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Original article

Structural optimization of indole based compounds for highly promising anti-cancer activities: Structure activity relationship studies and identification of lead molecules

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ABSTRACT

Based on the anti-cancer data of previous compounds, 27 more compounds were synthesized and subjected to anti-cancer screening. Compounds were tested over 60 human tumor cell lines of different types of cancer. As per the data available, some compounds exhibited appreciable anti-cancer properties over certain cell lines with their GI_{50} in nM range. With the help of UV–vis spectral studies, enzyme immunoassay and molecular modeling studies, dihydrofolate reductase was found to be the probable cellular target of the compounds under present investigation.

1. Introduction

As per the latest information available, it is estimated that if the spread of cancer continues at its present rate, it may cause over 13.1 million deaths in 2030 worldwide [1]. Irrespective of the un-tired efforts and screening of myriads of compounds for anti-cancer activities [2–4], the uncertainty about the cause of origin of cancer, limitations in its detection at early stage, its direct connection with process of cell division, metastatic nature of cancer cells and lack of drug penetration to cancer tissue are some of the features of this disease which prove as hurdles in the successful treatment of cancer [5,6]. Amongst the various approaches to treatment of cancer, chemotherapy finds a wide use during both pre-operative and post-operative conditions [7,8]. The main focus of chemotherapeutic agents is to block/slow down propagation of cancer cells for which enzymes associated with cell division are the primary targets. Ribonucleotide reductase (RNR), thymidylate synthase (TS), thymidylate phosphorylase (TP), dihydrofolate reductase (DHFR) are primarily concerned with generation of raw material for cell division [9,10] and hence they were made the targets during the design and development of anti-cancer agents. As a result some highly efficacious drugs like 5-fluorouracil [11], methotrexate [12], pemetrexed [13] etc appeared in the market with some hope for the cancer patients. In continuation to earlier reports for development of anti-cancer agents [14–17], particularly indole based compounds [14,15], here we report another set of compounds with appreciable anti-cancer activity over certain human tumor cell lines.

2. Results and discussion

2.1. Chemistry

A simple synthetic methodology was undertaken to synthesize conjugates of indole, pyrazole and barbituric acid. Compounds **2a** and **2b** were synthesized by condensation of **1a** and **1b** (1 mmol) with 1-(3-chlorophenyl)-3-methyl-2-pyrazolin-5-one (1.2 mmol) under microwave irradiation. Similarly, compounds **4a**, **b** and **5a**, **b** were prepared by irradiating the mixture of **1a**, **b** with barbituric acid and **1a**, **b** with oxindole (1,3-dihydroindol-2-one), respectively under microwaves. Compound **3a** and **b** were procured from reaction of **1a** and **b** with indolinone (1-[2,6-dichlorophenyl]-1,3-dihydroindol-2-one) in ethanol-water (1:2) in presence of NaOH (Scheme 1).

It was also planned to introduce a substituent at N-1 position of indole in compounds **2**–**5**, for which indoles **1a** and **b** were first

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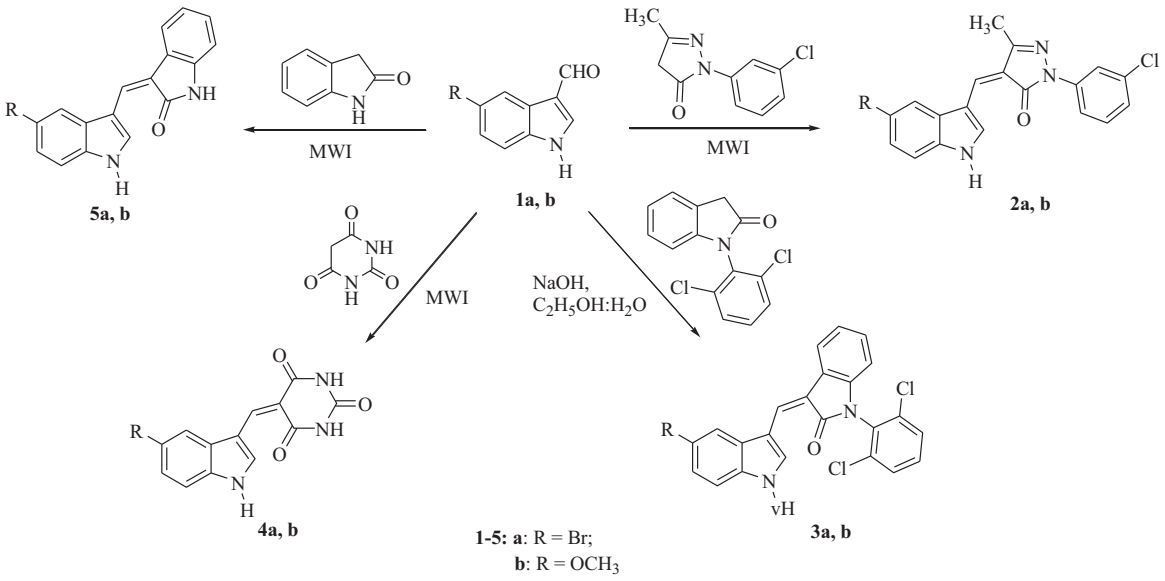


Scheme 1. Synthesis of compounds **2**–**5**.

alkylated/acylated in presence of NaH and thereby compounds **6**–**10** were prepared (Scheme 2).

Treatment of compound **6**–**9** with 1-(3-chlorophenyl)-3-methyl-2-pyrazolin-5-one/indolinone/barbituric acid/oxindole under microwave irradiation provided compounds **11**–**21** (Scheme 3).

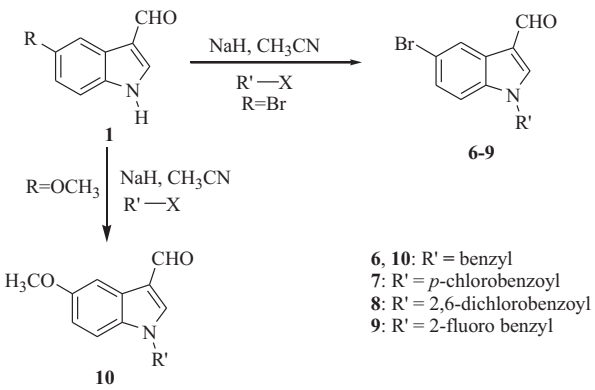
Similarly, reactions of compound **10** with 1-(3-chlorophenyl)-3-methyl-2-pyrazolin-5-one/indolinone/barbituric acid/oxindole resulted into the formation of compounds **22**–**25** (Scheme 4).

Since trifluoromethyl group is an active component of a number of drugs, compounds **26**–**28** were prepared through condensation of 2-trifluoromethylbenzaldehyde with oxindole, indolinone and 1-(3-chlorophenyl)-3-methyl-2-pyrazolin-5-one, respectively. Further treatment of compound **26** with *p*-chlorobenzoyl chloride and 2-fluorobenzyl bromide in presence of NaH provided compounds **29** and **30** (Scheme 5). Therefore, through a very convenient synthetic protocol, a library of compounds was procured in almost quantitative yield.

2.2. Anti-cancer activity

In vitro tumor growth inhibitory activities of compounds **3a**, **3b**, **4a**, **4b**, **11**, **12**, **15**, **16**, **18**, **19**, **21**, **23**, **24**, **25**, **27**, **28** and **29** were investigated on 60 cell line panel of human cancer cells at National Cancer Institute (NCI), Bethesda, MD, USA. The compounds were found to be selective for different cell lines and anti-cancer data of compounds with significant activity over certain cancer cell lines is given in Table 1. Compound **3b** showed specificity for various cell lines of non-small cell lung cancer, colon cancer and prostate cancer. Compound **11** exhibited high selectivity for MDA-MB-468 cell line of breast cancer with GI_{50} 120 nM. Compound **12** was identified as the most potent amongst those screened here. It exhibited excellent specificity for various cell lines of leukemia along with non-small cell lung cancer, colon cancer, melanoma, prostate cancer and renal cancer. Remarkably, GI_{50} of compound **12** for NCI-H522 cell line of non-small cell lung cancer was 1 nM. Interestingly, presence of Br in compound **12** considerably increased its anti-cancer activity in comparison to its analog **31** (Chart 1), reported earlier [15]. Compounds **16**, **18**, **19** and **25** showed growth inhibition over certain cell lines with GI_{50} in μM (Table 1). Compounds **23** and **27** were more specific for various cell lines of leukemia. LC_{50} (50% lethal conc of compound) of these compounds was >100 μM indicating their appreciable selectivity indices. Therefore, results of anti-cancer screening experiments clearly indicate the potency of these compounds for controlling cancer growth over certain cell lines.

Looking at the structure activity relationship of these compounds, it is quite evident that a small variation in the structure of the compound made a dramatic change in its biological activity. Compounds **3b** and **23** with OCH_3 substituent at C-5 of indole showed better anti-cancer activity than their analogs **3a** and **15** carrying Br at C-5 of indole. 5-Unsubstituted indole-pyrazole adduct (**32**, Chart 1) [15] did not show anticancer activity but with introduction of Br at C-5 position of indole, the resulting compound **2a** exhibited appreciable anticancer activity [16]. Moreover, far better GI_{50} of compound **12** in comparison to that of compound **2a** indicates the role of substituent at N-1 of indole in increasing the anti-cancer potency of compound **12**. The substituent specificity was observed by replacing *p*-chlorobenzoyl group of compound **12** with 2,6-dichlorobenzoyl in compound **14** which made compound **14** inactive towards anti-cancer activity. Similarly, comparison of compounds **11** and **22** indicates that presence of Br in compound **11** made it selective towards MDA-MB-468 cell line of breast cancer. It means instead of a single substituent, the biological activity of the compound is decided by the combination of all the



Scheme 2. Synthesis of compounds **6**–**10**.