Cytotoxic and acute toxicity studies of isoniazid derivatives

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Abstract: Cancer is ultimately the result of cells that hysterically grow and do not die. Cells can experience uncontrolled growth if there are mutations to DNA, and therefore, alterations to the genes involved in cell division. Cancer occurs when a cell's gene mutations make the cell unable to correct DNA damage and is unable to destroy itself. There are over 100 different types of cancer each classified by the type of initially affected cell. Isoniazid, a well-known antitubercular agent has been reported to exhibit some cytotoxic activity. This finding prompt us to carry out this study where isoniazid and its sixteen derivatives were studied for any possible cytotoxic activity against Human astrocytoma SNB-19 cells, human Dukes' type C colorectal adenocarcinoma HCT-15 cells, human Dukes' type D colorectal adenocarcinoma COLO-205 cells, and human prostate adenocarcinoma (grade IV) PC-3 cells. Among the test compounds, SN-07 (a phenacyl derivative with *para* phenyl substitution) demonstrated slight cytotoxic effects on two types of human colorectal adenocarcinoma cells HCT-15 and COLO-205. Moreover, the acute toxicity of the compounds was also estimated in which some compounds were evaluated with more LD_{50} values than isoniazid.

Keywords: Isoniazid, Cytotoxicity, Acute toxicity, LD_{50}

INTRODUCTION

Neoplasia commonly known as cancer can conveniently be described as a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. Normal cells in the body follow a precise and sequential path of growth, division, and death. A programmed cell death called apoptosis is crucial in regulating this order and when this process is hampered, neoplasms begin to be formed. Similarly, cancer can be a result of mutations that inhibit any of the oncogene or tumor suppressor gene functions thereby leading to uncontrollable cell growth. Worldwide, one in seven deaths is due to cancer; in fact cancer causes more deaths than AIDS, tuberculosis, and malaria combined. While grouping countries according to their socioeconomic status, cancer is the second leading cause of death in high-income countries (following cardiovascular diseases) and the third one in low- and middle-income countries (following cardiovascular diseases and infectious and parasitic diseases).

In order to combat this disease burden on health system there is an urgent need of new drugs applicable to treat this condition which can present itself in almost any part or system of the body. Keeping in view this requirement, an important strategy for drug discovery named drug repositioning has been developed recently which is defined as the study aimed at the application of presently available drugs for other diseases (Liu *et al.*, 2013; Ma *et al.*, 2013).

Isoniazid, the leading drug for treatment of tuberculosis is an analogue of isonicotinic acid, an isomer of nicotinic acid (de Souza, 2006; de Souza *et al.*, 2008) . Though the relevance of isoniazid in tuberculosis treatment is strongly established and clinically applied worldwide, this drug has not been studied well enough for its other possible pharmacological therapeutic actions.

Malhotra and co-workers (Kumar *et al.*, 2011) have illustrated the possible promising perspectives of some analogues of isoniazid in the field of neoplasm's treatment and several other research studies have also determined the cytotoxic activity of synthetic isoniazid (INH) analog (Vigorita *et al.*, 1992; Nerkar *et al.*, 2009; Shabani *et al.*, 2010; Kumar *et al.*, 2014; Rodrigues *et al.*, 2014; Laxmi *et al.*, 2016). This study of isoniazid and its synthetic analog (fig. 1) (Naeem *et al.*, 2014; Naeem *et al.*, 2016) is particularly aimed at determining their cytotoxic activity against some specific human cancer cell lines in an attempt to discover new treatment agents for the rapidly progressing disease. Furthermore, the acute toxicity studies were also carried out to discern the safety index (LD_{50}) of these derivatives for their therapeutic use even at high doses.

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MATERIALS AND METHODS

Determination of cytotoxic activity

Chemicals

Dulbecco's modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin and trypsin 0.25% were purchased from Hyclone (GE Healthcare Life Science, Pittsburgh, PA, USA). Phosphate buffered saline (PBS) was purchased from Invitrogen GIBCO (Grand Island, NY, USA). Dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich, USA.

Cell lines and cell culture

Four cancer cell lines were selected to determine the cytotoxicity of the compounds. The human astrocytoma SNB-19 cell line, human Dukes' type C colorectal adenocarcinoma HCT-15 cell line, human Dukes' type D colorectal adenocarcinoma COLO-205 cell line, and human prostate adenocarcinoma (grade IV) PC-3 cell line were purchased from the American Type Culture Collection (ATCC, Manassas, VA). All the cell lines were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin in a humidified incubator at 37°C with 5% CO_2.

MTT cytotoxicity assay

The cytotoxicity of INH and compounds SN-01 – SN-16 to cultured cancer cells was tested by MTT colorimetric assay. The assay assesses cell viability by detecting the formazan product formed from the reduction of 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyltetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase of metabolically active cells (Carmichael *et al.*, 1987). Cells were seeded in 96-well plates at 5000cells/well. After 24 hours of incubation, various concentrations of the 17 compounds were added respectively to the cells for 72 hours continuous drug incubation. At the end of the 68th hour of incubation, MTT reagent (4mg/mL) was added and the plates were incubated at 37°C for 4 hours till the whole drug incubation ended. Subsequently, the supernatant was removed and 100µl of DMSO were added to dissolve the formazan crystals. The plates were well shaken for 5 minutes and the absorbance was determined at 570nm by the OPSYS microplate reader (Dynex Technology, Chantilly, VA). The IC_{50} (concentration that inhibited the survival of cells by 50%) values were calculated to represent the cytotoxicity of the compounds.

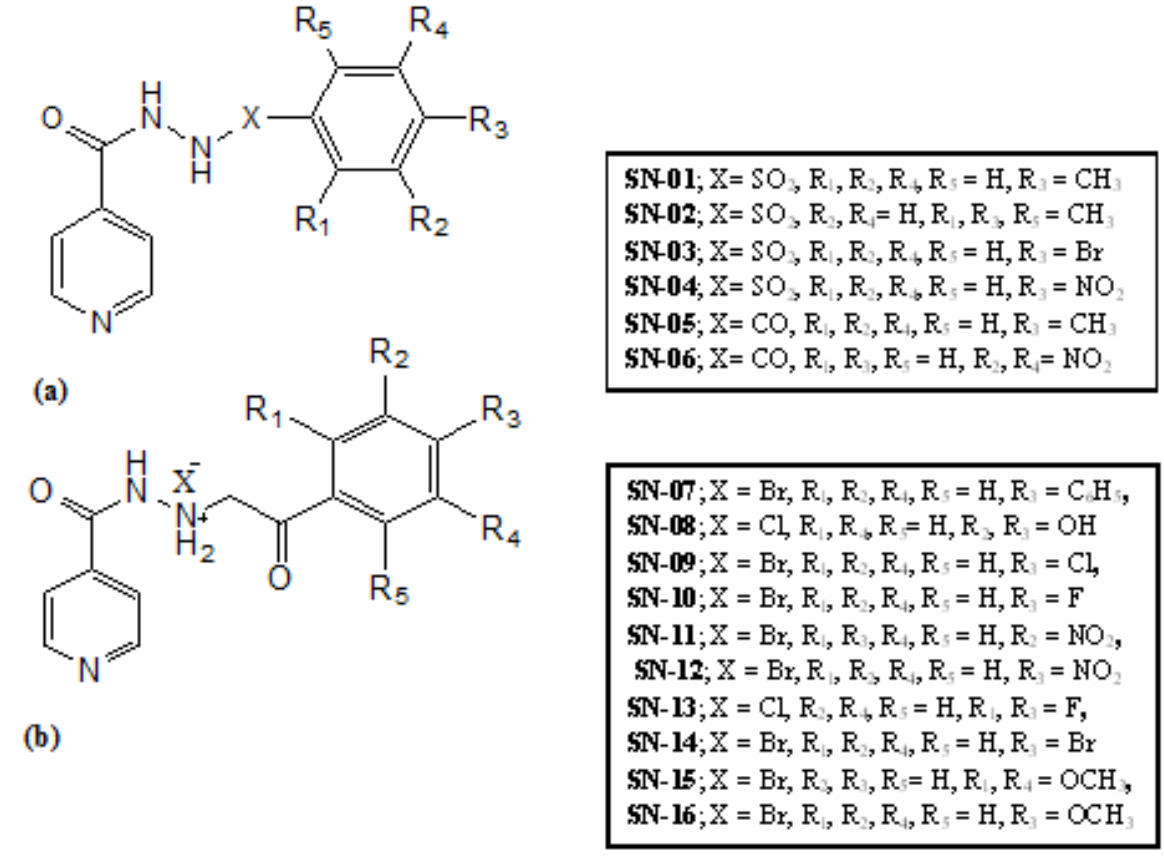

Fig. 1: Synthetic INH derivatives a) Sulphonyl and Benzoyl b) Phenacyl.

Table 1: Cytotoxicity of Isoniazid and its Derivatives on Four Human Cancer Cell Lines

Compound	IC_{50} ± SD (μM)			
	SNB-19	HCT-15	COLO-205	PC-3
SN-01	>100	>100	>100	>100
SN-02	>100	>100	>100	>100
SN-03	>100	>100	>100	>100
SN-04	>100	>100	>100	>100
SN-05	>100	>100	>100	>100
SN-06	>100	>100	>100	>100
SN-07	>100	78.85 ± 14.69	81.3 ± 16.57	>100
SN-08	>100	>100	>100	>100
SN-09	>100	>100	>100	>100
SN-10	>100	>100	>100	>100
SN-11	>100	>100	>100	>100
SN-12	>100	>100	>100	>100
SN-13	>100	>100	>100	>100
SN-14	>100	>100	>100	>100
SN-15	>100	>100	>100	>100
SN-16	>100	>100	>100	>100
INH	>100	>100	>100	>100

Data represents the mean IC_{50} values for each cell line ± SD obtained from three independent sets of experiments.

Table 2: Acute Toxicity of INH and its derivatives

Compound	Percentage Mortality of mice at various doses (%)					LD_{50} (mg/Kg)
	100mg/Kg	250mg/Kg	500mg/Kg	750mg/Kg	1000mg/Kg	
INH	100					100
SN-01	0	33.33	100			353.55
SN-02	0	33.33	100			353.55
SN-03	0	33.33	100			353.55
SN-04	0	33.33	100			353.55
SN-05	0	0	0	33.33	100	866.02
SN-06	0	33.33	100			353.55
SN-07	0	33.33	100			353.55
SN-08	0	0	0	0	100	1000
SN-09	0	0	0	0	0	> 1000
SN-10	0	0	0	0	0	> 1000
SN-11	0	0	0	0	0	> 1000
SN-12	0	0	0	33.33	100	866.02
SN-13	0	0	33.33	100		613.37
SN-14	0	0	0	0	100	1000
SN-15	100					100
SN-16	0	100				250

Determination of acute toxicity (LD_{50})

Mice of the same weight (25±2gm) were taken and kept for three days with proper diet and water prior to activity. Then they were grouped in a set of three mice each. INH and its analogs were administered orally in different test doses of 100, 250, 500, 750 and 1000mg/Kg using Water for Injection (WFI) as a solvent. Control group treated with WFI only always run parallel to the study.

Acute toxicity was performed following method reported by Lorke in which each set of mice was treated with a single dose and kept observed for 24-48 hours for lethality. The process was repeated for all doses of all the test compounds (Lorke, 1983). LD_{50} was then calculated by the given formula:

$LD_{50} = \sqrt{}$ (Lowest lethal dose × Highest lethal dose)

RESULTS

The results of cytotoxic activity on different cancer cells lines were summarized in table 1. The findings of the acute toxicity were presented in Table 2 in terms of $LD_{50.}$

DISCUSSION

Overall, most compounds showed low cytotoxicity to the tested cancer cell lines. Only SN-07 (a phenacyl derivative with *para* phenyl substitution) had slight cytotoxic effects on the two types of human colorectal adenocarcinoma cells HCT-15 and COLO-205, with IC_{50} values (mean ± standard deviation) of 78.85±14.69µM and 81.3± 16.57µM respectively. However, the activity was not observed in case of parent compound isoniazid. This may be related to the increase of lipophilicity resulted from an extra phenyl substitution among phenacyl derivatives. However, SNB-19 and PC-3 cells that treated with SN-07 did not show an IC_{50} lower than 100µM.

Determination of acute toxicity is considered as the preliminary step in the estimation and evaluation of toxic properties of a substance and expressed as "the adverse effect(s) occurring within a short time of administration of a single dose or multiple doses given within 24 hours". Acute toxicity is often related to the determination of lethal dose i.e., LD_{50} (dose causing fatality of 50% of the treated animals in a given period) which then can be used to establish the therapeutic index of a chemical compound (Walum, 1998; Shetty Akhila and Alwar, 2007).

The parent compound INH displayed LD_{50} value of 100mg/Kg while the synthetic analogs possessed LD_{50} values greater than INH. Phenacyl derivatives SN-09, SN-10 with *para* chloro and fluoro substituents respectively possessed LD_{50} values greater 1000mg/Kg. Another phenacyl derivative SN-11 with *meta* nitro group showed the same $LD_{50.}$ The compounds, SN-08 and SN-14 containing dihyroxy and bromo groups respectively displayed LD_{50} of 1000mg/Kg. Therefore, it is predicted that the presence of electron withdrawing groups on the phenacyl ring might be increasing their safety.

CONCLUSION

From the study we can conclude that lipophilicity in a compound can induce cytotoxic effects as phenacyl derivative of INH with *para* phenyl substitution exhibited cytotoxicity on two human colon cancer cell lines HCT-15 and COLO-205. Similarly presence of electron withdrawing groups (such as chloro, bromo, fluoro and nitro) in phenacyl derivatives of INH presented less acute toxicity as observed through greater LD_{50} values than isoniazid. Hence the synthetic compounds can be used safely as therapeutic agents even at higher doses but further structure-activity relationship analysis should be conducted for confirmation and other potential mechanisms.

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