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Naproxen and ibuprofen based acyl hydrazone derivatives: Synthesis, structure analysis and cytotoxicity studies

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ABSTRACT

Several acyl hydrazone derivatives based on naproxen and ibuprofen were synthesized via a three step method in good yields. All the compounds synthesized were found to exist as a mixture of two rotameric forms in solution e.g. antiperiplanar (ap) and synperiplanar (sp) as indicated by their ¹H NMR spectra. This was supported by the analysis of 1D ¹H NMR and ¹H-¹H 2D Homo COSY spectra of a representative compound. The X-ray diffraction study of the single crystal of another representative compound indicated that the molecule exists as an H-bonded symmetrical dimeric ap form in the solid state. This study also confirmed the E-geometry of the C=N bond unambiguously. Many of these compounds were found to be potent when tested against human prostate cancer (Pc-3) cell line in vitro. The ibuprofen based hydrazones showed superior cytotoxicity than that of naproxen.

Keywords: Acid hydrazide, naproxen, ibuprofen, cytotoxicity, single crystal, X-ray.

INTRODUCTION

Hydrazones have attracted considerable attention in medicinal chemistry due to their distinctive structural features and a wide range of pharmacological activities [1]. This is exemplified by the synthesis and pharmacological evaluation of a large number of hydrazone derivatives against various pharmacological targets [2-4]. While a number of hydrazones have been reported to possess promising antitumoral activities [1], the synthesis and cytotoxicities of hydrazone derivatives derived from anti-inflammatory agents have remained unexplored until recently. For example hydrazones (**A**, Figure 1) derived from diclofenac

acid have shown antimycobacterial activities when tested in vitro and in vivo [5]. Very recently, we have reported synthesis of a series of acyl hydrazones based on mefenamic acid some of which showed cytotoxic properties in vitro especially at higher doses [6]. In continuation to our previous efforts to identify more potent cytotoxic agents, we decided to explore the structural features of other anti-inflammatory agents that can be incorporated in our target molecules. Accordingly, we selected two potent anti-inflammatory agents such as naproxen [7] (**1**, Figure 1) and ibuprofen [8] (**2**, Figure 1), representatives of a group of NSAIDs (non steroidal anti-inflammatory drugs) that are commonly used to treat pain and other inflammatory diseases. We anticipated that combination of structural features of these NSAIDs with substituted hydrazones in a single molecule would provide novel agents possessing potent cytotoxic activities. Herein, we report the synthesis, structure analysis and in vitro pharmacological evaluation of a series of hybrid molecules based on hydrazone.

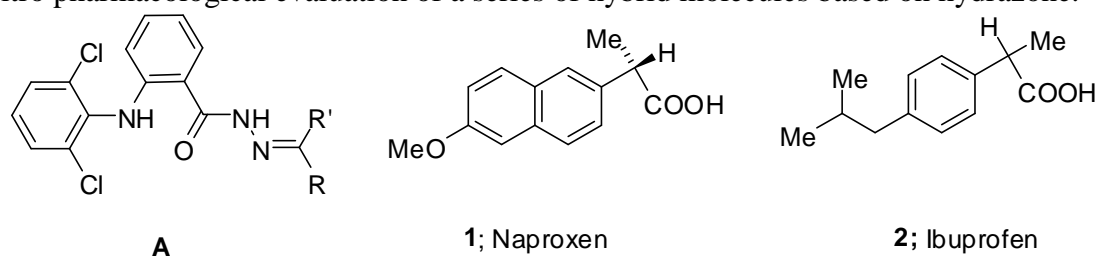


Figure 1. Structures of diclofenac acid based hydrazones (**A**), naproxen (**1**) and ibuprofen (**2**).

EXPERIMENTAL SECTION

General methods: Melting points were all determined by open glass capillary method on a Cintex melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer spectrometer in KBr pellets. ¹H NMR spectra were recorded on a Bruker ACF-300 machine or a Varian 300 or 400 MHz spectrometer using either DMSO-*d*₆ or CDCl₃ as a solvent with tetramethylsilane as internal reference (TMS, δ = 0.00). Chemical shift (δ) values of rotameric hydrogens whenever identified are presented within the parenthesis by assigning asterisk (*) mark along with that of other form. Elemental analyses were performed by Varian 3LV analyzer series CHN analyzer. Mass spectra were recorded on a Jeol JMCD-300 instrument. All solvents used were commercially available and distilled before use. All reactions were monitored by TLC on pre-coated silica gel plates (60 F 254; Merck). Column chromatography was performed on 100-200 mesh silica gel (SRL, India) using 10-20 fold excess (by weight) of the crude product. The organic extracts were dried over anhydrous Na₂SO₄. (*S*)-Naproxen, (±)-Ibuprofen and all the aldehydes used are commercially available. Methyl ester of naproxen and ibuprofen was prepared according to the known methods [9, 10].

Preparation of acid hydrazide **3** (and **4**)

Synthesis of 2-(6-methoxy-naphthalen-2-yl)-propionic acid hydrazide (3**):** To a solution of 2-(6-methoxy-naphthalen-2-yl)-propionic acid methyl ester (500 mg, 0.01 mol) in ethanol (20 mL) was added a few drops of conc. H₂SO₄ drop wise and the mixture was stirred at 85-90 °C for 12 h. After the completion of the reaction (indicated by TLC) ethanol was distilled off and the residue was treated with water. The solid separated was filtered and dried to give the desired product as a colorless solid (yield 96%); mp 104-106 °C (lit [11] 106-108 °C); ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.22 (s, NH, 1H, D₂O exchangeable), 7.78-7.69 (m, 3H, ArH), 7.45 (dd, *J* = 8.3 and 1.6 Hz, 1H), 7.28-7.24 (m, 1H), 7.13 (dd, *J* = 8.8 and 2.5 Hz, 1H), 4.21

(bs, NH, 2H, D₂O exchangeable), 3.86 (s, 3H, OMe), 3.66 (q, 1H, *J* = 6.4 Hz), 1.41 (d, 3H, *J* = 6.5 Hz); IR (KBr, cm⁻¹) 3310, 3250, 1656, 1623; MS (ES): *m/z*: 245.2 (M+1, 100%).

Synthesis of 2-(4-*i*-butyl-phenyl)-propionic acid hydrazide (4): To a solution of 2-(4-*i*-butyl-phenyl)-propionic acid methyl ester (1.0 gm, 0.251 mol) in ethanol (5mL) was added hydrazine hydrate (3.0 mL) with vigorous stirring at room temperature. The mixture was then stirred at 85-90 °C for 12 h. After completion of the reaction (indicated by TLC) ethanol was distilled off and the residue was treated with water. The solid separated was filtered and dried to give the desired product as a colorless solid (yield 89%); mp 76-78 °C (lit [12] 77-78 °C); ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.16 (NH, 1H, D₂O exchangeable), 7.32 (d, *J* = 8.3 Hz, 2H, ArH), 7.14 (d, *J* = 8.3 Hz, 2H, ArH), 4.20 (NH, 2H, D₂O exchangeable), 3.50 (q, *J* = 7.5 Hz, 1H), 2.40 (d, *J* = 6.6 Hz, 2H), 1.82 (m, 1H), 1.33 (d, *J* = 7.2 Hz, 3H), 0.62 (d, *J* = 6.6 Hz, 6H); IR (KBr, cm⁻¹) 3308, 3284, 1635, 1606; MS (ES): *m/z*: 221.2 (M+1, 100%).

General method for the preparation of acyl hydrazones 6 (and 7)

A mixture of acid hydrazide **3** (or **4**) (0.01 mol) and aromatic aldehyde **5** (0.011 mol) in PEG-400 (10 mL) was stirred at room temperature for the time indicated in Table 1. The reaction mixture was poured into crushed ice. The solid separated was filtered and purified by column chromatography to give the desired compound.

2-(6-Methoxy-naphthalen-2-yl)-propionic acid (4-chloro-benzylidene)-hydrazide (6a).

Off white solid; (yield 69%); mp 66-68 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 11.64 (11.36*, s, NH, 1H, D₂O exchangeable), 8.18 (7.88*, s, =CH, 1H), 7.79-7.13 (m, ArH, 10H), 4.76 (3.84*, q, CH, 1H, *J* = 6.6 Hz), 3.86 (3.83*, s, OMe, 3H), 1.47 (1.45*, d, Me, 3H, *J* = 6.6 Hz) (*ap*: *sp* rotamer ratio 9:8); IR (KBr, cm⁻¹) 3430, 3189, 3024, 2937, 1667 (CO_{amide} I), 1606 (C=N), 1544; MS (ES): *m/z*: 367.2 (M⁺, 100%); Elemental analysis found: C, 68.51; H, 5.28; N, 7.73; C₂₁H₁₉ClN₂O₂ requires C, 68.76; H, 5.22; N, 7.64.

2-(6-Methoxy-naphthalen-2-yl)-propionic acid (3-nitro-benzylidene)-hydrazide (6b)

Off white solid; (yield 62%); mp 66-68 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 11.85 (11.50*, s, NH, 1H, D₂O exchangeable), 10.10 (8.69*, s, =CH, 1H), 8.55-7.13 (m, ArH, 10 H), 4.55 (3.85*, m, CH, 1H), 3.86 (3.83*, s, OMe, 3H), 1.50 (1.48*, m, Me, 3H) (*ap*: *sp* rotamer ratio 1:1); IR (KBr, cm⁻¹) 1691, 1677 (CO_{amide} I), 1607 (C=N), 1533; MS (ES): *m/z*: 376.2 (M-1⁺, 90%); Elemental analysis found: C, 66.69; H, 5.10; N, 11.16; C₂₁H₁₉N₃O₄ requires C, 66.83; H, 5.07; N, 11.13.

2-(6-Methoxy-naphthalen-2-yl)-propionic acid (3-hydroxy-benzylidene)-hydrazide (6c).

Off white solid; (yield 70%); mp 102-104 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 11.49 (11.20*, bs, NH, 1H, D₂O exchangeable), 9.60 (9.58*, OH, 1H, D₂O exchangeable) 8.06 (7.79*, s, 1H, =CH), 7.78-6.75 (m, ArH, 10 H), 4.73 (3.79*, q, CH, 1H, *J* = 7.5 Hz), 3.84 (3.82*, s, 3H, OMe), 1.47 (1.40*, d, 3H, Me, *J* = 7.5 Hz) (*ap*: *sp* rotamer ratio 1:1); IR: (KBr, cm⁻¹) 3418, 3281, 3058, 3020, 2893, 1650 (CO_{amide} I), 1605 (C=N), 1585, 1541; MS (ES): *m/z*: 348 (M⁺, 90%); Elemental analysis found: C, 72.28; H, 5.82; N, 8.07; C₂₁H₂₀N₂O₃ requires C, 72.40; H, 5.79; N, 8.04.

2-(6-Methoxy-naphthalen-2-yl)-propionic acid (2-hydroxy-benzylidene)-hydrazide (6d).

Off white solid; (yield 72%); mp 162-164 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 11.77 (11.05, bs, NH, 1H, D₂O exchangeable), 11.23 (11.25*, s, OH, 1H, D₂O exchangeable) 8.36 (8.17*, s, 1H, =CH), 7.80-6.82 (m, ArH, 10H), 4.68 (3.65*, q, CH, 1H, *J* = 6.9 Hz), 3.85 (3.82*, s, 3H, OMe), 1.49 (1.45*, d, 3H, Me, *J* = 6.9 Hz); ¹H NMR (CDCl₃, 300 MHz): δ

11.36 (10.05*, bs, NH, 1H, D₂O exchangeable), 9.28 (8.53*, bs, OH, 1H, D₂O exchangeable) 8.19 (7.18*, 1H, s, =CH), 7.74-6.84 (m, ArH, 10H), 4.51 (3.97*, q, 1H, CH, *J* = 6.9 Hz), 3.95 (3.91*, s, 3H, OMe), 1.70 (1.63*, d, 3H, Me, *J* = 6.9 Hz) (*ap*: *sp* rotamer ratio 6:3); IR: (KBr, cm⁻¹) 3425, 3210, 3055, 2954, 2933, 1651 (CO_{amide I}), 1624, 1604 (C=N), 1574, 1548; MS (ES): *m/z*: 349 (M⁺, 90%); Elemental analysis found: C, 72.52; H, 5.76; N, 8.01; C₂₁H₂₀N₂O₃ requires C, 72.40; H, 5.79; N, 8.04.

2-(6-Methoxy-naphthalen-2-yl)-propionic acid (2-nitro-benzylidene)-hydrazide (6e).

Off white solid; (yield 60%); mp 156-158 °C; ¹HNMR (400 MHz, DMSO-*d*₆): δ 11.90 (11.60*, bs, NH, 1H, D₂O exchangeable), 8.62 (8.30*, s, 1H, =CH), 8.20-7.12 (m, ArH, 10 H), 4.76 (3.82*, q, CH, 1H, *J* = 6.9 Hz), 3.86 (3.84*, s, 3H, CH₃), 1.48 (1.45*, d, 3H, CH₃, *J* = 6.9 Hz) (*ap*: *sp* rotamer ratio 9:8); IR: (KBr, cm⁻¹) 3189, 3011, 2953, 2977, 2936, 1662 (CO_{amide I}), 1633, 1606 (C=N), 1566, 1524; MS (ES): *m/z*: 375.9 (M⁺-1, 94%); Elemental analysis found: C, 66.96; H, 5.09; N, 11.08; C₂₁H₁₉N₃O₄ requires C, 66.83; H, 5.07; N, 11.13.

2-(6-Methoxy-naphthalen-2-yl)-propionic acid-benzylidene-hydrazide (6f).

Off white solid; (yield 80%); mp 134-136 °C; ¹HNMR (300 MHz, DMSO-*d*₆): δ 11.55 (11.27*, bs, NH, 1H, D₂O exchangeable), 8.17 (7.87*, s, 1H, =CH), 7.80-7.07(m, ArH, 11H), 4.76 (4.12*, q, CH, 1H, *J* = 6.9 Hz), 3.84 (3.82*, s, 3H, OCH₃), 1.49 (1.47*, d, 3H, CH₃, *J* = 6.9 Hz) (*ap*: *sp* rotamer ratio 1:1); IR: (KBr, cm⁻¹) 3238, 3209, 3061, 3034, 2968, 2927, 2853, 1668 (CO_{amide I}), 1635, 1609 (C=N), 1548; MS (ES): *m/z*: 333.0 (M⁺, 93%); Elemental analysis found: C, 75.97; H, 6.10; N, 8.28; C₂₁H₂₀N₂O₂ requires C, 75.88; H, 6.06; N, 8.43.

2-(6-Methoxy-naphthalen-2-yl)-propionic acid (4-methoxy-benzylidene)-hydrazide (6g).

Light brown solid; (yield 75%); mp 148-150 °C; ¹HNMR (300 MHz, DMSO-*d*₆): δ 11.41 (11.13*, bs, NH, 1H, D₂O exchangeable), 8.09 (7.81*, s, 1H, =CH), 7.81-6.94 (m, ArH, 10H), 4.73 (3.80*, q, CH, *J* = 6.9 Hz), 3.84 (3.82*, s, 3H, OCH₃), 3.78 (3.77*, s, 3H, OMe), 1.47 (1.45*, d, 3H, CH₃, *J* = 6.9 Hz) (*ap*: *sp* rotamer ratio 1:1); IR: (KBr, cm⁻¹) 3197, 3004, 2961, 2937, 2907, 2836, 1656 (CO_{amide I}), 1606 (C=N), 1573, 1513; MS (ESI): *m/z*: 363.0 (M⁺, 97%); Elemental analysis found: C, 72.79; H, 6.17; N, 7.88; C₂₂H₂₂N₂O₃ requires C, 72.91; H, 6.12; N, 7.73.

2-(6-Methoxy-naphthalen-2-yl)-propionic acid (2-chloro-benzylidene)-hydrazide (6h).

Pale yellow solid; (yield 78%); mp 152-154 °C; ¹HNMR (300 MHz, DMSO-*d*₆): δ 11.64 (11.25*, bs, NH, 1H, D₂O exchangeable), 8.35 (7.95*, s, 1H, =CH), 7.85-7.15 (m, ArH, 10H), 4.80 (3.80*, q, CH, 1H, *J* = 6.9 Hz), 3.86 (3.84*, s, 3H, OCH₃), 1.62 (1.60*, d, CH₃, 3H, *J* = 6.9 Hz) (*ap*: *sp* rotamer ratio 1:1); IR: (KBr, cm⁻¹) 3187, 3055, 2937, 1682 (CO_{amide I}), 1633, 1606 (C=N), 1564; MS (ES): *m/z*: 367.17 (M⁺, 93%); Elemental analysis found: C, 68.89; H, 5.16; N, 7.51; C₂₁H₁₉ClN₂O₂ requires C, 68.76; H, 5.22; N, 7.64.

2-(6-Methoxy-naphthalen-2-yl)-propionic acid (furan-2-ylmethylene-hydrazide (6i).

1:1 mixture of rotamers; Pale brown solid; (yield 61%); mp 148-150 °C; ¹HNMR (300 MHz, DMSO-*d*₆): δ 11.55 (11.21*, bs, NH, 1H, D₂O exchangeable), 8.10 (7.86*, 1H, =CH, s), 7.81-6.60 (m, ArH, 9H), 4.76 (3.85*, q, CH, 1H, *J* = 6.9 Hz), 3.86 (3.84*, s, 3H, OCH₃), 1.45 (1.43*, d, CH₃, 3H, *J* = 6.9 Hz) (*ap*: *sp* rotamer ratio 1:1); IR: (KBr, cm⁻¹) 3205, 3061, 2968, 2936, 1666 (CO_{amide I}), 1606 (C=N), 1560, 1542; MS (ES): *m/z*: 323.01 (M⁺, 95%); Elemental analysis found: C, 70.61; H, 5.72; N, 8.74; C₁₉H₁₈N₂O₃ requires C, 70.79; H, 5.63; N, 8.69.

2-(4-*i*-Butyl-phenyl)-propionic acid (4-chloro-benzylidene)-hydrazide (7a).

White solid; (yield 77%); mp 175-178 °C; ¹HNMR (300 MHz, DMSO-*d*₆): δ 11.59 (11.33*, bs, NH, 1H, D₂O exchangeable), 8.18 (7.90*, 1H, =CH), 7.69-7.05 (m, ArH, 8H), 4.63 (3.65*, q, CH, 1H, *J* = 7.2 Hz), 2.40 (2.37*, d, 2H, CH₂, *J* = 6.6 Hz), 1.76 (m, CH, 1H), 1.38 (1.35*, d, 3H, CH₃, *J* = 7.2 Hz), 0.84 (0.82*, 6H, d, *J* = 6.6 Hz) (*ap*: *sp* rotamer ratio 1:1); IR: (KBr, cm⁻¹) 3202, 3066, 2953, 2913, 2867, 2845, 1668 (CO_{amide} I), 1606 (C=N), 1561, 1509; MS (ES): *m/z*: 343.17 (M⁺, 100%); Elemental analysis found: C, 70.21; H, 6.72; N, 8.03; C₂₀H₂₃ClN₂O requires C, 70.06; H, 6.76; N, 8.17.

2-(4-*i*-Butyl-phenyl)-propionic acid (3-nitro-benzylidene)-hydrazide (7b).

White solid; (yield 67%); mp 160-162 °C; ¹HNMR (300 MHz, DMSO-*d*₆): δ 11.78 (11.51*, bs, NH, 1H, D₂O exchangeable), 8.94 (8.49*, 1H, =CH), 8.44-7.68 (m, ArH, 4H), 7.29-7.07 (m, ArH, 4H), 4.61 (3.70*, q, CH, 1H, *J* = 7.5 Hz), 2.40 (2.36*, d, 2H, CH₂, *J* = 6.6 Hz), 1.75 (m, CH, 1H), 1.40 (1.38*, d, 3H, CH₃, *J* = 7.5 Hz), 0.84 (0.79*, 6H, d, *J* = 6.6 Hz) (*ap*: *sp* rotamer ratio 1:1); IR: (KBr, cm⁻¹) 3036, 2954, 2928, 2867, 1662 (CO_{amide} I), 1608 (C=N), 1567, 1533; MS (ES): *m/z*: 354.18 (M⁺, 90%); Elemental analysis found: C, 67.90; H, 6.63; N, 12.05; C₂₀H₂₃N₃O₃ requires C, 67.97; H, 6.56; N, 11.89.

2-(4-*i*-Butyl-phenyl)-propionic acid (3-hydroxy-benzylidene)-hydrazide (7c).

White solid; (yield 81%); mp 113-115 °C; ¹HNMR (300 MHz, DMSO-*d*₆): δ 11.47 (11.21*, bs, NH, 1H, D₂O exchangeable), 9.01 (8.96*, bs, OH, 1H, D₂O exchangeable), 8.09 (7.83, s, 1H, =CH), 7.28-6.78 (m, ArH, 8H), 4.63 (3.64*, q, CH, 1H, *J* = 7.2 Hz), 2.40 (2.37*, d, 2H, CH₂, *J* = 6.6 Hz), 1.76 (m, CH, 1H), 1.38 (1.35*, d, 3H, CH₃, *J* = 7.2 Hz), 0.84 (0.82*, 6H, 2CH₃, d, *J* = 6.6 Hz) (*ap*: *sp* rotamer ratio 1:1); IR: (KBr, cm⁻¹) 3601, 3342, 3183, 3082, 2959, 2923, 2869, 1668 (CO_{amide} I), 1604 (C=N), 1578, 1547; MS (ES): *m/z*: 325.19 (M⁺, 96%); Elemental analysis found: C, 73.87; H, 7.52; N, 8.79; C₂₀H₂₄N₂O₂ requires C, 74.04; H, 7.46; N, 8.64.

2-(4-*i*-Butyl-phenyl)-propionic acid (2-hydroxy-benzylidene)-hydrazide (7d)

White solid; (yield 85%); mp 168-170 °C; ¹HNMR (300 MHz, DMSO-*d*₆): δ 11.93 (11.63*, bs, NH, 1H, D₂O exchangeable), 8.88 (8.45*, bs, OH, 1H, D₂O exchangeable), 8.64 (8.35*, s, 1H, =CH), 8.22-7.09 (m, ArH, 8H), 4.74 (3.67*, q, CH, 1H, *J* = 6.9 Hz), 2.41 (2.36*, d, 2H, CH₂, *J* = 7.2 Hz), 1.73 (m, CH, 1H), 1.41 (1.35*, d, 3H, CH₃, *J* = 6.9 Hz), 0.85 (0.78*, 6H, 2CH₃, d, *J* = 6.6 Hz) (*ap*: *sp* rotamer ratio 1:1); IR: (KBr, cm⁻¹) 3611, 3332, 3167, 3073, 2949, 2927, 2866, 1666 (CO_{amide} I), 1605 (C=N); MS (ES): *m/z*: 325.23 (M⁺, 100%); Elemental analysis found: C, 74.18; H, 7.54; N, 8.48; C₂₀H₂₄N₂O₂ requires C, 74.04; H, 7.46; N, 8.64.

2-(4-*i*-Butyl-phenyl)-propionic acid (2-nitro-benzylidene)-hydrazide (7e)

light green solid; (yield 74%); mp 138-140 °C; ¹HNMR (300 MHz, DMSO-*d*₆): δ 11.85 (11.55*, bs, NH, 1H, D₂O exchangeable), 8.59 (8.29*, s, 1H, =CH), 8.06-7.09 (m, ArH, 8H), 4.58 (3.67*, q, CH, 1H, *J* = 6.0 Hz), 2.39 (m, 2H, CH₂), 1.75 (m, CH, 1H), 1.38 (m, 3H, CH₃), 0.84 (0.81*, 6H, 2CH₃, d, 2CH₃, *J* = 6.9 Hz) (*ap*: *sp* rotamer ratio 1:1); IR: (KBr, cm⁻¹) 3184, 3013, 2954, 2928, 2868, 1675 (CO_{amide} I), 1658, 1608 (C=N), 1562, 1527; MS (ES): *m/z*: 354.19 (M⁺, 100%); Elemental analysis found: C, 67.87; H, 6.50; N, 11.98; C₂₀H₂₃N₃O₃ requires C, 67.97; H, 6.56; N, 11.89.

2-(4-*i*-Butyl-phenyl)-propionic acid benzylidenehydrazide (7f).

White solid; (yield 87%); mp 135-138 °C; ¹HNMR (300 MHz, DMSO-*d*₆): δ 11.50 (11.25*, bs, NH, 1H, D₂O exchangeable), 8.19 (7.91*, s, 1H, =CH), 7.65-7.06 (m, ArH, 9H), 4.63

(3.65*, q, CH, 1H, $J = 6.9$ Hz), 2.40 (2.37*, d, 2H, CH₂, $J = 7.2$ Hz), 1.75 (m, CH, 1H), 1.38 (1.36*, d, 3H, CH₃, $J = 6.9$ Hz), 0.84 (0.82*, 6H, d, 2CH₃, $J = 7.2$ Hz) (*ap*: *sp* rotamer ratio 1:1); IR: (KBr, cm⁻¹) 3182, 2966, 2951, 1665 (CO_{amide I}), 1612, 1602 (C=N), 1573; MS (ES): *m/z*: 309.27 (M⁺, 100%); Elemental analysis found: C, 77.75; H, 7.90; N, 9.17; C₂₀H₂₄N₂O requires C, 77.89; H, 7.84; N, 9.08.

2-(4- *i*-Butyl-phenyl)-propionic acid (4-methoxy-benzylidene)-hydrazide (7g).

White solid; (yield 85%); mp 146-148 °C; ¹HNMR (300 MHz, DMSO-*d*₆): δ 11.38 (11.13*, NH, bs, 1H, D₂O exchangeable), 8.13 (7.85*, s, 1H, =CH), 7.61-6.97 (m, ArH, 8H), 4.62 (3.63*, q, CH, 1H, $J = 6.9$ Hz), 3.79 (3.75*, 3H, s, OMe), 2.40 (2.37*, d, 2H, CH₂, $J = 7.2$ Hz), 1.75 (m, CH, 1H), 1.38 (1.36*, d, 3H, CH₃, $J = 6.9$ Hz), 0.84 (0.82*, 6H, d, 2CH₃, $J = 7.2$ Hz) (*ap*: *sp* rotamer ratio 13:12); IR: (KBr, cm⁻¹) 3184, 3013, 2954, 2928, 2868, 1666 (CO_{amide I}), 1614, 1604 (C=N), 1572, 1510; MS (ES): *m/z*: 339.20 (M⁺, 100%); Elemental analysis found: C, 74.67; H, 7.67; N, 8.15; C₂₁H₂₆N₂O₂ requires C, 74.52; H, 7.74; N, 8.28.

2-(4- *i*-Butyl-phenyl)-propionic acid (2-chloro-benzylidene)-hydrazide (7h).

Off white solid; (yield 79%); mp 144-146 °C; ¹HNMR (300 MHz, DMSO-*d*₆): δ 11.74 (11.45*, bs, NH, 1H, D₂O exchangeable), 8.58 (8.30*, s, 1H, =CH), 7.94-7.05 (m, ArH, 8H), 4.63 (3.65*, q, CH, 1H, $J = 6.9$ Hz), 2.39 (2.36*, d, 2H, CH₂, $J = 7.2$ Hz), 1.75 (m, CH, 1H), 1.38 (1.35*, d, 3H, CH₃, $J = 6.9$ Hz), 0.83 (0.80*, 6H, d, 2CH₃, $J = 6.9$ Hz) (*ap*: *sp* rotamer ratio 1:1); IR: (KBr, cm⁻¹) 3201, 3058, 2953, 2952, 2906, 1668 (CO_{amide I}), 1595 (C=N), 1568; MS (ES): *m/z*: 343.26 (M⁺, 100%); Elemental analysis found: C, 69.84; H, 6.83; N, 8.05; C₂₀H₂₃ClN₂O requires C, 70.06; H, 6.76; N, 8.17.

2-(4- *i*-Butyl-phenyl)-propionic acid (furan-2-ylmethylene)-hydrazide (7i).

Light brown solid; (yield 74%); mp 148-150 °C; ¹HNMR (300 MHz, DMSO-*d*₆): δ 11.45 (11.22*, bs, NH, 1H, D₂O exchangeable), 8.07 (7.82*, s, 1H, =CH), 7.26-6.60 (m, ArH, 7H), 4.59 (3.61*, q, CH, 1H, $J = 6.9$ Hz), 2.40 (2.38*, d, 2H, CH₂, $J = 7.5$ Hz), 1.77 (m, CH, 1H), 1.37 (1.35*, d, 3H, CH₃, $J = 6.9$ Hz), 0.83 (0.80*, 6H, 2CH₃, d, $J = 7.5$ Hz) (*ap*: *sp* rotamer ratio 9:8); IR: (KBr, cm⁻¹) 3182, 3082, 2966, 2951, 1674 (CO_{amide I}), 1609 (C=N); MS (ES): *m/z*: 299.1 (M⁺, 100%); Elemental analysis found: C, 72.31; H, 7.49; N, 9.51; C₁₈H₂₂N₂O₂ requires C, 72.46; H, 7.43; N, 9.39.

Crystallographic study of 2-(6-methoxy-naphthalen-2-yl)-propionic acid (4-methoxy-benzylidene)-hydrazide (6g) : C₂₂H₂₂N₂O₃, $M_r = 362.42$, triclinic, P1, $a = 6.746$ (4), $b = 8.014$ (4), $c = 17.267$ (9) Å, $\alpha = 95.18$ (1), $\beta = 96.21$ (1), $\gamma = 96.72$ (1)°, $V = 916.6$ (8) Å³, $D_x = 1.316$ g cm⁻³, $Z = 2$, $\mu = 0.088$ mm⁻¹, $T = 100$ (2) K. A colorless needle prepared by slow evaporation from methanol was used for data collection in an Bruker SMART CCD area-detector diffractometer with graphite monochromated MoK α radiation ($\lambda = 0.71073$ Å). Cell parameters were determined from a least-squares refinement of the setting angles of 716 reflections ($2.76 < \theta < 18.65^\circ$). A total of 4851 reflections were collected. The data were corrected for Lorentz and polarisation effects. Absorption correction based on multi-scan ($T_{min} = 0.949$, $T_{max} = 0.996$) was applied. The structure was solved by direct methods and refined on F^2 using SHELXS 97 [13] and SHELXL 97 [14]. The displacement parameters of all non-hydrogen atoms were refined anisotropically. The hydrogen atoms were positioned geometrically and were treated as riding. The isotropic displacement parameters of H-atoms was set to 1.5 $U_{eqv}(C)$ for methyl H-atoms and 1.2 $U_{eqv}(C/N)$ for other H-atoms. The final full-matrix least-squares refinement based on 2242 observed reflections [$I > 2\theta(I)$] converged to led to convergence with a $R = 0.076$, $wR = 0.160$. Full crystallographic details have been deposited at the Cambridge Crystallographic Data Centre (CCDC No. 757172).

Pharmacology

Chemicals and reagents: Dulbecco's modified eagle medium (DMEM), L-glutamine, streptomycin and penicillin were obtained from Sigma-Aldrich, USA. Foetal bovine serum was procured from PAA Biotech, Germany. All other fine chemicals/reagents used in this study were of cell culture grade and obtained from Sigma-Aldrich and/or Merck.

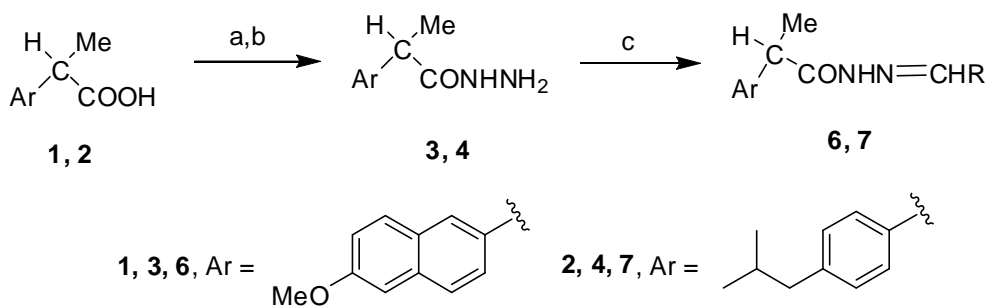
Cell line and culture conditions: Pc-3 (human prostate cancer cell line) was obtained from National Centre for Cell Science, Pune, India. The cells were grown in DMEM culture medium supplemented with 2 mM L-glutamine, 10% FBS, penicillin (50 IU/mL) and streptomycin (50 µg/mL) at a temperature of 37 °C in a humidified incubator with a 5% CO₂ atmosphere and passaged twice weekly to maintain a sub confluent state.

Preparation of test compounds: Test compounds were dissolved in DMSO and were diluted appropriately with culture media prior to treatment of cells. The final concentration of DMSO used in the culture medium was less than 0.2%.

MTT assay for cytotoxicity: The viability of the cells was assessed by MTT [3, 4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, which is based on the reduction of MTT by the mitochondrial dehydrogenase of intact cells to a purple formazan product [15]. Etoposide, a known anticancer drug was used as a reference compound in this assay. Cells (1×10^4) were plated in a 96-well plate. After 24 h, they were treated with different concentration (0 – 10 µM) of different test compounds diluted appropriately with culture media for 48 h. Cells grown in media containing equivalent amount of DMSO served as positive control and cells in medium without any supplementation were used as negative control. After the treatment, media containing compound were carefully removed by aspiration. 100 µL of 0.4mg/ml MTT in PBS was added to each well and incubated in the dark for 4 h. 100 µL of DMSO was added to each well and kept in an incubator for 4 h for dissolution of the formed formazan crystals. Amount of formazan was determined by measuring the absorbance at 540 nm using an ELISA plate reader. The data were presented as percent dead cells, whereas absorbance from non-treated control cells was defined as 100% live cells. The percent dead cells was plotted (Y-axis) against concentration (X-axis) of compounds, where IC₅₀ values could be interpolated from the graph.

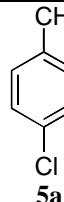
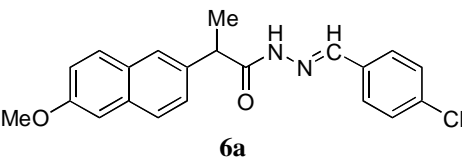
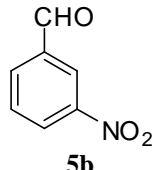
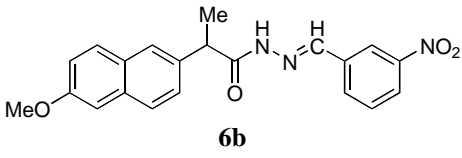
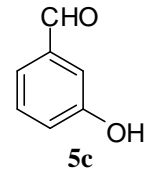
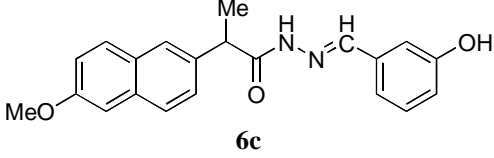
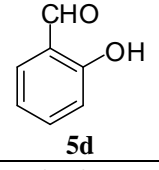
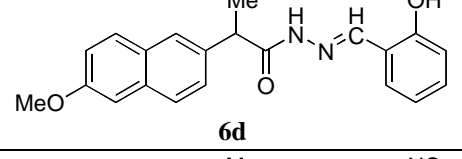
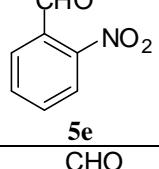
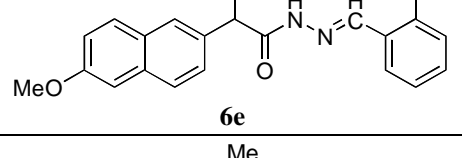
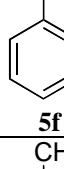
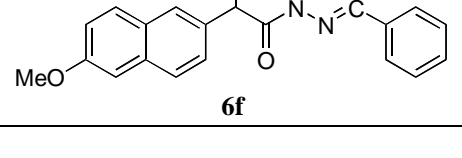
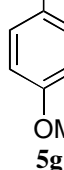
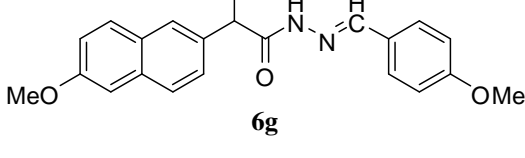
RESULTS AND DISCUSSION

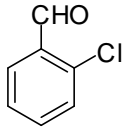
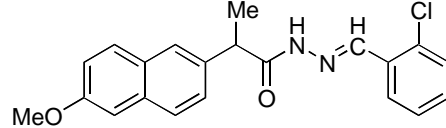
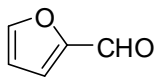
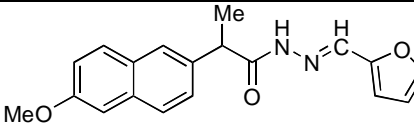
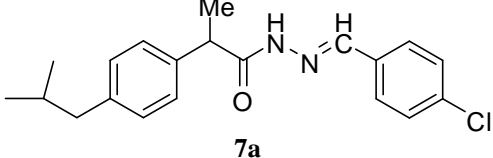
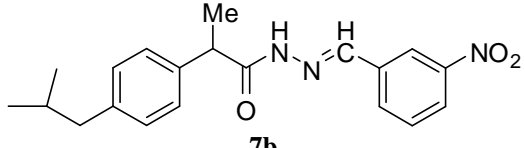
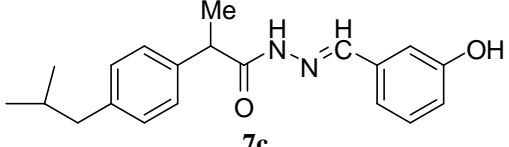
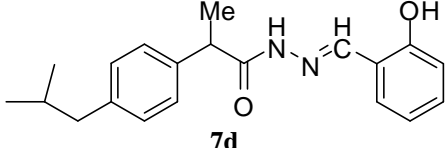
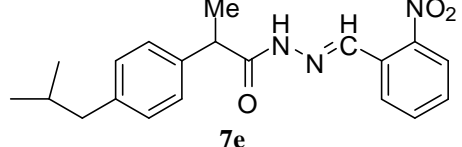
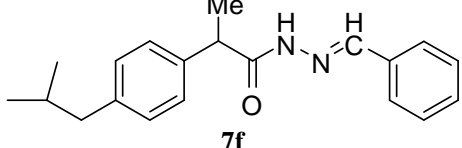
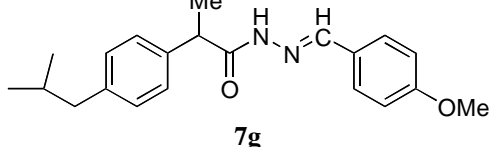
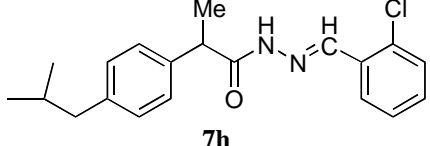
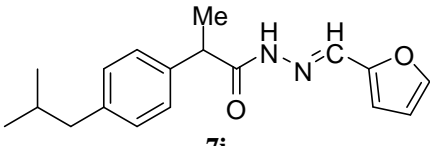
Chemistry: All target compounds were prepared via direct condensation of the key intermediate i.e. hydrazide with a range of commercially available aldehydes (Scheme 1). Thus esterification [9,10] of commercially available (*S*)-naproxen (**1**) or (\pm)-ibuprofen (**2**) with methanol in the presence of catalytic amount of concentrated sulphuric acid followed by treating the resulting methyl ester with hydrazine hydrate in ethanol afforded the key intermediate **3** or **4** respectively in good yield. The hydrazide **3** was then reacted with a series of commercially available aldehydes (**5a-h**) in PEG-400 at room temperature for 5-6 h to give the desired compounds **6a-h**. Compounds **7a-h** were prepared from other hydrazide **4** following a similar method. Specific reaction conditions for each reaction and the product yield are summarized in Table 1.



Scheme 1. Synthesis of 6 and 7. Reaction conditions: (a) MeOH/ Conc. H₂SO₄; (b) N₂H₄. H₂O; (c) RCHO (5)/ PEG-400, rt.

Table 1. Preparation of hydrazone 6 or 7 via the reaction of aldehyde 5 with hydrazide 3 or 4 respectively.

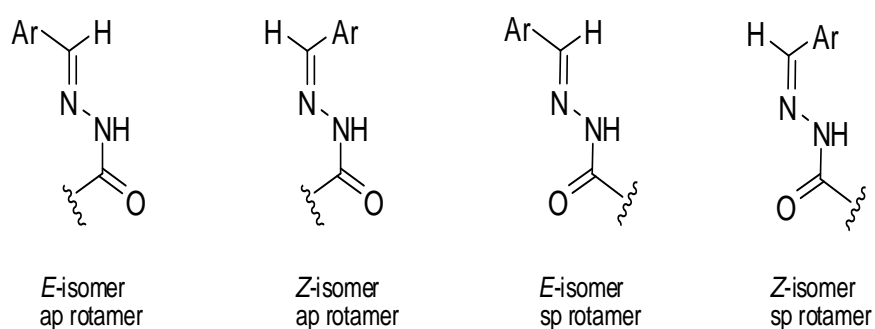
Entry	RCHO (5)	Products (6 or 7)	Time (h)	Yield (%)
1			5.0	69
2			7.0	62
3			5.0	70
4			5.0	72
5			7.0	60
6			5.0	80
7			5.0	75

8	 5h	 6h	5.0	78
9	 5i	 6i	6.0	61
10	5a	 7a	5.0	77
11	5b	 7b	6.0	67
12	5c	 7c	5.0	81
13	5d	 7d	5.0	85
14	5e	 7e	6.5	74
15	5f	 7f	5.0	87
16	5g	 7g	5.0	85
17	5h	 7h	6.0	79
18	5i	 7i	6.0	74

Compound characterization and structure analysis: All the new compounds prepared were initially characterized by spectral (IR, NMR and Mass) and analytical data [16-20]. While the presence of C=O and C=N groups [21] were indicated by the appearance of stretching frequencies in the range 1675-1640 and 1610-1590 cm^{-1} respectively in the IR spectra of compounds **6** and **7**, determination of *E*- or *Z*- geometry of C=N bond by ^1H NMR remained inconclusive. However, based on the earlier report that *N*-acylhydrazones derived from aromatic aldehydes in solution remained in the *E* form because of the hindered rotation on the imine bond [22] we considered *E*- geometry in our cases. Moreover, all compounds were found to exist as a mixture of two rotameric forms in solution [23] e.g. antiperiplanar (*ap*) and synperiplanar (*sp*) as indicated by their ^1H NMR spectra. Two sets of signals were observed for all groups in the ^1H NMR spectra of each compound indicating the possibility of equilibrium and interconversion between rotamers (and/or configurational isomers) in solution.

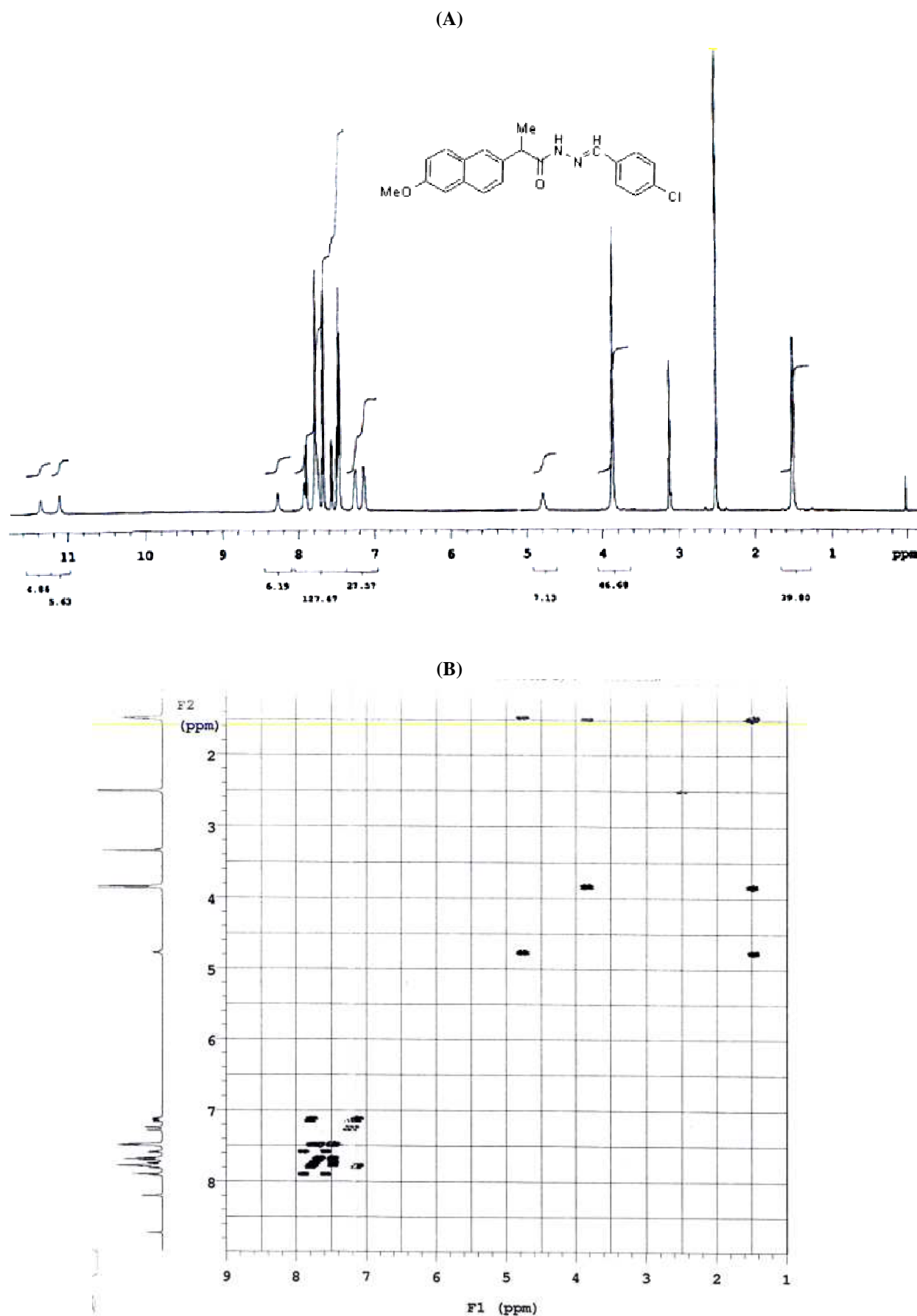
Overall, the present acylhydrazone derivatives of **6** and **7** can exist in four possible forms e.g. two rotameric forms for each geometrical isomer as shown in Scheme 2. The *Z* configuration around N-N bond can be ruled because it is not observed even in case of hydrazones of aldehydes due to the steric crowding [16]. Similarly, the existence of non-planar form of C=N-NH moiety can be ruled out as it would disturb the $n-\pi$ conjugation thereby the energy of stabilization [18,19]. Thus the *ap* form with respect to the C-N bond appeared to be the preferred form for the acylhydrazone derivatives of **6** and **7**. Indeed, in solid state these compounds were found to exist in *ap* form as indicated by the X-ray single crystal data of a representative compound **6g** (vide infra for further discussion).

The *E*-geometry of the C=N bond was also established unambiguously by this study. These observations are in consistent with the earlier report on relative free energy calculations (performed at an ab initio level of theory using the electronic structure code, Gaussian 03) of all possible isomers derived from 2-fold rotations about the C(O)-N, N-N and C=N bonds [20]. The two most stable isomers identified were found to possess *E*-geometry according to this optimization studies.



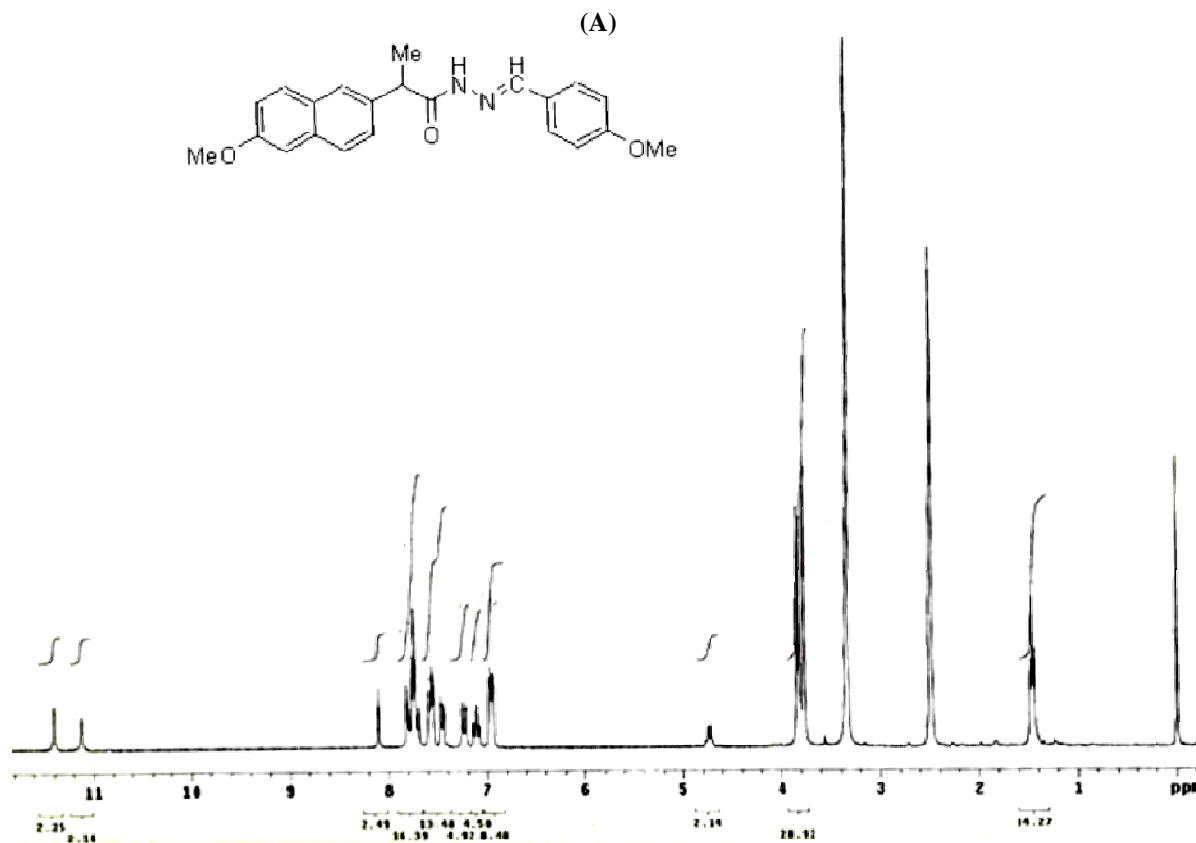
Scheme 2. Four possible forms of acylhydrazone derivatives of **6** and **7**.

The presence of possible tautomeric forms was ruled out on the basis of ^1H NMR spectra recorded for compound **6d** in $\text{DMSO}-d_6$ and CDCl_3 (the only compound that was found to be soluble in both the solvents, see the Experimental section). Except from the solvent dependent shift of the signals, the overall pattern of both spectra was found to be same. The 1D ^1H NMR and $^1\text{H}-^1\text{H}$ 2D Homo COSY spectra of a representative compound **6a** is shown in Figure 2.



It was evident from the 1D spectrum that the methoxy groups of both forms along with the benzylic proton of one form appeared at 3.87 δ (the integration value accounts for more than 3.0 H), whereas the benzylic proton of other form appeared at 4.79 δ (the integration value accounts for less than 1.0 H). This was further supported by the ^1H - ^1H 2D *Homo COSY* spectra. An entry through the diagonal peak at the extreme low frequency portion of this spectrum (1.51 δ) followed by and then tracing either directly to the left or directly down would intersect two cross peaks at 3.87 and 4.79 δ . This indicates protons at 3.87 and 1.51 δ are ^3J coupled. Similarly, protons of 4.79 and 1.51 δ are also ^3J coupled.

It is evident from the structure of **6a** that the CH proton at 4.79 δ of one rotamer is coupled with the methyl group at 1.51 δ . In another rotamer, the CH proton at 3.87 δ is coupled with the methyl group at 1.51 δ . This can be clearly verified by considering the diagonal peak at 3.87 and 4.79 δ in the 2D spectrum. Both of them have off diagonal peak for methyl signal at 1.51 δ . Notably, the presence of both rotameric forms was not detected in the solid state as evaporation of solutions containing both rotamers consistently provided only one solid hydrazone. Crystallization of **6a** from methanol provided fine white crystals whose sharp melting point of 66-68 $^\circ\text{C}$ (corr.) likewise indicated the presence of one isomer. But when crystalline **6a** was re-dissolved in DMSO- d_6 for ^1H NMR study presence of both rotamers was detected thereby confirming their presence in solution. The ratio of rotamers present in solution can be calculated from the ^1H NMR spectra of each compound and was found to be approximately 1:1 for most of the cases. For example, the molar composition i.e. 1:1 ratio approximately of rotamers of **6a** was determined by ^1H NMR (300 MHz, DMSO- d_6): the protons (singlet) of $-\text{CO}-\text{NH}-$ and $-\text{N}=\text{CH}-$ appeared at δ 11.64 and 8.18 were deshielded (due to the effect of carbonyl group present on the same side) and belong to *ap* rotamer whereas those appeared at δ 11.36 and 7.88 were shielded and belong to the *sp* rotamer.



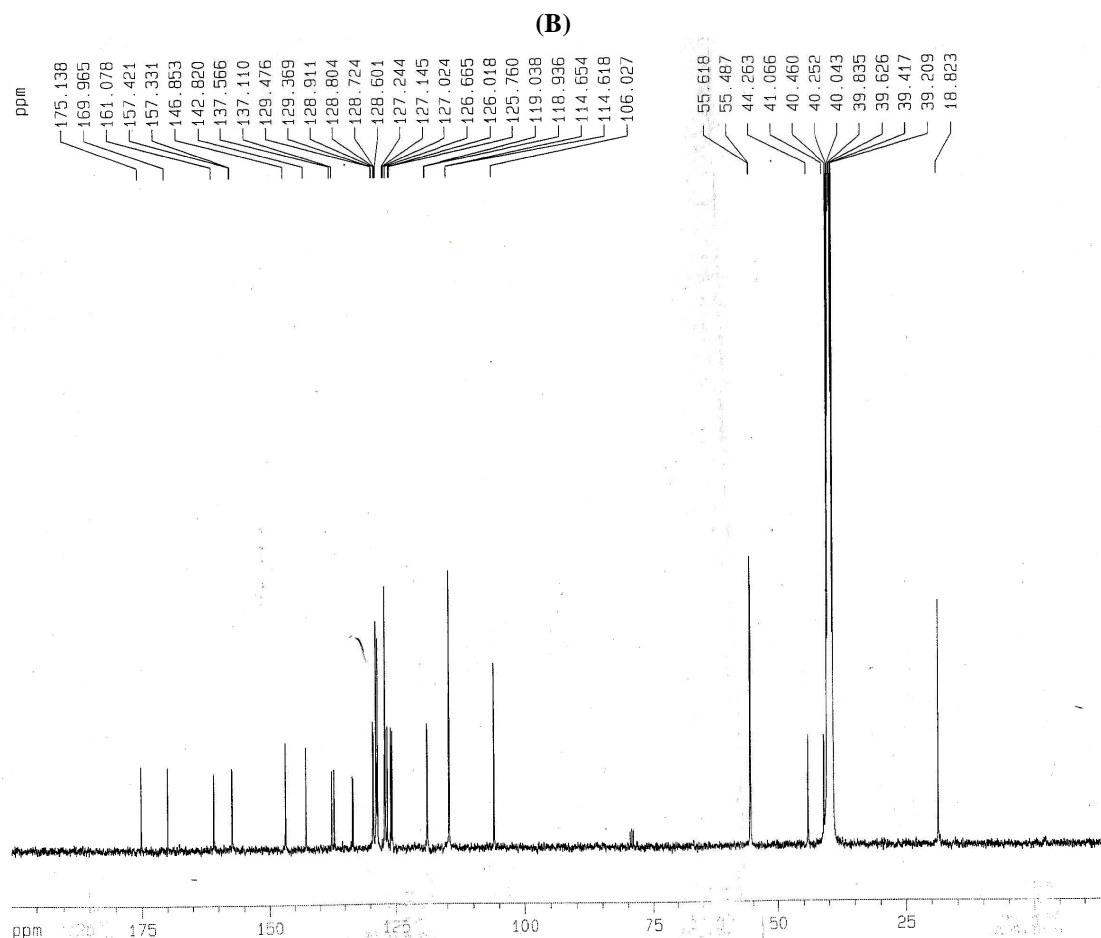


Figure 3. (A) ^1H NMR and (B) ^{13}C NMR spectra of compound **6g** in $\text{DMSO-}d_6$

Since *ap-sp* rotational equilibration in solution at ambient temperature has been reported earlier [24] and *E-Z* equilibration is very unlikely, it can be concluded that the two acylhydrazones observed in solution are the *E*-isomers. These results also indicate that rotational interconversion of rotamers occurs in solution at ambient temperatures at a rate which is slower than the NMR timescale. The ^1H and ^{13}C NMR spectra of another compound e.g. **6g** are shown in Figure 3. In the ^{13}C NMR spectra of **6g** the chemical shift of amidic carbonyl group of *ap* form appeared at lower value e.g. 169.9 ppm due to the up-field shifting caused by the enhanced contribution of the conjugate form $-\text{N}^+=\text{C}-\text{O}^-$ [25] in compared to the down-field shifting (175.1 ppm) in case of *sp* form. It needs to be mentioned here that (*S*)-naproxen (**1**) and (\pm)-ibuprofen (**2**) was used for the preparation of compound **6** and **7** respectively. Thus the same chirality is maintained in products **6** and **7** as the chiral center present in **1** or **2** was not involved in any of the steps of reaction sequence carried out (Scheme 1). This also explains the complexity observed in the ^1H NMR spectra of **7** due to being a racemic mixture instead of a single antipode like compound **6**.

An OPTEP [26] view of the asymmetric unit of **6g**, comprising of two molecules A and B, with atom numbering scheme is shown in Figure 4. In each molecule, the methoxyphenyl and methoxynaphthyl groups are linked through $-\text{CHNNHC}(\text{O})\text{CH}(\text{CH}_3)-$ a chain with a fold about the C10-C11 bond. The *E*-configuration of molecules is established by the torsion angle N2-N1-C8-C5 which assumes a value of -178.9 (7) \AA in A and -177.6 (7) \AA in B. In the crystal packing of **6g**, the molecules exist as hydrogen-bonded symmetry dimers in *ap* forms.

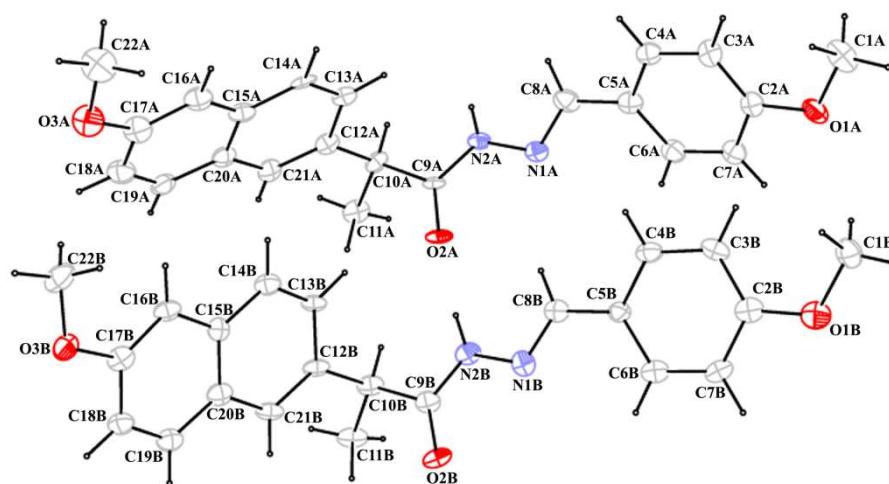


Figure 4. ORTEP view of compound 6g.

Pharmacology: All the substituted hydrazones e.g. chiral **6** and racemic **7** synthesized were tested in vitro against the human prostate cancer cell line (Pc-3) based on an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Table 2) [15]. The percentage of cell death was measured for each compound at various concentrations and finally the IC_{50} (half maximal inhibitory concentration) values were determined to measure the cytotoxic activities (Chart 1). IC_{50} is inversely proportional to the cytotoxicity of a compound, i.e. lower is the IC_{50} value, higher is the activity. In general, the majority of compounds were found to be active against the Pc-3 cancer cell line. Notable among them are **6c** (Entry 3, Table 2), **6d** (Entry 4, Table 2), **7b** (Entry 11, Table 2), **7d** (Entry 13, Table 2), **7f** (Entry 15, Table 2), **7h** (Entry 17, Table 2), and **7i** (Entry 18, Table 2). Indeed, the IC_{50} values were found to be less than 3.0 μ M for compound **7b**, **7c** and **7f**.

Table 2. Cytotoxic activity of compounds **6** and **7** for Pc-3 cancer cell line determined by MTT assay protocol

Entry	Compounds	% of cell death at various concentrations					IC_{50} (μ M)
		1.0 μ M	2.0 μ M	5.0 μ M	10.0 μ M	25.0 μ M	
1	6a	10.24	17.6	42.21	68.03	68.85	6.4
2	6b	4.9	6.50	10.24	69.67	75.81	8.0
3	6c	6.9	23.30	51.63	72.54	75.0	4.8
4	6d	4.56	29.30	53.42	65.71	77.19	4.6
5	6e	6.09	15.85	34.55	50.81	69.51	10.0
6	6f	20.54	41.27	48.96	65.76	76.88	6.0
7	6g	8.45	30.14	39.75	49.31	66.82	11.0
8	6h	7.45	29.06	36.24	49.96	63.54	10.0
9	6i	2.84	9.34	29.26	46.34	73.98	11.6
10	7a	8.16	21.73	50.62	76.51	78.94	5.0
11	7b	19.21	40.03	65.4	68.4	78.17	2.5
12	7c	7.42	16.0	49.21	75.78	76.56	5.0
13	7d	22.69	51.56	61.32	72.36	76.56	2.0
14	7e	6.8	23.80	53.42	73.12	75.34	4.8
15	7f	40.25	50.64	61.47	66.6	67.96	2.0
16	7g	9.95	27.70	38.96	47.61	63.20	12.0
17	7h	14.63	26.42	54.47	74.79	75.60	4.6
18	7i	11.07	16.61	57.65	72.31	79.47	4.5

^aEtoposide ($IC_{50} = 9.85 \mu$ M) was used as a reference compound.

A careful analysis of the data presented in Table 2 reveals that the ibuprofen based hydrazones were generally superior to that of naproxen. On the other hand, the nature of

arylidine moiety present in these molecules played an important role in cytotoxic activities shown by the corresponding compound. For example, 4-methoxybenzylidene, 2-chlorobenzylidene and furanylidene moieties were found to be inferior in compared to that containing simple benzylidene moiety. The results of dose response studies for of/with the two most potent compounds e.g. **7d** and **7f** are presented in Chart 2 by plotting the compound concentrations and % of cell death on X- and Y-axis respectively.

It is known that a number of acyl hydrazone derivatives that showed activities in standard growth inhibition assays were found to inhibit tubulin polymerization [27] (which is the most probable primary mechanism of the action of these compounds). Thus, cytotoxicity shown by the present series of compounds (**6** and **7**) could be due to their covalent binding with β -tubulin thereby inhibiting the tubulin polymerization.

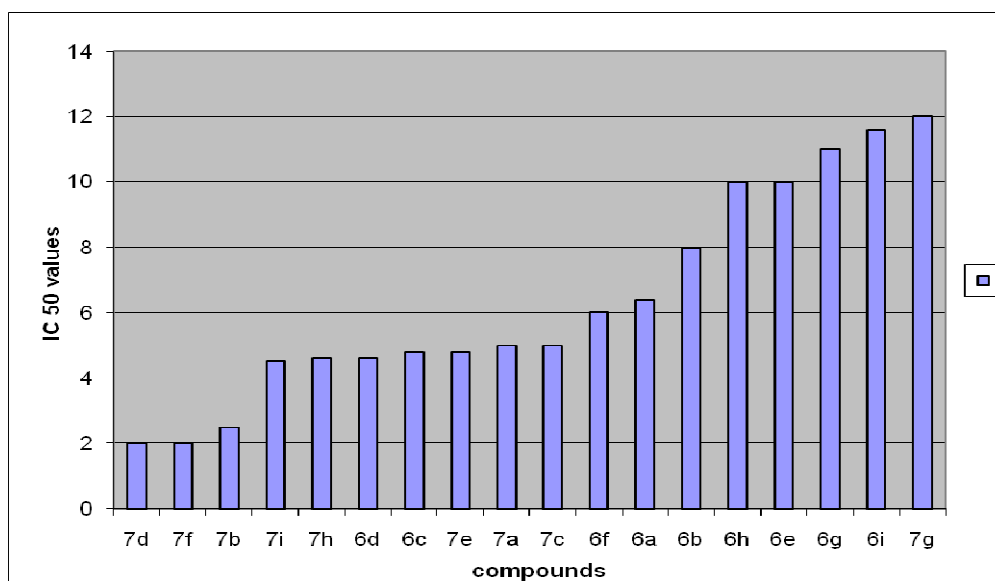


Chart 1. Bar diagram of IC₅₀ values of all the compounds (**6** and **7**) prepared.

(A)

(B)

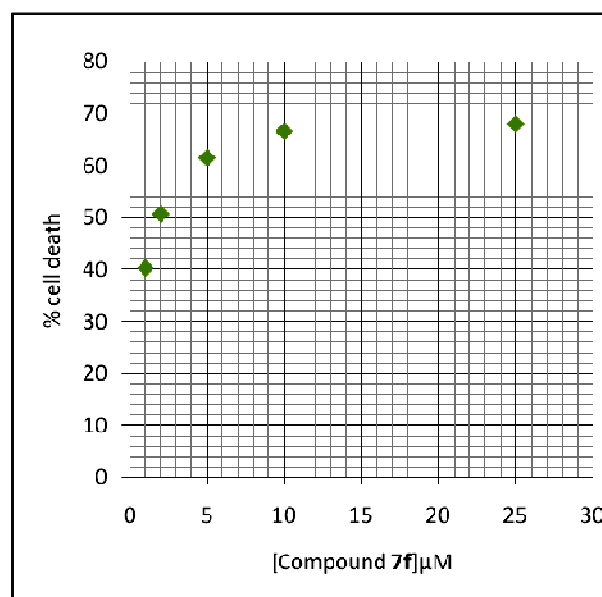
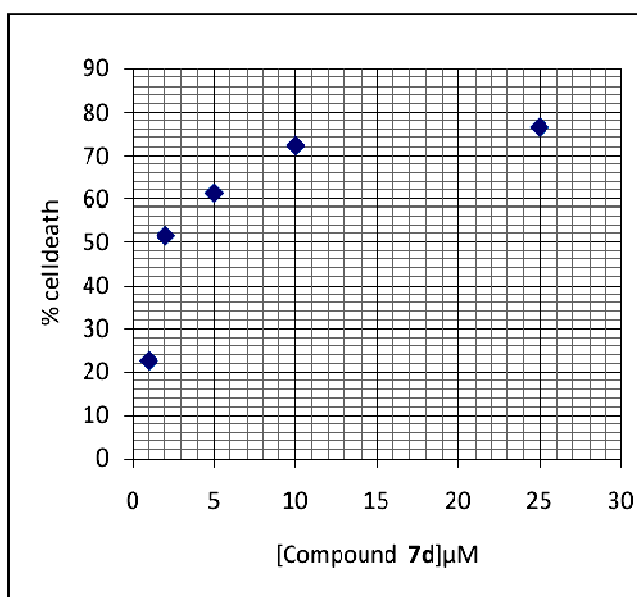


Chart 2. Dose response studies for of/with **7d** and **7f**.

CONCLUSION

In conclusion, we have described the synthesis, structure analysis and in vitro pharmacological properties of a number of acyl hydrazone derivatives based on naproxen and ibuprofen. All compounds were synthesized from readily available reactants and reagents via a simple three step method in good yields. The compounds were found to exist as a mixture of two rotameric forms in solution e.g. antiperiplanar (*ap*) and synperiplanar (*sp*) as indicated by their ¹H NMR spectra. This was supported by the analysis of 1D ¹H NMR and 2D ¹H-¹H 2D *Homo COSY* spectra of a representative compound. Single crystal X-ray structure analysis of a representative compound indicated that the molecule exists as an H-bonded symmetrical dimeric *ap* form in the solid state. This present study also established an *E*-configuration of the C=N bond unambiguously. In general, all compounds were found to be active when tested against human prostate cancer (Pc-3) cell line in vitro and many of them were found to be potent in the same assay. The ibuprofen based hydrazones showed superior cytotoxicity than that of naproxen. Finally, we believe that this class of hydrazone derivatives presents an interesting profile for further experimental investigations especially in the area of anticancer research.

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