ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Novel Series of Substituted Heterocycles derived from α,β–Unsaturated Ketones for Anticancer Evaluation.

Rasha S Gouhar[*1] and Mohamoud Youns[2].

[1]Therapeutic Chemistry Department, National Research Centre, El-Buhouth St., Dokki, P.O. 12622, Giza, Egypt
[2]Biochemistry and Molecular Biology Department, Faculty of Pharmacy, Helwan University, Egypt.

ABSTRACT

The aldol condensation of 2-acetyl (5,6,7,8-tetrahydro-naphthalene) with 1-naphthaldehyde afforded chalcone derivative **1** that consider as excellent starting material for the synthesis of many Heterocycles derivatives. On consideration of **1** with hydrazine hydrate, methyl hydrazine, and / or phenyl hydrazine afforded the pyrazole ring system **2a-c** and also on condensation of **1** with hydroxyl amine hydrochloride gave isooxazole derivative **3**. The condensation of **1** with substituted semicarbazide or substituted thiosemeicarbazide gave the corresponding pyrazole urea or thiourea derivatives **4a-d**. On the other hand, cyclo condensation of **1** with urea, thourea and / or guanidine gives pyrimidine derivatives **5a-c**. The addition reaction of different primary amines to chalcone **1** gave the corresponding addition products **6a-c**. The cyclo condensation of **1** with ethyl acetoacetate, malononitrile and / or ethyl cyanoacetate gave the pyrane ring system **7-9** respectively. Cyclization of **4b** with phenacyl bromide and / or 2-acetyl 5,6,7,8-tetrahydronaphthalene gave substituted thiazole **10**, **11** respectively. Many of these newly synthesized compounds are evaluated as anticancer agent in two cell lines Hep-G2 cells and CaCO.2 cells

Keywords: α,β-Unsaturated Ketone. Pyrazoles. Oxazoles. Pyrimidines. Pyranes. Thiazoles. anticancer agent

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Chalcones (α,β–Unsaturated Ketones) represent active intermediate for the preparation of several heterocyclic ring systems [1] such as pyrazoles [2], isooxazoles [3], pyrimidines [4], pyranes and also, fused heterocyclic derivatives which one of great biological interest especially as anticancer [5], and antioxidant [6]. The available naphthalene containing drugs such as Nafacillin, Naftifine, Tolnaftate and Terbinafine play vital roles in the central of many diseases. On the other side, the chemistry of 1,2,3,4-tetrahydronaphthalene compounds especially those incorporated into different Heterocycles has been of increasing interest since many of these compounds have found useful applications as anticancer reagents [7]. Encouraged by these observations, the present study aimed to synthesized new series of compounds structurally containing naphthalene and tetrahydronaphthalene backbone that incorporated with different biologically active heterocycles hopping to get more active and less toxic compounds might possess a better biological scope as antitumor agents.

MATERIALS AND METHODS

General

All melting points were uncorrected and were taken in open capillary tubes using an Electro thermal IA 9100 digital melting point apparatus. Elemental microanalyses were carried out on Elementar, Vario EL, at Micro analytical laboratory, central services laboratory, National Research center, Dokki, Cairo, Egypt, and were found within ±0.5% of the theoretical values. Infrared spectra were recorded on a FT/IR 6100, Fourier transform infrared spectrometer (Japan) at cm^{-1} scale by using KBr disc technique. ^{1}HNMR spectra was determined by using a JEOI EX-270 NMR spectrometer and measured in δ scale using TMS as an internal standard. The mass spectra were measured with a Finnegan MAT SSQ-7000 mass spectrometer. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminum sheets (Type 60, f 254,Merck,Darmstadt,Germany) and the spots were detected by exposure to UV lamp at λ_{254} nm for few seconds. The chemical names given of the prepared compounds are according to the IUPAC system.

Synthesis of 3-(naphthalen-1-yl)-1-(5,6,7,8-tetrahydronaphthalen-2-yl)prop-2-en-1-one (1)

A mixture of 2-acetyl-5,6,7,8-tetrahydronaphthalene (17.4 mL, 0.01 mol) and 1-naphthaldehyde (15.6 g, 0.1 mol) in 10% alcoholic sodium hydroxide (50 mL) was stirred for 3 h at room temperature. The reaction mixture was left over night, filtered, and the precipitate washed several times with water, dried and crystallized from the ethanol to give compound **1** as yellow crystals, yield 88%; m.p. 90 ^{o}C. IR (KBr): $\tilde{\nu}$ = 2928 (CH_2, tetrahydronaphthalene), 1660 (C=O) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.60 (4 H, 2 CH_2, tetrahydronaphthalene, m), 2.85(4 H, 2 CH_2, tetrahydronaphthalene, m), 7.32-7.90 (12 H, m, Ph) ppm; MS (EI, 70 eV): *m/z* (%) = 312.25 (15%) $[M]^+$; Anal. for $C_{23}H_{20}O$ (312.40): calcd. C, 88.43; H, 6.45; found: C, 87.93; H, 5.95.

General Synthesis of 2a-2c

A mixture of compound **1** (0.62 g, 0.002 mol) and the appropriate hydrazines namely hydrazine hydrate, methyl hydrazine, and / or phenyl hydrazine (0.004 mol) in absolute eth-

anol (10 mL) was refluxed for 3 h. The formed precipitate on cooling was filtered off, dried and crystallized from ethanol to give compounds **2a**-**2c** respectively.

5-(Naphthalen-1-yl)-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-4,5-dihydro-1H-pyrazole (2a)

Buff crystals, yield 60%; m.p. 77 ^{o}C; IR (KBr): $\tilde{\nu}$ = 3047 (NH), 2926 (CH_2, tetrahydronaphthaline) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.72 (4 H, 2 CH_2, tetrahydronaphthaline, m), 2.74(4 H, 2 CH_2, tetrahydronaphthaline, m), 3.96 (3H, m, pyrazole), 7.16-8.18 (10 H, m, Ph) ppm; MS (EI, 70 eV): *m/z* (%) = 326.33 (100%) $[M]^+$; Anal. for $C_{23}H_{22}N_2$ (326.43): calcd.C,84.63; H, 6.79; N, 8.58; found: C, 84.88; H, 7.26; N, 9.08.

1-Methyl-5-(naphthalen-1-yl)-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-4,5-dihydro-1H-pyrazole (2b)

Pale yellow crystals, yield 65%; m.p. 80 ^{o}C; IR (KBr): $\tilde{\nu}$ = 2923 (CH_2, tetrahydronaphthaline) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.60 (4 H, 2 CH_2, tetrahydronaphthaline, m), 2.47 (3H, s, CH_3), 2.85(4 H, 2 CH_2, tetrahydronaphthaline, m), 3.9 (3H, m, pyrazole), 7.25-7.68 (10 H, m, Ph) ppm; MS (EI, 70 eV): *m/z* (%) = 340.39 (100%) $[M]^+$; Anal. for $C_{24}H_{24}N_2$ (340.46): calcd.C,84.67; H, 7.11; N, 8.23; found: C, 84.39; H, 6.77; N, 7.89.

1-Phenyl-5-(naphthalen-1-yl)-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-4,5-dihydro-1H-pyrazole (2c)

Yellow crystals; yield 68%; m.p. 110 ^{o}C; IR (KBr): $\tilde{\nu}$ = 2924 (CH_2, tetrahydronaphthaline) cm^{1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.72 (4 H, 2 CH_2, tetrahydronaphthaline, m), 2.47 (4 H, 2 CH_2, tetrahydronaphthaline, m), 3.92 (2H, m, CH_2 pyrazole), 5.19 (1H, m, CH pyrazole), 6.23-8.50 (15 H, m, Ph) ppm; MS (EI, 70 eV): *m/z* (%) = 402 (20%) $[M]^+$; Anal. for $C_{29}H_{26}N_2$ (402.53): calcd.C,86.53; H, 6.51; N, 6.96; found: C, 86.17; H, 6.29; N, 6.84.

Synthesis of 3-(naphthalen-1-yl)-5-(5,6,7,8-tetrahydronaphthalen-2-yl) -2,3- dihydroisoxazole (3)

A mixture of compound **1** (0.62 g, 0.002 mol) and hydroxylamine hydrochloride (0.14 g, 0.002 mol) in 5% ethanolic sodium hydroxide (15 mL) was refluxed for 10 h, then the reaction mixture was cooled, poured onto ice/cold water and acidified with dilute hydrochloric acid. The formed precipitate was filtered off, dried and crystallized from ethanol to give compound **3** as yellow crystals; yield 75%; m.p. 125 ^{o}C; IR (KBr): $\tilde{\nu}$ = 2936 (CH_2, tetrahydronaphthaline) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.72 (4 H, 2 CH_2, tetrahydronaphthaline, m), 2.47 (4 H, 2 CH_2, tetrahydronaphthaline, m), 4.60 (1H, d, CH oxazole), 6.60 (1H, d, CH oxazole), 6.80-7.90 (10 H, m, Ph) ppm; MS (EI, 70 eV): *m/z* (%) = 327 (9%) $[M]^+$; Anal. for $C_{23}H_{21}NO$ (327.42): C calcd.,84.37; H, 6.46; N, 4.28; found: C, 83.93; H, 6.24; N, 4.02.

General Synthesis of 4a-4d

A mixture of compound **1** (0.62 g, 0.002 mol) and the appropriate carbazides namely semicarbazide, thiosemicarbazide, ethyl thiosemicarbazide or phenyl thiosemicarbazide (0.002 mol) in 1% alcoholic sodium hydroxide (20 mL) was refluxed for 10 h, then the reac-

tion mixture was cooled, poured onto ice/cold water and acidified with dilute hydrochloric acid. The formed precipitate was filtered off, dried and crystallized from the diluted acetic acid to give compounds **4a**-**4d** respectively.

4,5-Dihydro-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-5-(naphthalen-4-yl)pyrazole-1-carboxamide (4a)

Yellowish white crystals; yield 70%; m.p. 93 ^{o}C; IR (KBr): $\tilde{\nu}$ = 3400-3325 (NH_2), 2927 (CH_2, tetrahydronaphthaline), 1685 (amide C=O) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.72 (4 H, 2 CH_2, tetrahydronaphthaline, m), 2.47 (4 H, 2 CH_2, tetrahydronaphthaline, m), 4.00 (2H, m, CH_2 pyrazole), 5.00 (1H, t, CH pyrazole), 7.16-8.00 (10 H, m, Ph) ppm; MS (EI, 70 eV): *m/z* (%) = 396 (5%) $[M]^+$; Anal. for $C_{24}H_{23}N_3O$ 369.46): calcd.C,78.02; H, 6.27; N, 11.37; found: C, 77.84; H, 5.94; N, 10.87.

4,5-Dihydro-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-5-(naphthalen-4-yl)pyrazole-1-carbothioamide (4b)

Pale brown crystals; yield 78%; m.p. 145 ^{o}C; IR (KBr): $\tilde{\nu}$ = 3370-3264 (NH_2), 2935 (CH_2, tetrahydronaphthaline) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.72 (4 H, 2 CH_2, tetrahydronaphthaline, m), 2.00 (2H, d, CH_2 pyrazole), 2.47 (4 H, 2 CH_2, tetrahydronaphthaline, m), 3.90 (1H, t, CH pyrazole), 7.20-7.70 (10 H, m, Ph) ppm; MS (EI, 70 eV): *m/z* (%) = 385 (5%) $[M]^+$; Anal. for $C_{23}H_{23}N_3S$ (385.52): calcd.C,74.77; H, 6.01; N, 10.90; found: C, 75.13; H, 6.26; N, 11.34.

N-ethyl-4,5-dihydro-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-5-(naphthalen-4-yl)pyrazole-1-carbothioamide (4c)

Brown crystals; yield 52%; m.p. 65 ^{o}C; IR (KBr): $\tilde{\nu}$ = 3446(NH), 2926 (CH_2, tetrahydronaphthaline) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.00 (3H, m, CH_3 ethyl), 1.72 (4 H, 2 CH_2, tetrahydronaphthaline, m), 2.00 (2H, d, CH_2 pyrazole), 2.47 (4 H, 2 CH_2, tetrahydronaphthaline, m), 3.50 (2H, m, CH_2 ethyl), 4.00 (1H, t, CH pyrazole), 7.25-7.90 (10 H, m, Ph) ppm; MS (EI, 70 eV): *m/z* (%) = 413 (5%) $[M]^+$; Anal. for $C_{26}H_{27}N_3S$ (314.58): calcd.C,75.51; H, 6.58; N, 10.16; found: C, 75.67; H, 6.69; N, 10.42.

4,5-Dihydro-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-5-(naphthalen-4-yl)-N-phenylpyrazole-1-carbothioamide (4d)

Yellow crystals; yield 50%; m.p. 75 ^{o}C; IR (KBr): $\tilde{\nu}$ = 3425 (NH), 2925 (CH_2, tetrahydronaphthaline) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.72 (4 H, 2 CH_2, tetrahydronaphthaline, m), 2.00 (2H, d, CH_2 pyrazole), 2.47 (4 H, 2 CH_2, tetrahydronaphthaline, m), 4.00 (1H, t, CH pyrazole), 7.25-7.70 (15 H, m, Ph) ppm; MS (EI, 70 eV): *m/z* (%) = 461 (2%) $[M]^+$; Anal. for $C_{30}H_{27}N_3S$ (461.62): calcd.C,78.06; H, 5.90; N, 9.10; found: C, 77.81; H, 5.40; N, 8.74.

General Synthesis of 5a-5c

A mixture of compound **1** (0.31 g, 0.001 mol) and the appropriate urea namely urea, thiourea or guanidine sulphate (0.002 mol) in 1% ethanolic sodium hydroxide (20 mL) was

refluxed for 10 h. After cooling, the mixture was poured onto ice/cold water and acidified with dilute hydrochloric acid. The formed precipitate was filtered off, washed several times with water, dried and crystallized from ethanol to give compounds **5a**-**5c** respectively.

3,4-Dihydro-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-4-(naphthalen-4-yl)pyrimidin-2(1H)-one (5a)

Yellow crystals; yield 80%; m.p. 109 ^{o}C; IR (KBr): $\tilde{\nu}$ = 3395, 3279 (NH), 2926 (CH_2, tetrahydronaphthaline), 1661 (cyclic C=O) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.72 (4 H, 2 CH_2, tetrahydronaphthaline, m), 2.47 (4 H, 2 CH_2, tetrahydronaphthaline, m), 6.00 (2H, m, CH pyrimidine), 7.00-7.80 (10 H, m, Ph) ppm; MS (EI, 70 eV): *m/z* (%) = 354 (8%) $[M]^+$; Anal. for $C_{24}H_{22}N_2O$ (354.44): calcd.C,81.33; H, 6.26; N, 7.90; found: C, 81.75; H, 6.37; N, 8.40.

3,4-Dihydro-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-4-(naphthalen-4-yl)pyrimidine-2(1H)-thione (5b)

Yellow crystals; yield 78%; m.p. 120 ^{o}C; IR (KBr): $\tilde{\nu}$ = 3424 (NH), 2926 (CH_2, tetrahydronaphthaline) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.72 (4 H, 2 CH_2, tetrahydronaphthaline, m), 2.47 (4 H, 2 CH_2, tetrahydronaphthaline, m), 6.70 (2H, d, 2CH pyrimidine), 7.00-7.80 (10 H, m, Ph) ppm; MS (EI, 70 eV): *m/z* (%) = 370 (10%) $[M]^+$; Anal. for $C_{24}H_{22}N_2S$ (370.51): calcd.C,77.80; H, 5.98; N, 7.56; found: C, 77.55; H, 5.62; N, 7.22.

4-(5,6,7,8-tetrahydronaphthalen-2-yl)-6-(naphthalen-4-yl)pyrimidin-2-amine (5c)

White crystals; yield: 85%; m.p. 178 ^{o}C; IR (KBr): $\tilde{\nu}$ = 3468, 3291 (NH_2), 2924 (CH_2, tetrahydronaphthaline) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.72 (4 H, 2 CH_2, tetrahydronaphthaline, m), 2.47 (4 H, 2 CH_2, tetrahydronaphthaline, m), 7.00-7.70 (12 H, m, Ph, CH pyrimidine) ppm. MS (EI, 70 eV): *m/z* (%) = 351 (100%) $[M]^+$; Anal. for $C_{24}H_{21}N_3$ (351.44): calcd.C,82.02; H, 6.02; N, 11.96; found: C, 82.25; H, 6.46; N, 12.34.

General Synthesis of 6a-6c

A mixture of compound **1** (0.62 g, 0.002 mol) and the appropriate amine or aromatic amines namely cyclohexyl amine, *p*-amino pyridine or *p*-bromo aniline (0.002 mol) in absolute ethanol (20 mL) was refluxed for 8 h. The formed precipitate on cooling was filtered off, dried and crystallized from diluted acetic acid to give compounds **6a**-**6c** respectively.

3-(Cyclohexylamino)-1-(5,6,7,8-tetrahydronaphthalen-2-yl)-3-(naphthalen-4-yl)propan-1-one (6a)

Yellow crystals; yield 55%; m.p. 86 ^{o}C; IR (KBr): $\tilde{\nu}$ = 3424 (NH), 2926 (CH_2, tetrahydronaphthaline), 1670 (C=O) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.20-2.80 (19 H, cyclohexyl, tetrahydronaphthaline, m), 3.20 (2 H, CH_2, d), 4.50 (1H, CH, s), 7.00-7.90 (10 H, m, Ph) ppm; MS (EI, 70 eV): *m/z* (%) = 411 (5%) $[M]^+$; Anal. for $C_{29}H_{33}NO$ (411.58): calcd.C,84.63; H, 8.08; N, 3.40; found: C, 84.89; H, 8.58; N, 3.81.

1-(5,6,7,8-tetrahydronaphthalen-2-yl)-3-(naphthalen-4-yl)-3-(pyridin-4-ylamino)propan-1-one (6b)

Yellow crystals; yield 63%; m.p. 105 ^{o}C; IR (KBr): $\tilde{\nu}$ = 3420 (NH), 2929 (CH_2, tetrahydronaphthaline), 1660 (C=O) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.72 (4 H, 2 CH_2, tetrahydronaphthaline, m), 2.47 (4 H, 2 CH_2, tetrahydronaphthaline, m), 3.20 (2 H, CH_2, d), 4.50 (1H, CH, t), 7.00-8.50 (14 H, m, Ph, pyridine) ppm; MS (EI, 70 eV): *m/z* (%) = 406 (3%) $[M]^+$; Anal. for $C_{28}H_{26}N_2O$ (406.52): calcd. C,82.73; H, 6.45; N, 6.89; found: C, 82.26; H, 6.18; N, 6.39.

3-(4-Bromophenylamino)-1-(5,6,7,8-tetrahydronaphthalen-2-yl)-3-(naphthalen-4-yl)propan-1-one (6c)

Yellow crystals; yield: 61%; m.p. 95 ^{o}C; IR (KBr): $\tilde{\nu}$ = 3413 (NH), 2928 (CH_2, tetrahydronaphthaline), 1660 (C=O) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.72 (4 H, 2 CH_2, tetrahydronaphthaline, m), 2.47 (4 H, 2 CH_2, tetrahydronaphthaline, m), 3.20 (2 H, CH_2, d), 4.50 (1H, CH, t), 6.60-8.50 (14 H, m, Ph) ppm; MS (EI, 70 eV): *m/z* (%) = 403, 405 (5%) $[M]^+$; Anal. for $C_{29}H_{26}BrNO$ (484.43): calcd. C, 71.90; H, 5.41; N, 2.89; found: C, 71.40; H, 5.19; N, 2.49.

Synthesis of 1-(6-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-hydroxy-4-(naphthalen-4-yl)-4H-pyran-3-yl)ethanone (7)

A mixture of compound **1** (0.62 g, 0.002 mol) and ethyl acetoacetate (0.26 mL, 0.002 mol) in 1% ethanolic sodium hydroxide (20 mL) was refluxed for 6 h. The formed precipitate on hot was filtered off, washed several times with water, dried and crystallized from acetic acid to give compound **7** as yellow crystals; yield 85%; m.p. 209 ^{o}C; IR (KBr): $\tilde{\nu}$ = 3406 (br. OH), 2928 (CH_2, tetrahydronaphthaline), 1650 (C=O); ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.72 (4 H, 2 CH_2, tetrahydronaphthaline, m), 2.30 (3 H, CH_3, s), 2.47 (4 H, 2 CH_2, tetrahydronaphthaline, m), 5.00 (2H, CH pyrane, d), 6.90-8.20 (10 H, m, Ph) ppm; MS (EI, 70 eV): *m/z* (%) = 396 (5%) $[M]^+$; Anal. for $C_{27}H_{24}O_3$ (396.48): calcd. C,81.79; H, 6.10; found: C, 82.02; H, 6.34.

Synthesis of 2-amino-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-4-(naphthalen-4-yl)-4H-pyran-3-carbonitrile (8)

A mixture of compound **1** (0.62 g, 0.002 mol) and malononitrile (1.3 g, 0.002 mol) in 1% ethanolic sodium hydroxide (15 mL) was refluxed for 6 h. The formed precipitate on hot was filtered off, washed several times with water, dried and crystallized from acetic acid to give compound **8** as yellow crystals; yield 79%; m.p. 180 ^{o}C; IR (KBr): $\tilde{\nu}$ = 3418 (NH_2), 2920 (CH_2, tetrahydronaphthaline), 2195 (CN) cm^{-1}; MS (EI, 70 eV): *m/z* (%) = 378 (4%) $[M]^+$; Anal. for $C_{26}H_{22}N_2O$ (378.47): calcd. C, 82.51; H, 5.85; N, 7.40; found: C, 82.15; H, 5.51; N, 7.06.

Synthesis of 6-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-hydroxy-4- (naphthalen-4-yl)-4H-pyran-3-carbonitrile (9)

A mixture of compound **1** (0.62 g, 0.002 mol) and ethyl cyanoacetate (0.23 mL, 0.002 mol) in 1% ethanolic sodium hydroxide (15 mL) was refluxed for 4 h. The formed precipitate on hot was filtered off, washed several times with water, dried and crystallized from acetic

acid to give compound **9** as yellow crystals; yield: 74%; m.p. 240 ^{o}C; IR (KBr): $\tilde{\nu}$ = 3420 (br. OH), 2200 (CN) ppm; MS (EI, 70 eV): *m/z* (%) = 379 (3%) $[M]^{+}$; Anal. for $C_{26}H_{21}NO_2$ (379.45): calcd. C, 82.30; H, 5.58; N, 3.69; found: C, 81.99; H, 5.19; N, 3.32.

General Synthesis of 10 and 11

A mixture of compound **4b** (0.38 g, 0.001 mol) and the appropriate ketones namely phenacyl bromide or 2-bromoacetyl-5,6,7,8-tetrahydronaphthalene (0.001 mol) in absolute ethanol (10 mL) was refluxed for 6 h. The formed precipitate on cooling was filtered off, dried and crystallized from ethanol to give compounds **10** and **11** respectively.

4,5-Dihydro-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-5-(naphthalen-4-yl)-1-(4-phenylthiazol-2-yl)-1H-pyrazole (10)

Yellow crystals; yield 55%; m.p. 83 ^{o}C; MS (EI, 70 eV): *m/z* (%) = 485 (3%) $[M]^{+}$. Anal. for $C_{32}H_{27}N_3S$ (485.64): calcd C,79.14; H, 5.60; N, 8.65; found: C, 78.87; H, 5.16; N, 8.15.

4,5-Dihydro-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-1-(4-(5,6,7,8-tetrahydronaphthalen-2-yl)thiazol-2-yl)-5-(naphthalen-4-yl)-1H-pyrazole (11)

Yellow crystals; yield 50%; m.p. 72 ^{o}C; MS (EI, 70 eV): *m/z* (%) = 539 (4%) $[M]^{+}$. Anal. for $C_{36}H_{33}N_3S$ (539.73): calcd. C,80.11; H, 6.16; N, 7.79; found: C, 80.44; H, 6.43; N, 7.98.

ANTICANCER EVALUATION

Cell culture and treatment

All reagents were handled in a sterile fume hood. DMEM medium, and fetal bovine serum (FBS) were purchased from Gibco; phosphate-buffered saline pH 7.4 (PBS) and trypsin-EDTA were obtained from Sigma–Aldrich. Alamar Blue or Resazurin (Promega, Mannheim, Germany) reduction assay was used to assess the cytotoxicity of the studied samples. The growth medium (DMEM medium with 10% FBS, 100 U/ml penicillin, and 100 mg/L streptomycin), and alamar blue were stored at 48^{o}C, while trypsin–EDTA and FBS were stored frozen at -208^{o}C and thawed before use; PBS was stored at room temperature. The Hep-G2, and CaCo-2 were obtained from the German Cancer Research Center (DKFZ). Cells were cultured in 50 cm^2 culture flasks (Corning) using DMEM medium supplemented with 10% FBS, penicillin (100 IU/ml), and streptomycin (100 mg/ml). The culture was maintained at 37ºC in an atmosphere of 5% CO_2 and 95% relative humidity. The cells were transferred to a new flask every 2 days and treated with trypsin–EDTA to detach them from the flask. Cells were counted under a microscope using a hemacytometer (Hausser Scientific). Cell solutions were diluted with growth medium to a concentration of 1 x 10^5 cells/ml and transferred to a 96-well plate, and treated with gradient concentrations of test compounds.

Resazurin cell growth inhibition assay

Alamar Blue or Resazurin (Promega, Mannheim, Germany) reduction assay was used to assess the cytotoxicity of the studied samples. The assay tests cellular viability and mito-

chondrial function. Briefly, adherent cells were grown in tissue culture flasks, and then harvested by treating the flasks with 0.025% trypsin and 0.25 mM EDTA for 5 min. Once detached, cells were washed, counted and an aliquot (5×10^3 cells) was placed in each well of a 96-well cell culture plate in a total volume of 100 µl. Cells were allowed to attach overnight and then treated with samples. The final concentration of samples ranged from 0-100 µM. After 48 h, 20 µl Resazurin 0.01% w/v solution was added to each well and the plates were incubated at 37°C for 1–2 h. Fluorescence was measured on an automated 96-well Infinite M2000 Pro™ plate reader (Tecan, Crailsheim, Germany) using an excitation wavelength of 544 nm and an emission wavelength of 590 nm. After 48 h incubation, plates were treated with resazurin solution as above mentioned. Doxorubicin was used as positive control. Each assay was done at least three times, with two replicates each. The viability was compared based on a comparison with untreated cells. IC_{50} (on cancer cells) were the concentration of sample required to inhibit 50% of the cell proliferation and were calculated from a calibration curve by a linear regression using Microsoft Excel.

Caspase-glo 3/7 assay

The influence of our test samples on caspase 3/7 activity in pancreatic cancer resistant cells (Panc-1) was detected using Caspase-Glo 3/7 Assay kit (Promega). Cells cultured in DMEM were seeded in 96-well plates and treated with the sample ($2 \times IC_{50}$; IC_{50}; $½ \times IC_{50}$) or DMSO (solvent control). After 24 h treatment, 100 µl of caspase 3/7 reagent were added to each well, mixed and incubated for 1 h at room temperature. Luminescence was measured using well Infinite M2000 Pro™ instrument (Tecan). Caspase 3/7 activity was expressed as percentage of the untreated control.

RESULTS AND DISCUSSION

Chemistry

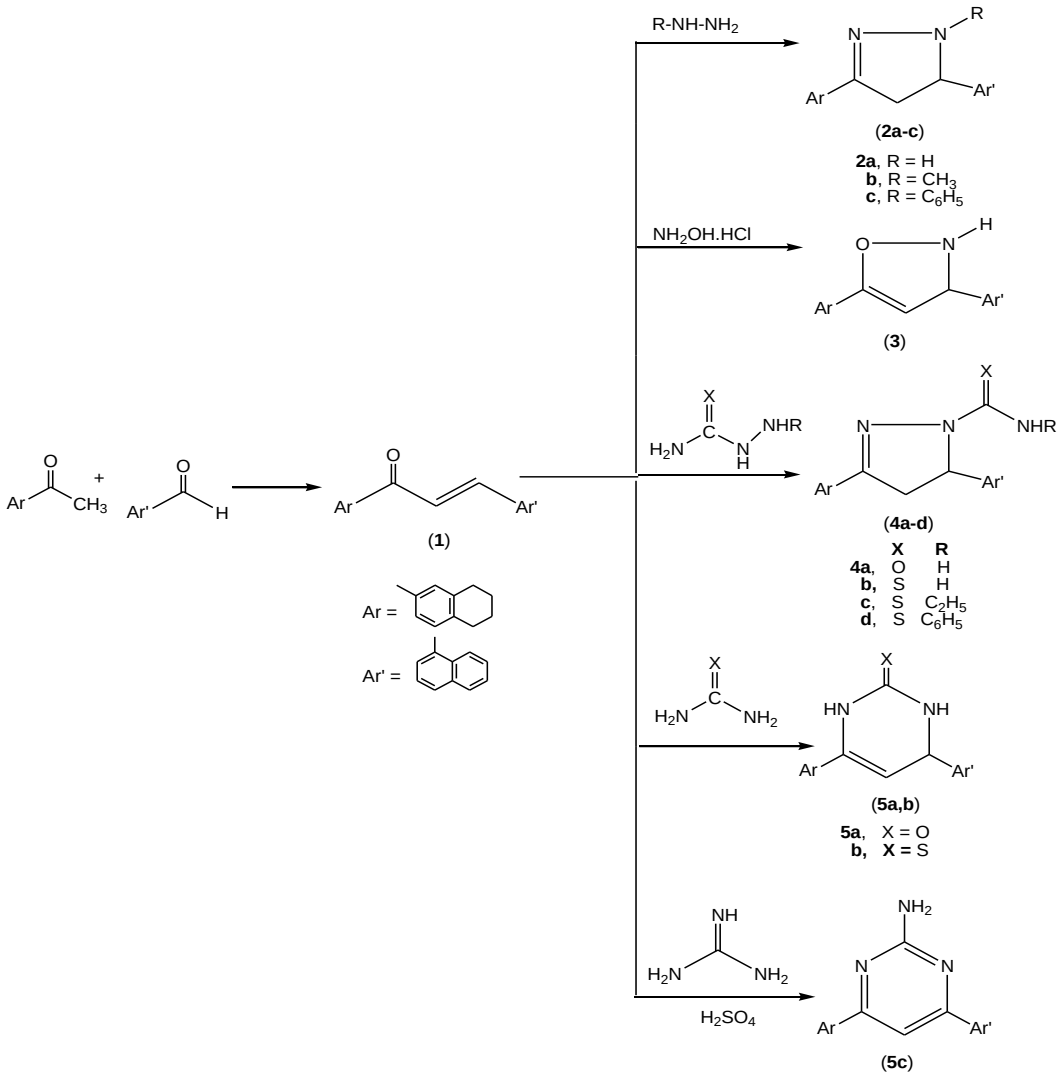
Chalcones are well known as intermediates for synthesizing various heterocyclic compounds [8]. The desired 3-(naphthalen-1-yl)-1-(5,6,7,8-tetrahydronaphthalen-2-yl)prop-2-en-1-one (**1**) was prepared by condensation of 1-naphthaldehyde with 2-acetyl(5,6,7,8-tetrahydronaphthalene) in 5% ethanolic sodium hydroxide. Upon reaction of **1** with hydrazine hydrate, methyl hydrazine, and / or phenyl hydrazine in refluxing ethanol gave the corresponding 5-(naphthalen-1-yl)-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-4,5-dihydro-1*H*-pyrazole (**2a**), 1-methyl-5-(naphthalen-1-yl)-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-4,5-dihydro-1*H*-pyrazole (**2b**) and 1-phenyl-5-(naphthalen-1-yl)-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-4,5-dihydro-1*H*-pyrazole (**2c**) respectively. Further, cyclo condensation of chalcone **1** with hydroxylamine hydrochloride in alcoholic sodium hydroxide afforded 3-(naphthalen-1-yl)-5-(5,6,7,8-tetrahydronaphthalen-2-yl)-2,3-dihydroisoxazole (**3**) (Scheme 1).

Also, the key compound **1** was allowed to react with substituted semicarbazide and / or substituted thiosemicarbazide namely semicarbazide, thiosemicarbazide, ethyl thiosemicarbazide and / or phenyl thiosemicarbazide in ethanolic sodium hydroxide gave 4,5-dihydro-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-5-(naphthalen-4-yl)pyrazole-1-carboxamide (**4a**), 4,5-dihydro-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-5-(naphthalen-4-yl)pyrazole-1-

carbothioamide (**4b**), *N*-ethyl-4,5-dihydro-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-5-(naphthalen-4-yl)pyrazole-1-carbothioamide (**4c**) and 4,5-dihydro-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-5-(naphthalen-4-yl)-*N*-phenylpyrazole-1-carbothioamide (**4d**) (Scheme 1).

In addition, reaction of chalcone **1** with urea and / or thiourea gave the corresponding 3,4-dihydro-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-4-(naphthalen-4-yl)pyrimidin-2(1*H*)-one (**5a**), and 3,4-dihydro-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-4-(naphthalen-4-yl)pyrimidine-2(1*H*)-thione (**5b**) while condensation of **1** with guanidine sulphate gave 4-(5,6,7,8-tetrahydronaphthalen-2-yl)-6-(naphthalen-4-yl)pyrimidin-2-amine (**5c**) (Scheme 1).

Scheme 1



The addition reaction of primary amine named cyclohexyl amine, *p*-amino pyridine and / or *p*-bromo aniline on parent chalcones **1** gave the corresponding addition products namely 3-(cyclohexylamino)-1-(1,2,3,4-tetrahydronaphthalen-5-yl)-3-(naphthalen-4-yl)propan-1-one (**6a**), 1-(1,2,3,4-tetrahydronaphthalen-5-yl)-3-(naphthalen-4-yl)-3-(pyridin-

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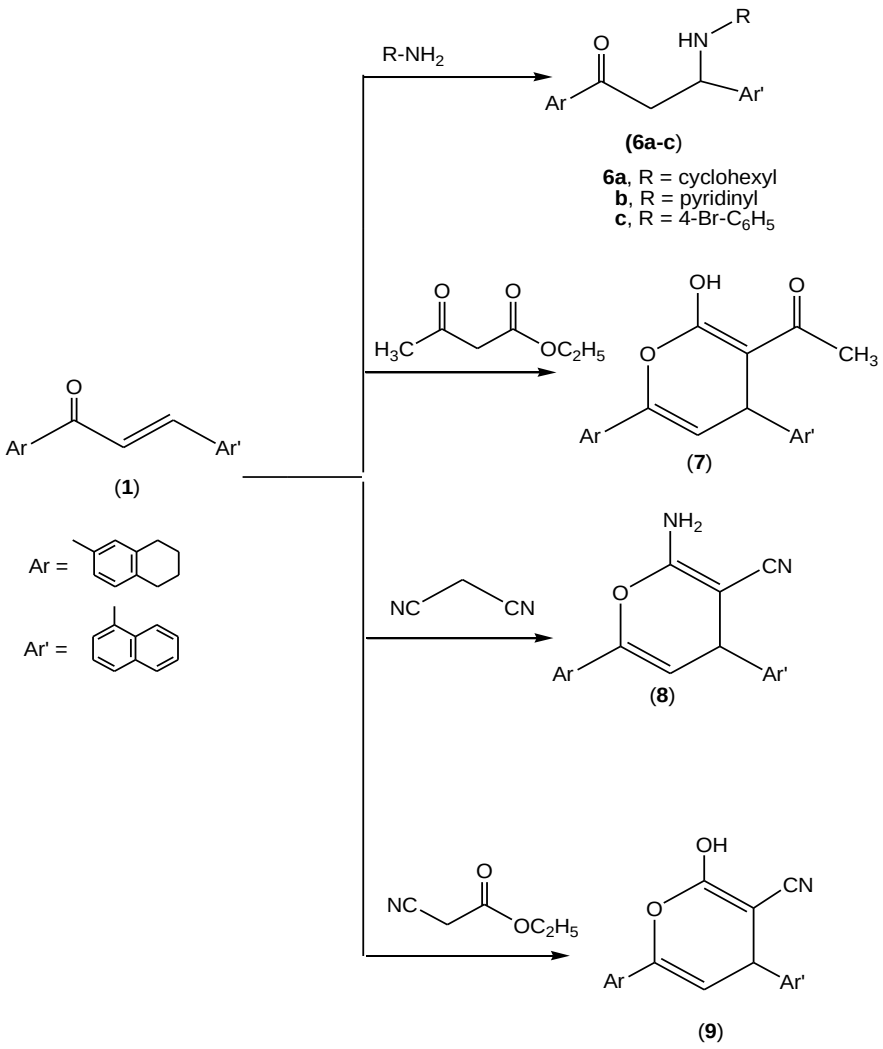
4-ylamino)propan-1-one (**6b**), and 3-(4-bromophenylamino)-1- (1,2,3,4 - tetrahydronaphthalen-5-yl)-3-(naphthalen-4-yl)propan-1-one (**6c**) respectively (Scheme 2).

Furthermore, cyclo condensation of *α,β*-unsaturated Ketones **1** with active methylene compounds namely ethyl acetoacetate, malononitrile, and / or ethyl cyanoacetate in sodium ethoxide afforded pyrane derivatives 1-(6-(1,2,3,4-tetrahydronaphthalen-5-yl)-2-hydroxy-4-(naphthalen-4-yl)-4*H*-pyran-3-yl)ethanone (**7**), 2-amino-6- (1,2,3,4- tetrahydronaphthalen-5-yl)-4-(naphthalen-4-yl)-4*H*-pyran-3-carbonitrile (**8**) and 6-(1,2,3,4-tetrahydronaphthalen-5-yl)-2-hydroxy-4-(naphthalen-4-yl)-4*H*-pyran-3-carbonitrile (**9**) (Scheme 2).

Scheme 2



One of our targets in this work is introduce thiazole ring system incorporated into the parent compounds. As it has been reported in many literatures, the potent biological activities of such compounds [9-11] and accordingly the pyrazole derivatives **4b** was allowed to condense with phenacyl bromide and / or 2-bromoacetyl-5,6,7,8-tetrahydronaphthalene to give 4,5-dihydro-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-5-(naphthalen-4-yl)-1-(4-phenylthiazol-2-yl)-1*H*-pyrazole (**10**) and 4,5-dihydro-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-1-(4-(1,2,3,4-tetrahydronaphthalen-7-yl)thiazol-2-yl)-5-(naphthalen-4-yl)-1*H*-pyrazole (**11**) respectively (Scheme 3).

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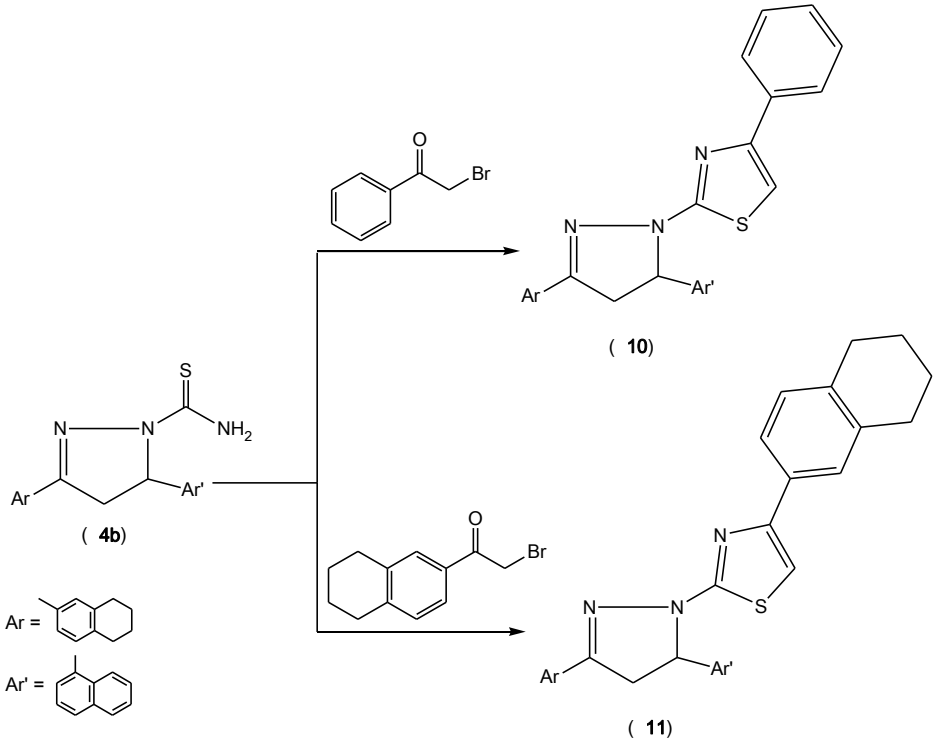
Scheme 3



Biological Activity

Some synthesized compounds were screened for their *in vitro* cytotoxic and growth inhibitory activities against two different cell lines of two different tumor types [12-16], namely Hep-G2 and CaCo-2 cells in comparison with the activity of the known anticancer reference drug DOX (reference standard). The cytotoxic activities of our tested compounds were expressed as IC_{50} values (the dose that reduces survival to 50%).

Regarding Hep-G2 cell, it is evident that all of the tested compounds showed anti-tumor activities with IC_{50} values ranging from 13.83-37.60 μg/ml. The activity of the tested compounds against Hep-G2 cell line had the following descending order (**6b**> **9**>**8**> **1**> **4b**> **2c**> **5b**)

Table 1: *In vitro* anticancer activity of selected compounds against Hep-G2 and CaCo-2 cell lines.

Compound	IC_{50}(μM)	
	Hep-G2 cells	CaCo.2 cells
1	19.34 ± 2.56	13.99 ± 0.28
2c	14.88 ± 5.74	9.99 ± 0.13
4b	15.15 ± 0.40	11.64 ± 1.29
5b	13.83 ± 6.83	5.07 ± 0.60
6b	37.6 ± 0.03	14.58 ±0.14
8	19.89 ± 1.37	10.21 ± 0.01
9	36.46 ± 0.86	10.56 ± 0.86
Doxo	16.2 ± 4.1	6.90 ± 1.01

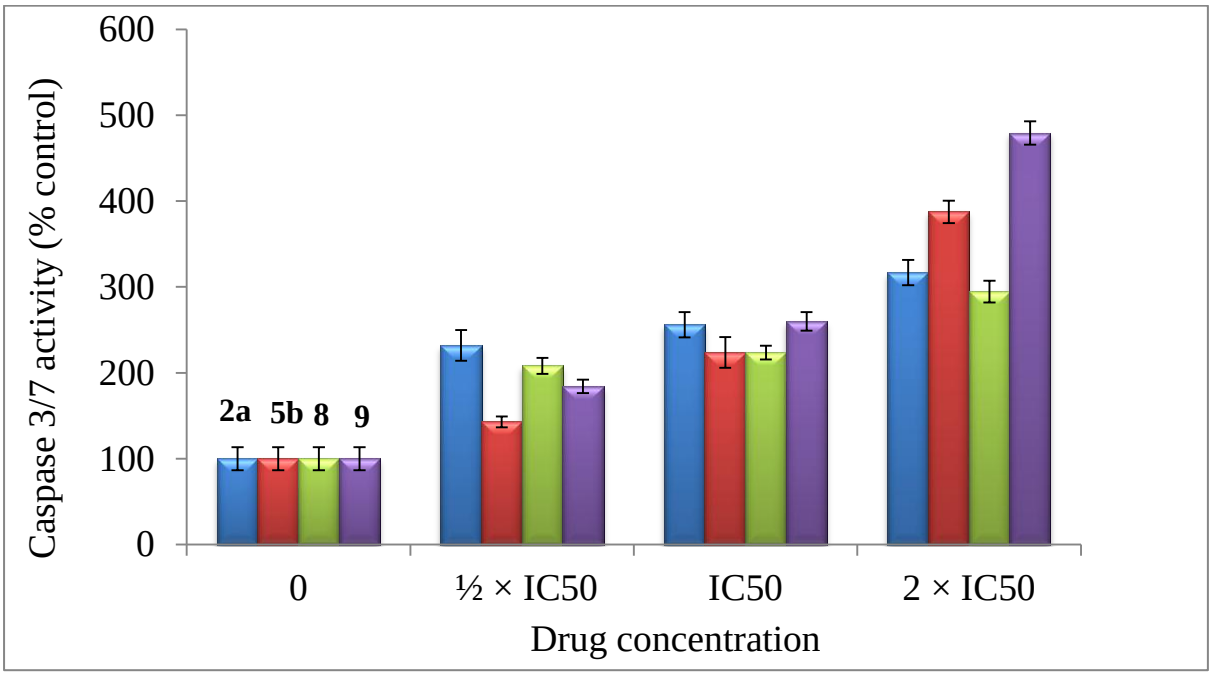
ISSN: 0975-8585

Concerning the activity of our compounds against Caco-2 cell line, table 1 showed very interesting results. The IC_{50} values ranged from 5.07-14.58 µg/ml. Compound **5b** possessed the highest degree of cytotoxicity, recording 5.07 µg/ml **(Fig. 1)**, which were three times more active than DOX (IC_{50}: 6.90 µg/ml). It is clear from the above results and from table 1, that the compound **5b** was the most potent anticancer agents among all the synthesized compounds in our study (**Fig. 1**).

Caspase 3/7 assay

It is well established that the induction of the apoptotic cascade is one of the main mechanisms of chemotherapy-induced cell death [12-16]. To determine whether the chemosensitizing effect of our compounds demonstrated above is secondary to their ability to activate the apoptotic cascade, the pancreatic resistant cell line Panc-1 was treated with test samples (2 × IC_{50}; IC_{50}; ½ × IC_{50}) or DMSO (solvent control). Six hours after treatment, the activity of caspase 3/7 was measured using the Caspase-Glo 3/7 assay. **Fig. 1** shows that our tested compounds caused significant increase in activation of caspase- 3/7 in a dose dependent manner. These results suggest that samples-induced apoptosis was, in part, due to activation of caspases 3/7.

Figure 1: Caspase 3/7 assay result of compounds 2a, 5b, 8 and 9.



DISCUSSION

The results displayed in table 1 showed that all the synthesized compounds had broad spectrum antitumor activity against all screened cancer cell lines regardless of tumour type. Some of our compounds exhibited potent antiproliferative activity against CaCo-2 and Hep-G2 cell lines (Table 1). These results suggest that the tetrahydronaphthalene backbone is an interesting antitumor pharmacophore. Moreover, some of our compounds were even more potent than the standard drug Doxorubicin. At the cellular level, the results of caspase

3/7 assay suggest that **2a**, **5b**, **8** and **9** samples-induced apoptosis is, in part, due to activation of caspases 3/7. Apoptosis is a programmed cell death and is an important controller of physiological growth. In addition, apoptosis regulates tissue homeostasis. The stimulation of apoptosis signal transduction pathways in cancer cells is the main mechanism for the activity of currently available chemotherapy and/or immunotherapy [12-16]. It is known that the stimulation of the apoptotic cascade is one of the main mechanisms of chemotherapy induced cell death [12-16].

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