Synthesis and Biological Evaluation of Some Hydrazone Derivatives as Anti-inflammatory Agents

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Abstract: In the present study, some hydrazone derivatives were synthesized *via* the reaction of 3-cyclohexylpropionic acid hydrazide with various benzaldehydes. The chemical structures of the compounds were elucidated by spectroscopic techniques such as IR, ^{1}H-NMR and FAB-MS and elemental analyses. The compounds were evaluated for their anti-inflammatory and cytotoxic activities. Anti-inflammatory activity was determined in terms of inhibition of NF-κB, ROS generation and iNOS activity. Several derivatives inhibited NF-κB and iNOS, but no effect was observed on intracellular ROS generation. Furthermore no cytoxicity was observed. Biological activity compared with the chemical structural information suggests that different functional groups on the phenyl ring influence the physicochemical properties and thus modulate biological activity.

Keywords: Hydrazone, Anti-inflammatory activity, Cytotoxicity, Antioxidant activity.

INTRODUCTION

Inflammation, a biological stress response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants, is a protective attempt by the organism to remove the injurious stimuli and then initiate the healing response [1,2].

Nonsteroidal anti-inflammatory drugs (NSAIDs) act primarily by inhibiting cyclooxygenase (COX) enzymes, which catalyze the first step in the prostaglandin biosynthesis. These agents are currently used as first-line therapeutics in the treatment of osteoarthritis, rheumatoid arthritis, systemic lupus erythematosis, and other inflammatory syndromes to reduce inflammation and control pain. In each case, the treatment is palliative rather than disease modifying [3].

The frequent use of NSAIDs as over-the-counter (OTC) drugs has been rising in recent years and therefore the adverse effects associated with the widespread use of these agents have become an inevitable problem. In order to overcome this serious problem, the search for new effective anti-inflammatory agents has gained great importance [4-6].

Hydrazones have received considerable attention due to their biological importance in medicinal chemistry. Many studies have confirmed that hydrazone derivatives exhibit a wide spectrum of biological effects including anti-inflammatory activity [7-25]. Fraga and co-workers discovered new effective analgesic, anti-inflammatory and antithrombotic agents bearing hydrazone moiety and carried out considerable research for identifying the pharmacophoric contribution of N-acylhydrazone moiety to modulation of biological activity [25].

In this study, we described the synthesis of some hydrazone derivatives and focused on their anti-inflammatory activity and cytotoxicity.

MATERIALS AND METHODS

Chemistry

All reagents were purchased from commercial suppliers and were used without further purification. Melting points (m.p.) were determined on a Electrothermal 9100 melting point apparatus (Weiss-Gallenkamp, Loughborough, UK) and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on Bruker 500 MHz spectrometer (Bruker, Billerica, MA, USA). Chemical shifts were expressed in parts per million (ppm) and tetramethylsilane was used as an internal standard. Mass spectra were recorded on a VG Quattro Mass spectrometer (Agilent, Minnesota, USA). Elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyzer (Perkin-Elmer, Norwalk, CT, USA).

General Procedure for Synthesis of the Compounds

3-cyclohexylpropionic acid hydrazide (1)

The compound was prepared by reacting ethyl 3-cyclohexylpropionate with hydrazine hydrate according to the literature [26].

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N-(benzylidene)-3-cyclohexylpropionic acid hydrazide derivatives (2a-j)

A mixture of 3-cyclohexylpropionic acid hydrazide (**1**) (30 mmol) and appropriate benzaldehydes in absolute ethanol (25 mL) was refluxed for 3-5 hours. The resulting solid was filtered and crystallized from ethanol.

N-(benzylidene)-3-cyclohexylpropionic acid hydrazide (2a)

IR (KBr) ν_{max} (cm^{-1}): 3450-3246 (NH), 1680 (C=O), 1600-1542 (C=N, C=C).

^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 0.87-1.75 (11H, m, cyclohexane), 2.19-2.22 (2H, cyclohexane-CH_2), 2.60-2.65 (2H, t, CH_2-CO), 7.40-8.18 (6H, m, phenyl and CH=N protons), 11.19-11.35 (1H, two s, N-H).

MS (FAB); *m*/*z*: 259 [M + 1]

Anal. Calc. for $C_{16}H_{22}N_2O$: C, 74.38; H, 8.58; N, 10.84. Found: C, 74.40; H, 8.57; N, 10.88.

N-(4-nitrobenzylidene)-3-cyclohexylpropionic acid hydrazide (2b)

IR (KBr) ν_{max} (cm^{-1}): 3468-3255 (NH), 1685 (C=O), 1605-1530 (C=N, C=C).

^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 0.88-1.75 (11H, m, cyclohexane), 2.24-2.27 (2H, m, cyclohexane-CH_2), 2.65-2.68 (2H, t, CH_2-CO), 7.91-8.30 (5H, m, phenyl and CH=N protons), 11.53-11.66 (1H, two s, N-H).

MS (FAB); *m*/*z*: 304 [M+1]

Anal. Calc. for $C_{16}H_{21}N_3O_3$: C, 63.35; H, 6.98; N, 13.85. Found: C, 63.39; H, 6.95; N, 13.83.

N-(4-methylbenzylidene)-3-cyclohexylpropionic acid hydrazide (2c)

IR (KBr) ν_{max} (cm^{-1}): 3475-3239 (NH), 1684 (C=O), 1625-1510 (C=N, C=C).

^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 0.85-1.75 (11H, m, cyclohexane), 2.18-2.21 (2H, m, cyclohexane-CH_2), 2.33-2.34 (3H, CH_3), 2.60-2.63 (2H, t, CH_2-CO), 7.24-8.12 (5H, m, phenyl and CH=N protons), 11.13-11.27 (1H, two s, N-H).

MS (FAB); *m*/*z*: 273 [M+1]

Anal. Calc. for $C_{17}H_{24}N_2O$: C, 74.96; H, 8.88; N, 10.28. Found: C, 74.99; H, 8.90; N, 10.27.

N-(4-bromobenzylidene)-3-cyclohexylpropionic acid hydrazide (2d)

IR (KBr) ν_{max} (cm^{-1}): 3440-3222 (NH), 1688 (C=O), 1621-1511 (C=N, C=C).

^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 0.87-1.74 (11H, m, cyclohexane), 2.19-2.23 (2H, m, cyclohexane-CH_2), 2.60-2.65 (2H, t, CH_2-CO), 7.59-8.14 (5H, m, phenyl and CH=N protons), 11.28-11.41 (1H, two s, N-H).

MS (FAB); *m*/*z*: 339 [M+2]

Anal. Calc. for $C_{16}H_{21}BrN_2O$: C, 56.98; H, 6.28; N, 8.31. Found: C, 56.95; H, 6.25; N, 8.31.

N-(4-florobenzylidene)-3-cyclohexylpropionic acid hydrazide (2e)

IR (KBr) ν_{max} (cm^{-1}): 3472-3225 (NH), 1679 (C=O), 1588-1502 (C=N, C=C).

^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 0.87-1.74 (11H, m, cyclohexane), 2.19-2.22 (2H, m, cyclohexane-CH_2), 2.60-2.63 (2H, t, CH_2-CO), 7.26-8.17 (5H, m, phenyl and CH=N protons), 11.21-11.35 (1H, two s, N-H).

MS (FAB); *m*/*z*: 277 [M+1]

Anal. Calc. for $C_{16}H_{21}FN_2O$: C, 69.54; H, 7.66; N, 10.14. Found: C, 69.54; H, 7.68; N, 10.11.

N-(4-hydroxybenzylidene)-3-cyclohexylpropionic acid hydrazide (2f)

IR (KBr) ν_{max} (cm^{-1}): 3458-3228 (NH), 1681 (C=O), 1595-1508 (C=N, C=C).

^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 0.85-1.74 (11H, m, cyclohexane), 2.15-2.19 (2H, m, cyclohexane-CH_2), 2.57-2.60 (2H, t, CH_2-CO), 6.80-8.04 (5H, m, phenyl and CH=N protons), 9.86-9.89 (1H, O-H), 10.98-11.12 (1H, two s, N-H).

MS (FAB); *m*/*z*: 275 [M+1]

Anal. Calc. for $C_{16}H_{22}N_2O_2$: C, 70.04; H, 8.08; N, 10.21. Found: 70.01; H, 8.11; N, 10.24.

N-(4-methoxybenzylidene)-3-cyclohexylpropionic acid hydrazide (2g)

IR (KBr) ν_{max} (cm^{-1}): 3468-3215 (NH), 1688 (C=O), 1612-1535 (C=N, C=C).

^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 0.87-1.74 (11H, m, cyclohexane), 2.17-2.20 (2H, m, cyclohexane-CH_2), 2.59-2.62 (2H, t, CH_2-CO), 3.79-3.80 (3H, OCH_3), 6.99-8.10 (5H, m, phenyl and CH=N protons), 11.07-11.20 (1H, two s, N-H).

MS (FAB); *m*/*z*: 289 [M+1]

Anal. Calc. for $C_{17}H_{24}N_2O_2$: C, 70.80; H, 8.39; N, 9.71. Found: C, 70.84; H, 8.41; N, 9.73.

N-(4-chlorobenzylidene)-3-cyclohexylpropionic acid hydrazide (2h)

IR (KBr) ν_{max} (cm^{-1}): 3442-3216 (NH), 1686 (C=O), 1590-1526 (C=N, C=C).

^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 0.85-1.74 (11H, m, cyclohexane), 2.19-2.23 (2H, m, cyclohexane-CH_2), 2.61-2.65 (2H, t, CH_2-CO), 7.49-8.15 (5H, m, phenyl and CH=N protons), 11.27-11.40 (1H, two s, N-H).

MS (FAB); *m*/*z*: 293 [M+1]

Anal. Calc. for $C_{16}H_{21}ClN_2O$: C, 65.63; H, 7.23; N, 9.57. Found: C, 65.65; H, 7.25; N, 9.56.

N-(4-isopropylbenzylidene)-3-cyclohexylpropionic acid hydrazide (2i)

IR (KBr) ν_{max} (cm^{-1}): 3452-3240 (NH), 1688 (C=O), 1635-1555 (C=N, C=C).

^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 0.85-1.74 (17H, m, cyclohexane, isopropyl $(CH_3)_2$, 2.18-2.21 (2H, m,

cyclohexane-CH_2), 2.60-2.65 (2H, t, CH_2-CO), 2.89-2.95 (1H, m, isopropyl CH), 7.30-8.12 (5H, m, phenyl and CH=N protons), 11.15-11.27 (1H, two s, N-H).

MS (FAB); *m*/*z*: 301 [M+1]

Anal. Calc. for $C_{19}H_{28}N_2O$: C, 75.96; H, 9.39; N, 9.32. Found: C, 75.97; H, 9.41; N, 9.34.

N-(4-dimethylaminobenzylidene)-3-cyclohexylpropionic acid hydrazide (2j)

IR (KBr) ν_{max} (cm^{-1}): 3440-3215 (NH), 1685 (C=O), 1610-1500 (C=N, C=C).

^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 0.84-1.75 (11H, m, cyclohexane), 2.15-2.18 (2H, m, cyclohexane-CH_2), 2.57-2.59 (2H, t, CH_2-CO), 2.92-3.05 (6H, $N(CH_3)_2$), 6.73-8.00 (5H, m, phenyl and CH=N protons), 10.90-11.02 (1H, two s, N-H).

MS (FAB); *m*/*z*: 302 [M+1]

Anal. Calc. for $C_{18}H_{27}N_3O$: C, 71.72; H, 9.03; N, 13.94. Found: C, 71.74; H, 9.05; N, 13.97.

Biological Assays

In vitro cytotoxicity and anti-inflammatory activities were determined at National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, The University of Mississippi.

Anti-Inflammatory Assay

Anti-inflammatory activity was determined in terms of inhibition of NF-*κ*B-mediated transcription and inhibition of intracellular generation of reactive oxygen species (ROS) and nitric oxide (NO). Free radicals are formed in pathological events, such as oxidative stress during inflammatory processes, which are often correlated with increased levels of inducible nitric oxide synthase (iNOS)-derived nitric oxide. Nitrite and nitrate, nitric oxide stable metabolites, can react with superoxide radical, thus generating peroxynitrites which, in turn, may activate NF-*κ*B. Inhibition of NF-*κ*B mediated transcription was determined in human chondrosarcoma (SW1353) cells by a reporter gene assay as described earlier [27]. In brief, at about 75% confluency, cells were harvested and transfected with NF-*κ*B reporter luciferase plasmid construct at 160 V and one 70-ms pulse in a BTX Electro Square Porator T 820. Transfected cells were plated in 96-well plates ($1x10^5$ cells/well) and incubated for 24 h. After 24 h, cells were exposed to test samples for 30 min and then incubated for 8 h with PMA (70 ng/mL) for the activation of NF-*κ*B. After removing medium, cells were lysed by adding 40 µL of a 1:1 mixture of LucLite reagent and PBS containing 1 mM calcium and magnesium. Luciferase activity was measured as light output on a SpectraMax plate reader. IC_{50} values were obtained from dose response curves. Sp-1 was used as a control transcription factor to evaluate the toxicity of tested compounds in the same assay. Parthenolide was used as the positive control. Inhibition of intracellular NO production as a result of iNOS activity was assayed in mouse macrophages (RAW 264.7 cells) as described [27]. Cells were seeded in 96-well plates at a density of 50,000 cells/well and grown for 24 h for a confluency of 75% or more. Test samples were added at various concentrations and after 30 minutes LPS (5 µg/mL) was added and cells were further incubated for 24 h. NO concentration was determined by measuring the level of nitrite in the cell culture supernatant with Griess reagent. The degree of inhibition of nitrite production was calculated in comparison to the vehicle control. IC_{50} values were obtained from dose response curves. Cytotoxicity of test samples to macrophages was also determined in parallel to check if the inhibition of iNOS is due to cytotoxic effects. Parthenolide was used in each assay as the positive control.

Inhibition of intracellular ROS generation (antioxidant activity) was assayed in human promyelocytic leukemia (HL-60) cells. Cells were seeded in 96-well plates (100,000 cells/well) and treated with different concentrations of test samples for 30 min. Cells were then stimulated with PMA (100 ng/mL) for 30 min. ROS generation is determined by using DCFH-DA as described previously [27]. DCFH-DA is a non-fluorescent probe that diffuses into the cells. Cytoplasmic esterases hydrolyze the DCFH-DA to 2',7'-dichlorofluorescein (DCFH). The reactive oxygen species (ROS) generated within cells oxidize DCFH to 2',7'-dichlorofluorescein (DCF) that fluoresces. The ability of the test compounds to inhibit production of DCF in PMA treated HL-60 cells was measured in comparison to the vehicle control. The IC_{50} values were calculated from dose curves of the % DCF production versus test concentrations. Trolox was used as positive control.

Cytotoxicity Assay

Cytotoxicity was determined against a panel of four human tumor cell lines [SK-MEL (malignant melanoma); KB (oral epidermal carcinoma); BT-549 (breast ductal carcinoma); and SK-OV-3 (ovary carcinoma)]; and two noncancerous cell lines Vero (African green monkey kidney fibroblasts) and LLC-PK1 (pig kidney epithelial cells)] as described earlier [27]. Cells were seeded at a density of 25,000 cells/well in 96-well plates and grown for 24 hours. Samples were added and plates were incubated for 48 hours. Cell viability was determined by using Neutral Red. IC_{50} values were obtained from dose response curves of percent viability versus test concentrations. Doxorubicin was used as a positive control.

RESULTS AND DISCUSSION

Chemistry

Initially, 3-cyclohexylpropionic acid hydrazide (**1**) was obtained by the reaction of ethyl 3-cyclohexylpropionate with hydrazine hydrate [26]. The target compounds (**2a-j**) were synthesized *via* the nucleophilic addition-elimination reaction of 3-cyclohexylpropionic acid hydrazide with various benzaldehydes. These reactions are summarized in Scheme **1** and some properties of the compounds are listed in Table **1**.

The structures of the compounds (**2a-j**) were confirmed by IR, ^{1}H- NMR and mass spectral data and elemental analyses.

In the IR spectra, all derivatives (**2a-j**) had a strong, characteristic band in the region 1700-1650 cm^{-1} due to the C=O stretching vibration. The N-H stretching vibration of

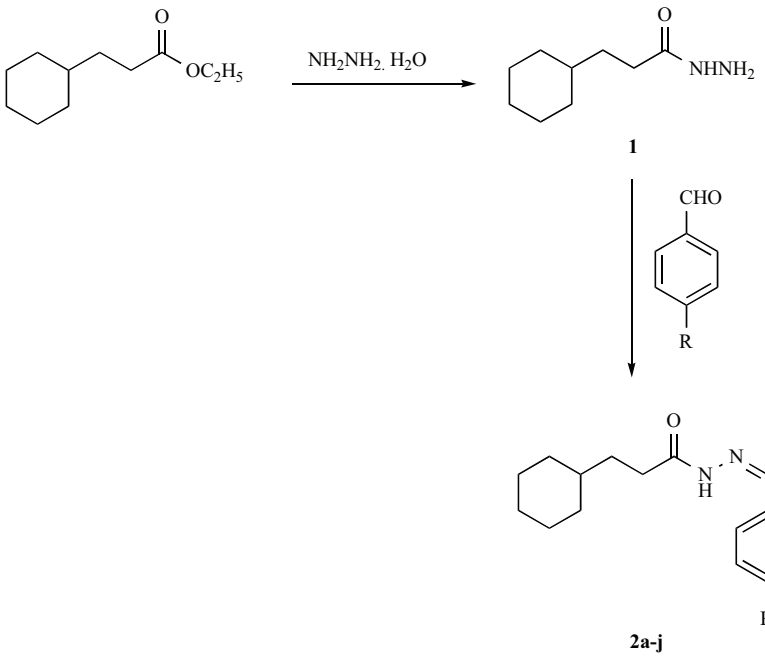

Fig. (1). The synthesis of hydrazone derivatives (**2a-2j**).

Table 1. Some Properties of Hydrazone Derivatives (2a-2j)

Compound	R	Yield (%)	M.p. (°C)	Molecular formula	Molecular weight
2a	H	85	109-112	$C_{16}H_{22}N_2O$	258
2b	NO_2	95	164-168	$C_{16}H_{21}N_3O_3$	303
2c	CH_3	75	113-116	$C_{17}H_{24}N_2O$	272
2d	Br	85	180-183	$C_{16}H_{21}BrN_2O$	337
2e	F	85	110-113,5	$C_{16}H_{21}FN_2O$	276
2f	OH	80	174-178	$C_{16}H_{22}N_2O_2$	274
2g	OCH_3	80	122-125	$C_{17}H_{24}N_2O_2$	288
2h	Cl	85	149-152	$C_{16}H_{21}ClN_2O$	292
2i	$CH(CH_3)_2$	80	83-86	$C_{19}H_{28}N_2O$	300
2j	$N(CH_3)_2$	80	116-118	$C_{18}H_{27}N_3O$	301

the compounds (**2a-j**) gave rise to a band at 3475-3215 cm^{-1}. The stretching bands for C=C and C=N groups were observed at 1635-1500 cm^{-1}.

In the ^{1}H-NMR spectra of the compounds (**2a-j**), the cyclohexyl protons were observed at 0.84-1.75 ppm. The CH_2-cyclohexyl protons were observed at 2.15-2.27 ppm. The signal due to CH_2-CO protons appeared at 2.57-2.68 ppm as a triplet. The N=CH and phenyl protons were observed at 6.73-8.30 ppm. The hydrazone proton signal appeared as two singlet peaks at 10.90-11.66 ppm.

The mass spectra of all compounds (**2a-j**) showed M+1 peaks, in agreement with their molecular formula. All compounds (**2a-j**) gave satisfactory elemental analysis.

Biological Activity

All synthesized compounds (**2a-j**) were evaluated for their anti-inflammatory effects and cytotoxicity in mammalian cells.

Anti-inflammatory activity was determined by monitoring the effects of these compounds on cellular targets for inflammation. Three targets were selected for this study which includes NF-κB, reactive oxygen species (ROS) and inducible nitric oxide synthase (iNOS).

Inhibition of NF-κB mediated transcription and inhibition of intracellular generation of ROS and nitric oxide (NO) were determined in cell based assays. Inhibition of NF-κB mediated transcription was seen in SW1353 cells with IC_{50} values in the range of 6-14 μg/mL (Table **2**). The considerable inhibition of NF-κB activity in SW1353 cells was observed for compounds **2a, 2c** and **2h** with IC_{50} values of 6.9, 7.7 and 6.4 μg/mL, respectively. This outcome confirms that methyl and chloro groups have a considerable influence on the inhibition of NF-κB mediated transcription. Other compounds (**2d**, **2e**, **2g**, **2i**) inhibited the NF-κB activity to a much lesser extent, and their IC_{50} values were in the range 10-14 μg/mL.

Table 2. Anti-Inflammatory and Antioxidant Activities of the Target Compounds (2a-2j)

Cytotoxicity (anti cell proliferation activity) in a panel of cell lines							Inhibition of NF-κB activity in SW1353 cells		Antioxidant Activity (inhibition of ROS generation) in HL-60 cells		Inhibition of iNOS activity (NO production) in RAW 264.7 cells	
	IC_{50} (μg/mL)						IC_{50} (μg/mL)		IC_{50} (μg/mL)		IC_{50} (μg/mL)	
Compound	**SK-MEL[1]**	**KB[1]**	**BT-549[1]**	**SK-OV-3[1]**	**VERO[2]**	**LLC-PK1[2]**	**NF-κB**	**SP-1**	**Antioxidant activity**	**Cytotoxicity**	**iNOS**	**Cytotoxicity**
2a	NA	NA	NA	NA	NA	NC	6.9	NA	NA	NA	11	NC
2b	NA	NA	NA	NA	NA	NC	NA	NA	NA	NA	>25	NC
2c	17	18	18.5	12	NA	25	7.7	19	NA	NA	8.9	NC
2d	NA	NA	NA	NA	NA	NC	14	NA	NA	NA	>25	NC
2e	NA	NA	NA	NA	NA	NC	12	NA	NA	NA	9.5	NC
2f	NA	NA	NA	NA	NA	NC	NA	NA	NA	NA	12	8.6
2g	NA	NA	NA	NA	NA	NC	10	NA	NA	NA	12	6.7
2h	NA	NA	NA	NA	NA	NC	6.4	NA	NA	NA	10	>25
2i	NA	NA	NA	NA	NA	17.5	10	14	NA	NA	9.5	NC
2j	NA	NA	NA	NA	NA	NC	NA	NA	NA	NA	20	NC
Doxorubicin[3]	0.8	1.45	1.35	1.3	>5	0.9				0.24		
Parthenolide[3]							1.3	9			0.4	13.5
Trolox[3]									0.12			

[1]Cancer cells. [2]Noncancer cells. SK-MEL – Human malignant melanoma; KB – Human oral epidermal carcinoma; BT-549 – Human Breast ductal Carcinoma; SK-OV-3 – Human ovary carcinoma; Vero – Monkey Kidney Fibroblasts; LLC-PK1 – Pig kidney epithelial cells. [3]Standard compounds with known biological activities. NA – no activity up to 25 μg/mL. NC – no cytotoxicity up to 25 μg/mL.

Compounds **2c** and **2i** affected SP-1 activity as well, indicating that their effect on NF-κB was not specific. It can be concluded that compounds bearing aliphatic groups (methyl and isopropyl) on phenyl ring show anti-inflammatory activity *via* non-selective inhibition of NF-κB mediated transcription.

Inhibition of iNOS activity was also observed in LPS-induced RAW 264.7 cells with IC_{50} values in the range of 9.5 to 25 μg/mL (Table **2**). Compounds **2c**, **2e** and **2i** were more active than the other compounds. It is apparent that there is a positive correlation between the inhibition of iNOS activity and three functional groups, namely methyl, floro, and isopropyl substituents on phenyl ring. Compounds **2b**, **2d** and **2j** were comparatively less active than other compounds or not effective in the inhibition of NF-κB and iNOS activities. Cytotoxicity to RAW cells was also measured as part of iNOS assay to determine if the cell viability was affected by test samples during the assay and if the inhibition of iNOS could be as a result of toxicity. Compounds **2f**, **2g** and **2h** were toxic to RAW cells. Toxicity could result from the effects of hydroxy, methoxy, and chloro groups, respectively.

Inhibition of reactive oxygen species (ROS) was determined in HL-60 cells by DCFH-DA method to examine antioxidant activity of these compounds. DCFH-DA is a non-fluorescent probe that diffuses into the cells. Cytoplasmic esterases hydrolyse the DCFH-DA to 2',7'-dichlorofluorescin (DCFH). The reactive oxygen species (ROS) generated within HL-60 cells oxidize DCFH to 2',7'-dichlorofluorescin (DCF) fluorescence of which is measured as an indicator of ROS generation. None of the compounds showed any inhibition of intracellular ROS generation indicating that they did not reduce the oxidative stress. None of the compounds showed any cytotoxicity to the panel of tested HL-60 cells.

The cytotoxic effects of the compounds (**2a-2j**) were determined against selected human cancer cell lines (SK-MEL, KB, BT-549, SKOV-3, VERO, and LLC-PK1). No cytotoxicity was observed for any of the samples up to 25 μg/mL except compound **2c**. The compound bearing methyl substituent (**2c**) was weakly active towards to all cancer cell lines with IC_{50} values of 12 to 18.5 μg/mL and was inactive against mammalian Vero (monkey kidney fibroblast) cell. However compound **2c** was toxic to LLC-PK1 (pig kidney epithelial) cell (IC_{50}: 25 μg/mL). Although compound **2i** was inactive to all these cancer lines, it showed toxicity to LLC-PK1 (IC_{50}: 17.5 μg/mL) cell. Toxicity could be due to increased lipophilicity associated with isopropyl group.

CONCLUSION

In the present paper, we synthesized a series of hydrazone derivatives and evaluated their anti-inflammatory activity and cytotoxicity. These observations clearly indicated that functional groups at the para position on the phenyl ring have a crucial influence on anti-inflammatory activity and toxicity. In particular, compound **2a**, which is the unsubstituted derivative, can be identified as the most promising anti-inflammatory agent due to its inhibitory

effect on NF-κB mediated transcription with an IC_{50} value of 6.9 μg/mL. In addition, no effect was seen on the growth of solid tumor cell lines and kidney epithelial and kidney fibroblast cells. In the view of this study, further research can be carried out on the development of new effective anti-inflammatory agents bearing hydrazone moiety by the modification of compound **2a**.

CONFLICT OF INTEREST

The authors have reported no conflict of interest.

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