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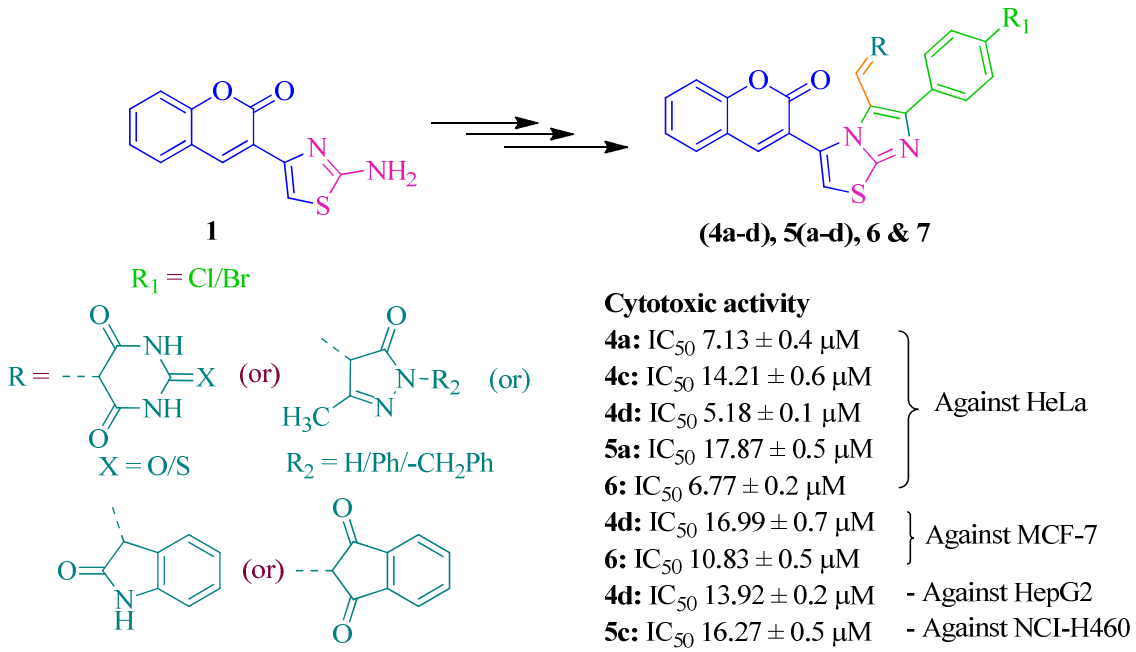
Graphical abstract

Synthesis and *in vitro* cytotoxic activity of novel coumarinylimidazo[2,1-*b*]thiazole derivatives

Rajitha Gali,[a] Janardhan Banothu,[a] Ravibabu Velpula,[a] Mahendar Porika,[b] Rajitha Bavantula,[a]* Sadanandam Abbagani[b]



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Synthesis and *in vitro* cytotoxic activity of novel coumarinylimidazo[2,1-*b*]thiazole derivatives

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Abstract

A series of novel coumarinylimidazo[2,1-*b*]thiazole derivatives were synthesized by the treatment of 3-(2-aminothiazol-4-yl)-2*H*-chromen-2-one with phenacyl bromides followed by Vilsmeier–Haack and Knoevenagel condensation reactions. Structures of all the newly synthesized compounds were confirmed by their spectral and analytical studies. All the synthesized compounds were screened for their *in vitro* cytotoxic activity against Breast cancer cell line (MCF-7), Hepatocellular liver carcinoma cell line (HepG2), Cervical carcinoma cell line (HeLa) and Lung cancer cell line (NCI-H460). Cytotoxic activity results revealed that, the compound **4d** has shown broad spectrum activity against MCF-7, HepG2 & HeLa with IC_{50} values 16.99 ± 0.7, 13.92 ± 0.2 & 5.18 ± 0.1 μM respectively. Compound **6** against MCF-7 (IC_{50} 10.83 ± 0.5 μM) and HeLa (IC_{50} 6.77 ± 0.2 μM), **4a**, **4c** & **5a** against HeLa and **5c** against NCI-H460 with IC_{50} values 7.13 ± 0.4, 14.21 ± 0.6, 17.87 ± 0.5 & 16.27 ± 0.5 μM respectively have shown good activity. Remaining compounds have shown moderate activity against all the tested cell lines.

Keywords: Coumarinylimidazo[2,1-*b*]thiazole, Cytotoxic activity, Knoevenagel condensation, Vilsmeier–Haack reaction.

Introduction

Cancer is the most frightening disease of human worldwide. According to the statistics, cancer is the second leading cause of human mortality after cardiovascular disease.[1] The World

Cancer Congress has released a report stating that 7.6 million cancer deaths (around 13% of all deaths) in 2008, that are projected to continue rising, with an estimated 13.1 million deaths in 2030. Conventional treatments include surgery, radiation, drugs and other therapies. Anticancer drugs such as Cisplatin, 5-Fluorouracil, Paclitaxel and Docetaxel are some of the major chemotherapeutic agents currently being used to treat cancer. Many of the available anticancer drugs exhibit undesirable side effects such as reduced bioavailability, toxicity and drug-resistance.[2] Therefore, the development of novel anticancer agents remains a major challenge in the field of cancer chemotherapy.

Imidazo[2,1-*b*]thiazoles are important scaffold in highly significant bioactive molecules and pharmaceuticals. For instance, imidazo[2,1-*b*]thiazole constitutes the main part of Tetramisole[3] and Levamisole[4] which are well known for their antihelminthic and immunomodulatory properties. Imidazo[2,1-*b*]thiazole derivatives occupied a prominent place in medicinal chemistry because of their therapeutic properties, such as antitumor,[5] anticancer,[6] anticoccidial,[7] anthelmintic,[4] antimicrobial,[8] antioxidant,[9] antitubercular,[10] antiviral,[11] anti-inflammatory[12] and cytotoxic[13] activities. They also act as inhibitors of acetylcholinesterase, butyrylcholinesterase[14] and mitochondrial NADH dehydrogenase[15] enzymes. Recently, some pyrimidinyl substituted imidazo[2,1-*b*]thiazole derivatives were reported as RAF kinases inhibitors.[16] Natural and synthetic coumarin derivatives have attracted intense interest in recent years because of their diverse biological and pharmacological properties such as anticancer, anticoagulant, anti-inflammatory, antimicrobial, antioxidant, antiviral and cardiovascular activities.[17] Coumarins are also widely used as additives in foods, perfumes, cosmetics, optical brighteners, dispersed fluorescence and lasers dyes.[18]

Owing to the remarkable pharmacological properties of imidazo[2,1-*b*]thiazole and coumarin derivatives, we aimed to design a moiety that embodied both the active pharmacophores in a single molecule and to evaluate their biological activities especially anticancer activity. Therefore, in the present investigation and in continuation of our work on thiazole and coumarin containing heterocycles,[19] we report the synthesis of novel coumarinylimidazo[2,1-*b*]thiazole derivatives and evaluation of their *in vitro* cytotoxic activity.

Results and discussion

3-(2-Aminothiazol-4-yl)-2*H*-chromen-2-one (**1**) was prepared by the reaction of 3-(2-bromoacetyl)-2*H*-chromen-2-one[20] with thiourea in the presence of catalytic NaF at ambient

temperature.[19b] Compound **1** on treatment with 4-bromo phenacyl bromide/4-chloro phenacyl bromide furnished the corresponding imidazo[2,1-*b*]thiazoles (**2a,b**) in 80-82% yields. Vilsmeier–Haack formylation on **2a,b** followed by Knoevenagel condensation with barbituric acid, thiobarbituric acid, 3-methyl-1*H*-pyrazol-5(4*H*)-ones, indolin-2-one and 1,3-indandione afforded the target compounds (**4a-d, 5a-d, 6 & 7**) in good yields. The synthetic strategy adopted to obtain the target compounds has shown in **Scheme 1**.

(Scheme 1)

All the synthesized compounds were characterized by IR, NMR and mass spectral data. The appearance of a broad band ranging from 3103-3179 cm^{-1} for the NH of **4a-d**, **5a**, **6** and a sharp band in the range of 1630-1719 cm^{-1} for the C=O (barbituric acid/ thiobarbituric acid/3-methyl-1*H*-pyrazol-5(4*H*)-ones/indolin-2-one/1,3-indandione carbonyl group) of **4a-d**, **5a-d**, **6** & **7** from the IR spectra confirm the products formation. From the ^{1}H NMR spectra, the absence of a singlet at 9.78 ppm (aldehyde proton) and presence of a singlet in the range of 8.31-8.39 ppm (ethylene proton), and from ^{13}C NMR spectra, the absence of aldehyde carbonyl carbon signal at 189.12 ppm and the presence of ethylene carbon signal in the region of 132.32-135.73 ppm indicative of the products formation. In addition, the molecular ion peak from the mass spectra as well as the elemental analyses data further confirmed the formation of products.

In vitro cytotoxic activity was carried out against human Breast cancer cell line (MCF-7), Hepatocellular liver carcinoma cell line (HepG2), Cervical carcinoma cell line (HeLa) and Lung cancer cell line (NCI-H460). Doxorubicin was used as a reference drug. Cell viability in the presence of the test samples were measured by the MTT-microcultured tetrazolium assay.[21] The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of MCF-7 (**Fig. 1**), HepG2 (**Fig. 2**), HeLa (**Fig. 3**) and NCI-H460 (**Fig. 4**). The response parameter calculated was the IC_{50} value, which corresponds to the concentration required for 50% inhibition of cell viability. The IC_{50} values for compounds (**4a-d, 5a-d, 6 & 7**) were presented in (**Table 1**).

(Table 1)

(Fig. 1)

(Fig. 2)

(Fig. 3)

(Fig. 4)

In vitro cytotoxic activity results revealed that (**Table 1**), all the compounds were active against all the four tested cell lines. Among them, the compound derived from 4-chloro phenacyl bromide and thiobarbituric acid *i.e.* **4d** has shown broad spectrum activity against MCF-7, HepG2 & HeLa with IC_{50} values 16.99 ± 0.7, 13.92 ± 0.2 & 5.18 ± 0.1 μM respectively. The Compound derived from 4-bromo phenacyl bromide and indolin-2-one *i.e.* **6** against MCF-7 & HeLa with IC_{50} values 10.83 ± 0.5 & 6.77 ± 0.2 μM respectively, the compounds derived from 4-bromo phenacyl bromide and barbituric acid (**4a**)/ thiobarbituric acid (**4c**)/3-methyl-1*H*-pyrazol-5(4*H*)-one (**5a**) against HeLa with IC_{50} values 7.13 ± 0.4, 14.21 ± 0.6 & 17.87 ± 0.5 μM respectively and the compound derived from 4-bromo phenacyl bromide and 1-benzyl-3-methyl-1*H*-pyrazol-5(4*H*)-one *i.e.* **5c** against NCI-H460 with IC_{50} value 16.27 ± 0.5 μM have displayed prominent activity when compared with the standard drug Doxorubicin. Remaining compounds have shown moderate activity with IC_{50} values ranging from 21.00 - 185.14 μM against the four cell lines. On overall comparison, compounds derived from barbituric acid, thiobarbituric acid and indolin-2-one *i.e.* **4a-d** & **6** have shown promising activity against all the tested cell lines.

Conclusion

In conclusion, a novel series of coumarinylimidazo[2,1-*b*]thiazole derivatives were synthesized *via* Vilsmeier–Haack formylation and Knoevenagel condensation reactions. All the compounds were screened for their *in vitro* cytotoxic activity against MCF-7, HepG2, HeLa and NCI-H460 cancer cell lines. Compound **4d** has shown broad spectrum activity against MCF-7, HepG2 and HeLa. Compound **6** against MCF-7 & HeLa, **4a, 4c** & **5a** against HeLa and **5c** against NCI-H460 have shown prominent activity when compared with the standard drug. The synthesized compounds may serve as a lead compounds for the design and development of new cytotoxic agents.

Experimental

Melting points were determined in open capillaries using Stuart SMP30 apparatus and are uncorrected. The progress of the reactions as well as purity of the compounds was monitored by thin layer chromatography with F_{254} silica-gel precoated sheets using hexane/ethyl acetate (7/3) as eluent. IR spectra were recorded on Perkin-Elmer 100S spectrophotometer using KBr pellet. NMR spectra were recorded on Bruker 400 MHz spectrometer using DMSO-d_6 as solvent and TMS as internal standard. Elemental analyses were performed on a Carlo Erba modal EA1108 and mass spectra were recorded on a Jeol JMSD-300 spectrometer.

Synthesis of 3-(6-arylimidazo[2,1-*b*]thiazol-3-yl)-2*H*-chromen-2-ones (2a,b)

A mixture of 3-(2-aminothiazol-4-yl)-2*H*-chromen-2-one (**1**, 20 mmol) and 4-bromo phenacylbromide/4-chloro phenacylbromide (20 mmol) were taken in 100 mL of ethanol and refluxed for 8-10 h. After completion of the reaction (monitored by TLC), the solid separated out was filtered and washed with cold ethanol to afford the analytically pure intermediates in good yields.

3-(6-(4-Bromophenyl)imidazo[2,1-*b*]thiazol-3-yl)-2*H*-chromen-2-one (2a)

Yellow solid; Yield: 82%; mp: 231-233 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.40-7.49 (m, 4H), 7.55 (s, 1H), 7.65-7.69 (m, 2H), 7.82 (d, J = 7.6 Hz, 2H), 8.53 (s, 1H), 8.65 (s, 1H); MS (ESI) *m/z*: 423 $[M]^+$; Anal. Calcd. for $C_{20}H_{11}BrN_2O_2S$: C, 56.75; H, 2.62; N, 6.62. Found: C, 56.63; H, 2.71; N, 6.74.

3-(6-(4-Chlorophenyl)imidazo[2,1-*b*]thiazol-3-yl)-2*H*-chromen-2-one (2b)

Yellow solid; Yield: 80%; mp: 228-230 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.42-7.48 (m, 4H), 7.57 (s, 1H), 7.60-7.70 (m, 2H), 7.87 (d, J = 7.6 Hz, 2H), 8.55 (s, 1H), 8.65 (s, 1H); MS (ESI) *m/z*: 378 $[M]^+$; Anal. Calcd. for $C_{20}H_{11}ClN_2O_2S$: C, 63.41; H, 2.93; N, 7.39. Found: C, 63.55; H, 2.82; N, 7.26.

Synthesis of 3-(2-oxo-2*H*-chromen-3-yl)-6-arylimidazo[2,1-*b*]thiazole-5-carbaldehydes (3a,b)

Vilsmeier reagent was prepared at 0-5 ^{o}C by dropping $POCl_3$ (54 mmol) into a stirred solution of DMF (65 mmol) in $CHCl_3$ (5 mL). Compound (**2a,b**, 5 mmol) was suspended in $CHCl_3$ (20 mL) and dropped into the Vilsmeier reagent while maintaining stirring and cooling. The mixture was kept at room temperature for 3 h and at reflux for 10-12 h. After completion of the reaction shown by TLC, the excess of chloroform was removed under reduced pressure and the resulting oily mixture was poured onto ice cold water. The crude precipitate was collected by filtration and crystallized from chloroform.

6-(4-Bromophenyl)-3-(2-oxo-2*H*-chromen-3-yl)imidazo[2,1-*b*]thiazole-5-carbaldehyde (3a)

Yellow solid; Yield: 89%; mp: 240-242 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.42-7.44 (m, 2H), 7.49 (d, J = 8.0 Hz, 2H), 7.68-7.71 (m, 2H), 7.84-7.86 (m, 2H), 8.48 (s, 1H), 8.68 (s, 1H), 9.78 (s, 1H); MS (ESI) *m/z*: 451 $[M]^+$; Anal. Calcd. for $C_{21}H_{11}BrN_2O_3S$: C, 55.89; H, 2.46; N, 6.21. Found: C, 55.78; H, 2.51; N, 6.27.

6-(4-Chlorophenyl)-3-(2-oxo-2*H*-chromen-3-yl)imidazo[2,1-*b*]thiazole-5-carbaldehyde (3b)

Yellow solid; Yield: 90%; mp: 236-238 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.41-7.51 (m, 4H), 7.69 (t, J = 7.2 Hz, 2H), 7.84-7.88 (m, 2H), 8.41 (s, 1H), 8.68 (s, 1H), 9.78 (s, 1H); MS (ESI) *m/z*: 406 $[M]^{+}$; Anal. Calcd. for $C_{21}H_{11}ClN_2O_3S$: C, 62.00; H, 2.73; N, 6.89. Found: C, 62.13; H, 2.79; N, 6.81.

Synthesis of 3,5,6-trisubstituted imidazo[2,1-*b*]thiazole derivatives (4a-d, 5a-d, 6 & 7)

A mixture of 3-(2-oxo-2*H*-chromen-3-yl)-6-arylimidazo[2,1-*b*]thiazole-5-carbaldehydes (**3a,b**, 10 mmol) and barbituric acid/thiobarbituric acid/3-methyl-1*H*-pyrazol-5(4*H*)-ones/2-oxo-indole/indan-1,3-dione (10 mmol) were dissolved in 20 mL of ethanol having catalytic amount of glacial acetic acid and refluxed for 2-4 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the solid separated out was filtered and washed with hot ethanol which afforded the analytically pure product in good yields.

5-((6-(4-Bromophenyl)-3-(2-oxo-2*H*-chromen-3-yl)imidazo[2,1-*b*]thiazol-5-yl)methylene) pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (4a)

Red solid; Yield: 92%; mp: 273-275 ^{o}C; IR (KBr, cm^{-1}) υ_{max} : 3179 (NH), 1730, 1698 (C=O), 1608 (C=N), 508 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.45 (t, J = 7.6 Hz, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.75 (t, J = 8.0 Hz, 2H), 7.85 (d, J = 7.2 Hz, 2H), 8.06 (s, 1H), 8.36 (s, 1H), 8.84 (s, 1H), 10.99 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 103.68, 116.35, 118.26, 119.16, 121.29, 125.11, 129.33, 133.35, 143.09, 145.98, 150.31, 153.63, 158.33, 163.52, 163.75, 166.08, 171.98, 177.47; MS (ESI) *m/z*: 561 $[M]^{+}$; Anal. Calcd. for $C_{25}H_{13}BrN_4O_5S$: C, 53.49; H, 2.33; N, 9.98. Found: C, 53.36; H, 2.42; N, 9.87.

5-((6-(4-Chlorophenyl)-3-(2-oxo-2*H*-chromen-3-yl)imidazo[2,1-*b*]thiazol-5-yl)methylene) pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (4b)

Red Solid; Yield: 90%; mp: 269-271 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3166 (NH), 1722, 1684 (C=O), 1608 (C=N), 754 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.45 (t, J = 7.6 Hz, 2H), 7.53 (d, J = 8.0 Hz, 2H), 7.73-7.77 (m, 2H), 7.84 (d, J = 7.6 Hz, 2H), 8.06 (s, 1H), 8.36 (s, 1H), 8.83 (s, 1H), 10.98 (s, 2H); MS (ESI) *m/z*: 516 $[M]^{+}$; Anal. Calcd. for $C_{25}H_{13}ClN_4O_5S$: C, 58.09; H, 2.53; N, 10.84. Found: C, 58.17; H, 2.62; N, 10.90.

5-((6-(4-Bromophenyl)-3-(2-oxo-2*H*-chromen-3-yl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (4c)

Reddish brown solid; Yield: 91%; mp: 280-282 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3103 (NH), 1690,1630 (C=O), 1607 (C=N), 1568 (C=C), 1250 (C=S), 515 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.44-7.55 (m, 4H), 7.76 (t, *J* = 8.0 Hz, 2H), 7.85 (d, *J* = 7.2 Hz, 2H), 8.06 (s, 1H), 8.39 (s, 1H), 9.10 (s,1H), 12.11 (s, 2H); MS (ESI) *m/z*: 577 $[M]^{+}$; Anal. Calcd. for $C_{25}H_{13}BrN_4O_4S_2$: C, 52.00; H, 2.27; N, 9.70. Found: C, 52.13; H, 2.19; N, 9.64.

5-((6-(4-Chlorophenyl)-3-(2-oxo-2*H*-chromen-3-yl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (4d)

Reddish brown solid; Yield: 89%; mp: 278-280 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3161 (NH), 1703, 1687 (C=O), 1609 (C=N), 1573 (C=C), 1249 (C=S), 757 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.46 (t, *J* = 7.2 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.74-7.78 (m, 2H), 7.86 (t, *J* = 6.4 Hz, 2H), 8.06 (s, 1H), 8.39 (s, 1H), 9.09 (s, 1H), 12.11 (s, 2H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 103.66, 116.35, 118.19, 120.12, 121.02, 125.12, 129.38, 133.46, 143.62, 146.19, 153.66, 158.20, 161.27, 161.93, 167.78, 177.65, 178.19; MS (ESI) *m/z*: 532 $[M]^{+}$; Anal. Calcd. For $C_{25}H_{13}ClN_4O_4S_2$: C, 56.34; H, 2.46; N, 10.51. Found: C, 56.47; H, 2.41; N, 10.58.

4-((6-(4-Bromophenyl)-3-(2-oxo-2*H*-chromen-3-yl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-3-methyl-1*H*-pyrazol-5(4*H*)-one (5a)

Reddish brown solid; Yield: 92%; mp: 286-288 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3146 (NH), 1715 (C=O), 1607 (C=N), 1585 (C=C), 523 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.90 (s, 3H), 7.39-7.49 (m, 5H), 7.69 (t, *J* = 7.6 Hz, 2H), 7.84 (d, *J* = 7.6 Hz, 2H), 8.31 (s, 1H), 8.40 (s, 1H), 10.90 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 12.64, 116.10, 118.39, 118.66, 118.86, 121.15, 124.57, 124.79, 129.06, 129.19, 132.70, 132.85, 144.71, 145.20, 153.49, 153.66, 155.61, 158.66, 158.96, 165.88, 172.08, 172.28, 174.48; MS (ESI) *m/z*: 531 $[M]^{+}$; Anal. Calcd. for $C_{25}H_{15}BrN_4O_3S$: C, 56.51; H, 2.85; N, 10.54. Found: C, 56.39; H, 2.73; N, 10.63.

4-((6-(4-Bromophenyl)-3-(2-oxo-2*H*-chromen-3yl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (5b)

Reddish brown solid; Yield: 86%; mp: 298-300 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 1715 (C=O), 1606 (C=N), 1557 (C=C), 571 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.12 (s, 3H), 7.15 (t, *J* = 7.2 Hz, 1H), 7.38-7.51 (m, 6H), 7.65 (s, 1H), 7.72 (t, *J* = 7.2 Hz, 2H), 7.85 (d, *J* = 6.8 Hz, 2H), 7.92 (d, *J* = 8.0 Hz, 2H), 8.36 (s, 1H), 8.63 (s,1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 12.72, 114.29, 116.16, 117.76, 118.48, 118.86, 121.69, 123.92, 124.78, 128.74, 129.16, 132.86, 136.29, 138.71,

144.72, 145.54, 150.86, 153.75, 158.61, 163.20, 175.55; MS (ESI) *m/z*: 607 $[M]^+$; Anal. Calcd. for $C_{31}H_{19}BrN_4O_3S$: C, 61.29; H, 3.15; N, 9.22. Found: C, 61.14; H, 3.06; N, 9.36.

1-Benzyl-4-((6-(4-bromophenyl)-3-(2-oxo-2*H*-chromen-3-yl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-3-methyl-1*H*-pyrazol-5(4*H*)-one (5c)

Red solid; Yield: 85%; mp: 243-245 ^oC; IR (KBr, cm^{-1}) υ_{max}: 1733, 1719 (C=O), 1605 (C=N), 1571 (C=C), 560 (C-Br); ^1H NMR (400 MHz, DMSO-d_6): δ 1.98 (s, 3H), 4.79 (s, 2H), 7.22-7.27 (m, 3H), 7.33 (t, *J* = 7.2 Hz, 2H), 7.41 (t, *J* = 7.2 Hz, 2H), 7.49 (d, *J* = 8.4 Hz, 2H), 7.54 (s, 1H), 7.70 (t, *J* = 8.0 Hz, 2H), 7.84 (d, *J* = 7.6 Hz, 2H), 8.33 (s,1H), 8.49 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 12.51, 46.78, 114.51, 116.13, 118.45, 118.85, 121.77, 124.74, 127.09, 127.43, 128.39, 129.10, 132.77, 135.20, 137.94, 145.36, 148.77, 153.70, 158.63, 161.87, 163.05, 171.97, 174.98; MS (ESI) *m/z*: 621 $[M]^+$; Anal. Calcd. for $C_{32}H_{21}BrN_4O_3S$: C, 61.84; H, 3.41; N, 9.01. Found: C, 61.75; H, 3.53; N, 9.11.

1-Benzyl-4-((6-(4-chlorophenyl)-3-(2-oxo-2*H*-chromen-3-yl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-3-methyl-1*H*-pyrazol-5(4*H*)-one (5d)

Orange solid; Yield: 84%; mp: 239-241 ^oC; IR (KBr, cm^{-1}) υ_{max}: 1719 (C=O), 1606 (C=N), 1571 (C=C), 752 (C-Cl); ^1H NMR (400 MHz, DMSO-d_6): δ 1.98 (s, 3H), 4.79 (s, 2H), 7.21-7.27 (m, 3H), 7.33 (t, *J* = 7.2 Hz, 2H), 7.41 (t, *J* = 7.6 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.54 (s, 1H), 7.69 (d, *J* = 7.6 Hz, 2H), 7.83 (d, *J* = 8.0 Hz, 2H), 8.33 (s,1H), 8.49 (s, 1H); MS (ESI) *m/z*: 577 $[M]^+$; Anal. Calcd. for $C_{32}H_{21}ClN_4O_3S$: C, 66.60; H, 3.67; N, 9.71. Found: C, 66.47; H, 3.59; N, 9.82.

3((6-(4-Bromophenyl)-3-(2-oxo-2*H*-chromen-3-yl)imidazo[2,1-*b*]thiazol-5-yl)methylene)indolin-2-one (6)

Reddish brown solid; Yield: 93%; mp: 316-318 ^oC; IR (KBr, cm^{-1}) υ_{max}: 3159 (NH), 1714, 1689 (C=O), 1608 (C=N), 1573 (C=C), 1516 (C-Br); ^1H NMR (400 MHz, DMSO-d_6): δ 6.77 (d, *J* = 7.6 Hz, 1H), 6.83 (t, *J* = 7.2 Hz, 1H), 7.07 (t, *J* = 7.6 Hz, 1H), 7.40-7.49 (m, 4H), 7.64-7.70 (m, 3H), 7.81-7.87 (m, 4H), 8.23 (s, 1H), 10.43 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 109.00, 116.10, 116.22, 118.80, 119.02, 119.13, 120.49, 122.81, 124.58, 125.14, 126.88, 127.11, 128.89, 132.32, 139.48, 144.61, 145.10, 153.69, 155.94, 159.06, 167.69, 172.28; MS (ESI) *m/z*: 566 $[M]^+$; Anal. Calcd. for $C_{29}H_{16}BrN_3O_3S$: C, 61.49; H, 2.85; N, 7.42. Found: C, 61.41; H, 2.72; N, 7.55.

2-((6-(4-Chlorophenyl)-3-(2-oxo-2*H*-chromen-3-yl)imidazo[2,1-*b*]thiazol-5-yl)methylene)-1*H*-indene-1,3(2*H*)-dione (7)

Reddish brown solid; Yield: 87%; mp: 306-308 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 1739, 1712, 1672 (C=O), 1608 (C=N), 1567 (C=C), 726 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.46 (t, J = 7.2 Hz, 2H), 7.54 (d, J = 8.4 Hz, 2H), 7.62 (s, 1H), 7.74-7.87 (m, 8H), 8.39 (s, 1H), 8.87 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 116.17, 116.31, 118.69, 119.22, 120.78, 121.99, 122.76, 124.89, 124.98, 129.29, 133.05, 133.23, 133.50, 134.74, 135.73, 145.11, 145.78, 146.24, 151.47, 153.57, 159.16, 160.06, 160.88, 185.06; MS (ESI) *m/z*: 534 [M]$^{+}$; Anal. Calcd. for $C_{30}H_{15}ClN_2O_4S$: C, 67.35; H, 2.83; N, 5.24. Found: C, 67.21; H, 2.92; N, 5.36.

Anticancer activity

All the synthesized compounds were evaluated for their *in vitro* cytotoxic activity against four different cancer cell lines *i.e.* breast cancer cell line (MCF-7), hepatocellular liver carcinoma cell line (HepG2), cervical carcinoma cell line (HeLa) and lung cancer cell line (NCI-H460). Cell viability in the presence of the test samples were measured by the MTT-microcultured tetrazolium assay. This assay is a quantitative colorimetric method for determination of cell cytotoxicity. The assessed parameter is the metabolic activity of viable cells. Metabolically active cells reduce pale yellow tetrazolium salt (MTT) to a dark blue water-insoluble formazan, which can be directly quantified after solubilization with DMSO. The absorbance of the formazan directly correlates with the number of viable cells. MCF-7, HepG2, HeLa and NCIH-460 cells were plated into a 96-well plate at a density of 1x10^4 cells/well. Cells were grown overnight in the full medium and then switched to the low serum media. DMSO was used as control. After 48 h of treatment with different concentrations of test compounds, the cells were incubated with MTT (2.5 mg/mL) in the CO_2 chamber for 2 h. The medium was then removed and 100 µL of DMSO was added into each well to dissolve formazan crystals. After thoroughly mixing, the plates were read at 570 nm for optical density which is directly correlated with cell quantity. The results were represented as percentage of cytotoxity/viability. All the experiments were carried out in triplicates. The IC_{50} values were calculated from the percentage of cytotoxicity and compared with the reference drug Doxorubicin (**Table 1**).

Determination of the IC_{50} Values

The IC_{50} values were determined from plot of dose response curve between compound concentration and % of cell viability. The IC_{50} values were derived using curve fitting methods

with *GraphPad Prism* as statistical software (Ver. 5.02). The average of three (triplicates manner) were taken in determination. Graph was plotted by keeping concentration of drug on X-axis and % of cell viability on Y-axis. The dose-response profile for compounds on human MCF-7, HepG2, HeLa and NCI-H460 cell line were shown in **Figure 1-4**. The IC_{50} values of compounds against four different cell lines were calculated by plotting a graph of % of cell viability vs compound concentration. Statistical significance values (P values) for all compounds against all the cell lines were provided at the bottom of the figures.

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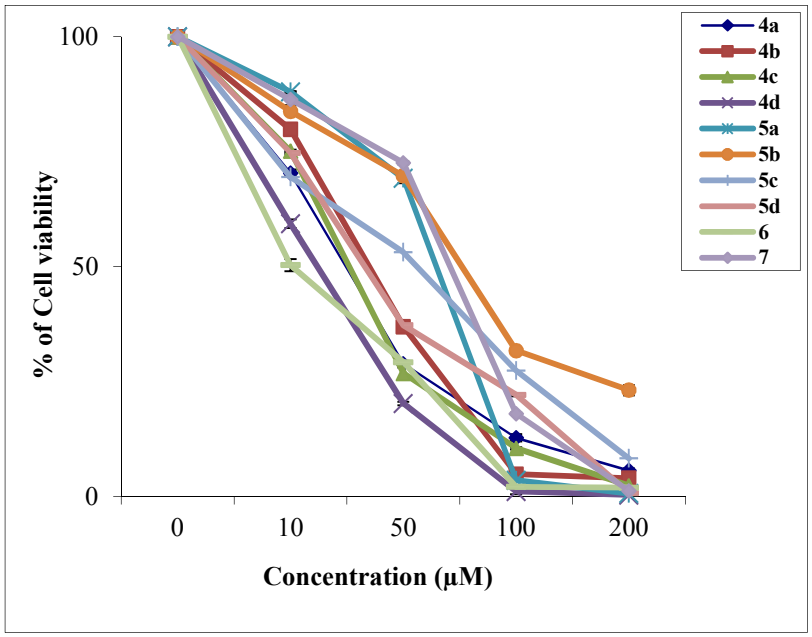


Fig. 1. Survival curves of MCF-7 for coumarinylimidazo[2,1-*b*] thiazoles (4a-d, 5a-d, 6 & 7). Analysis of variance for Compounds: P<0.01: 4d, 6; P<0.05: 4a, 4b, 4c, 5d.

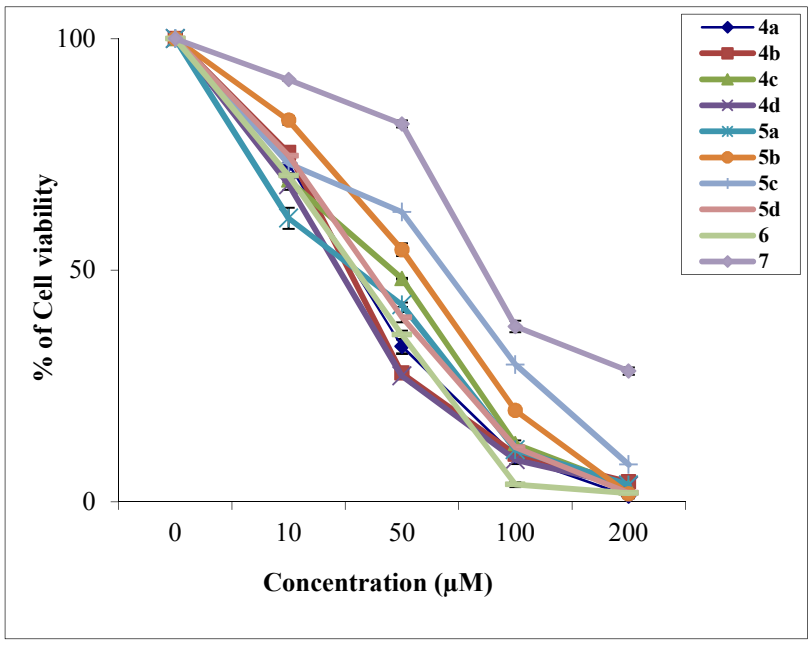


Fig. 2. Survival curves of HepG-2 for coumarinylimidazo[2,1-*b*] thiazoles (4a-d, 5a-d, 6 & 7). Analysis of variance for Compounds: P<0.05: 4a, 4b, 4c, 4d, 5a, 5d, 6.

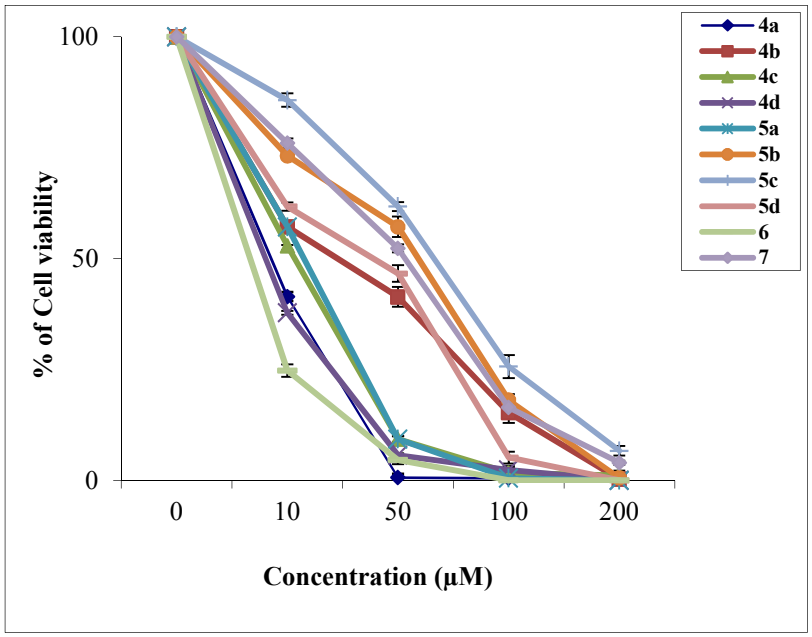


Fig. 3. Survival curves of Hela for coumarinylimidazo[2,1-*b*] thiazoles (4a-d, 5a-d, 6 & 7). Analysis of variance for Compounds: P<0.01: 4a, 4b, 4c, 4d, 5a, 5d, 6; P<0.05: 5b, 5c, 7.

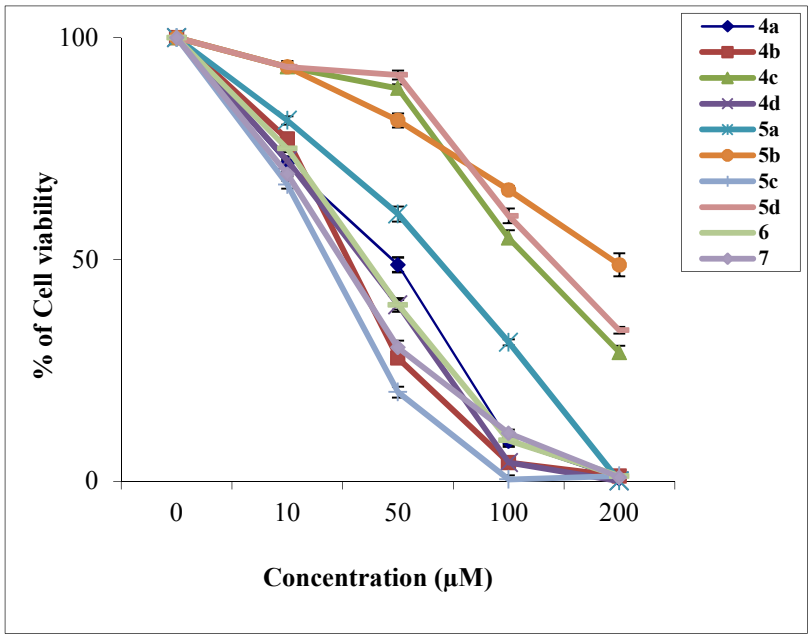
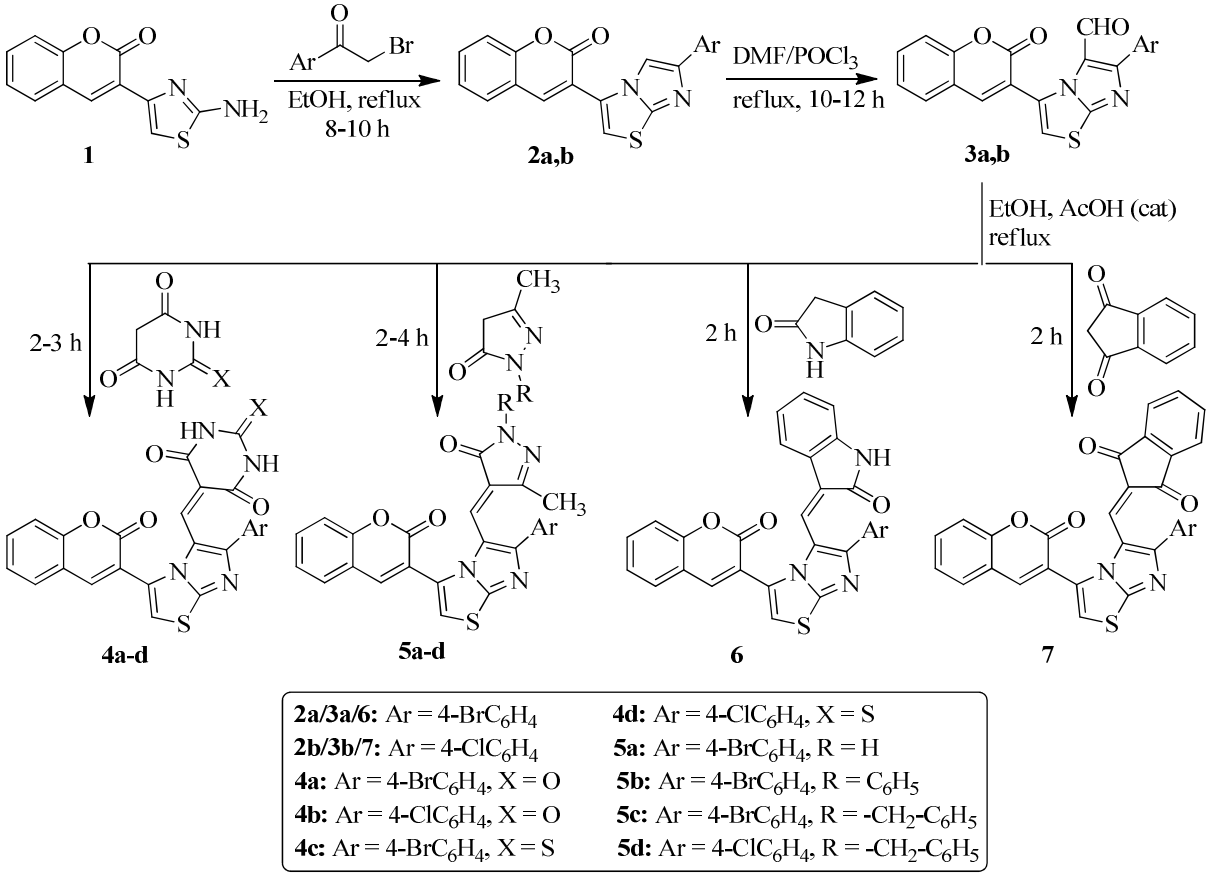
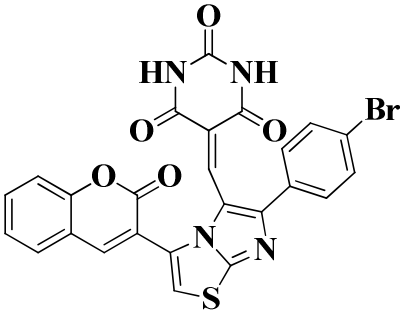
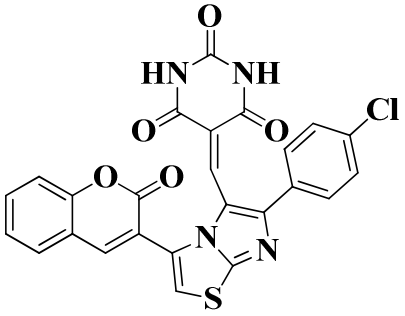
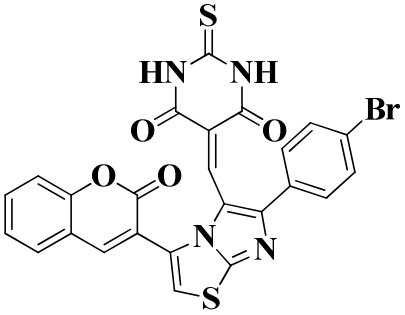
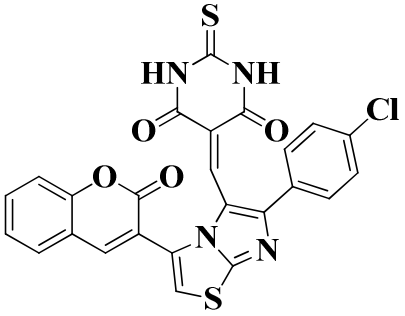
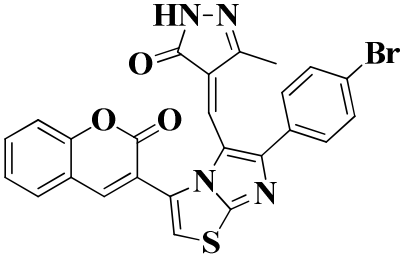


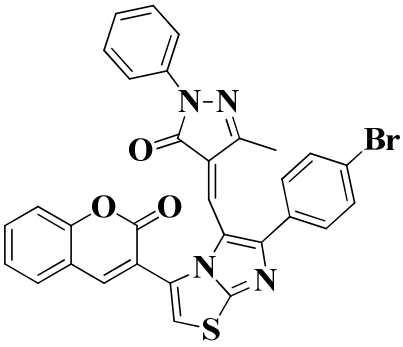
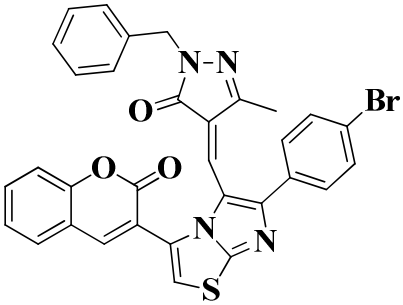
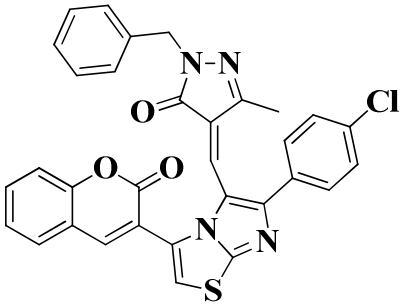
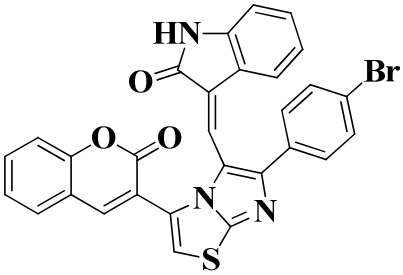
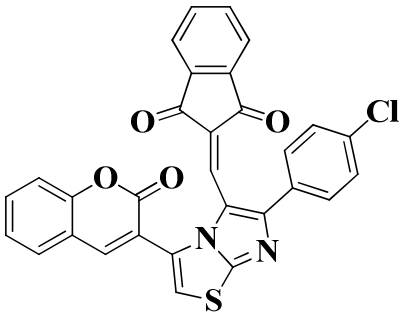
Fig. 4. Survival curves of NCI-H460 for coumarinylimidazo[2,1-*b*] thiazoles (4a-d, 5a-d, 6 & 7). Analysis of variance for Compounds: P<0.01: 5c; P<0.05: 4a, 4b, 4d, 6, 7.



Scheme 1. Synthesis of coumarinylimidazo[2,1-*b*]thiazole derivatives (**4a-d, 5a-d, 6 & 7**).

Table 1. Inhibition values (IC_{50} in μM) of coumarinylimidazo[2,1-*b*] thiazoles (**4a-d**, **5a-d**, **6** & **7**) on human tumor cell lines MCF-7, HepG2, HeLa and NCI-H460.

Analogs	Product	IC_{50} values (μM)			
		MCF-7	HepG-2	HeLa	NCI-H460
4a		21.00 ± 0.8	30.00 ± 0.5	7.13 ± 0.4	43.25 ± 0.7
4b		39.45 ± 0.3	27.01 ± 0.7	45.18 ± 0.8	26.29 ± 0.1
4c		25.99 ± 1.0	42.07 ± 0.5	14.21 ± 0.6	136.04 ± 0.6
4d		16.99 ± 0.7	13.92 ± 0.2	5.18 ± 0.1	32.37 ± 0.9
5a		69.93 ± 0.4	22.55 ± 0.8	17.87 ± 0.5	74.21 ± 1.1

5b		96.37 ± 0.9	64.89 ± 0.6	70.06 ± 0.6	185.14 ± 1.0
5c		57.66 ± 0.7	71.38 ± 0.3	83.99 ± 0.9	16.27 ± 0.5
5d		44.12 ± 0.6	40.05 ± 0.3	48.31 ± 0.3	148.67 ± 0.5
6		10.83 ± 0.5	25.08 ± 0.7	6.77 ± 0.2	38.79 ± 0.4
7		78.38 ± 0.5	117.78 ± 0.4	68.72 ± 1.0	21.45±0.6
Standard	Doxorubicin	3.00 ± 0.3	1.10 ± 0.06	1.33 ± 0.11	0.83 ± 0.03