J Chem Biol (2016) 9:57–63
DOI 10.1007/s12154-015-0148-y

ORIGINAL ARTICLE

Photochemical synthesis and anticancer activity of barbituric acid, thiobarbituric acid, thiosemicarbazide, and isoniazid linked to 2-phenyl indole derivatives

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Received: 29 June 2015 / Accepted: 3 November 2015 / Published online: 17 November 2015

Abstract 2-Phenyl-1*H*-indole-3-carbaldehyde-based barbituric acid, thiobarbituric acid, thiosemicarbazide, isoniazid, and malononitrile derivatives were synthesized under photochemical conditions. The antitumor activities of the synthesized compounds were evaluated on three different human cancer cell lines representing prostate cancer cell line DU145, Dwivedi (DWD) cancer cell lines, and breast cancer cell line MCF7. All the screened compounds possessed moderate anticancer activity, and out of all the screened compounds, 5-{1[2-(4-chloro-phenyl)2-oxo-ethyl]-2-phenyl-1*H*-indole-3-ylmethylene}-2-thioxo-dihydro-pyrimidine-4,6-dione (2b) and 5-{1[2-(4-methoxy-phenyl)2-oxo-ethyl]-2-phenyl-1*H*-indole-3-ylmethylene}-2-thioxo-dihydro-pyrimidine-4,6-dione (2d) exhibited marked antitumor activity against used cell lines. Additionally, barbituric acid derivatives were selective to inhibit cell line DWD and breast cancer cell lines.

Keywords Anticancer activity · 2-Phenyl-1*H*-indole-3-carbaldehyde · Photochemical condition · Thiobarbituric acid

Electronic supplementary material The online version of this article (doi:10.1007/s12154-015-0148-y) contains supplementary material, which is available to authorized users.

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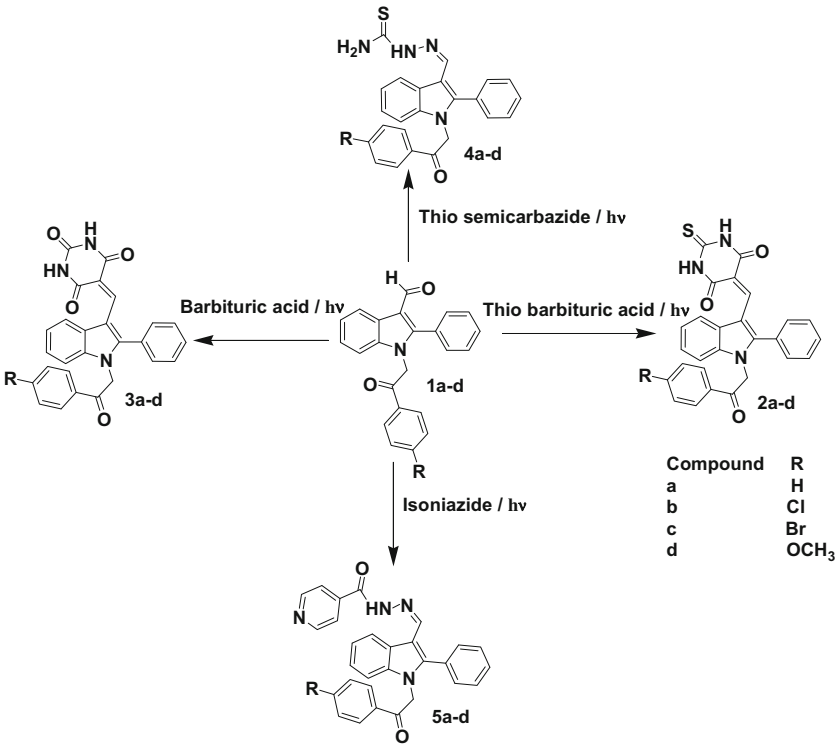
Introduction

It is an alarming situation to know that over a million cases of cancer occur in the USA annually, and cancer related deaths are estimated to reach 12 million worldwide in the year 2015 [1]. Breast cancer is the most common cancer among women [2]. Therefore, there is an urgent need for potential, selective anticancer drugs in modern oncology.

Chemotherapy is one of the principal modes of treatment for cancer patients. Clinically, many tumors present a satisfactory response when they are first exposed to the chemotherapeutic drugs. In continuation of our studies on biologically active heterocyclics [3, 4], we have now focused on anticancer activity of 2-phenyl-1*H*-indole-3-carbaldehyde derivatives. Indoles display an important role in biological activities such as antimalarial [5], antibacterial [6], anti-HIV [7], and antifungal [8]. Recent literature survey reveals that antitumor activities of indole derivatives [9–11] possessed a unique photoresponsive activity [12] used in light-emitting electrochemical cells [13], solid-state lasers, fluorescent labels, etc. 2-Phenyl-1*H*-indole-3-carbaldehyde derivatives inhibited the growth of MDA-MB-231 and MCF7 breast cancer cells by combining indole and barbituric acid, and new hybrid molecules were synthesized and evaluated for anticancer activity [14–18]. Fan Zhang et al. synthesized and evaluated in vitro antitumor activity of 2-amino-3-cyano-6-(1*H*-indole-3-yl)-4-phenylpyridine derivatives. These derivatives were screened for their cytotoxic activity against four human cell lines (A549, H460, HT-29, and SMMC-7721) and displayed an excellent antitumor activity against these cell lines [19]. Among 700 barbituric acid analogues, we observed that only few thiobarbiturates have shown anticancer activity. Merbarone has shown a curative activity against L1210 leukemia and also possessed an important activity against some other murine tumors [20, 21], but unfortunately, further

Scheme 1 Synthesis of 1-aryl-2-phenyl indole derivatives linked to barbituric acid, thiobarbituric acid, thiosemicarbazide, and isoniazid



studies were discontinued due to nephrotoxicity as well as to a general lack of antitumor activity [22]. Recent literature reports on biological activity and molecular docking studies reveal that small molecules possess greater activity as compared to bulkier groups [23]. On the other hand, isoniazid [24] and thiosemicarbazide exhibited marked biological activities.

The above-discussed literature study increases the need of developing new chemotherapeutic agents for more effective and economical treatment of cancer. For the synthesis of title compounds, several methodologies were reported in the literature [25] but we adopted the Knoevenagel condensation under photochemical

Scheme 2 Synthesis of 1-alkyl-2-phenyl indole derivatives linked to barbituric acid, thiobarbituric acid, thiosemicarbazide, and isoniazid

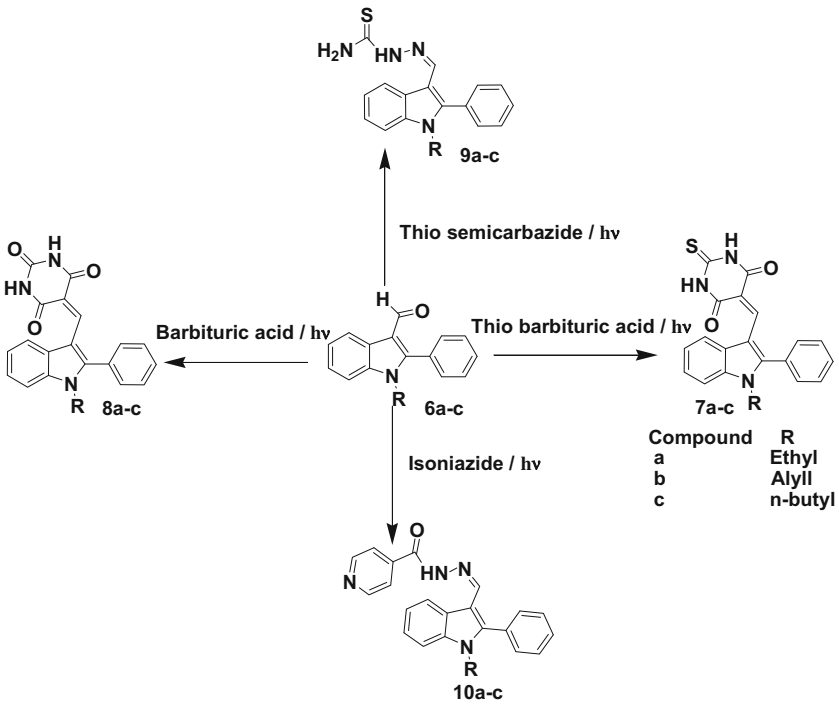
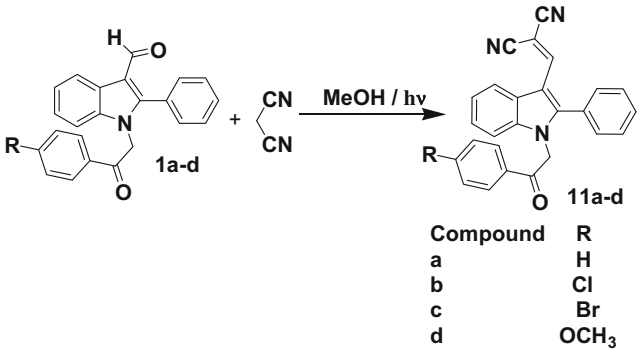
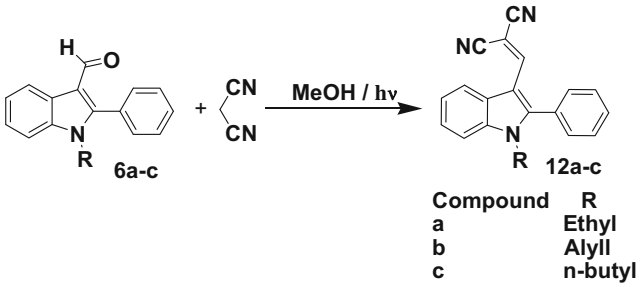


Table 1 Antitumor activity of screened compounds on three human cancer cell lines at four different concentrations

Molar drug concentration	% control growth										
	3b	3d	3c	8b	2b	2d	9a	5d	5b	10c	ADR
Human breast cancer cell line MCF7											
10^{-7}	100	100	100	100	100	100	91.4	90	89.3	99.8	56.8
10^{-6}	98.3	99.4	99.6	100	100	99	89.8	86	87.1	94.3	23.7
10^{-5}	48.6	82.4	69.4	100	51.5	24.6	88.1	75.5	76.3	79	
10^{-4}	22.9	23.6	16.5	36.5	−29.8	−25	49	60.3	33.2	25.5	−20.2
GI50 (μM)	55.31	64.35	56.14	80.91	38.21	28.43	98.73	>100	68.8	64.4	<0.1
Human prostate cancer cell line DU145											
10^{-7}	99.7	100	100	100	100	100	100	100	100	100	99.8
10^{-6}	100	100	100	100	100	100	100	100	100	100	38.7
10^{-5}	81.9	98.1	92.8	100	98.3	46.4	100	97.1	100	90.5	30.9
10^{-4}	35.5	30.1	31.4	46	−2.9	−9.7	88.5	96.4	75.7	41.9	10
GI50 (μM)	76.2	73.6	73.9	94.5	51.3	38	>100	>100	>100	86.4	18.8
Human Dwivedi (DWD) cancer cell line											
10^{-7}	100	100	100	100	100	100	100	100	100	100	65.1
10^{-6}	100	99.6	98.5	100	97.1	100	100	100	100	99.9	−21.8
10^{-5}	39.5	94.1	99	71.8	95.6	89.5	98.8	96.2	99.4	98.5	−26.5
10^{-4}	2.2	2.9	4.2	51.2	−2.1	−1.4	74.1	79.8	39.4	39.7	−30.5
GI50 (μM)	41	63.3	54.7	>100	50.8	50.4	>100	>100	>100	84.7	<0.1



Scheme 3 Synthesis of 1-aryl-2-phenyl indole derivatives linked to malononitrile



Scheme 4 Synthesis of 1-alkyl-2-phenyl indole derivatives linked to malononitrile

conditions and synthesized these hybrid molecules in good yields.

Experimental part

Chemistry

The barbituric acid, thiobarbituric acid, isoniazid, and thiosemicarbazide were purchased from Merck Company with 98 % purity. All melting points were determined with a Quimis apparatus, Q-340s 13 model, and are uncorrected. Thin-layer chromatography (TLC) was performed on 2.0× 6.0 cm aluminum sheets covered with silica gel (sorbent, 200 μm thickness) under ultraviolet radiation. Infrared (IR) spectra were obtained with an ABB spectrophotometer, FTLA 2000-100 model, using KBr pellets. ^{1}H NMR was measured on a Brucker 300-MHz spectrometer using DMSO as a solvent and TMS as an internal standard, and splitting patterns are as follows: s, singlet; d, doublet; and m, multiplet (chemical shifts in δ ppm). Mass spectra were recorded on a JEOL JMS-D-300 spectrometer and QSTAR XL, and elemental analysis was performed on a Carlo Erba model EA1108.

Anticancer evaluation

Experimental procedure for sulforhodamine B assay

The sulforhodamine B (SRB) assay is used for cell density determination, based on the measurement of cellular protein content. This method not only allows a large number of samples to be tested within a few days but also requires only simple equipment and inexpensive reagents. In general, the SRB assay represents a better method of assuming in vitro chemosensitivity. The SRB assay is therefore an efficient and highly cost-effective method for screening [26]. The cell lines were grown in a RPMI 1640 medium containing 10 % fetal bovine serum and 2 mM L-glutamine. For present screening experiment, the cells were inoculated into 96-well microtiter plates in 90 μl at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5 % CO_2, 95 % air, and 100 % relative humidity for 24 h prior to addition of experimental drugs. After 24 h, one plate of each cell line was fixed in situ with trichloroacetic acid (TCA) to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs were solubilized in an appropriate solvent at 400-fold the desired final maximum test

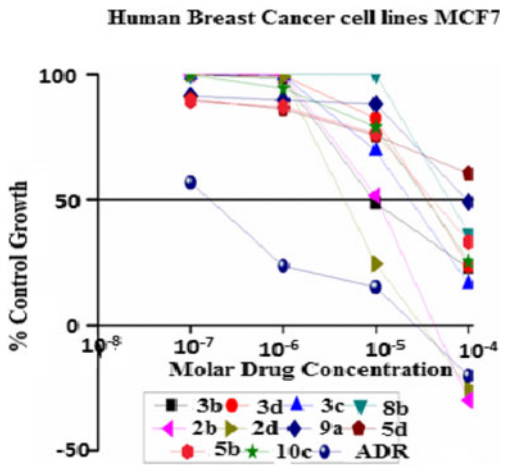


Fig. 1 Human breast cancer cell line MCF7

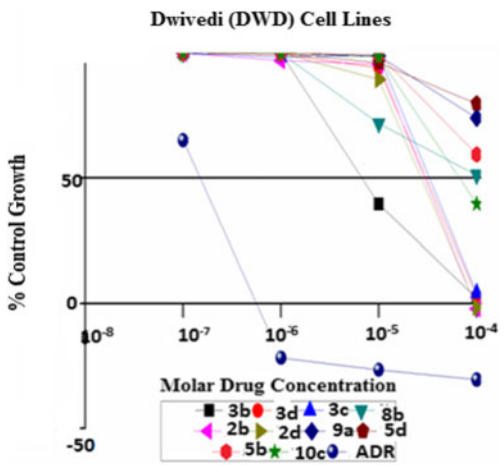


Fig. 2 Dwivedi (DWD) cell lines

concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to 10 times the desired final maximum test concentration with the complete medium containing a test article at a concentration of 10^{-3}. Additional three 10-fold serial dilutions were made to provide a total of four drug concentrations plus control. Aliquots of 10 μl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 μl of the medium, resulting in the required final drug concentrations.

Endpoint measurement

After compound addition, plates were incubated at standard conditions for 48 h and assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 μl of cold 30 % (*w*/*v*) TCA (final concentration, 10 % TCA) and incubated for 60 min at 4 °C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 μl) at 0.4 % (*w*/*v*) in 1 % acetic acid was added to each of the wells, and the plates were incubated for 20 min at room temperature. After staining, the unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried. The bound stain was subsequently eluted with 10 mM Trizma base, and the absorbance was read on an ELISA plate reader at a wavelength of 540 nm with a reference wavelength of 690 nm. Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent growth was expressed as the ratio of the average absorbance of the test well to the average absorbance of the control wells × 100.

Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as

$$[(\mathrm{Ti}-\mathrm{Tz})/(\mathrm{C}-\mathrm{Tz})] \times 100 \text{ for concentrations for which } \mathrm{Ti} \geq \mathrm{Tz}, \ (\mathrm{Ti}-\mathrm{Tz}) \text{ is positive or zero}$$

$$[(\mathrm{Ti}-\mathrm{Tz})/\mathrm{Tz}] \times 100 \text{ for concentrations for which } \mathrm{Ti} < \mathrm{Tz}, \ (\mathrm{Ti}-\mathrm{Tz}) \text{ is negative}$$

The dose-response parameters were calculated for each test article. Growth inhibition of 50 % (GI_{50}) was calculated from $[(\mathrm{Ti}-\mathrm{Tz})/(\mathrm{C}-\mathrm{Tz})] \times 100 = 50$, which is the drug concentration resulting in a 50 % reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The LC_{50} (concentration of drug resulting in a 50 % reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from $[(\mathrm{Ti}-\mathrm{Tz})/\mathrm{Tz}] \times 100 = -50$. The values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested.

Results and discussion

The present work aimed to synthesize, characterize, and investigate the potential anticancer effects of indole-based isoniazid, thiosemicarbazide, and thiobarbituric acid analogue by in vitro studies. The general method for the synthesis of 2-phenyl indole linked to thiobarbituric acids 2a–d and 7a–c, barbituric acids 3a–d and 8a–c, thiosemicarbazides 4a–d and 9a–c, isoniazids 5a–d and 10a–c, and malononitrile series of compounds 11a–d and 12a–c is depicted in Schemes 1, 2, 3, and 4. The synthesis of analogs 1a–d and 6a–c were carried out according to our earlier reports [27]. Compounds 1a–d or 6a–d were dissolved in methanol; an equimolar amount of barbituric acid, thiobarbituric acid, isoniazid, thiosemicarbazide, and malononitrile was added separately; the mixture was exposed to natural sunlight for 5 to 6 h; and product formation was confirmed by TLC. The solid obtained was filtered and washed well with cold methanol. Recrystallization with methanol yielded pure compounds. We have ascribed structures for all the synthesized compounds confirmed by spectral analysis.

Anticancer activity

Among the series, 10 compounds were evaluated for anticancer activity on three different human cancer cell lines representing prostate cancer cell line DU145, Dwivedi (DWD) cancer cell lines, and breast cancer cell line MCF7 with the standard drug Adriamycin (ADR) for comparison and the data is presented in Table 1. The data indicates that most of the screened compounds have shown an anticancer activity at 10^{-4} M concentration and the activity reduced with increased dilutions (10^{-7}, 10^{-6}, and 10^{-5} M concentrations). Among all the compounds, 5-{1[2-(4-chloro-phenyl)2-oxo-ethyl]-2-phenyl-1*H*-indole-3-ylmethylene}-2-thioxo-dihydro-pyrimidine-4,6-dione (2b) and 5-{1[2-(4-methoxy-phenyl)2-oxo-ethyl]-2-phenyl-1*H*-indole-3-ylmethylene}-2-thioxo-dihydro-pyrimidine-4,6-dione (2d) exhibited a greater activity than the standard drug ADR in % control growth inhibition on the human breast cancer cell line (Fig. 1) and human prostate cancer cell line.

Cocco et al. [28] reported more prominent anticancer activity of thioxopyrimidine in comparison to the oxopyrimidine derivatives. In structure activity relationship (SAR) studies, we also observed that thioxopyrimidine derivatives have shown a greater anticancer activity than the oxopyrimidine. We assessed that the C=S group possessed higher lipid solubility than the corresponding oxybarbiturates. Among oxopyrimidine derivatives, 3b–d hold a selective activity on human DWD cell lines and breast cancer cell lines (Figs. 1 and 2). Other thiosemicarbazide 4a–d and 9a–c and isoniazid 5a–d and 10a–c derivatives displayed moderate activity.

Conclusions

In summary, a series of thiosemicarbazide, isoniazid, barbituric acid, thiobarbituric acid, and malononitrile linked to indole derivatives were synthesized using an environmentally benign method with quantitative yields. Anticancer activity on three different human cancer cell lines has been evaluated on the prostate cancer cell line DU145, Dwivedi (DWD) cancer cell line, and breast cancer cell line MCF7. Compounds 5-{1[2-(4-chloro-phenyl)2-oxo-ethyl]-2-phenyl-1*H*-indole-3-ylmethylene}-2-thioxo-dihydro-pyrimidine-4,6-dione (2b) and 5-{1[2-(4-methoxy-phenyl)2-oxo-ethyl]-2-phenyl-1*H*-indole-3-ylmethylene}-2-thioxo-dihydro-pyrimidine-4,6-dione (2d) exhibited more anticancer response, and from SAR studies, we have assessed that the presence of C=S group at second position on pyrimidine ring on 5-{1[2-(4-chloro-phenyl)2-oxo-ethyl]-2-phenyl-1*H*-indole-3-ylmethylene}-2-thioxo-dihydro-pyrimidine-4,6-dione (2b) and 5-{1[2-(4-methoxy-phenyl)2-oxo-ethyl]-2-phenyl-1*H*-indole-3-ylmethylene}-2-thioxo-dihydro-pyrimidine-4,6-dione (2d) is more tolerated on the three used human cancer cell lines than compounds with C=O groups (3b–d and 8b). This study will give a scope for further investigations on this scaffold to develop potential anticancer agents.

Acknowledgments One of the authors (S.V.) thanks the Ministry of Human Resource Development for providing the fellowship. The authors are also thankful to the Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Mumbai, for screening the samples.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests. The authors alone hereby stand responsible for the contents of this scientific paper.

Ethical statements This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

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