 of imidazole); EI-MS (m/z): 537 ($M^+ + 1$). Anal. Calcd for $C_{20}H_{13}Br_2Cl\ N_4O_2$: C, 44.77; H, 2.44; N, 10.04; Found: C, 44.37; H, 2.78; N, 10.40.

2.2. In-vitro anticancer screening

In vitro cytotoxicity was determined using a standard MTT assay with protocol appropriate for the individual test system (Denizot and Lang, 1986). The four human cancer cell lines HeLa, MCF-7, HL-60 and HepG2, were cultured in the MEM medium supplemented with 10% FBS, 1% glutamine and 50 mM/ml gentamicin sulfate in a CO_2 incubator in a humidified atmosphere of 5% CO_2 and 95% air. The test compounds were prepared prior to the experiment by dissolving in 0.1% DMSO and diluted with medium. The cells were then exposed to different concentrations of drugs (1–100 mM) in the volume of 100 mM/well. Cells in the control wells received the same volume of medium containing 0.1% DMSO. After 24 h, the medium was removed and cell cultures were incubated with 100 ml MTT reagent (1 mg/ml) for 5 h at 37 °C. The known number of cells (1.0 ± 10^5) was incubated in a 5% CO_2 incubator at 37 °C in the presence of different concentrations of test compounds. After 48 h of drug incubation, the MTT solution was added in each well and absorbance was recorded at 540 nm by an ELISA reader. The experiment was performed in triplicate. Cell survival was calculated as the percentage of MTT inhibition as % growth inhibition = 100 – (mean OD of individual test Group/Mean OD of each Control Group) × 100.

The IC_{50} values of the synthesized compounds **4a–j** and **5a–d** for four cell lines are summarized in Table 1.

3. Results and discussion

3.1. Synthesis

All the synthesized compounds were characterized by physiochemical and spectral data. The characteristic functional groups present in the synthesized imidazolone fused quinazolinone derivatives are C═O (imidazoline ring), C═O (quinazolinone ring), C═N, C═C—H, C—H—Ar, C—O—Furan, C—Br, C—CH_3 and aromatic ring vibration. IR spectra showed the appearance of peak at 3300–3475 cm^{-1} indicating the presence of the N—N group in imidazolone fused quinazolinone ring in all the compounds. The appearance of peak

Table 1 *In-vitro* anti cancer screening of the synthesized compounds against various human cancer cell lines.

Compound no.	$IC_{50} \pm$ SE (μM)			
	HeLa[a]	MCF-7[b]	HL-60[c]	HepG2[d]
4a	78.12 ± 0.76	89.1 ± 0.91	75.2 ± 1.2	71.1 ± 0.65
4b	67.45 ± 0.10	81.2 ± 0.87	56.1 ± 0.3	67.23 ± 0.55
4c	81.4 ± 0.14	89.7 ± 0.52	89.1 ± 0.7	91.5 ± 0.78
4d	79.1 ± 0.12	76.76 ± 0.34	85.2 ± 0.4	77.2 ± 0.65
4e	41.8 ± 0.20	8.9 ± 0.29	6.6 ± 0.51	9.6 ± 0.45
4f	67.7 ± 0.44	56.13 ± 0.4	29.7 ± 0.74	45.2 ± 0.67
4g	71.4 ± 0.50	51.4 ± 0.32	45.7 ± 0.80	47.1 ± 0.41
4h	60.1 ± 0.41	61.8 ± 0.71	46.9 ± 0.21	40.2 ± 1.10
4i	57.1 ± 0.78	46.2 ± 0.88	17.4 ± 0.11	32.8 ± 0.91
4j	81.1 ± 0.67	70.1 ± 0.18	65.1 ± 0.13	76.1 ± 0.41
5a	89.1 ± 0.90	79.2 ± 0.34	46.1 ± 0.12	65.1 ± 0.50
5b	72.5 ± 0.43	51.9 ± 0.79	34.6 ± 0.31	41.7 ± 0.56
5c	75.7 ± 0.76	59.4 ± 0.61	56.1 ± 0.50	51.1 ± 0.67
5d	78.2 ± 0.30	63.1 ± 0.78	64.2 ± 0.81	52.6 ± 0.12
Cisplatin	34.9 ± 0.32	26.2 ± 0.68	6.9 ± 0.51	22.1 ± 0.19

[a] HeLa (cervical cancer).
[b] MCF-7 (breast cancer).
[c] HL-60 (human promyelocytic leukemia).
[d] HepG2 (hepatocellular carcinoma).

at 1500–1700 cm^{-1} confirmed C═O in imidazoline and the quinazolinone group in final products. The peaks at 1300–1500 cm^{-1} ascertained the presence of the C═N group. ^1H NMR spectra showed an intense signal at 6.83–7.86 due to the presence of olefinic proton in all the compounds. The methyl group of quinazolinone ring led to a sharp singlet at 3–3.32 ppm which signified 3 protons. The proton present in the aromatic ring was confirmed by multiplet at 6–8 ppm. In addition to these common groups, the proton of N $(CH_3)_2$ and OCH_3 was also identified by the signal at 3–4 ppm in compounds **4e**, **4f** and **4i**. In mass spectra of the synthesized compounds, the molecular ion was evident and its accurate mass was measured. From the physiochemical, spectral and elemental analysis data, all the synthesized compounds were in conformity with structures envisaged.

3.2. In vitro anticancer screening

The synthesized compounds were evaluated for *in vitro* cytotoxic activity against various cell lines such as HeLa (cervical cancer), MCF-7 (breast cancer), HL-60 (Human promyelocytic leukemia) and HepG2 (Hepatocellular carcinoma) by the MTT assay method. Cisplatin, one of the most effective anticancer agents was used as a reference drug in this study. The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of all the cancer cell lines HeLa, MCF-7, HL-60 and HepG2. The response parameter calculated was the IC_{50} values, which responds to the concentration required for 50% inhibition of cell viability. The *in vitro* cytotoxic activity of the synthesized compounds is summarized in Table 1.

In *in vitro* cytotoxic study, the compounds substituted by both the electron withdrawing groups like NO_2, Cl and electron donating groups such as OCH_3, OH, and N─$(CH_3)_2$ had shown remarkable influence in anticancer effect. It was found that electron donating groups have profound effect than the electron withdrawing groups. Among the tested compounds, **4e** exhibited maximum cytotoxic activity against HeLa (IC_{50} 41.8 μM) and is close to standard Cisplatin. The *para* substituted analog had shown more effect than *meta* substituted one. Among the compounds with electron withdrawing group, **4g** (IC_{50} 71.4 μM) was more effective. The cell viability was tested against MCF-7 and most of the compounds showed significant inhibitory concentration. Most surprisingly, the compound **4e** exhibited maximum cytotoxic activity with IC_{50} 8.9 μM. Apart from **4e**, **4g** (IC_{50} 51.4 μM) was also found to be active. Compounds **4e**, **4i**, **4f**, **5b**, **4g**, **5a** and **4h**, showed inhibitory concentration less than 50% which showed that these compounds are active against **HL-60** cell line. This result indicated that synthesized compound **4e** (IC_{50} 6.6 μM) has potency equivalent to the standard drug Cisplatin. All quinazolinone derivatives showed potent to moderate anti cancer activity against HepG2 cell line. The inhibitory concentration for more than five compounds was less than 50% (i.e. **4e**, **4i**, **4h**, **5b**, **4f** and **4g**) and among them, compound **4e** IC_{50} was 9.6 μM.

The following mechanism of action can be proposed for quinazolinone derivatives since their chemical structure, in some respect resembles Raltitrexed and Thymitaq which inhibits EGFR tyrosine kinase by binding to the adenosine triphosphate (ATP)-binding site of the enzyme. The epidermal growth factor receptor (EGFR) is cellular trans-membrane tyrosine kinases that is over-expressed in a significant number of human tumors (e.g., breast, ovarian, colon, and prostate), their expression levels often correlate with vascularity and is associated with poor prognosis in patients (Kersemaekers et al.,1999) Thus the function of the EGFR tyrosine kinase in activating the anti-apoptotic rap signal transduction cascade is inhibited, and malignant cells are inhibited. The synthesized quinazolinone derivatives may be a selective inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase over expression through the inhibition of EGFR autophosphorylation and EGF-stimulated signal transduction (Griffin et al., 1998). Furthermore, it can also be proposed that quinazolinones may exert their antitumor activity through inhibition of the DNA repair enzyme system. Enzyme-mediated repair of strand lesions in DNA is an established mechanism for resistance toward antitumor DNA-damaging drugs and radiotherapy (Fricker, 2006; Griffin et. al., 1998). Hence it can be expected that these derivatives may inhibit the tumor cells by both or any one of these mechanisms. However, a detailed receptor study and DNA analysis are necessary to reach a concrete conclusion.

4. Conclusions

Comprehensively, it is summarized that some of the quinazolinone derivatives are possessing prominent activity against cervical cancer, breast cancer, leukemia and hepatocellular cancer. Among the cell lines tested, these derivatives have shown profound effect against HL-60 and HepG2. Most remarkably, compound **4e** is having three folds higher activity than Cisplatin against breast cancer, twofold greater than that of standard against liver cancer whereas against cervical and blood cancer the activity is equivalent to standard. Structure activity relationship of the compounds showed that the electron donating group enhances the activity while the electron withdrawing group decreases the activity. The prominent cytotoxic effect of **4e** may be due to the presence of electron donor at *para* carbon as compared to other derivatives except **4f**. It can also be justified that the *para* substituent may not have more steric hindrance as the *ortho* group does in general. However, extensive pharmacological and toxicological studies will explore the therapeutic benefit of the tested quinazolinone derivatives.

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