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Synthesis and *In-vitro* Anti-Cancer Screening of N^1[(Substituted Phenyl)Benzylidene]Benzohydrazides

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Abstract

A series of novel benzohydrazides having 2-chloro, 3-nitro, 4-hydroxy, 2, 4-dichloro substitution have been synthesized and characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectra. The synthesized compounds have been screened for their *in-vitro* anti cancer activity by MTT assay against human lung cancer lines (A-549). All the compounds have shown moderate to significant activity against human lung cancer cell lines.

Key words-Benzylidene, hydrazones/ hydrazides, lung cancer.

INTRODUCTION

Hydrazone/hydrazide derivatives are molecules containing highly reactive azomethine group and thus useful in new drug development. They are derived from aldehydes and ketones by reaction with different hydrazines derivatives to give the grouping R_1R_2 C=N-NHR. Hydrazines and their derivatives constitute an important class of compounds that has found wide utility in organic synthesis, while hydrazines have traditionally been employed as reagents for the derivatization and characterization of carbonyl compounds [1-3]. Recently hydrazides-hydrazones have gained great important due to their diverse biological properties including anti-bacterial, anti-fungal, anti-inflammatory, anti-malarial and anti-tubercular activities [4-9].

Due to the capability to react with electrophilic and nucleophilic reagents, hydrozones are widely used in organic synthesis, especially for the preparation of heterocyclic compounds. It is worth mentioning the synthesis of indoles according to the fischer reaction, the synthesis of 4-thiazolidine-4-one, the synthesis of azetidines by cyclo addition and different synthesis of various membered heterocyclic compounds. Earlier we reported the synthesis of heterocycles such as 1,3,4-oxadiazoles, 1,2,4-triazoles via the hydrazone intermediates [10-13].

A number of investigations have demonstrated that hydrazides and hydrazones are having various biological activities. But limited data are available for the anti lung cancer activity of hydrazone/hydrazide derivatives. On the basis of this background the present work has been aimed to synthesize N^1 - [(substituted phenyl) benzylidene] benzohydrazides and to evaluate their cytotoxic potential against human lung cancer cell lines.

MATERIALS AND METHODS

All the synthetic work was done by procuring available laboratory grade reagents and analytical grade solvents. The solvents and reagents were purified and dried according to the given in vogels text book of practical organic chemistry.TLC were performed to monitor the reaction and to determine the purity of the products. Further the compounds were purified by recrystallization using suitable solvents. The melting point of the synthesized compounds were determined in open capillaries using veego VMP-1 apparatus and expressed in ˚C and are uncorrected. The IR spectrum of compounds was recorded on Shimadzu FT-IR spectrometer using KBr pellet technique and is expressed in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on BRUKER (400MHz FT-NMR) using $CDCl_3$ as solvent and TMS as internal standard. Mass spectra were recorded by Shimadzu LC-MS.

Synthesis of benzoic acid hydrazide from methyl benzoate (1)

Methyl benzoate (1.36 ml, 0.01M) is taken in a round bottom flask. To that hydrazine hydrate (0.70 ml, 0.15M) is added and refluxed for 16 hours. The total volume of solution is reduced to half and it is cooled in ice water. The solid is precipitated out and recrystallized with ethanol.

General prodecure for the Synthesis of N^1 (substituted phenyl) benzylidene] benzohydrazides (2a-e)

Benzoic acid hydrazide (1.36 gm, 0.01M) dissolved in 50 ml of ethanol is taken in round bottom flask .To that a mixture of different aromatic aldehyde (0.01 M) and 2 drops of glacial acetic acid is added and refluxed for 2 hours. The precipitate is filtered, washed, dried and purified by recrystallization by using ethanol. The physical data of the synthesized compounds are given in **Table 1& 2**.

Analytical data

Compound 2b

IR (KBr) 3179.76 (Ali-N-H str), 3020.63 (C = C str), 2847.99 (C- C str), 1736.96 (CONH str), 1646.30 (C=N str), 1593.28 (N-N str), 759.01 (C- Cl str), 697.29 (aromatic str). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.4-7.9 (m, 9H, Ar-H); 8.9 (s, 1H, NH); 8.0 (d, 2H, CH). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 163.14, 143.64, 133.18, 133.13, 131.90, 131.59. 131.46, 129.91, 128.48, 127.65,127.61,126.86. FAB-MS: 258.04 (M^+, 100).

Table 1. List of compounds synthesized

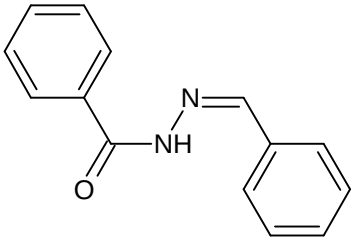
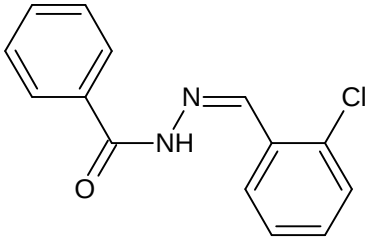
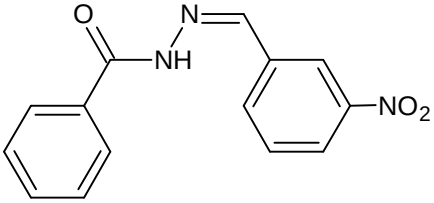
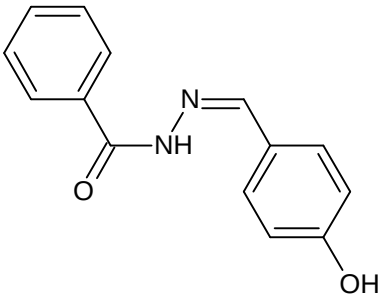
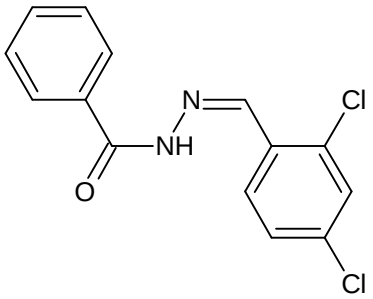
S. NO	COMPOUND CODE	STRUCTURE AND IUPAC NAME
1	2a	 *N*'-[(*Z*)-phenylmethylidene]benzohydrazide
2	2b	 *N*'-[(*Z*)-(2-chlorophenyl)methylidene]benzohydrazide
3	2c	 *N*'-[(*Z*)-(3-nitrophenyl)methylidene]benzohydrazide
4	2d	 *N*'-[(*Z*)-(4-hydroxyphenyl)methylidene]benzohydrazide
5	2e	 *N*'-[(*Z*)-(2,4-dichlorophenyl)methylidene]benzohydrazide

Table 2. Physical parameters of synthesized compounds

Compound no	Molecular Formula	Molecular weight	Melting point (˚C)	Rf values[a]
2a	$C_{14}H_{12}N_2O$	224.257	192 -195	0.78
2b	$C_{14}H_{11}N_2OCl$	258.702	195 - 197	0.44
2c	$C_{14}H_{11}N_3O_3$	269.255	197 - 199	0.88
2d	$C_{14}H_{12}N_2O_2$	240.257	238 - 240	0.60
2e	$C_{14}H_{10}N_2OCl_2$	293.148	221 - 224	0.44

[a]Ethyl acetate : chloroform (6 : 4)

Compound 2c

IR (KBr) 3224.12 (Ali-N-H str), 3032.20 (C=C str), 2823.88 (C- C str), 1768.78 (CONH str), 1645.33 (C=N str), 1579.75 (N-N str), 1526.71 (Ar NO_2), 692.47 (aromatic str). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.5-7.9 (m, 9H, Ar-H); 8.5 (s, 1H, NH); 8.2 (d, 2H, CH). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 163.36, 148.20, 145.29, 136.20, 133.36, 133.11, 130.42, 128.48, 127.69, 120.85. FAB-MS: 269.09 (M^+, 100).

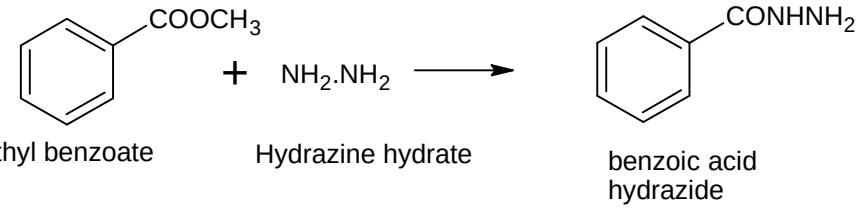
***In-vitro* anticancer screening**

The cytotoxicity assay was carried out using 100 µl of cell suspension, containing 10,000 cells/well of a 96 well microtitre plate. Fresh medium containing different concentrations of the test samples were added after 24 h of partial monolayer. Control cells were incubated without test samples and with Dulbecco's Modified Eagle's Medium (DMEM). The microtitre plates were incubated at 37 ºC in a humidified incubator with 5 % CO_2 for a period of 72 h. Four wells were used for each concentration of the sample. The morphology of the cells was inspected daily. The cytopathogenic effect (CPE) was scored. The 50 percent cytotoxic concentration (CTC_{50}) was determined by the standard MTT assay (1). After 72 h. 10 µl of MTT solution (2mg/ml in PBS) was added to the plates and was incubated for 4 h at 37 ºC. MTT-formazon crystals formed were dissolved in 100 µl of iso propanol and optical density was read with a microtitre plate reader (Biorad) at 540 nm [14]. The experiments were each carried out in triplicate and the results are tabulated in **Table 3**.

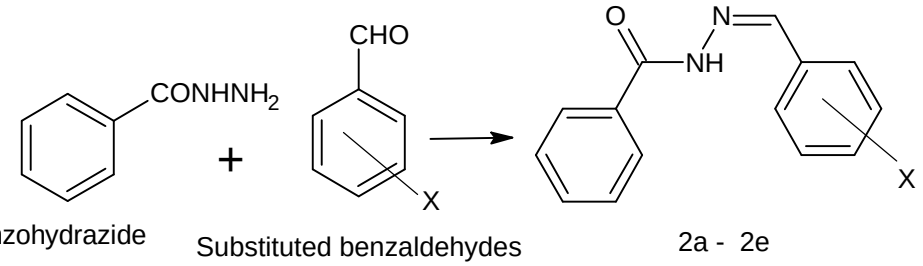
Table 3. Cytotoxicity studies of synthesized compounds

Sl.No	Compound No	CTC_{50} µg/ml
1.	2a	40
2.	2b	65
3.	2c	105
4.	2d	70
5.	2e	55

STEP - 1



STEP – 2



COMPOUND	X
• 2a	H
• 2b	2-Cl
• 2c	3-NO_2
• 2d	4-OH
• 2e	2,4-Cl_2

Scheme 1. Synthetic route of titled compounds

RESULTS AND DISCUSSION

Methyl benzoate was converted in to benzoic acid hydrazide by the reaction with hydrazine hydrate using small quantity of alcohol as a solvent. The benzoic acid hydrazide was reacted with different substituted aromatic aldehydes to derive the corresponding hydrazones. All the synthesized final compounds were first purified by successive recrystallization using appropriate solvents. The purity of the synthesized compounds was checked by performing thin layer chromatography and determining its melting point. IR, ^{1}HNMR and ^{13}C NMR spectra were consistent with the assigned structures. The synthesized compounds were screened for Cytotoxicity activity against A-549 human lung cancer cell lines by determination of CTC_{50} (concentration of the sample required to kill 50% of the cells) value by MTT assay. All of the tested compounds were able to inhibit the growth of the tested human lung carcinoma cell line in a dose-dependent manner. The results indicated in the Table 3 revealed that compound 2a showed the highest inhibitory effect against the human lung carcinoma cell line. The rest of the compounds 2b, 2d and 2e showed moderate growth inhibitory effect. The inhibitory effects showed by all the compounds may be due to the presence of (-CONH-N=CH-) group. It is also noted that the substituents such as chloro, nitro groups had not influenced the inhibitory activity of the synthesized compounds.

CONCLUSION

The synthesized compounds have been screened for cytotoxicity against human lung carcinoma cell lineby MTT assay. All the compounds showed moderate to significant inhibitory activities. The synthesized compound hydrazone (2a) analogue is having significant anti lung cancer activity. However, it can be supported by further *in-vivo* studies.

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