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Indolylmethylene benzo[*h*]thiazolo[2,3-*b*]quinazolinones: Synthesis, characterization and evaluation of anticancer and antimicrobial activities

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ABSTRACT

A series of novel 10-((1*H*-indol-3-yl)methylene)-7-aryl-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-ones (**8a**–**t**) have been synthesized in good yields by the reaction of benzo[*h*]quinazoline-2(1*H*)-thiones (**4a**–**f**) with 2-chloro-*N*-phenylacetamide (**5**) followed by Knoevenagel condensation with various indole-3-carbaldehydes (**7a**–**d**) under conventional method. All the synthesized compounds were characterized by spectral studies and screened for their in vitro anticancer and antimicrobial activities. Compound **8c** has exhibited excellent activity against MCF-7 (breast cancer cell line) than the standard drug Doxorubicin. Compound **8d** against both the cancer cell lines, **8q** against MCF-7 and **8c**, **8h** against HepG2 have also shown good activity. Remaining compounds have shown moderate activity against both the cell lines. Antimicrobial activity revealed that, the compound **8q** and **8t** against *Staphylococcus aureus* and **8i**, **8k**, **8l**, **8q** & **8t** against *Klebsiella pneumoniae* have shown equipotent activity on comparing with the standard drug Streptomycin. Remaining compounds have shown significant antibacterial and comparable antifungal activities against all the tested microorganisms.

Cancer is one of the deathliest diseases in the medical field worldwide, characterized by uncontrolled, rapid, and pathological proliferation of abnormal cells. It represents the second leading cause of human mortality after cardiovascular diseases.[1] According to information from the World Health Organization (WHO), it is estimated that there will be 12 million deaths from cancer in 2030. World statistics on cancers have revealed that hepatocarcinoma is the sixth most common malignancy which is highly resistant to chemotherapeutic treatment resulting in increased mortality rates.[2] Breast cancer is the most commonly diagnosed malignant tumor in women and accounting for approximately 23% of all female cancers and the second most lethal cancer in women worldwide today.[3] Therefore, the search for potent, safe, and selective anticancer compounds is a crucial aspect of modern cancer research. On the other hand bacterial infections have increased at an alarming rate causing deadly diseases. The treatment of infectious diseases remains as an important issue because of increasing number of multi-drug resistant microbial pathogens.[4] Considering this, the development of newer chemotherapeutics which selectively acts on the target without side effects has become a primary objective of medicinal chemists.

Recently, thiazoloquinazolines have emerged as an important target molecules due to their therapeutic and pharmacological properties such as antibiofilm,[5] anticancer,[6] anticonvulsant,[7] anti-HSV-1,[8] antitubercular,[9] anti-inflammatory,[10] antimicrobial,[11] analgesic, antioxidant,[12] antinociceptive,[13] and antiparkinsonian[14] activities. They also serve as inhibitors of Bcl-2 family proteins[15] and CDC25B phosphatase enzymes.[16] Indole ring system is one of the most ubiquitous heterocycles in nature, with wide range of biological activities[17] and also found as an active ingredient in various drugs, pharmaceuticals, natural products and agrochemicals.[18]

Prompted by the above facts and in continuation of our work on thiazoloquinazolines[19] herein, we report the synthesis of novel indolylmethylene benzo[*h*]thiazolo[2,3-*b*]quinazolinones and evaluated for their in vitro anticancer and antimicrobial activities.

Benzo[*h*]quinazoline-2(1*H*)-thiones (**4a**–**f**) which were obtained by a modified Biginelli reaction involving 1-tetralone (**1a**, **b**), arylaldehydes (**2a**–**d**) and thiourea (**3**) in the presence of poly (4-vinylpyridinium)hydrogen sulfate,[20] on cyclization with

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Table 1
Synthesis of indolylmethylene benzo[*h*]thiazolo[2,3-*b*]quinazolinones (**8a–t**)[a]

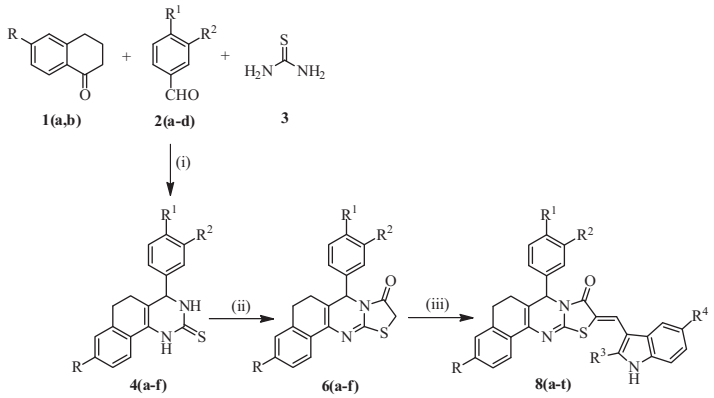
Analog	R	R^1	R^2	R^3	R^4	Time (h)	Yield[b] (%)
8a	OCH_3	Cl	H	H	H	3.5	80
8b	OCH_3	Cl	H	CH_3	H	3.5	78
8c	OCH_3	Cl	H	H	Br	3.0	82
8d	OCH_3	OCH_3	H	H	Br	2.0	84
8e	H	Cl	H	CH_3	H	3.5	77
8f	H	Cl	H	H	H	4.0	75
8g	H	Cl	H	H	OCH_3	3.0	79
8h	H	Cl	H	H	Br	2.5	84
8i	OCH_3	OCH_3	H	H	H	3.0	79
8j	OCH_3	H	NO_2	CH_3	H	3.0	76
8k	OCH_3	H	NO_2	H	CH_3	3.5	79
8l	OCH_3	H	NO_2	H	Br	2.0	85
8m	OCH_3	H	NO_2	H	H	2.5	76
8n	OCH_3	OCH_3	H	CH_3	H	3.0	80
8o	OCH_3	OCH_3	H	H	OCH_3	2.5	83
8p	H	H	NO_2	H	H	3.0	79
8q	OCH_3	F	H	H	Br	2.0	87
8r	OCH_3	F	H	H	OCH_3	3.0	81
8s	OCH_3	F	H	CH_3	H	2.5	82
8t	OCH_3	F	H	H	H	3.0	80

[a] Reaction conditions: Benzo[*h*]thiazolo[2,3-*b*]quinazolinones (**6a–f**, 1 mmol), 1*H*-indole-3-carbaldehyde (**7a–d**, 1 mmol), ethanol (10 mL) and piperidine (cat.), reflux.
[b] Isolated yields.

2-chloro-*N*-phenylacetamide (**5**) furnished benzo[*h*]thiazolo[2,3-*b*]quinazolinones (**6a–f**).[19] Compounds **6a–f** on Knoevenagel condensation with various indole-3-carbaldehydes[21] (**7a–d**) in ethanol with catalytic amount of piperidine under reflux condition afforded the title compounds (**8a–t**) in good yields (Table 1). The synthetic pathway has shown in Scheme 1.

Structures of all the synthesized compounds were established on the basis of their analytical and spectral studies. The appearance of a broad band at 3400 cm^{-1} (indole NH stretching), a sharp band at 1690 cm^{-1} (thiazole C=O stretching) and a medium band at 1634 cm^{-1} (pyrimidine C=N stretching) from IR spectrum, disappearance of a multiplet at 4.01–4.12 ppm (thiazole $-CH_2-$ protons of intermediate **6**) and presence of a singlet at 8.00 ppm (indolylmethylene =CH— proton) from the ^1H NMR spectrum, and appearance of a signal at 138.99 ppm (indolylmethylene =CH— carbon) from ^{13}C NMR confirmed the formation of product (**8g**). Molecular ion peak from the mass spectrum as well as elemental analysis further confirmed the formation of the product (**8g**).

In vitro anticancer activity was carried out against human breast cancer cell line (MCF-7) and hepatocellular liver carcinoma cell line (HepG2). Doxorubicin was used as a reference drug. Cell viability in the presence of the test samples were measured by the MTT-microcultured tetrazolium assay.[22] The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of MCF-7 and HepG2 (Fig. 1). The response parameter calculated was the IC_{50} value, which



Scheme 1. Synthesis of indolylmethylene benzo[*h*]thiazolo[2,3-*b*]quinazolinones (**8a–t**). Reaction conditions: (i) P(4-VPH)HSO_4 (0.015 g), solvent-free, 120 °C, 10–20 min (yield: 88–94%); (ii) 2-chloro-*N*-phenylacetamide (**5**), acetic acid, reflux, 4–6 h (yield: 64–73%); (iii) 1*H*-indole-3-carbaldehyde (**7a–d**), ethanol, piperidine (cat.) reflux, 2–4 h (yield: 75–87%).

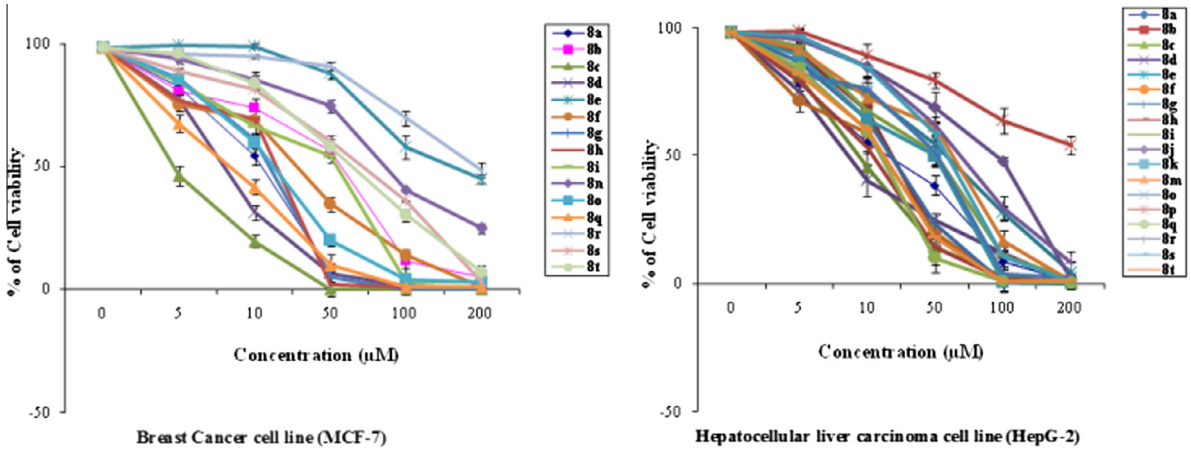


Figure 1. Survival curves of MCF-7 and HepG2 for indolylmethylene benzo[*h*]thiazolo[2,3-*b*]quinazolinones (**8a–t**).

Table 2
Inhibition values (IC_{50}) of indolylmethylene benzo[*h*]thiazolo[2,3-*b*]quinazolinones (**8a–t**) on human tumor cell lines MCF-7 and HepG2

Entry	Product	MCF-7 (IC_{50})	HepG2 (IC_{50})
1	**8a**	23.53	34.91
2	**8b**	61.90	38.76
3	**8c**	2.59	11.55
4	**8d**	7.72	8.60
5	**8e**	166.45	69.84
6	**8f**	42.83	16.26
7	**8g**	20.74	27.65
8	**8h**	18.85	13.73
9	**8i**	54.36	61.11
10	**8j**	>200	94.53
11	**8k**	>200	51.62
12	**8l**	>200	>200
13	**8m**	>200	70.26
14	**8n**	112.46	>200
15	**8o**	30.62	57.52
16	**8p**	>200	199
17	**8q**	8.96	22.1
18	**8r**	192.78	86.75
19	**8s**	82.41	73.77
20	**8t**	67.34	25.82
21	Doxorubicin	3.00	1.10

corresponds to the concentration required for 50% inhibition of cell viability (Table 2).

Activity results (Table 2) revealed that, the compound derived from 6-methoxy-1-tetralone, 4-chloro benzaldehyde and 5-bromo indole-3-carbaldehyde, that is, **8c** has exhibited excellent activity against MCF-7 (IC_{50} 2.59 μM, 1.15 fold potent) than the standard drug Doxorubicin (IC_{50} 3.00 μM). Compound **8d** against both the cancer cell lines (IC_{50} 7.72 & 8.60 μM), **8q** against MCF-7 (IC_{50} 8.96 μM) and **8c**, **8h** against HepG2 (**8c**: IC_{50} 11.55 μM; **8h**: 13.73 μM) have shown good activity. Compounds **8a**,**b**, **8f–i**, **8o**, **8s**,**t** against MCF-7 and **8a**,**b**, **8e–g**, **8i–k**, **8m**, **8o**, **8q–t** against HepG2 have exhibited promising activity with IC_{50} values ranging from 16.26 to 94.53 μM. Remaining compounds have shown moderate activity against both the cell lines.

All the synthesized compounds were screened for their in vitro antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes* (Gram-positive), *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Gram-negative) bacterial strains, and in vitro antifungal activity against *Candida albicans*, *Candida glabrata*, *Aspergillus niger* and *Aspergillus parasiticus* fungal strains using agar well diffusion method.[23] Streptomycin for bacteria and Clotrimazole for fungi are taken as standard drugs.

Antibacterial activity results (Table 3) revealed that, the compounds possessing 4-fluoro phenyl on pyrimidine ring and 5-bromo indol-3-ylmethylene/indol-3-ylmethylene on thiazole ring, that is, **8q** and **8t** have shown excellent activity against gram-positive bacterial strain *S. aureus* and gram-negative bacterial strain *P. aeruginosa* on comparing with the standard drug Streptomycin. These compounds (**8q** & **8t**) have also shown good activity against *K. pneumoniae*. Similarly, compounds **8e** & **8f** against *P. aeruginosa*, **8i–l** against both gram positive bacterial strains and **8k**,**8l** against *K. pneumoniae* have shown good activity. Remaining compounds have shown moderate activity against all the tested bacterial strains. From the antifungal activity results (Table 3) we noticed that, the compound possessing 3-nitro phenyl on pyrimidine ring and 2-methyl-5-bromo indol-3-ylmethylene on thiazole ring (**8j**) is highly active against *A. niger* on comparing with the standard drug Clotrimazole. Remaining compounds are moderately active against all the tested fungal strains with ZOI ranging from 8 to 20 mm.

In conclusion, we have synthesized a series of novel indolylmethylene benzo[*h*]thiazolo[2,3-*b*]quinazolinones via Knoevenagel condensation of benzo[*h*]thiazolo[2,3-*b*]quinazolinones with various indole-3-carbaldehydes under conventional method in good yields. All the compounds were screened for their in vitro anticancer and antimicrobial activities. Among the series, compound **8c**

Table 3
Antimicrobial activity of indolylmethylene benzo[*h*]thiazolo[2,3-*b*]quinazolinones (**8a–t**)

Analog	Antibacterial activity								Antifungal activity			
	S. aureus		*S. pyogenes*		*P. aeruginosa*		*K. pneumoniae*		*C. albicans*	*C. glabrata*	*A. niger*	*A parasiticus*
	ZOI	MIC	ZOI	MIC	ZOI	MIC	ZOI	MIC	ZOI			
8a	8	200	8	200	8	200	8	200	8	14	8	10
8b	8	200	8	200	8	200	8	200	8	8	8	12
8c	8	200	8	200	8	200	8	200	8	10	10	10
8d	8	200	8	200	8	200	8	200	8	12	8	10
8e	13	100	8	200	17	50	8	200	16	19	14	14
8f	12	100	8	200	18	50	8	200	8	12	12	12
8g	14	100	8	200	14	100	8	200	15	16	18	18
8h	13	100	8	200	15	100	8	200	8	10	8	8
8i	17	50	18	50	8	200	15	50	18	20	18	18
8j	16	50	15	50	13	100	14	100	17	14	20	18
8k	17	50	17	50	8	200	17	50	8	12	8	8
8l	19	50	16	50	8	200	17	50	8	8	10	10
8m	8	200	8	200	8	200	8	200	8	8	12	8
8n	8	200	8	200	8	200	8	200	8	8	8	10
8o	8	200	8	200	8	200	8	200	8	8	14	10
8p	8	200	8	200	8	200	8	200	8	10	12	12
8q	22	25	8	200	22	25	18	50	8	10	8	8
8r	8	200	8	200	8	200	15	100	8	10	10	10
8s	13	100	8	200	13	100	16	100	8	8	12	8
8t	21	25	8	200	21	25	17	50	8	8	10	10
Streptomycin	22	25	21	12.5	20	12.5	22	50	—	—	—	—
Clotrimazole	—	—	—	—	—	—	—	—	24	22	20	20

Zone of inhibition values (mm) for analogs (**8a–t**) and positive control drugs (Streptomycin and Clotrimazole) were measured at 150 and 30 μg/mL, respectively. MIC values were given in μg/mL.
Bacterial strains: *S. aureus*—*Staphylococcus aureus*, *S. pyogenes*—*Streptococcus pyogenes*, *P. aeruginosa*—*Pseudomonas aeruginosa* and *K. pneumoniae*—*Klebsiella pneumonia.*
Fungal strains: *C. albicans*—*Candida albicans*, *C. glabrata*—*Candida glabrata*, *A. niger*—*Aspergillus niger*, *A. parasiticus*—*Aspergillus parasiticus.*
'—' Not performed.

bearing 4-chlorophenyl on pyrimidine ring and 5-bromo indol-3-ylmethylene on thiazole ring has shown 1.15 fold potent activity against breast cancer cell line-MCF-7 than the standard drug Doxorubicin, remaining compounds have shown moderate activity against both MCF-7 and HepG2 cell lines. Compounds **8q** and **8t** bearing 4-fluoro phenyl on pyrimidine ring and 5-bromo indol-3-ylmethylene/indol-3-ylmethylene on thiazole ring have shown equipotent antibacterial activity against *S. aureus* whereas compound **8j** bearing 3-nitrophenyl on pyrimidine ring and 2-methyl-5-bromo indol-3-ylmethylene on thiazole ring has shown good antifungal activity against *A. niger*. These results are suggesting that the synthesized compounds can be better candidates for future investigations.

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Supplementary data

Supplementary data (experimental procedures and characterization data of compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.07.030.

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