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Synthesis and Cytotoxicity Evaluation of Some New Pyrimidinethione and Thiazolopyrimidine Derivatives Linked to N-Propylpiperidone

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ABSTRACT

New pyrimidinethione derivatives were synthesized in good yields from 2,6-bisarylmethylene-N-propylpiperidone as starting material. These derivatives were allowed to react with chloroacetic acid and 2-bromopropanoic acid to give thiazolopyrimidine derivatives. Bisarylmethylene was also reacted with 2-amino thiouracil to yield pyridothiopyrimidine derivative, which reacted with halo acids to give pyridothiazolopyrimidine derivatives. The structures were elucidated on the basis of NMR, MS, FTIR and elemental analyses. Further, the cytotoxic activity of the synthesized compounds were evaluated in vitro against human HepG-2 (liver carcinoma), PC-3 (prostate adenocarcinoma) and HCT116 (colorectal carcinoma) cell lines using MTT assay.

Key words: Piperidone, pyimidinethione, thiazolpyrimidine, cytotoxic activity.

INTRODUCTION

Pyrimidine is the parent substance of a large group of heterocyclic compounds and plays a significant role in many biological processes, as found in nucleic acids, some vitamins, co-enzymes and purines. Also, pyrimidinthione derivatives have significant interest in medicinal chemistry as they have a wide range of pharmaceutical and pharmacological industries. Various biological applications have been reported for pyrimidinethiones such as antibactrial, antifungal, anticancer [1-3], antimicrobial, anti-tubercular [4,5], analgesic, anti-inflammatory [6-8] and anti-HIV [9]. Thiazolopyrimidines are also of pharmacological interest due to their anti-inflammatory [10,11], antiperkinsonian [12], antimicrobial [13], anticancer [14] and antiviral activity as inhibitors of HIV-1[15].

Based on the above observations and in continuation of our research work [16-21] we reported here the synthesis of some new fused pyrimidine derivatives containing *N*-propylpiperidone moiety. The synthesized compounds were screened for their antitumor activities using the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.

MATERIALS AND METHODS

Chemistry

The melting points were determined on the Electrothermal 9100 melting point apparatus (Electrothermal, UK) and were uncorrected. Thin layer chromatography were performed using HF254 fluorescent silica gel plates (Merck), which were examined under UV254 and 365 nm light. The elemental analysis for C, H, N and S were performed by Micro analytical center, Cairo University, Cairo, Egypt. Infrared spectra (ν, cm^{-1}) were recorded on Jasco FT-IR

4100 instruments using KBr Disks. The NMR spectra were measured with Bruker 600 MHz (^{1}H, 600 MHz; ^{13}C, 150 MHz, Switzerland) and Jeol ECA 500 (^{1}H, 500 MHz; ^{13}C, 125.7 MHz, Japan) spectrometers in DMSO-d_6 or $CDCl_3$ and chemical shifts were recorded in δ ppm relative to TMS. The mass spectra were run at: ESI-TOF-MS (micr OTOFII, Bruker) and EI-MS, 70 eV with a Finnigan SSQ 7000 spectrometer (Cairo university, Egypt).

Synthesis of 3,5-bisarylmethylene-1-propyl-4-piperidone (2a,b)

To a mixture of *N*-propyl-4-piperidone **1** (10 mmol) and aromatic aldehydes, namely, 4-methoxybenzaldehyde or 4-fluorobenzaldehyde (20 mmol) in EtOH (100 mL), 1 g KOH in H_2O (1ml) was added. The mixture was stirred at room temperature for 1 hr. The obtained solid was filtered off, dried and crystallized from the proper solvent to give the corresponding arylmethylene derivatives **2a,b**, respectively.

3,5-Bis-(4-methoxybenzylidene)-1-propyl-piperidin-4-one (2a)

Yield 85%; mp 145–147°C (EtOH); IR (KBr, cm^{-1}): 1675; ^{1}H NMR ($CDCl_3$): δ 7.85 (s, 2H, benzylic proton), 7.40-6.95 (m, 8H, ArH), 3.80 (s, 6H, 2OCH_3), 3.75 (s, 4H, *N*-$(CH_2)_2$), 2.55 (t, 2H, *N*-CH_2-propyl), 1.53 (m, 2H, CH_2-propyl), 0.93 (t, 3H, CH_3-propyl); ^{13}C NMR ($CDCl_3$): δ 187.4, 160.2, 135.9, 132.3, 114, 59.4, 55.3. 20.5, 11.8; HRESI-MS: *m/z* 378.2072 $[M + H]^+$, calculated $[M+H]^+$ ion for $C_{24}H_{27}NO_3$, *m/z* 378.4813.

3,5-Bis-(4-fluorobenzylidene)-1-propyl-piperidin-4-one (2b)

Yield 90%; mp 125–127°C (EtOH); IR (KBr, cm^{-1}): 1680; ^{1}H NMR ($CDCl_3$): δ 7.77 (s, 2H, benzylic proton), 7.42-7.13 (m, 8H, ArH), 3.79 (s, 4H, *N*-$(CH_2)_2$), 2.51 (t, 2H, *N*-CH_2-propyl), 1.48 (m, 2H, 2-CH_2-propyl), 0.89 (t, 3H, CH_3-propyl); EI-MS: *m/z* 352 [M - 1] (9), 324 (10), 261 (8), 95 (100); Anal. Calcd for $C_{22}H_{21}FNO$: C, 74.77; H, 5.99; N, 3.96. Found: C, 74.65; H, 5.86; N, 3.87.

Synthesis of pyrimidinthione derivatives (3a,b)

Method A

A mixture of compound **1** (10 mmol), aromatic aldehydes, namely, 4-methoxybenzaldehyde or 4-fluorobenzaldehyde (20 mmol) and thiourea (0.76 g, 10 mmol) in ethanolic potassium hydroxide (1 g in 100 mL ethanol) was refluxed for 6 h. The reaction mixture was allowed to cool then poured onto cold water and the solid formed was filtered off, washed with water, dried and crystallized from the proper solvent to give the corresponding pyrimidinethione derivatives **3a,b**, respectively.

Method B

A mixture of compound **2a,b** (10 mmol) and thiourea (0.76 g, 10 mmol) in ethanolic potassium hydroxide (1 g in 100 mL ethanol) was refluxed for 3 h. The reaction mixture was allowed to cool and the solid formed was filtered off, washed with water, dried and crystallized from the proper solvent to give the corresponding pyrimidinethione derivatives **3a,b**, respectively, in better yield than method A.

8-(4-Methoxy-benzylidene)-4-(4-methoxy-phenyl)-6-propyl-3,4,5,6,7,8-hexahydro-1H-pyrido[4,3-d]pyrimidine-2-thione (3a)

Yield, [A] 60%, [B] 85%; mp 128–130°C (EtOH); IR (KBr, cm^{-1}): 3235; ^{1}H NMR ($CDCl_3$): δ 7.75, 7.68 (2s, 2H, NH exchangeable with D_2O), 7.45-6.59 (m, 9H, ArH + benzylic proton), 5.02 (s, 1H, CH-pyrimidine), 3.86, 3.82 (2s, 6H, 2OCH_3), 3.64, 3.28, 3.05, 2.57 (4br, 4H, N$(CH_2)_2$), 2.26 (m, 2H, CH_2-*N*-propyl), 1.33 (m, 2H, 2-CH_2-propyl), 0.78 (t, 3H, CH_3-propyl); ^{13}C NMR ($CDCl_3$): δ 159.8, 158.9, 153, 130.7, 128.5, 125.4, 125, 121.8, 114.3, 113.9, 59.2, 59.1, 55.3, 55.3, 52.6, 52, 20, 11.7; HRESI-MS: *m/z* 436.2067 $[M + H]^+$, calculated $[M+H]^+$ ion for $C_{25}H_{29}N_3O_2S$, *m/z* 436.5805.

8-(4-Fluoro-benzylidene)-4-(4-fluoro-phenyl)-6-propyl-3,4,5,6,7,8-hexahydro-1H-pyrido[4,3-d]pyrimidine-2-thione (3b)

Yield, [A] 65%, [B] 90%; mp 227–230°C (EtOH); IR (KBr, cm^{-1}): 3216; ^{1}H NMR ($CDCl_3$): δ 7.65, 7.57 (2s, 2H, NH exchangeable with D_2O), 7.27–6.92 (m, 9H, ArH+ benzylic proton), 5.10 (s, 1H, CH-pyrimidine), 3.46 (m, 4H, *N*-$(CH_2)_2$), 2.1 (m, 2H, *N*-CH_2-propyl), 1.25 (m, 2H, 2-CH_2-propyl), 0.74 (t, 3H, CH_3-propyl); EI-MS: *m/z* 410 [M - 1] (100), 302 (62), 287 (60), 229 (18); Anal. Calcd for $C_{23}H_{23}F_2N_3S$: C, 67.13; H, 5.63; N, 10.21; S, 7.79. Found: C, 67.08; H, 5.56; N, 10.12; S, 7.82.

Synthesis of pyrimidinone derivatives (4a,b)

A mixture of compound **2a,b** (10 mmol) and urea (10 mmol) in EtOH (50 ml) containing NaOH (0.5 g) was refluxed for 6-8 hr. After cooling, the reaction mixture was poured onto ice-water, the solid formed was collected by filtration, air dried and crystallized from the proper solvent to give the compounds **4a,b**.

8-(4-Methoxy-benzylidene)-4-(4-methoxy-phenyl)-6-propyl-3,4,5,6,7,8-hexahydro-1H-pyrido[4,3-d]pyrimidin-2-one (4a)

Yield 65%, mp 157–159°C (EtOH); IR (KBr, cm^{-1}): 3432, 1654; ^{1}H NMR ($CDCl_3$): δ 8.05., 7.75 (2s, 2H, NH exchangeable with D_2O), 7.45 – 6.85 (m, 9H, ArH+ benzylic proton), 4.90 (s, 1H, CH-pyrimidine), 4.15 & 3.95 (s, 6H, $2OCH_3$), 3.40 (s, 4H, *N*-$(CH_2)_2$), 2.35 (t, 2H, *N*-CH_2-propyl), 1.40 (m, 2H, 2-CH_2-propyl), 0.80 (t, 3H, CH_3-propyl); EI-MS: *m/z* 418 $[M - 1]^+$ (75), 453 (65), 285 (54); Anal. Calcd for $C_{25}H_{29}N_3O_3$: C, 71.57; H, 6.97; N, 10.02. Found: C, 71.51; H, 6.89; N, 10.09.

8-(4-Fluoro-benzylidene)-4-(4-fluoro-phenyl)-6-propyl-3,4,5,6,7,8-hexahydro-1H-pyrido[4,3-d]pyrimidin-2-one (4b)

Yield 70%, mp 228-230°C (EtOH); IR (KBr, cm^{-1}): 3435, 1702; ^{1}H NMR (DMSO-d_6): δ 8.20, 7.61 (2s, 2H, NH exchangeable with D_2O), 7.36–6.96 (m, 9H, ArH + benzylic proton), 4.97 (s, 1H, CH-pyrimidine), 3.76, 3.63 (2br, 4H, *N*-$(CH_2)_2$), 2.21 (2H, m, *N*-CH_2-propyl), 1.33 (m, 2H, 2-CH_2-propyl), 0.78 (t, 3H, CH_3-propyl); ^{13}C NMR (DMSO-d_6): δ 159.8, 158.9, 130.7, 128.5, 125.4, 125., 121.8, 114.3, 113.9, 59.2, 59.1, 55.3, 55.3, 52.6, 52, 20, 11.7; EI-MS: *m/z* 394 [M - 1] (95), 271 (100), 243 (58); Anal. Calcd for $C_{23}H_{23}F_2N_3O$: C, 69.86; H, 5.86; N, 10.63. Found: C, 69.79; H, 5.77; N, 10.57.

Synthesis of aminopyrimidine derivatives (5a,b)

To a mixture of compound **2a.b** (10 mmol) and guanidine hydrochloride (10 mmol) in EtOH (50 ml), NaOH (0.8 g) in H_2O (1 ml) was added. The mixture was refluxed for 5 hr., then allowed to cool. The solid formed was filtered off and crystallized from the proper solvent to give the corresponding aminopyrimidine derivatives **5a,b**.

8-(4-Methoxybenzylidene)-5,6,7,8-tetrahydro-4-(4-methoxyphenyl)-6-propylpyrido[4,3-d]pyrimidin-2-amine (5a)

Yield 75%; mp 190–192°C (EtOH); IR (KBr, cm^{-1}): 3480, 3319; ^{1}H NMR ($CDCl_3$): δ 8.10 (s, 1H, benzylic proton) 7.55-6.96 (m, 8H, ArH), 4.98 (s, 2H, NH_2 exchangeable with D_2O) 3.87, 3.86 (2s, 6H, $2OCH_3$), 3.76, 3.48 (2br, 4H, *N*$(CH_2)_2$), 2.45 (br, 2H, *N*CH_2-propyl), 1.44 (m, 2H, 2-CH_2-propyl), 0.84 (t, 3H, CH_3-propyl); ^{13}C NMR ($CDCl_3$): δ 165.2, 159.8, 159.2, 131.4, 130.1, 130.4, 129.2, 129, 115.2, 113.8, 113.4, 59.6, 55.37, 55.3, 54.2, 53.5, 50.8, 20.4, 11.8; HRESI-MS: *m/z* 417.2289 $[M + H]^+$, calculated $[M + H]^+$ ion for $C_{25}H_{28}N_4O_2$, *m/z* 417.5301.

8-(4-Fluorobenzylidene)-4-(4-fluorophenyl)-5,6,7,8-tetrahydro-6 propylpyrido[4,3-d]pyrimidin-2-amine (5b)

Yield 75%; mp 148–150°C (EtOH); IR (KBr, cm^{-1}): 3420, 3305; ^{1}H NMR ($CDCl_3$): δ 8.09 (s, 1H, benzylic proton) 7.56-7.08 (m, 8H, ArH), 5.07 (s, 2H, NH_2 exchangeable with D_2O), 3.70, 3.58 (2br, 4H, *N*-$(CH_2)_2$), 2.44 (t, 2H, *N*-CH_2-propyl), 1.25 (m, 2H, 2-CH_2-propyl), 0.83 (t, 3H, CH_3-propyl); EI-MS: *m/z* 391 $[M - 1]^+$ (100), 363 (90), 334 (30), 283 (38); Anal. Calcd for $C_{23}H_{22}F_2N_4$: C, 70.39; H, 5.65; N, 14.28. Found: C, 70.31; H, 5.58; N, 14.20.

General procedure for the synthesis of thiazolopyrimidine derivatives (6a,b) and (8a,b).

A mixture of compound **3a,b** (10 mmol), chloroacetic acid or 2-bromopropanoic acid (10 mmol), sodium acetate anhydrous (2 g) in glacial acetic acid (30 ml) and acetic anhydride (10 ml) was refluxed for 3 hr. After cooling, the reaction mixture was poured gradually with stirring onto cold water, the solid formed was filtered off, washed with water, dried and crystallized from proper solvent to give the corresponding thiazolopyrimidine derivatives **6a,b** and **8a,b**, respectively.

8-(4-Methoxy-benzylidene)-4-(4-methoxy-phenyl)-6-propyl-5,6,7,8-tetrahydro-4H-1-thia-3a,6,9-triaza-cyclopenta[b]naphthalen-3-one (6a)

Yield 65%; mp 158–160°C (EtOH); IR (KBr, cm^{-1}): 1725; ^{1}H NMR ($CDCl_3$): δ 7.45–6.88 (m, 9H, ArH + benzylic proton), 5.45 (s, 1H, CH-pyrimidine), 3.84, 3.80 (2s, 6H, $2OCH_3$), 3.83, 3.72 (2br, 2H, CH_2-thiazole), 3.70, 3.48, 3.08, 2.83 (2dd, *J* = 16 & 14 Hz, 4H, N$(CH_2)_2$), 2.33 (m, 2H, *N*-CH_2-propyl), 1.37 (m, 2H, 2-CH_2-propyl), 0.79 (t, *J* = 4.9 Hz, 3H, CH_3-propyl); ^{13}C NMR ($CDCl_3$): δ 170.6, 159.9, 158.4, 153.5, 133.6, 131.2, 130.6, 129.9, 129.3, 125, 114.1, 113.6, 113.4, 59.1, 58.1, 55.2, 55.2, 53.2, 52.9, 31.7, 20.3, 11.7; HRESI-MS: *m/z* 476.2010 $[M + H]^+$, calculated $[M+H]^+$ ion for $C_{27}H_{29}N_3O_3S$, *m/z* 476.6170.

8-(4-Fluoro-benzylidene)-4-(4-fluoro-phenyl)-6-propyl-5,6,7,8-tetrahydro-4H-1-thia-3a,6,9-triaza-cyclopenta [b]naphthalen-3-one (6b)

Yield 65%; mp 173–175°C (EtOH); IR (KBr, cm^{-1}): 1730; ^{1}H NMR ($CDCl_3$): δ 7.47–7.03 (m, 9H, ArH + benzylic proton), 5.48 (s, 1H, CH-pyrimidine), 3.76 (s, 2H, CH_2- thiazole), 3.62, 3.25 (br, 4H, N$(CH_2)_2$), 2.35 (m, 2H, *N*-CH_2-propyl), 1.34 (m, 2H, 2-CH_2- propyl), 0.82 (t, 3H, CH_3-propyl); EI-MS: *m/z* 450 $[M - 1]^+$ (100), 393 (4), 287 (9); Anal. Calcd for $C_{25}H_{23}F_2N_3OS$: C, 66.50; H, 5.13; N, 9.31; S, 7.10 Found: C, 66.35; H, 5.28; N, 9.50; S, 7.21.

8-(4-Methoxy-benzylidene)-4-(4-methoxy-phenyl)-2-methyl-6-propyl-5,6,7,8-tetrahydro-4H-1-thia-3a,6,9-triaza-cyclopenta[b]naphthalen-3-one (8a)

Yield 55%; mp 238–240°C (EtOH); IR (KBr, cm^{-1}): 1724; ^{1}H NMR ($CDCl_3$): δ 7.55-6.90 (m, 9H, ArH + benzylic proton), 5.38 (s, 1H, CH-pyrimidine), 3.85, 3.81 (2s, 6H, $2OCH_3$), 4.13 (m, 1H, CH-thiazole), 3.35 (m, 4H, *N*-$(CH_2)_2$), 2.35 (m, 2H, *N*- CH_2-propyl), 1.51 (br, 3H, CH_3-thiazole), 1.51 (m, 2H, 2-CH_2-propyl), 0.74 (br, 3H, CH_3-propyl); ^{13}C NMR ($CDCl_3$): δ 173.9, 160.4, 159.8, 155, 133, 131, 130.7, 129, 128.4, 127, 118, 114.8, 114.3, 58, 55.4, 54, 51, 41.7, 19.3, 10.9; HRESI-MS: *m/z* 490.2154 [M + H], calculated $[M + H]^+$ ion for $C_{28}H_{31}N_3O_3S$, *m/z* 490.6438.

8-(4-Fluoro-benzylidene)-4-(4-fluoro-phenyl)-2-methyl-6-propyl-5,6,7,8-tetrahydro-4H-1-thia-3a,6,9-triaza-cyclopenta[b]naphthalen-3-one (8b)

Yield 55%; mp 165–167°C (EtOH); IR (KBr, cm^{-1}): 1733; ^{1}H NMR ($CDCl_3$): δ 7.46–7.02 (m, 9H, ArH + benzylic proton), 5.45 (s, 1H, CH-pyrimidine), 3.77 (m, 1H, CH, thiazole), 3.46 (m, 4H, *N*-$(CH_2)_2$), 2.33 (m, 2H, *N*-CH_2-propyl), 1.47 (d, 3H, CH_3-thiazole), 1.27 (m, 2H, 2-CH_2-propyl), 0.82 (t, 3H, CH_3-propyl); EI-MS: *m/z* 464 [M - 1] (100), 376 (6), 287 (11), 133 (33); Anal. Calcd for $C_{26}H_{25}F_2N_3OS$: C, 67.08; H, 5.41; F, 8.16; N, 9.03; S, 6.89 Found: C, 67.15; H, 5.29; N, 9.12; S, 6.81

Synthesis of arylmethylene thiazolopyrimidine derivatives (7a,b)

Method A

A mixture of compound **3a,b** (10 mmol), chloroacetic acid (10 mmol), sodium acetate anhydrous (2 g) in glacial acetic acid (30ml) and acetic acid anhydride (10 ml) was refluxed for 12 min., then equimolecular amount of the appropriate aromatic aldehydes, namely, 3,4-drimethoxybenzaldehyde or 3,4,5-trimethoxybenzaldehyde (2 mmol) was added. The reaction mixture was refluxed for 2 h, allowed to cool, poured onto cold water; the formed precipitate was filtered off, dried and crystallized from proper solvent to give the corresponding arylmethylene thiazolopyrimidine derivatives **7a,b**, respectively.

Method B

A mixture of compound **6a,b** (10 mmol), equimolecular amount of appropriate aromatic aldehydes, namely, 3,4-dimethoxybenzaldehydes or 3,4,5-trimethoxybenzaldehyde (10 mmol), sodium acetate anhydrous (2 g) in a mixture of acetic acid (30 ml) and acetic anhydride (10 ml) was refluxed for 2 hr. After cooling, the reaction mixture was poured onto cold water, the formed solid was collected by filtration and crystallized from the proper solvent to give the corresponding arylmethylene thiazolopyrimidine derivatives **7a,b**, respectively. The products were identified by their m.p. and R_f-values in comparison with authentic samples previously obtained by method A.

2-(3,4-Dimethoxy-benzylidene)-8-(4-methoxy-benzylidene)-4-(4-methoxy-phenyl)-6-propyl-5,6,7,8-tetrahydro-4H-1-thia-3a,6,9-triazacyclopenta[b]naphthalen-3-one (7a)

Yield [A] 80%, [B] 65%; mp 165–167°C (EtOH); IR (KBr, cm^{-1}): 1695; ^{1}H-NMR ($CDCl_3$): δ 7.62, 7.48 (2s, 2H, benzylic protons), 7.38-6.88 (m, 11H, ArH), 5.60 (s, 1H, CH-pyrimidine), 3.95, 3.84, 3.79 (3s, 12H, $4OCH_3$), 3.74, 3.50, 3.15, 2.88 (2dd, 4H, $N(CH_2)_2$), 2.35 (br, 2H, *N*-CH_2-propyl), 1.38 (m, 2H, 2-CH_2-propyl), 0.80 (br, 3H, CH_3-propyl); ^{13}C NMR ($CDCl_3$): δ 170.6, 165,7, 159.8, 158.4, 150.7, 149.2, 133.9, 131.3, 129.36, 130.6, 129.8, 129.3, 126.6, 125, 124.4, 118.1, 114.2, 113.6, 111.8, 111.3, 59.1, 58.1, 56, 55.9, 55.2, 53.2, 53, 31.7, 20.3, 11.7; HRESI-MS: *m/z* 624.2533 $[M + H]^+$, calculated $[M+H]^+$ ion for $C_{36}H_{37}N_3O_5S$, *m/z* 624.7772.

8-(4-Fluoro-benzylidene)-4-(4-fluoro-phenyl)-6-propyl-2-(3,4,5-trimethoxy-benzylidene)-5,6,7,8-tetrahydro-4H-1-thia-3a,6,9-triazacyclopenta[b]naphthalen-3-one (7b)

Yield [A] 85%, [B] 70%; mp 208–210°C (EtOH); IR (KBr, cm^{-1}): 1698; ^{1}H NMR ($CDCl_3$): δ 7.60, 7.45 (2s, 2H, benzylic protons), 7.40–6.90 (m, 10H, ArH), 5.65 (s, 1H, CH-pyrimidine), 3.91 (s, 9H, $3OCH_3$), 3.65 (s, 4H, *N*-$(CH_2)_2$), 2.34 (m, 2H, *N*-CH_2-propyl), 1.27 (m, 2H, 2-CH_2-propyl), 0.81 (t, 3H, CH_3-propyl); EI-MS: *m/z* 628 [M - 1] (80), 585 (10), 376 (25); Anal. Calcd for $C_{35}H_{33}F_2N_3O_4S$: C, 66.76; H, 5.28; N, 6.67; S, 5.09; Found: C, 66.65; H, 5.24; N, 6.62; S, 5.34

2-[N'-(4-Fluoro-phenyl)-hydrazino]-8-(4-methoxy-benzylidene)-4-(4-methoxy-phenyl)-6-propyl-5,6,7,8-tetrahydro-4H-1-thia-3a,6,9-triaza-cyclopenta[b]naphthalen-3-one (9)

A solution of *p*-fluoroaniline (10 mmol) in hydrochloric acid (3 ml) and water (10 ml) was rapidly cooled below 0ºC and diazotized by the addition of sodium nitrite (10 mmol) in water (5 ml) under vigorous stirring. After an hour, the diazonium salt was added to a well cooled, stirred mixture of compound **6a** (2 mmol) in 10% aqueous NaOH (10 ml) containing excess sodium acetate. The mixture was kept at room temperature for one day. The precipitate was filtered off, washed with water, dried and recrystallized from the proper solvent to give compound **9**.

Yield 70%; mp 223–225°C (EtOH); IR (KBr, cm^{-1}): 1723; ^{1}H NMR (DMSO-d_6): δ 11.34 (br, 2H, 2NH exchangeable with D_2O), 7.61 (br, 1H, benzylic proton), 7.36–6.84 (m, 12H, ArH), 5.85 (s, 1H, CH-pyrimidine), 5.68 (s, 1H, *N*-CH-thiazole), 3.79, 3.74 (2s, 6H, $2OCH_3$), 3.50 (m, 4H, *N*-$(CH_2)_2$), 2.49 (t, 2H, *N*-CH_2-propyl), 1.56 (m, 2H, CH_2-propyl), 0.76 (t, 3H, CH_3-propyl), ^{13}C NMR (DMSO-d_6): δ 170, 160.5, 159.4, 158.9, 156.2, 150.6, 139.9, 130.7, 128.5, 127.3, 121.3, 120.6, 115.6, 115.4, 114.2, 114.1, 55.9, 55.7, 50.4, 48.2, 31.7, 16.8, 10.6; EI-MS: *m/z* 598 $[M - 1]^+$ (15), 530 (30), 474 (75); Anal. Calcd for $C_{33}H_{34}FN_5O_3S$: C, 66.09; H, 5.71; N, 11.68; S, 5.35. Found: C, 66.17; H, 5.62; N, 11.74; S, 5.26.

(Z)-9-(4-fluorobenzylidene)-5-(4-fluorophenyl)-2,3,6,7,8,9-hexahydro-7-propyl-2-thioxopyrimido[4,5-b][1,6] naphthyridin-4(1H)-one (11)

A mixture of compound **2** (10 mmol) and (10 mmol) of 6-amino-thiouracil **(10)**, which was prepared according to literature [22] in glacial acetic acid (40 mL) was refluxed for 10 h. The reaction mixture was cooled and poured onto ice-cold water and the solid product was collected by filtration and crystallized to give compound **11**.

Yield 70%; mp 208–210°C (Dioxan); IR (KBr, cm^{-1}): 3421, 3244; ^{1}H NMR (DMSO-d_6): δ 12.27, 12.93 (2s, 2H, NH exchangeable with D_2O), 8.05 (s, 1H, benzylic proton), 7.42–7.20 (m, 8H, ArH), 3.50 & 3.00 (m, 4H, *N*-$(CH_2)_2$), 2.27 (t, 2H, *N*-CH_2-propyl), 1.20 (m, 2H, CH_2-propyl), 0.85 (t, 3H, CH_3-propyl); EI-MS: *m/z* 476 $[M]^+$ (100), 447, 418, 367; Anal. Calcd for $C_{26}H_{22}F_2N_4OS$: C, 65.53; H, 4.65; F, 7.97; N, 11.76; S, 6.73. Found: C, 65.42; H, 4.76; N, 11.65; S, 6.62.

Synthesis of Compounds 12 and 13

A mixture of compound **11** (10 mmol) , bromoacetic acid or *α*-bromopropanoic acid (10 mmol), and fused sodium acetate (2 g) in glacial acetic acid (30 mL) and acetic anhydride (10 mL) was refluxed for 3 h, left to cool, then poured gradually with stirring onto cold water, the solid formed was filtered off, washed with water and crystallized from ethanol to give compounds **12** and **13**, respectively.

9-(4-Fluoro-benzylidene)-5-(4-fluoro-phenyl)-7-propyl-6,7,8,9-tetrahydro-1-thia-3a,7,10,11-tetraazacyclo penta[b]anthrace-3,4-dione (12)

Yield 55%; mp 258–260°C (EtOH); IR (KBr, cm^{-1}): 1710; ^{1}H NMR ($CDCl_3$): δ 7.51 (s, 1H, benzylic proton), 7.28–6.96 (m, 8H, ArH), 3.92 (s, 2H, CH_2-thiazole), 3.47 (s, 4H, *N*-$(CH_2)_2$), 2.77 (m, 2H, *N*-CH_2-propyl), 1.07 (m, 2H, 2-CH_2-propyl), 0.71 (t, 3H, CH_3-propyl); EI-MS: *m/z* 517 $[M+1]^+$ (68), 475 (82), 447 (87); Anal. Calcd for $C_{28}H_{22}F_2N_4O_2S$: C, 65.10; H, 4.29; N, 10.85; S, 6.21. Found: C, 65.17; H, 4.36; N, 10.75; S, 6.29.

9-(4-Fluoro-benzylidene)-5-(4-fluoro-phenyl)-2-methyl-7-propyl-6,7,8,9-tetrahydro-1-thia-3a,7,10,11-tetraaza cyclopenta[b]anthrace-3,4-dione (13)

Yield 65%; mp 264–267°C (EtOH); IR (KBr, cm^{-1}): 1690; ^{1}H NMR (DMSO-d_6): δ 8.02–7.08 (m, 9H, ArH+ benzylic proton), 4.29 (d, 1H, CH-thiazole), 3.77 (m, 4H, *N*-$(CH_2)_2$), 2.89 (m, 2H, *N*-CH_2-propyl), 1.52 (m, 3H, CH_3-thiazole); 1.29 (m, 2H, 2-CH_2-propyl), 0.72 (t, 3H, CH_3-propyl); ^{13}C NMR (DMSO-d_6): δ 173.4, 170, 160.1, 153.4, 145.3, 131.7, 129.3, 125.5, 115.1, 114.7, 57.4, 52.2, 18.9, 11.1; EI-MS: *m/z* 531 $[M + 1]^+$ (38), 476 (75), 447 (60). 381 (73); Anal. Calcd for $C_{29}H_{24}F_2N_4O_2S$: C, 65.65; H, 4.56; N, 10.56; S, 6.04. Found: C, 65.58; H, 4.43; N, 10.63; S, 6.12.

2-(4-Chloro-benzylidene)-9-(4-fluoro-benzylidene)-5-(4-fluoro-phenyl)-7-propyl-6,7,8,9-tetrahydro-1-thia-3a,7,10,11-tetraazacyclopenta[b]anthracene-3,4-dione (14)

Method A

A mixture of compound **11** (10 mmol), chloroacetic acid (10 mmol), sodium acetate anhydrous (2 g) in glacial acetic acid (30 ml) and acetic acid anhydride (10 ml) was refluxed for 12 min., then equimolecular amount of *p*-cholrobenzaldehyde (2 mmol) was added. The reaction mixture was refluxed for 2 h, allowed to cool, poured onto cold water; the formed precipitate was filtered off, dried and crystallized from proper solvent to give the corresponding arylmethylene thiazolopyrimidine derivative **14**.

Method B

A mixture of compound **12** (10 mmol), equimolecular amount of *p*-chlorobenzaldehyde (10 mmol), sodium acetate anhydrous (2 g) in a mixture of acetic acid (30 ml) and acetic anhydride (10 ml) was refluxed for 2 h. After cooling, the reaction mixture was poured onto cold water; the formed solid was collected by filtration and crystallized from the proper solvent to give the compound **14**. The products were identified by their m.p. and R_f-values in comparison with authentic samples previously obtained by method A.

Yield [A] 80%, [B] 65%; mp 203–205°C (EtOH); IR (KBr, cm^{-1}): 1668; ^{1}H NMR [DMSO-d_6]: δ 7.95 (s, 1H, benzylic proton), 7.43–6.97 (m, 13H, ArH + benzylic proton), 3.40 (m, 4H, *N*-$(CH_2)_2$), 2.34 (m, 2H, N-CH_2-propyl),

1.36 (m, 2H, 2-CH_2-propyl), 0.82 (t, 3H, CH_3-propyl); EI-MS: *m/z* 640 $[M + 1]^+$ (20), 596 (35), 476 (83); Anal. Calcd for $C_{35}H_{25}ClF_2N_4O_2S$: C, 65.77; H, 3.94; N, 8.77; S, 5.02 Found: C, 65.65; H, 3.86; N, 8.66; S, 5.10.

In-vitro antitumor activity

Cell culture of HepG-2 (human liver carcinoma), PC-3 (human prostate adenocarcinoma) and HCT116 (human colorectal carcinoma) cell lines were purchased from the American Type Culture Collection (Rockville, MD) and maintained in RPMI-1640 medium which was supplemented with 10% heat-inactivated FBS (fetal bovine serum), 100U/mL penicillin and 100U/mL streptomycin. The cells were grown at 37°C in a humidified atmosphere of 5% CO_2.

MTT cytotoxicity assay

The antitumor activity against HepG-2, PC-3 and HCT-116 human cancer cell lines was estimated using the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, which is based on the cleavage of the tetrazolium salt by mitochondrial dehydrogenases in viable cells [23-25]. Cells were dispensed in a 96 well sterile microplate (5 x 10^4 cells/well), and incubated at 37°C with series of different concentrations, in DMSO, of each tested compound or doxorubicin® (positive control) for 48 h in a serum free medium prior to the MTT assay. After incubation, media were carefully removed, 40 µL of MTT (2.5 mg/mL) were added to each well and then incubated for an additional 4 h. The purple formazan dye crystals were solubilized by the addition of 200 µL of DMSO. The absorbance was measured at 590 nm using a SpectraMax® Paradigm® Multi-Mode microplate reader. The relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells. The IC_{50} values of the tested compounds were expressed as µg/mL in Table 1.

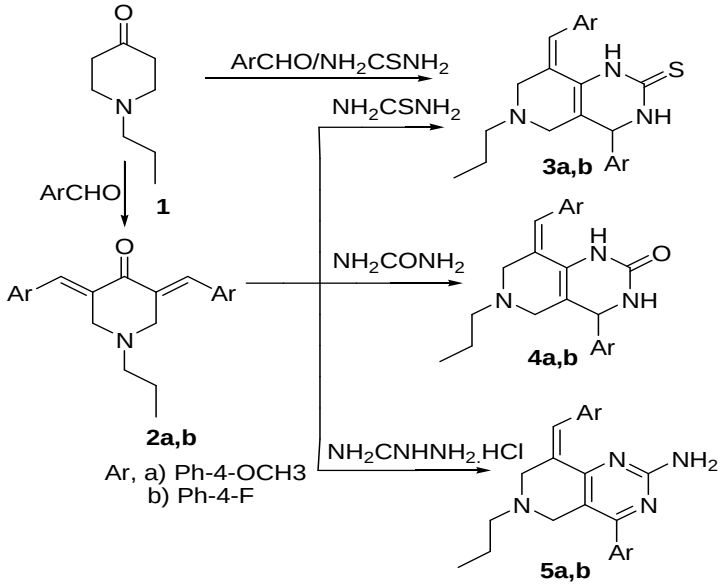
Statistical analysis

All experiments were conducted in triplicate and repeated in three different days. All the values were represented as mean ± SD. IC_{50}s were determined by profit analysis using SPSS software program (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Chemistry

Claisen-Schmidt condensation of *N*-propyl piperidone (**1**) with aromatic aldehydes, namely, 4-methoxybenzaldehyde or 4-fluorobenzaldehyde in ethanolic potassium hydroxide solution afforded 3,5-bisarylmethylene-1-propyl-4-piperidone (**2a,b**), respectively. Additionally, one pot reaction of compound **1** with aromatic aldehydes and thiourea in potassium hydroxide solution under reflux gave thiopyrimidine derivatives (**3a,b**), which were prepared also by cyclocondensation of bisarymethylene **2a,b** with thiourea (Scheme 1).



Scheme 1. Synthetic routes for compounds 2-5

The structures of the products (**3a,b**) were confirmed by NMR and MS spectral analysis. The ^{1}H-NMR spectrum of **3a** showed the methine pyrimidine proton signal at δ 5.02 ppm, and its methine carbon signal in ^{13}C NMR at δ 59.1 ppm (Figure 1). The compound **3a** was also confirmed by HRESI-MS with molecular weight *m/z* 436.2067 $[M + H]^+$. Furthermore, compounds **2a,b** were heated under reflux with urea or guandine hydrochloride in ethanolic sodium hydroxide solution to yield the corresponding pyrimidinone (**4a,b**) and aminopyrimidine (**5a,b**) derivatives

respectively, (Scheme 1). The ^{13}C-NMR spectra of these pyrimidine (**4a,b**) and (**5a,b**) derivatives revealed downfield carbon signals at δ 163 for carbonyl carbon and δ 165 for amino carbon, respectively, compared to the same carbon in thiopyrimidine **3a** which revealed at δ 153 ppm (Figure 2). Moreover, the structures of pyrimidine **3-5** derivatives were also confirmed by EI- and HRESI-MS.

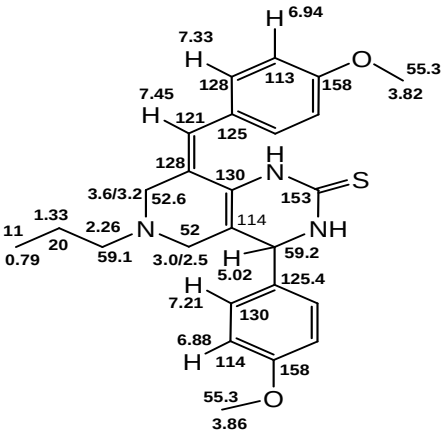


Figure 1. ^{1}H, ^{13}C-NMR data of compound 3a

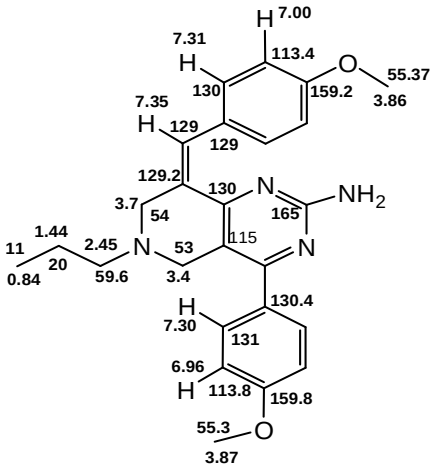
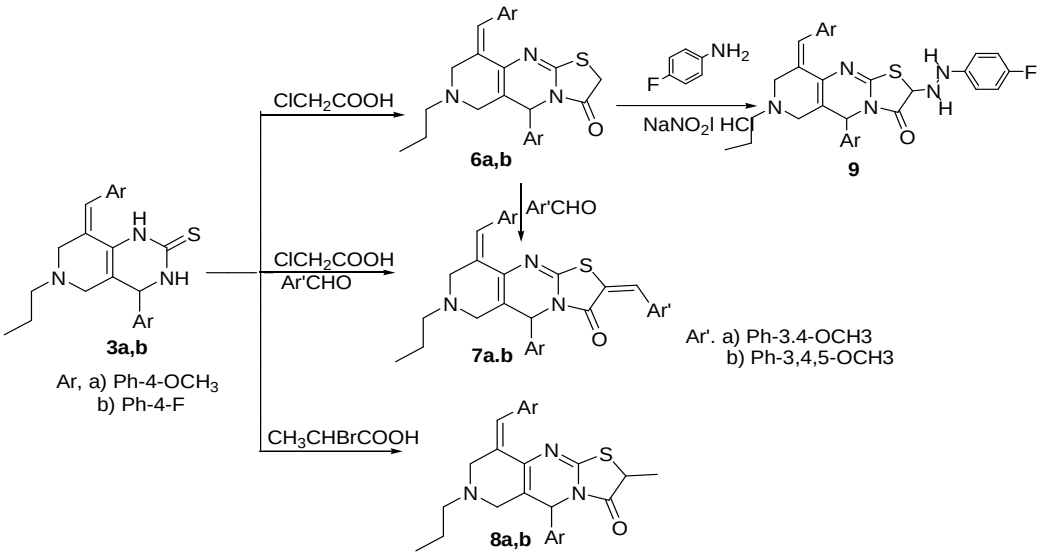


Figure 2. ^{1}H, ^{13}C-NMR data of compounds 5a

Thiopyrimidines **3a,b** were allowed to react with chloroacetic acid or 2-bromopropionic acid in the presence of sodium acetate anhydrous and glacial acetic acid/acetic anhydride mixture to give thiazolopyrimidine derivatives **6a,b** and **8a,b**, respectively, (Scheme 2). In the ^{1}H-NMR spectrum of compound **6a**, the methine pyrimidine proton was showed at δ 5.45 downfield by 0.43 ppm relative to CH-pyrimidine of compound **3a** due to thiazole ring, which delivered its methylene protons at δ 3.83 and 3.72 as a broad signals that confirmed by 2D-NMR experiments. The carbon resonance of this methylene group at δ 31 ppm, in ^{13}C-NMR was identified through the HSQC correlation with δ_H 3.83 and 3.72 ppm. Also, in the spectrum of ^{13}C-NMR, the most downfield carbon signal at δ 170.6 ppm, was assigned for the carbonyl carbon of thiazole ring with confirmed by the aid of HMBC experiment, where it is correlated with the methylene protons (δ_H 3.83 and 3.72 ppm) through ^{2}J coupling. Other HMBC correlations were used to confirm the structure of **6a** (Figure 3), and its molecular formula $C_{27}H_{29}N_3O_3S$ was deduced from its HRESI-MS at *m/z* 476.2010 $[M + H]^+$.



Scheme 2. Synthetic routes for compounds 6-9

Refluxing of thiazolopyrimidine derivatives **6a,b** with aromatic aldehydes in the presence of sodium acetate anhydrous and a mixture of glacial acetic acid/acetic anhydride led to formation of arylmethylene thiazolopyrimidine derivatives **7a,b**, which also prepared directly in a good yield from the reaction of compounds **3a,b** with chloroacetic acid and aromatic aldehydes (Scheme 2). The IR spectrum of compound **7a** showed band at 1695 cm^{-1} for (C=O) lower frequency relative to compound **6a** due to conjugation with exocyclic double bond, and the HRESI-MS of **7a** showed mass peak at *m/z* 624.2533 $[M + H]^+$ calculated for $C_{36}H_{37}N_3O_5S$, indicating additional aromatic ring to the compound **6a**. This confirmed in NMR spectra of compound **7a** (Figure 4), which revealed extra

NMR groups, benzylic (δ_H 7.62; δ_C 124 ppm), three aromatic (δ_H 7.01, 691 and 6.88; δ_C 131, 111.8 and 111.3 ppm), and two methyl ether (δ_H 6.90; δ_C 55.9 ppm) with disappearance to the methylene group in compound **6a**.

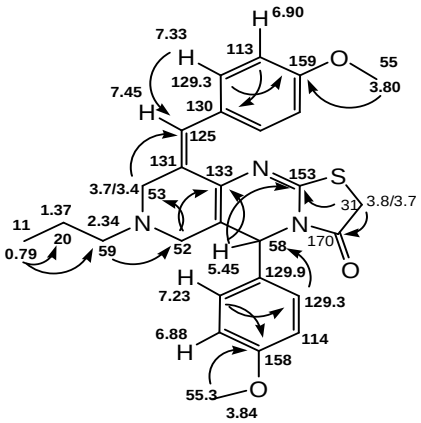


Figure 3. ^{1}H, ^{13}C-NMR and some selected

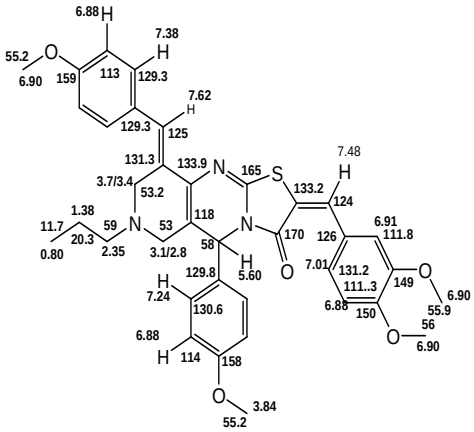
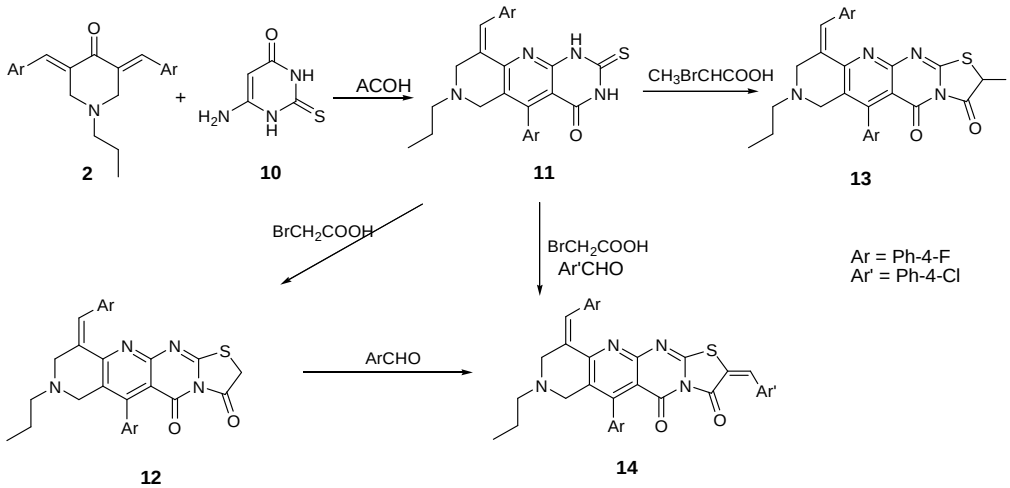


Figure 4. ^{1}H, ^{13}C-NMR spectral data of 7a HMBC correlations of 6a

In Scheme 2, arylazothiazolopyrimidine (**9**) was formed by the treatment of compound **6a** under cooling with diazonium salt of *p*-flouroaniline. The molecular formula of compound **9** was deduced from its EI-MS and elemental analysis as $C_{33}H_{32}FN_5O_3S$ of 596 amu, and with the NMR spectral data, the structure of **9** identified to have di-imino groups and aromatic ring additional to compound **6a**. The NMR spectrum of compound **9** delivered additional *p*-substituted benzene ring compared to the NMR of **6a**, as well as thiazole methine group signals (δ_H 5.68; δ_C 58 ppm) in compound **9**, instead of methylene group signals (δ_H 3.83 and 3.72; δ_C 31 ppm) in compound **6a**.

Bisarylmethylene **2b** was reacted with 6-amino-2-thioxo-pyrimidine-4 (3H)-one (**10**) in acetic acid under reflux to give(Z)-9-(4-fluorobenzylidene)-5-(4-fluorophenyl)-2,3,6,7,8,9-hexahydro-7-propyl-2-thioxopyrimido[4,5 b][1,6] naphthyridin-4(1H)-one (**11**). The ^{1}H-NMR spectrum of compound **11** showed the most two downfield signals at δ 12.27 and 12.93 ppm, as two singles assigned for two NH exchangeable with D_2O. The EI-MS of **11** (*m/z* 476 amu) and its elemental analysis was used to confirm the structure by its calculated molecular formula $C_{26}H_{22}F_2N_4OS$. Moreover, compound **11** was allowed to react with bromoacetic acid or 2-bromopropionic acid in acetic acid/acetic anhydride mixture in presence of sodium acetate anhydrous to give pyridothiazolopyrimidine (**12**) and methylpyridothiazolopyrimidine (**13**) derivatives, respectively. Refluxing of compound **12** with *p*-chlorobenzaldehyde afforded arylpyridothiazolopyrimidine **14**, which also prepared under the same reaction conditions from compound **11** by one pot reaction *via* 2-bromoacetic acid, *p*-chlorobenzaldehyde (Scheme 3). The thiazole rings which have been formed in compounds **12** and **13** were identified in the NMR spectral data of both compounds, characterized in ^{1}H-NMR by their groups of methylene (δ 3.92 ppm) and methine (δ 4.29 ppm), respectively. However, both groups were disappeared in the NMR spectra of compound **14**, and instead of aryl group was delivered, where its benzylic proton signal showed at δ 7.95 ppm, in addition to *p*-substituted benzene protons in the aromatic region compared to the same region in ^{1}H-NMR spectra of compounds **12** and **13**.



Scheme 3. Synthetic routes for compounds 11-14

Cytotoxic activity

Nineteen synthesized compounds were examined *in vitro* for their cytotoxic activities against HepG-2, PC-3 and HCT-116 human carcinoma cell lines using MTT assay. The percentage of the intact cells was measured and compared with that of the drug control doxorubicin (Figure 5). From the analysis reported in Table 1, HepG-2 liver cancer cells were found to be less sensitive to all tested compounds. The most active derivatives are **2a**, **2b**, **3a**, **3b**, **4b**, **5b**, **9** and **14**, which showed good cytotoxic activities against HCT-116 carcinoma cells with IC_{50} values ranging 54-60 µg/mL. Against the same cells, compounds **5a**, **6a**, **6b**, **7a**, **7b**, **8b**, **12** and **13** exhibited mild cytotoxic activities with IC_{50} values from 65 to 84 µg/mL, while the rest compounds showed weak activities. Additionally, The PC-3 cancer cells showed good sensitivity against compounds **2a**, **2b**, **3a**, **3b**, **4a** and **5b**; moderate with compounds **4b**, **12** and **14**, and weak or no cytotoxic activities with the rest of the compounds.

Table 1: The cytotoxicity values (IC_{50}) of the 19 compounds using MTT assay

Compound	HCT-116	PC-3	HepG-2
	IC_{50} (µg/mL) ± SD		
2a	54.18 ± 5.2	67.43 ± 2.9	124.25 ± 10.4
2b	56.17 ± 4.8	63.79 ± 3.6	176.67 ± 20.3
3a	55.976 ± 3.9	65.74 ± 5.1	256.71 ± 23.1
3b	55.59 ± 5.6	69.52 ± 5.6	191.67 ± 11.6
4a	90.14 ± 6.1	64.58 ± 4.9	280.15 ± 21.7
4b	55.68 ± 3.8	76.71 ± 5.1	154.37 ± 10.1
5a	83.85 ± 5.7	> 1000	174.99 ± 11.4
5b	56.99 ± 2.9	63.64 ± 4.8	974.53 ± 25.9
6a	74.56 ± 5.8	> 1000	161.79 ± 10.2
6b	79.87 ± 7.1	> 1000	238.28 ± 13.1
7a	68.22 ± 3.9	163.61 ± 11.2	376.39 ± 17.9
7b	71.28 ± 5.1	302.01 ± 13.9	501.73 ± 26.1
8a	155.49 ± 10.1	518.80 ± 20.9	156.73 ± 12.4
8b	65.81 ± 6.8	> 1000	413.89 ± 26.5
9	60.15 ± 4.8	123.59 ± 10.2	214.23 ± 14.7
11	92.81 ± 9.1	105.26 ± 9.9	430.30 ± 25.3
12	64.78 ± 5.7	76.99 ± 6.1	667.58 ± 14.6
13	84.96 ± 5.9	105.61 ± 10.3	583.23 ± 21.4
14	56.86 ± 3.6	77.53 ± 5.3	487.59 ± 19.8

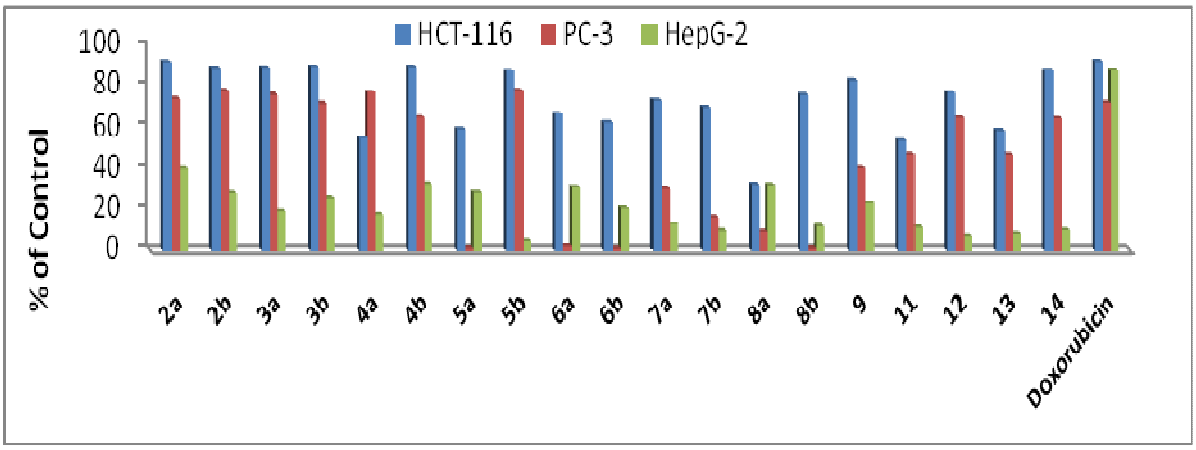


Figure 5. Cytotoxic activity of the nineteen compounds against three cancer types, using MTT assay at 100 ppm

It is observed from these results, the small molecules of bisarylmethylene (**2a,b**) and pyrimidinethione derivatives (**3a,b**) are most bioactive compounds against HCT-116 and PC-3 cancer cells as well as the fluorinated pyrimidinone (**4b**) and aminopyrimidine (**5b**) are also active against both cells. However the cancer cells HCT-116 are sensitive the collective halogenated derivatives of thiazolopyrimidine (**8b**), arylazothiazolopyrimidine (**9**), pyridothiazolopyrimidine (**12**) and arylpyridothiazolo-pyrimidine (**14**). Thus we suggested that the derivatives bearing halogen atoms were more active than 4-methyl ether group. Furthermore, the cytotoxic activity of small nitrogen heterocyclic molecules (**2-5**) was decreased by collective compounds with increasing the number of nitrogen atoms.

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