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Synthesis, Anti-Proliferative Activity and SAR Studies of Novel 5-(3-indolyl)-5H-thiazolo[4,3-b][1,3,4]thiadiazoles Tethered with Steroid Moieties

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Abstract

A new series of the fused thiazolo[4,3-*b*]-1,3,4-thiadiazoles **2a-e** have been synthesized *via* one-pot reaction of *N*-substituted indole-3-carboxaldehydes **1a-e** with thioglycolic acid and thiosemicarbazide under grinding condition. Condensation reaction of **2a-e** with acetylated epiandrosterone and progesterone afford the corresponding Schiff's bases **3a-e** and **4a,b**, respectively. Besides, the chloroacetylation of **2a-e** *in situ* by chloroacetyl chloride yielded the chloroacetamides **5a-e**. The reaction of **5a-e** with 3-amino-pyrazolopyridine derivative **6** provided the goal 2-[(steroids)-2*H*-pyrazolo[3,4-*b*]pyridin-3-ylamino]-5-(indoles)-thiazolo[4,3-*b*][1,3,4]thiadiazol-2-yl]-acetamides **7a-e**. The analytical and spectral data of the entire target compounds **3a-e**, **4a,b** and **7a-e** were compatible with their structures. Compounds **3a-e, 4a,b**, and **7a-e** were selected to be screened *in vitro* against different cancer cell lines, namely A-549, HC-T116, MCF-7, and PC3 using MTT assay. The anti-proliferative activity results implied that compounds **3a-e, 4a,** and **7e** showed excellent growth inhibitory activity toward the human colon cancer cell (HCT-116) with IC_{50} value ranging from 7.25-38.92 µM/ml in comparison to the reference drug doxorubicin with IC_{50} of 48.02 µM/ml. Interestingly, compound **3e** found to be the most active one towards A-549, HCT-116, and PC3 cancer cell lines with IC_{50} of 27.05, 12.69, and 24.61µM/ml in comparison with doxorubicin of IC_{50} 39.74, 48.02, and 34.77µM/ml, respectively. In addition, molecular docking studies helped to rationalize the binding interaction of the most active compounds toward human colon cancer cell (HCT-116) **3a-e**, **4a** and **7a** with the anti-apoptotic Bcl-2 and the result revealed that the docking of compounds was more potent compared co-crystalline ligand.

Keywords: Indol-3-carboxaldehyde; fused thiazolo[4,3-*b*][1,3,4]thiadiazoles; steroids anticancer; SAR; molecular docking.

1. Introduction

Cancer is a group of aggressive diseases and the topmost killer worldwide, which is recognized by the abnormal cell growth and metastasis to other parts of the body [1]. There are different ways of cancer treatments; however, chemotherapy remains a mainstay of treatment [2-4]. Due to the high prohibition for cancer disease and the rapid growing of drug resistance for anti-cancer drugs intense research are carried out worldwide in order to overcome this aggressive disease [1, 5].

Thiadiazole is a widespread and significant five membered heterocyclic system with two nitrogen atoms and a sulphur atom [6]. Thiadiazole has many isomers including 1,2,3 thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, and 1,3,4-thiadiazole. The latter isomer has investigated more than others [6]. 1,3,4-Thiadiazoles show a wide range of biological activities such as anti-cancer, antioxidant, anti-inflammatory, antimicrobial, antituberculosis, anticonvulsants, and anti-hypertensive, and antidepressant [7-14]. 1,3,4-Thiadiazole are found in various marketed drugs example acetazolamide that act as carbonic anhydrase inhibitor for treatment of glaucoma, high-altitude diseases, idiopathic intracranial hypertension, hemiplegic migraine, obstructive sleep apnea, and sulfamethizole used as an antibacterial agent [6-15].

On the other hand, steroids are a diverse set of biologically active polycyclic compounds. Steroids play an essential role in regulating normal physiological processes and are the mainstay for

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treatment of various diseases, including cancer [16,17]. Steroidal heterocycles have drawn great attention due to their interesting structural features as well as their biological profiles [18]. Progesterone, dehydroisoandrosterone, epiandrosterone, and 3-hydroxy-5a-androstan-17-one are some of the steroids that are widely used as a pharmaceutical prototype for constructing various biologically active compounds [19-23].

Furthermore, indole is a well-known natural compound attracts massive interest of researchers in the field of organic and medicinal chemistry, having a broad spectrum of pharmaceutical activities comprise antitumor, antimicrobial, anti-inflammatory, antiviral [24-26].

Scientific research studies revealed that a combination of two different bioactive molecules is of great medicinal interest [27]. These bioactive molecules can efficiently overcome most of the pharmacokinetic disadvantage of classical drugs where they can launch two or more mode of action in parallel to restrain tumor growth [27]. Based on the information provided and as our work continues to synthesize new anticancer agents [25, 26, 28-30], our efforts in this work have directed to synthesize a new series of thiazolo[4,3-*b*]-1,3,4-thiadiazoles united to steroid moieties to be exploited for further diversification and screened for anti-proliferative activity.

2. Experimental

2.1. Instruments and reagents

Solvent and reagents are of commercial grade. Melting points measured on the melting digital device; 9100-Electrothermal, serial No. 8694, Rochford, United Kingdom, and are uncorrected. The reaction advance was observed by TLC; thin layer chromatography using silica gel plates (POLYGRAM SILG/UV254, 0.20 mm), which were seen under ultra violet light 254 and 365 nm. Elemental analyses have been carried out on a Perkin-Elmer analyzer 2400 (USA), and were originated inside the range of ± 0.4 % of the calculated values. The infrared spectra (IR) realized by Beckman infrared spectrophotometer (PU 7712) using potassium bromide disc. The ^{1}HNMR and ^{13}C NMR spectra were performed on Mercury Varian and BrukerAvance spectrometer (300, 400 & 100 MHz) using TMS as the internal standard at Microanalytical Center, Cairo and Ain Shams University, Egypt. Mass spectra (EI) were recorded by Jeol JMS-AX 500 spectrometer, 70ev (Japan).

2.2. Chemistry

Synthesis of 2-amino-5-(*N*-substituted-1*H*-indol-3-yl)-5*H*-thiazolo[4,3-*b*][1,3,4]thiadiazoles (2a-e)

1*H*-Indole-3-carboxaldehydes **1a-e** (20mmol) and thioglycollic acid (1.84 ml, 20 mmol) were ground in a mortar with a pestle at room temperature (10-15min). To the reaction mixture, thiosemicarbazide (20mmol) was added with milling, and then (10 ml) of concentrated H_2SO_4 under cooling and milling was added in a small quantities. The reaction mixture was homogenized, and then left for 24h at -20 ºC. The mixture was transferred by ice-water to a beaker of 250 ml. The neutralization of the formed suspension takes place using aqueous solution of sodium hydroxide (40 %) to pH 7-8. The precipitate was filtered, dried, and re-crystallized from dioxane : H_2O (1:1).

2-Amino-5-(*N*-benzoyl-1*H*-indol-3-yl)-5*H*-thiazolo [4,3-*b*][1,3,4]thiadiazole (2a)

Yield 65%, m.p. 215-7°C; IR (KBr, cm^{-1}): 3365 (NH_2), 1705 (C=O), 1633 (C=N), 1575 (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.21 (s, NH_2), 8.38 (s, 1H, H-2 indole), 8.33 (s, 1H, CH thiazole), 8.25 (d, 1H, Ar-H), 8.15 (d, 1H, Ar-H), 8.06 (d, 1H, Ar-H), 7.83-7.43 (m , 5H, Ar-H), 7.21-7.14 (m, 2H, Ar-H); ^{13}C NMR (DMSO-d_6,100 MHz,) δ: 184.96 (CO), 138.47, 137.04, 124.07, 123.40, 112.07, 120.78, 118.09, 112.43 (Ar-C); EI-MS: *m/z* (%) =378 [M^{+}, 22]; Anal Calcd for $C_{19}H_{14}N_4OS_2$ (378.47): C, 60.30; H, 3.73; N, 14.80; found: C, 60.16; H, 3.64; N, 14.71.

2-Amino-5-(*N*-(2-chloro-benzoyl)-1*H*-indol-3-yl)-5*H*-thiazolo[4,3-*b*][1,3,4]Thiadiazole (2b)

Yield 56%, m.p. 224-6°C; IR (KBr, cm^{-1}): 3369 (NH_2), 1705 (C=O), 1628 (C=N), 1575 (C=C), 755 (C-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.20 (s, NH_2), 8.31 (s, 1H, H-2 indole), 8.23 (d, 1H, Ar-H), 8.04 (s, 1H, CH thiazole), 7.28 (d, 1H, Ar-H), 7.43 (d, 2H, Ar-H), 7.25-7.12 (m, 5H, Ar-H); ^{13}CNMR (DMSO-d_6,100 MHz,) δ: 176.40 (CO), 140.80, 137.00, 130.98, 123.98, 123.91, 122.61, 122.14, 122.14, 120.55, 111.71, 111.07 (Ar-C); Anal Calcd for $C_{19}H_{13}ClN_4OS_2$ (412.92): C, 55.27; H, 3.17; N, 13.57; found: , 55.01; H, 3.00; N, 13.31.

2-Amino-5-(*N*-(4-chloro-benzoyl)-1*H*-indol-3-yl)-5*H*-thiazolo[4,3-*b*][1,3,4]Thiadiazole (2c)

Yield 62%, m.p. 205-7°C; IR (KBr, cm^{-1}): 3312 (NH_2), 1721 (C=O), 1622 (C=N), 1522 (C=C), 755 (C-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.17 (s, NH_2), 8.30 (s, 1H, H-2 indole), 8.21 (d, 1H, Ar-H), 8.00 (s, 1H, CH thiazole), 7.94 (d, 2H, Ar-H), 7.80 (d, 1H, Ar-H), 7.57 (d, 2H, Ar-H), 7.20-7.13 (m, 3H); ^{13}C NMR (DMSO-d_6,100 MHz,) δ: 176.40, (CO), 166.45, 140.81, 138.47, 137.01, 130.99, 123.44, 122.61, 122.11, 120.80, 120.60, 112.41, 111.73, 111.08 (Ar-C); EI-MS: *m/z* (%) = 412/414 [M^{+}/M+2, 21/11]; Anal Calcd for $C_{19}H_{13}ClN_4OS_2$ (412.92): C, 55.27; H, 3.17; N, 13.57; found: C, 55.05; H, 3.01; N, 13.34.

2-Amino-5-(*N*-(methanesulphonyl)-1*H*-indol-3-yl)-5*H*-thiazolo[4,3-*b*][1,3,4]Thiadiazole (2d)

Yield 53%, m.p. 198-200°C; IR (KBr, cm^{-1}): 3405 (NH_2), 1628 (C=N), 1577 (C=C), 1365 & 1136 (N-SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.26 (s,

NH_2), 8.32 (s, 1H, H-2 indole), 8.29 (d, 1H,Ar-H), 8.05 (s, 1H, CH thiazole), 7.82 (d, 1H, Ar-H), 7.43 (d, 1H, Ar-H), 7.20-7.13 (m, 2H), 3.77 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 152.41, 145.81, 140.81, 137.0, 130.98, 130.13, 128.77, 122.60, 122.13, 120.05, 111.72, 111.08 (Ar-C); EI-MS: *m/z* (%) = 352 [M^+, 22]; Anal Calcd for $C_{13}H_{12}N_4O_2S_3$ (352.45): C, 44.30; H, 3.43; N, 15.90; found: C, 44.12; H, 3.25; N, 15.75.

2-Amino-5-(*N*-(4-bromo-benzenesulphonyl)-1*H*-indol-3-yl)-5*H*-thiazolo[4,3-*b*][1,3,4]Thiadiazole (2e)

Yield 65%, m.p. 235-7°C; IR (KBr, cm^{-1}): 3423 (NH_2), 1628 (C=N), 1586 (C=C), 1345, 1123 (N-SO_2), 781 (C-Br); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.21 (s, NH_2), 8.33 (s, 1H, H-2 indole), 8.23 (d, 1H, Ar-H), 8.06 (s, 1H, CH thiazole), 7.96 (d, 2H, Ar-H), 7.83 (d, 1H, Ar-H), 7.58 (m, 3H), 7.21-7.14 (m, 2H); ^{13}C NMR (DMSO-d_6,100 MHz,) δ: 156.81, 140.52, 137.25, 130.47, 130.01, 123.04, 122.61, 122.01, 120.51, 120.11, 112.61, 111.71, 111.09(Ar-C); EI-MS: *m/z* (%) = 492/494 [M^+/ M^++2, 12/12]; Anal Calcd for $C_{18}H_{13}BrN_4O_2S_3$ (493.42): C, 43.82; H, 2.66; N, 11.35; found: C, 43.61; H, 2.43; N, 11.04.

Synthesis of Schiff's base 3a-e

A mixture of **2a-e** (10mmol) and acetylated epiandrosterone (10mmol) was milled in a mortar with a pestle at room temperature for 10-15 min, and then transferred by 25 ml of ethanol 99% to a round bottomed flask. The reaction mixture was heated under reflux for 6-8. After completion of the reaction, the reaction mixture was cooled to room temperature, and the formed solid was collected by filtration, dried, and re-crystallized from ethyl acetate: cyclohexane (1:1).

(*3S,8R,9S,10S,13S,14S,E*)-17-((5-(1-benzoyl-1*H*-indol-3-yl)-5*H*-thiazolo[4,3-b][1,3,4]thiadiazol-2-yl)imino)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[a]phenanthren-3-yl acetate (3a)

Yield 54%, m.p. 175-7°C; IR (KBr, cm^{-1}): 1711 (2C=O), 1618 (C=N), 1596 (C=C), 1126, 1053 (C-O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.36 (s, 1H, H-2 indole), 8.31 (d, 1H, Ar-H), 8.23 (d, 1H, Ar-H), 7.81-6.91 (m, 9H, Ar-H), 4.13 (m, 1H, CH-3), 2.38 (s, 3H, $COCH_3$), 1.44 (s, 3H, CH_3-18), 2.33-0.85 (m, 22H, steroid moiety), 0.84 (s, 3H, CH_3-19); EI-MS: *m/z* (%) = 692 [M^+, 0.13]; Anal Calcd for $C_{40}H_{44}N_4O_3S_2$ (692.94): C, 69.33; H, 6.40; N, 8.09; found: C, 69.01; H, 6.21; N, 7.90.

(*3S,8R,9S,10S,13S,14S,E*)-17-((5-(1-(2-chloro benzoyl)-1*H*-indol-3-yl)-5*H*-thiazolo[4,3-b][1,3,4] thiadiazol-2-yl)imino)-10,13-dimethylhexadeca-hydro-1*H*-cyclopenta[a]phenanthren-3-yl acetate (3b)

Yield 57 %, m.p. 152-7 °C; IR (KBr, cm^{-1}): 1704 (2C=O), 1618 (C=N), 1590 (C=C), 742 (C-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.44 (s, 1H, H-2 indole), 8.29 (d, 1H, Ar-H), 7.81-6.44 (m, 9H, Ar-H), 4.60 (m, 1H, CH-3), 2.92 (s, 3H, $COCH_3$), 1.20 (s, 3H, CH_3-18), 2.07-0.85 (m, 22H, steroid moiety), 0.84 (s, 3H,CH_3-19); Anal Calcd for $C_{40}H_{43}ClN_4O_3S_2$ (727.25):C, 66.05; H, 5.96; N, 7.70; found: C, 66.10; H, 5.77; N, 7.75.

(*3S,8R,9S,10S,13S,14S,E*)-17-((5-(1-(4-chloro benzoyl)-1*H*-indol-3-yl)-5*H*-thiazolo[4,3-b][1,3,4] thiadiazol-2-yl)imino)-10,13-dimethylhexadeca-hydro-1*H*-cyclopenta[a]phenanthren-3-yl acetate (3c)

Yield 61%, m.p. 231-3 °C; IR (KBr, cm^{-1}): 1705 (2C=O), 1632 (C=N), 1575 (C=C), 775 (C-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.32-8.05 (m, 3H, Ar-H), 7.97-7.93 (d, 2H, Ar-H), 7.81-6.44 (m, 6H, Ar-H), 4.62 (m, 1H, CH-3), 3.36 (s, 3H, $COCH_3$), 1.31 (s, 3H, CH_3-18), 2.14-0.83 (m, 22H, steroid moiety), 0.78 (s, 3H, CH_3-19); EI-MS: *m/z* (%) =727/729 [M^+/M+2, 6.24/2.11]; Anal Calcd for $C_{40}H_{43}ClN_4O_3S_2$ (727.25):C, 66.05; H, 5.96; N, 7.70; found: C, 66.20; H, 5.81; N, 7.65.

(*3S,8R,9S,10S,13S,14S,E*)-10,13-dimethyl-17-((5-(1-(methylsulfonyl)-1*H*-indol-3-yl)-5*H*-thiazolo [4,3-b][1,3,4]thiadiazol-2-yl)imino)hexadecahydro -1*H*-cyclopenta[a]phenanthren-3-yl acetate (3d)

Yield 62%, m.p. 262-4°C; IR (KBr, cm^{-1}): 1710 (C=O), 1633 (C=N), 1569 (C=C), 1365, 1126 (N-SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.41 (s, 1H, H-2 indole), 8.29-8.24 (m, 2H, Ar-H), 7.85-7.13 (m, 4H, Ar-H), 4.55 (m, 1H, CH-3), 3.46 (s, 3H, NSO_2CH_3), 2.87 (s, 3H, $COCH_3$), 1.27 (s, 3H, CH_3-18), 2.03- 0.82 (m, 22H, steroid moiety), 0.81 (s, 3H, CH_3-19); EI-MS: *m/z* (%) = 666 [M^+, 14]; Anal Calcd for $C_{34}H_{42}N_4O_4S_3$ (666.91): C, 61.23; H, 6.35; N, 8.40; found: C, 61.05; H, 6.10; N, 8.26.

(*3S,8R,9S,10S,13S,14S,E*)-17-((5-(1-((4-bromo phenyl)sulfonyl)-1*H*-indol-3-yl)-5*H*-thiazolo[4,3-b][1,3,4]thiadiazol-2-yl)imino)-10,13-dimethyl hexadecahydro-1*H*-cyclopenta[a]phenanthren-3-yl acetate (3e)

Yield 55%, m.p. 277-9°C; IR (KBr, cm^{-1}): 1710 (C=O), 1634 (C=N), 1638 (C=C), 1362, 1145 (N-SO_2), 782 (C-Br); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.92 (d, 1H, Ar-H), 8.32 (dd, 1H, Ar-H), 8.21 (m, 2H, Ar-H), 7.91-6.64 (m, 7H, Ar-H), 4.40 (m, 1H, CH-3), 3.39 (s, 3H, $COCH_3$), 1.72 (s, 3H, CH_3-18), 2.27-0.94 (m, 22H, steroid moiety), 0.84 (s, 3H, CH_3-19); Anal Calcd for $C_{39}H_{43}BrN_4O_4S_3$ (807.88): C, 57.98; H, 5.36; N, 6.94;; found: C, 57.72; H, 5.21; N, 6.81.

Synthesis of Schiff's base 4a,b

A mixture of compound **2a** or **2e** (20mmol) and progesterone (10mmol) was ground in a mortar with a pestle at room temperature for 10-15 min, and then transferred by 25 ml of absolute ethanol to a round bottomed flask. The reaction mixture was refluxed for 6-8 h, and then cooled to room temperature. The separated solid was filtered off, washed with water, dried and re-crystallized from ethyl acetate–cyclohexan (1:1).

10,13-Dimethyl-17-((*E*)-1-(5-(*N*-benzoyl-1*H*-indol-3-yl)-5*H*-thiazolo[4,3-*b*][1,3,4]thiadiazol-2-ylimino)ethyl)-7,8,9,11-tetrahydro-1*H*-cyclopenta[a] phenan-thren-3(2*H*,6*H*,10*H*,12*H*,13*H*,14*H*,15*H*, 16*H*,17*H*)-ylidene)-5-(*N*-benzoyl-1*H*-indol-3-yl)-5*H*-thiazolo[4,3-b][1,3,4]thiadiazol-2-amine (4a)

Yield 54%, m.p. 290-2°C; IR (KBr, cm^{-1}): 1717 (C=O), 1629 (C=N), 1596 (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.57-8.36 (m, 3H, Ar-H), 8.23-8.07 (m, 4H, Ar-H), 7.97-6.65 (m, 17H, Ar-H); 5.62 (s, 1H, CH-4), 1.94 (s, 3H, CH_3), 1.23 (s, 3H, CH_3-19), 2.49-1.13 (m, 20H, steroid moiety), 1.01 (s, 3H, CH_3-18); Anal Calcd for $C_{59}H_{54}N_8O_2S_4$ (1034.33): C, 68.44; H, 5.26; N, 10.82; found: C, 68.21; H, 5.04; N, 10.62.

10,13-Dimethyl-17-((*E*)-1-(5-(*N*-(4-bromo-benzene sulfonyl)-1*H*-indol-3-yl)-5*H*-thiazolo[4,3-*b*][1,3,4] thiadiazol-2-ylimino)ethyl)-7,8,9,11-tetrahydro-1*H*-cyclo-penta[a]phenant-hren-3(2*H*,6*H*,10*H*, 12*H*,13*H*,14*H*,15*H*,16*H*,17*H*)-ylidene)-5-(*N*-(4-bromo-benzenesulfonyl)-1*H*-indol-3-yl)-5*H*-thiazolo[4,3-b][1,3,4]thiadiazol-2-amine (4b)

Yield 49%, m.p. 296-8 °C; IR (KBr, cm^{-1}): 1633, 1628 (C=N), 1587 (C=C), 1365, 1135 (SO_2-N), 782 (C-Br);^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.92 (d, 2H, Ar-H), 8.35-8.26 (m, 2H, Ar-H), 8.07-7.87 (m, 3H, Ar-H), 7.76-6.46 (m, 15H, Ar-H); 5.81 (s, 1H, CH-4), 1.94 (s, 3H, CH_3), 1.31 (s, 3H, CH_3-19), 2.41-1.17 (m, 20H, steroid moiety), 1.04 (s, 3H, CH_3-18); Anal Calcd for $C_{57}H_{52}Br_2N_8O_4S_6$ (1264.27): C, 54.11; H, 4.14; N, 8.86; found: C, 53.85; H, 4.01; N, 8.67.

Synthesis of *N*-(5-(*N*-Substituted-1*H*-indol-3-yl)-5*H*-thiazolo[4,3b][1,3,4]thiadiazol-2-yl)-2-chloro-acetamides (5a-e)

A solution of the chloroacetyl chloride (5 ml, 40 mmol) in dimethylformamide (20 ml) was added slowly to a stirred solution of compound **2a-e** (20 mmol) in dimethylformamide (60 ml) at 0-5 °C. After complete addition, the reaction mixture was refluxed for 3h. After completion of the reaction, the reaction mixture was quenched with cold water, and then neutralized with sodium hydrogen carbonate (5%). The formed solid was filtered off, washed with water, dried, and re-crystallized from chloroform.

***N*-(5-(*N*-benzoyl-1*H*-indol-3-yl)-5*H*-thiazolo[4,3b] [1,3,4]thiadiazol-2-yl)-2-chloroacetamide (5a)**

Yield 65%, m.p. 110-2°C;IR (KBr, cm^{-1}): 3175 (N-H), 1707, 1688 (C=O), 1620 (C=N), 1562 (C=C), 775 (C-Cl); ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 9.07 (s, NH), 8.21 (s, 1H, H-2 indole), 7.81(d, 2H, Ar-H), 7.45-7.40 (m, 4H, Ar-H), 7.28-7.09 (m, 5H, Ar-H), 4.27 (s, 2H, CH_2); EI-MS: *m/z* (%) =454/456 [M^+/M+2, 29/11]; Anal Calcd for $C_{21}H_{15}ClN_4O_2S_2$ (454.95): C, 55.44; H, 3.32; N, 12.31; found: C, 55.30; H, 3.25; N, 12.19.

***N*-(5-(*N*-(2-chlorobenzoyl)-1*H*-indol-3-yl)-5*H*-thiazolo[4,3b][1,3,4]thiadiazol-2-yl)-2-chloro-acetamide (5b)**

Yield 60%, m.p. 164-6°C; IR (KBr, cm^{-1}): 3240 (N-H), 1710, 1696 (C=O), 1618 (C=N), 1545 (C=C), 757 (C-Cl); ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 9.72 (s, NH), 8.33 (s, 1H, H-2 indole), 8.12 (d, 1H, Ar-H), 7.92 (m, 2H, Ar-H), 7.52-7.11 (m, 7H, Ar-H), 4.05 (s, 2H, CH_2); Anal Calcd for $C_{21}H_{14}Cl_2N_4O_2S_2$ (489.40): C, 51.54; H, 2.88; N, 11.45; found: C, 51.41; H, 2.69; N, 11.27.

***N*-(5-(*N*-(4-chlorobenzoyl)-1*H*-indol-3-yl)-5*H*-thiazolo[4,3b][1,3,4]thiadiazol-2-yl)-2-chloro-acetamide (5c)**

Yield 51%, m.p. 82 dec.; IR (KBr, cm^{-1}): 3302 (N-H), 1697, 1686 (C=O), 1620 (C=N), 1577 (C=C), 772 (C-Cl); ^{1}H NMR (DMSO-d_6, 300 MHz,) δ: 10.02 (s, NH), 8.02 (s, 1H, H-2 indole), 7.82 (dd, 2H, Ar-H), 7.71-7.65 (m, 4H, Ar-H), 7.45 (s, 1H), 7.35-7.18 (m, 3H, Ar-H), 4.15 (s, 2H, CH_2); EI-MS: *m/z* (%) =489/490/492 [M^+/M+2/M+4, 20/11/2]; Anal Calcd for $C_{21}H_{14}Cl_2N_4O_2S_2$ (489.40): C, 51.54; H, 2.88; N, 11.45; found: C, 51.37; H, 2.70; N, 11.24.

***N*-(5-(*N*-(methane sulphonyl)-1*H*-indol-3-yl)-5*H*-thiazolo[4,3b][1,3,4]thiadiazol-2-yl)-2-chloroacetamide (5d)**

Yield 49%, m.p. 96-8°C; IR (KBr, cm^{-1}): 3175 (N-H), 1688 (C=O), 1618 (C=N), 1553 (C=C), 725 (C-Cl); ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.95 (s, NH), 7.84 (d, 1H,Ar-H), 7.81 (d, 1H,Ar-H), 7.75 (s, 1H, H-2 indole), 7.45-6.91 (m, 4H, Ar-H), 4.17 (s, 2H, CH_2), 3.92 (s, 3H, SO_2CH_3); EI-MS: *m/z* (%) =428/430 [M^+/M+2, 12/4]; Anal Calcd for $C_{15}H_{13}ClN_4O_3S_3$ (428.94): C, 51.54; H, 2.88; N, 11.45; found: C, 51.31; H, 2.57; N, 11.06.

***N*-(5-(*N*-(4-bromo-benzenesulfonyl)-1*H*-indol-3-yl)-5*H*-thiazolo[4,3b][1,3,4]thiad-iazol-2-yl)-2-chloroacetamide (5e)**

Yield 45%, m.p. 117-9°C; IR (KBr, cm^{-1}): 3215 (N-H), 1687 (C=O), 1617 (C=N), 1577 (C=C), 782, 757 (C-Br, C-Cl); ^{1}H NMR (DMSO-d_6, 300 MHz,) δ: 9.56 (s, NH), 7.82 (d, 1H, Ar-H), 7.78 (s, 1H, Ar-H), 7.75-7.21 (m, 8H, Ar-H), 4.07 (s, 2H, CH_2); Anal Calcd for $C_{20}H_{14}BrClN_4O_3S_3$ (569.90): C, 42.15; H, 2.48; N, 9.83; found: C, 42.01; H, 2.30; N, 9.64.

Synthesis of 2-[6-(10,13-dimethyl-3-oxo-2,3,6,7,8, 9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclo penta[*a*]phenanthren-17-yl)-2*H*-pyrazolo[3,4-*b*] pyridine-3-ylamino]-5-(*N*-substituted-1*H*-indol-3-yl)-thiazolo[4,3-*b*][1,3,4]thiadiazol-2-yl] acetamide (7a-e)

The appropriate of **5a-e** (1 mmol) and the 3-amino-pyrazolopyridine derivative **6** (0.40 g, 1 mmol) was fused for 30 min, and then transferred by 25 ml of absolute ethanol to a round flask. The reaction mixture was heated under reflux for 6-8 h. The reaction mixture was cooled to room temperature. The separated solid was collected by filtration, washed with water, air dried and crystallized from ethyl acetate–cyclohexane (1:1).

2-[6-(10,13-Dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-2*H*-pyrazolo [3,4-*b*]pyridin-3-ylamino]-5-((*N*-benzoyl-1*H*-indol-3-yl)-thiazolo[4,3-*b*][1,3,4]thiadiazol-2-yl] acetamide (7a)

Yield 62%, m.p. 265-7°C; IR (KBr, cm^{-1}): 3265, 3196 (N-H), 1714, 1705, 1698 (C=O), 1633, 1628 (C=N), 1592 (C=C);^{1}H NMR (DMSO-d_6, 300 MHz) δ: 12.40, 11.07 (s, 2H, 2NH), 8.41 (s, 1H, H-2 indole), 8.15-7.52 (m, 5H, Ar-H), 7.45 (d, 2H, 2CH-pyridine),7.43-6.92 (m, 6H, Ar-H), 5.05 (s, 1H, CH-4), 4.12 (s, 2H, CH_2), 1.59 (s, 3H, CH_3-19), 2.50 - 1.03 (m, steroid moiety), 0.90 (s, 3H, CH_3-18); EI-MS: *m/z* (%) =822[M^+, 14]; Anal Calcd for $C_{46}H_{46}N_8O_3S_2$ (822.31): C, 67.13; H, 5.63; N, 13.61; found: C, 66.93; H, 5.42; N, 13.44.

2-[6-(10,13-Dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-2*H*-pyrazolo [3,4-*b*]pyridin-3-ylamino]-5-((*N*-(2-chloro-benzoyl) -1*H*-indol-3-yl)-thiazolo[4,3-*b*][1,3,4]thiadiazol-2-yl]-acetamide (7b)

Yield 49%, m.p. 213-5°C; IR (KBr, cm^{-1}): 3320, 3242 (N-H), 1724, 1711, 1695 (C=O), 1628 (C=N), 1575 (C=C), 775 (C-Cl); EI-MS: *m/z* (%) =857/859 [M^+/M^++2, 24/8]; Anal Calcd for $C_{46}H_{45}ClN_8O_3S_2$ (856.5): C, 64.43; H, 5.29; N, 13.07; found: C, 64.21; H, 5.06; N, 12.87.

2-[6-(10,13-Dimethyl-3-oxo-2,3,6,7,8,9,10,11,12, 13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*] phenanthren-17-yl)-2*H*-pyrazolo [3,4-*b*]pyridin-3-ylamino]-5-((*N*-(4-chloro-benzoyl)-1*H*-indol-3-yl)-thiazolo[4,3-*b*][1,3,4]thiadiazol-2-yl]-acetamide (7c)

Yield 46%, m.p. 141-3 °C; IR (KBr, cm^{-1}): 3287, 3145 (N-H), 1712, 1696 (C=O), 1623 (C=N), 1587 (C=C), 752 (C-Cl); ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 11.62, 10.01 (s, 2NH), 8.25 (s, 1H, H-2 indole), 8.03 (d, 2H, Ar-H), 7.92 (d, 1H, CH-pyridine), 7.85-7.71 (m, 3H, Ar-H), 7.57 (d, 1H, CH-pyridine), 7.35-6.87 (m, 5H, Ar-H), 5.68 (s, 1H, CH-4), 3.95 (s, 2H, CH_2), 2.50-1.26 (m, steroid moiety), 1.12 (s, 3H, CH_3-19), 0.90 (s, 3H, CH_3-18); Anal Calcd for $C_{46}H_{45}ClN_8O_3S_2$ (856.5): C, 64.43; H, 5.29; N, 13.07; found: C, 64.25; H, 5.11; N, 12.90.

2-[6-(10,13-Dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-2*H*-pyrazolo[3,4-*b*]pyridin-3-ylamino]-5-((*N*-(methanesulphonyl)-1*H*-indol-3-yl)-thiazolo[4,3-*b*][1,3,4]thiadiazol-2-yl]-acetamide (7d)

Yield 51%, m.p. 162-4°C; IR (KBr, cm^{-1}): 3224, 3167 (N-H), 1705, 1687 (C=O), 1629 (C=N), 1565 (C=C);^{1}H NMR (DMSO-d_6, 300 MHz) δ: 12.28, 9.92 (2s, 2NH), 8.25 (d, 1H, Ar-H), 8.01(s, 1H, H-2 indole), 7.89-7.80 (d, 2H, 2CH-pyridine), 7.62 (d, 1H, Ar-H), 7.42-6.91 (m, 4H, Ar-H), 5.50 (s, 1H, CH-4), 4.07 (s, 2H, CH_2), 3.75 (s, 3H, SO_2CH_3),1.29 (s, 3H, CH_3-19), 1.05-2.50 (m, steroid moiety), 0.95 (s, 3H, CH_3-18); EI-MS: *m/z* (%) =797 [M^+, 12]; Anal Calcd for $C_{40}H_{44}N_8O_4S_3$ (796.26): C, 60.28; H, 5.56; N, 14.06; found: C, 60.04; H, 5.47; N, 13.86.

2-[6-(10,13-Dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-2*H*-pyrazolo [3,4-*b*]pyridin-3-ylamino]-5-((*N*-(4-bromo-benzene sulfonyl)--1*H*-indol-3-yl)-thiazolo[4,3-*b*][1,3,4]thia-diazol-2-yl]-acetamide (7e)

Yield 62 %, m.p. 184-6 °C; IR (KBr, cm^{-1}): 3262, 3163 (N-H), 1696, 1682 (C=O), 1618 (C=N), 1581 (C=C), 785 (C-Br); EI-MS: *m/z* (%) =936/938 [M^+, 7.52/7.52]; Anal Calcd for $C_{45}H_{45}BrN_8O_4S_3$ (937.19): C, 57.62; H, 4.84; N, 11.95; found: C, 57.45; H, 4.61; N, 11.70.

2.3. Biological assays

2.3.1. Cell culture:

The cancer cell lines, namely A-549 (Lung cancer), HCT-116 (colorectal carcinoma), MCF-7 (breast adenocarcinoma), PC3 (prostate cancer) were purchased from Vacsera (Giza, Egypt). HCT-116, MCF-7cells were maintained in RPMI 1640 medium. PC3 and A549 cells were maintained in DMEM medium. The media were supplemented with 10 % fetal bovine serum and incubated at 37 °C in 5 % CO_2 and 95 % humidity.

2.3.2. Cell viability assay:

Cell viability was studied using MTT; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (Bio Basic Canada Inc., Canada) assay [31]. The steps were executed in a sterile laminar air flow cabinet Biosafety class II level (Baker, SG403INT; Sanford, ME, USA). All incubations were carried out at 37 °C in 5 % CO_2 incubator under humidified atmosphere 95% (Sheldon, TC2323; Cornelius, OR, USA). Cells were seeded into 96-well microtiter plastic plates with20000 (cells/well) in case of HCT-116, RPE1, and with 10000 (cells/well) in case of A549, MCF-7, PC3 then allowed to adhere for 24 h. The medium was aspirated and then added to the cells with the test compounds at a dose of 100µg/ml in DMSO. After incubationof the medium for 48 h, 40µl of MTT salt (2.5µg/ml) was added to each well and then incubated for an additional 4 h. To stop the reaction and dissolve any formed formazan crystals, 200 µl of 10 % sodium dodecyl sulfate (SDS) were added to each well and incubated overnight at 37 °C. The amount of formazan product was measured at 595 nm with a reference wavelength of 690 nm as a background using a microplate reader (Bio-Rad Laboratories, model 3350, USA). For the untreated cells (negative control), the medium was added instead of the tested compounds. A positive control Adrinamycin® (doxorubicin) (Mr=579.9) (Pharmacia India Pvt Ltd, Gurgaon, Haryana 122001, India) was used as a known cytotoxic natural agent giving 100%

inhibition. Dimethylsulfoxide (DMSO) was the vehicle used for dissolution of the testing compound and its final concentration on the cells was less than 0.2 %.

2.3.3 Determination of IC_{50}values:

The concentration required for 50 % inhibition of cell viability (IC_{50}) of the potent compounds that showed cytotoxic effect towards the cancer cell lines under study was calculated using the probit analysis using a simple ttest (SPSS statistical analysis software package/version 9.0, 1989SPSS Inc., Chicago, USA).

2.4. In silico molecular docking

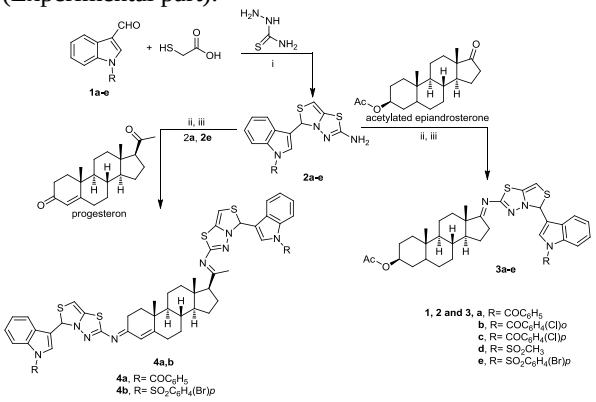
In *silico* molecular docking of the most anti-proliferative active compounds **3a-e**, **4a** and **7a** toward Bcl-2 (PDB ID: 2O2F) were performed using MOE program (Molecular Operating Environment, 2008.10. The computational experiments were performed on a Windows 10 pro with Intel R CoreTM i5-3210M CPU@4.00GHz processor and 12 GB RAM. The 3D structure of Bcl-2 protein was downloaded from RCSB Protein Data Bank in complex with 4-(4-benzyl-4-methoxypiperidin-1-yl)-n-[(4-{[1,1-dimethyl-2-(phenylthio)ethyl]amino}-3-nitrophenyl)sulfonyl]benzamide (LI0) [32]. The protein structure was prepared for docking process and the co-crystalline ligand was re-docked into the active bocket to validate the docking protocol with RMSD value less than 2Å. The 2D structure of **3a-e**, **4a** and **7a** were generated using ChemDraw Ultra 12.0 and converted into 3D by MOE programe. Hydrogen atoms was added, then partial charges was applied (based on MMFF94x force field), and minimized using the MMFF94x force field (eps= r, Cutoff until the RMS gradient of 0.01 kcal/mol/Å was achieved) [33]. Docking process was performed using the Triangle Matcher docking algorithm and London dG scoring function. A total of 30 most favorable binding sites and orientations were generated for each compound. The best docked pose was selected based on the docking score and binding interactions with the target. MOE program utilizing rigid/flexible (receptor/ligand) technique with five energy maps including H-bond interaction, electrostatic, two *Van der Waal* parameters and hydrophobicity.

3. Results and discussions

3.1. Chemistry

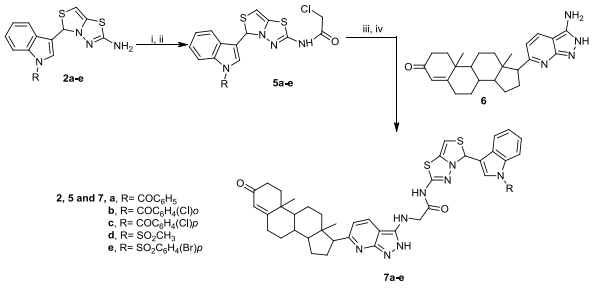
Aiming to synthesis of 5-(3-indolyl)-5*H*-thiazolo[4,3-*b*][1,3,4]thiadiazoles integrated with the steroid moieties, the starting materials *N*-substituted-1*H*-indole-3-carboxaldehydes **1a-e** were prepared as reported [34,35]. A one-pot reaction of **1a-e** with thioglycolic acid and thiosemicarbazide under grinding led to the formation of 5*H*-thiazolo[4,3-*b*][1,3,4]thiadiazole derivatives **2a-e** (**Scheme 1**). The ^{1}H-NMR spectra lack the singlet signal of the aldehydic protons of *N*-substituted indole-3-carboxaldehydes and revealed a new singlet signals at δ ranging from 11.17 - 11.26ppm, and 8.00 - 8.33ppm characteristic for NH_2 proton and methine proton of thiazoline ring, beside the other methine proton of the thiazoline ring within the aromatic protons (Experimental part).

Condensation of **2a-e** with the carbonyl group of steroid moieties, namely acetylated epiandrosterone and progesterone under grinding then reflux in absolute ethanol afforded Schiff's base **3a-e** and **4a,b**, correspondingly (**Scheme 1**). The ^{1}HNMR spectra of the new Schiff's base **3a-e** lack the singlet signal of two proton of NH_2 group and displayed a new multiple signals at δ 4.60- 4.13 for CH-3. Also, showed three singlet signals for 3 methyl group at δ ranging from 3.39 to 0.81ppm, beside multiple signals of the other steroid protons from 2.33-0.82ppm (Experimental part). The ^{1}HNMR spectra of the new Schiff's base **4a** and **4b** showed the absence of the singlet signal of NH_2 protons, and revealed a new singlet signals at δ 5.62, 5.81ppm for CH-4. Additionally revealed three new singlet signals for 3 methyl group at δ ranging from 1.94-1.01ppm, beside multiple signals of the other steroid protons from 2.49-1.13 and 2.41-1.17ppm, respectively (Experimental part).



Scheme 1. Synthesis of Schiff's bases 3a-e and 4a,b; reagents and conditions: i) grinding, 10-15 min, conc. H_2SO_4, -20°C, 24h; ii) grinding, 10-15 min; iii) EtOH, reflux, 6-8h.

Furthermore, the chloroacetylation of **2a-e** with the aid of chloroacetyl chloride under reflux in dimethylformamide for about 3h yielded the resultant chloroacetamide derivatives **5a-e**. Heating of compounds **5a-e** with 3-aminopyrazolo pyridine of progesterone **6 [**27**]** under reflux in absolute ethanol furnished the consequent 2-[(steroids)-2*H*-pyrazolo[3,4-*b*]pyridin-3-ylamino]-5-(indoles)-thiazolo[4,3-*b*][1,3,4]thiadiazol-2-yl]-acetamides **7a-e (Scheme 2)**. The ^{1}HNMR spectra of compounds **7a-e** revealed a new singlet signals at δ ranging from 5.68 to 5.05ppm for CH-4, two singlet signals for two methyl groups at δ from 1.59-0.95ppm, besides multiple signals of the other steroid protons from 2.50-1.05ppm (Experimental part).



Scheme 2. Synthesis of the acetamide derivatives 5a-e and thiazolo[4,3-b]-1,3,4-thiadiazoles7a-e; reagents and conditions: i) $ClCH_2COCl$, DMF, stirring, 0-5 °C; ii)reflux, 3h; iii) fusion, 30 min; iv) EtOH, reflux, 6-8h.

3.2. In vitro anti-proliferative activity evaluation

Assessment of *in-vitro* anti-proliferative activity

Anti-proliferative activity of the newly synthesized compounds **3a-e, 4a,b**, and **7a-e** were evaluated against A-549 (lung cancer), HCT-116 (colon cancer), MCF-7 (breast cancer), and PC3 (prostate cancer) at a single dose of 100µg/ml using MTT assay. The growth inhibition (%) results and the half-maximal inhibitory concentration (IC_{50}) values were recorded in Table **1** and **2**. Results referred that compounds **3a**, **3c**, **3d, 3e**, **4e,** and **7e** shown to be more potent towards HCT-116 cancer cell line than the reference drug doxorubicin of IC_{50} =48.02 µM/ml. Their activity was in order as follows of **7e** >**3a** > **4a** > **3e** > **3c** > **3d** > **3b** with IC_{50} of 7.25, 8.58, 8.6, 12.69, 15.88, 22.2 and 38.92µM/ml, respectively. Interestingly, the results implied that the 17-[5-(*N*-(4-bromo-benzenesulphonyl)-1*H*-indol-3-yl)-thiazolo[4,3-*b*][1,3,4]-thia-diazol-2-ylimino]-10,13-dimethyl-hexadecahydro-cyclopenta[*a*]phenanthren-3-ol (**3e)** was found to be the most active one towards the three studied cancer cell line; A-549, HCT-116, and PC3 with IC_{50} of 27.05, 12.69, and 24.61µM/ml, compared to doxorubicin of IC_{50} of 39.74, 48.02, and 34.77µM/ml respectively. None of the test compounds showed anti-proliferative towards MCF-7 cancer cell line.

Table 1: Anti-proliferative activity of the newly synthesized compounds against human carcinoma cell lines at 100µg ml^{-1}

Compd.[a]	Inhibition growth (%)			
	A-549	HCT-116	MCF-7	PC3
3a	58.6	94	54	71.5
3b	51.6	76	50	56.9
3c	56.3	76.2	49.6	62.0
3d	24.4	93	63.3	27.9
3e	75.1	86.6	40.9	85.6
4a	0	80.5	30.1	54.2
4b	0	33.2	3.6	18.0
7a	22.7	58	33.3	32.7
7b	35.2	84	0	21.4
7c	36.5	72.8	37.3	49.4
7d	5.7	82.2	57	82.2
7e	24.3	89.4	21.5	65.3
Negative control[b]	-	-	-	-
Doxorubicin	98	100	96.8	99

[a] concentration of the test compounds and positive control (doxorubicin) 100µg/ ml

[b] untreated cells in DMSO and its final concentration on the cells was less than 0.2%.

Table 2: IC_{50} of the highly anti-proliferative active compounds against human cancer cell lines

Compd.	IC_{50}µM/ml		
	A-549	HCT-116	PC3
3a	81.44	8.58	67.59
3b	110.76	38.92	113.48
3c	63.39	15.88	106.47
3d	-	22.2	-
3e	27.05	12.69	24.61
4a	-	8.6	67.21
7a	-	54.11	-
7b	-	46.58	-
7d	-	72.33	64.42
7e	-	7.25	80.34
Doxorubicin	39.74	48.02	34.77

[a] Results are the mean of three independent experiments.

3.3. Structure activity relationships

By checking the above-mentioned biological data, we can depict the structure activity relationships. The nature of substituent at *N*-position of 1*H*-indole moiety might play a vital role in the anti-proliferative activity. Meanwhile the existence of Schiff's bases bridge (CH=N) which characterize the most active anti-proliferative compounds against the HCT-116 cancer cell line, namely **3a**, **3c**, **3d**, **3e,** and **4a**. Regarding compound **3a** with *N*-benzoyl substituent at indole moiety was the most active member against the HCT-116 cancer cell line (IC_{50} of 8.58µM/ml) with 5.6-fold increased activity as compared to the reference drug doxorubicin of IC_{50} = 48.02µM/ml. It appears that electron withdrawing Cl substituent at *p*-position of the *N*-benzoyl **3c** decrease the activity than the un-substituted one **3a**, with keeping its activity towards HCT-116 cancer cell line (IC_{50} of 15.88µM/ml, 3.0-fold) than doxorubicin of (IC_{50}= 48.02µM/ml). Compounds **3d** and **3e** with sulphonyl substituent at the *N*-position of indole moiety revealed high anti-proliferative activity (IC_{50}=22.2, and 12.69µM/ml) with 2.16-, and 3.8-fold than doxorubicin (48.02µM/ml), and less than compound **3a**. Further observation of the effect of substitution pattern at *N*-indole of **4a** and **7a** with progesterone moiety are considered. Compounds **4a** substituted with *N*-benzoyl and **7a** substituted with *N*-(*p*)bromobenzene sulphonyl caused increase of activity against HCT-116 cancer cell line IC_{50} of 8.6 and 7.25 µM/ml, with 5.6-, and 6.6- fold than doxorubicin (48.02µM/ml).

In general, the epiandrosterone Schiff's base **3e** of *N*-(*p*)bromobenzene sulphonyl at the *N*-position of indole moiety was the most active among the tested compounds. It has excellent anti-proliferative activity towards A-549, HCT-116, and PC3 cancer cell lines with IC_{50} of 27.05, 12.69, and 24.61µM/ml, than the doxorubicin (IC_{50} = 39.74, 48.02 and 34.77µM/ml), respectively.

3.4. In silico molecular docking study

Bcl-2 belongs to proteins families that regulate programmed cell death or apoptosis and includes both death antagonists such as Bcl-2 and Bcl-xL and agonists as Bax, Bak, Bid, and Bad [36]. These proteins associate at least one of four homologous regions called Bcl homology (BH) domains (BH1 to BH4). High levels of Bcl-2 gene expression can be found in several different forms of human cancers [37]. Furthermore, Bcl-2 is involved in chemoresistance, since Bcl-2 overexpression can prevent the cell death effect of several anticancer drugs by stopping the apoptotic pathway. The levels of expression of Bcl-2 proteins are associated with relative tolerance to a wide variety of chemotherapeutic drugs and γ-irradiation [36]. Consequently, inhibiting the defensive role of overexpressed Bcl-2 protein in tumour cells is an enticing technique for either restoring the usual apoptotic mechanism or rendering these cells more responsive to traditional chemotherapy or radiotherapy. In this respect, Bcl-2 is a promising therapeutic target in the development of potential anti-cancer agents.

We have performed molecular docking studies to investigate the binding affinity of the most active anti-proliferative compounds toward human colon cancer cell (HCT-116) **3a-e**, **4a** and **7a** with target human Bcl-2 anti apoptotic protein (PDB ID: 2O2F) using program MOE 2008.10.

The data obtained (**Table 3**) revealed that all studied compounds **3a-e**, **4a,** and **7a** exhibited better docking score ranging from -22.98 to -29.57kcal/mol, compared to **LI0** of -25.71kcal/mol and RMSD 1.71 (**Table 3**). Also, all compounds showed effective fitting inside the protein active pocket *via* electrostatic, and H-bond interaction with Tyr105 and the same amino acid residue Arg134 as the co-crystalline ligand (**LI0**) (**Table 3, Fig 1**). Epiandrosterone derivative **3e** with powerful anti-proliferative activity towards the three studied cancer cell lines exhibited better docking score of -24.13 kcal/mol with good fitting inside the Bcl-2 active pocket *via* formation of H-bon acceptor and electrostatic interactions between SO group, indole moiety and the amino acid residue Tyr105, Arg134, respectively (**Table 3, Fig 2**).

Moreover, progesterone derivatives **4a** exhibited best docking score of -29.57 kcal/mol, higher than **LI0** (-25.71 kcal/mol), and displayed good fitting inside the Bcl-2 active site *via* H-bond acceptor with the amino acid residue of Arg134 (**Table 3**). While, the compound **7e** display good docking score of -23.50 kcal/mol, with excellent fitting inside the Bcl-2 active site *via* the two H-bonds acceptor and the two arene-cation interaction with the amino acid residue of Arg134 (**Table 3**).

Table 3: Docking results of the most active compounds 3a-e, 4,b and 7a-d into the active site of Bcl-2 (PDB ID: 2O2F).

Compd. No.	Binding Energy (kcal/mol)	Main atoms from the compounds	Amino acid residue	Type of interaction and bond length (Å)
LI0	-25.71 Rmsd: 1.71	O of SO_2 group Phenyl ring Phenyl ring	Tyr199 Tyr199 Arg143	H-acc (2.65) Arene-Arene Arene-Cation
3a	-22.98	CO of benzoyl moiety benzoyl moiety	Tyr105 Arg143 andTyr105	H-acc (2.57) Arene-Cation Arene- Arene
3b	-24.86	Indole moiety	Arg143	Arene-Cation
3c	-24.60	Phenyl ring	Arg143	Arene-Cation
3d	-23.88	SO Indole moiety	Tyr105 Arg143 andTyr105	H-acc (2.48) Two Arene-Cation Arene- Arene
3e	-24.13	SO Indole moiety	Tyr105 Arg143	H-acc (2.45) Two Arene-Cation
4a	-29.57	C=N	Arg143	H-acc (2.70)
7e	-23.50	SO SO Pyridine ring	Arg104 Arg104 Arg143	H-acc (2.71) H-acc (3.07) Arene-Cation

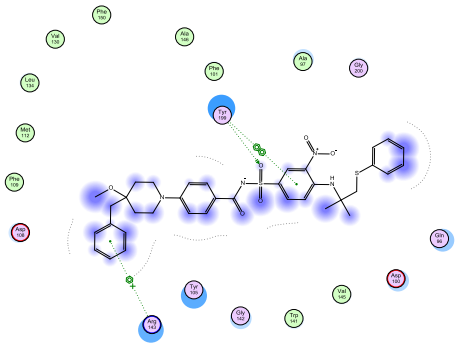


Fig 1a: The 2D binding mode of LI0 into the active site of Bcl-2 (PDB ID: 2O2F).

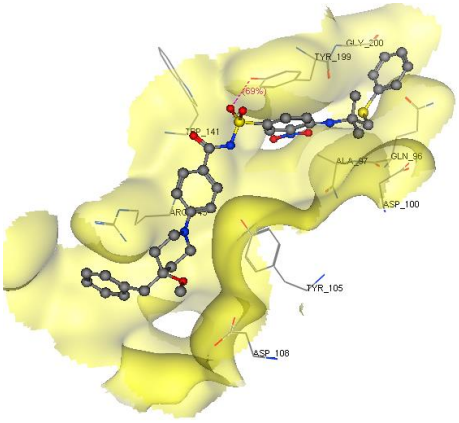


Fig 1b: The 3D docked confirmation of **of** LI0 into the active site of Bcl-2 (PDB ID: 2O2F).

4. Conclusion

To develop potent anti-proliferative agents, the indole and steroid moieties have been adopted to synthesize of novel series of *N*-substituted-3-indolyl-5*H*-thiazolo[4,3-*b*][1,3,4]thiadiazoles integrated with steroid moieties. Two new series of the Schiff's bases **3a-e** and **4a,b** have been synthesized *via* condensation reaction of the amino 5H-thiazolo[4,3-*b*]-1,3,4-thiadiazole derivatives **2a-e** with the acetylated epiandrosterone, and the progesterone. On the other hand, acetamide derivatives **7a-e** have been obtained through the reaction of chloroacetamides **5a-e** with the 3-aminopyrazolo pyridine of progesterone **6.** The anti-proliferative activity of the target compounds screened towards A549, HCT-116, MCF-7, and PC3 cancer cell lines have been studied. Cytotoxicity results indicated that the17-[5-(*N*-(4-bromo-benzenesulphonyl)-1*H*-indol-3-yl)-thiazolo[4,3-*b*][1,3,4]-thia-diazol-2-ylimino]-10,13-dimethyl-hexadecahydro-cyclopenta[*a*]phenanthren-3-ol (**3e)** emerged as a lead anti-proliferative agent among the examined series towards A-549, HCT-116, and PC3 cancer cell lines with IC_{50} of 27.05, 12.69, and 24.61µM/ml, respectively compared to the reference drug doxorobcine. In addition, molecular docking studies used for rationalize the binding interaction of the most active compounds **3a-e**, **4a,** and **7a** toward human colon cancer cell (HCT-116) with the anti-apoptotic Bcl-2. The result revealed that all docked compounds exhibited better docking score ranging from -22.53 to -29.57 kcal/mol, and showed effective fitting inside the protein active pocket *via* electrostatic and H-bond interaction with the same amino acid residue (Arg134) as the co-crystalline ligand **LI0** of -25.71 kcal/mol .

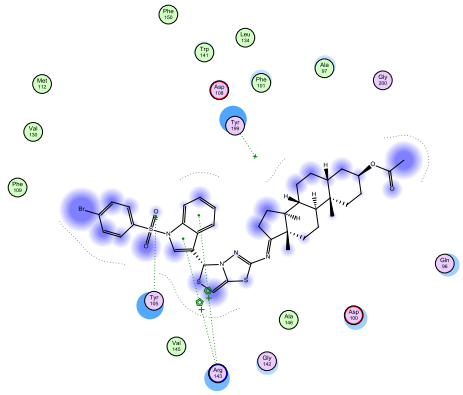


Fig 2a: The 2D binding mode of **3e** into the active site of Bcl-2 (PDB ID: 2O2F).

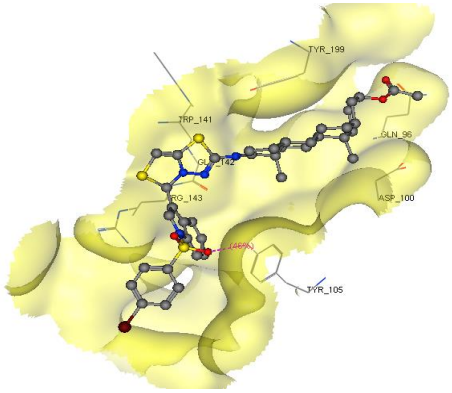


Fig 2b: The 3D docked confirmation of **3e** into the active site of Bcl-2 (PDB ID: 2O2F).

Conflicts of interest

The authors declare no conflict of interests, financial or otherwise.

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