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Research Article

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Design, Synthesis, Antitumor Activity, Cell Cycle Analysis and ELISA Assay for CDK-2 of a New (4-Aryl-6-Flouro-4H-Benzo [4, 5] Thieno [3, 2-b] Pyran) Derivatives

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ABSTRACT

A series of benzo [1] thiophene derivatives (3a-f), (4a-f) and (5a-f) were synthesized and characterized by spectroscopic and elemental analysis. All compounds were subjected to one dose anticancer screening in NCI-America, but the only high results were further subjected to five dose screening. An outstanding result of compound 4f (GI50 = 0.15, TGI= 1.14 µM) and 4c (GI50 = 1.09, TGI = 10.19, LC50 = 100 µM). To explore mechanism of cytotoxicity, compound 4f and 4c were allowed to affect cell cycle using HT-29 cell line for (24 and 48 hr). They caused induction of apoptosis causing preG1 apoptosis and cell growth arrest at G2/M in a time dependant manner inhibiting CDK-2. IC50 of compound 5d was 0.32 µM, IC50 of 6 was 0.15 µM while IC50 of erlotinib reference was 0.3 µM. Finally we synthesized a series of benzo [1] thiophene derivatives having a good cytotoxic activity suggesting promising anticancer derivatives.

Keywords: Benzo thienopyran; Anticancer; Cell cycle; Apoptosis; CDK-2

INTRODUCTION

The process of programmed cell death, or apoptosis, is generally characterized by distinct morphological characteristics [1]. Apoptosis is considered a vital component of various processes including normal cell turnover and proper development. Inappropriate apoptosis (either too little or too much) is a factor in types of cancer. Light microscopy has identified the various morphological changes that occur during apoptosis [2]. During the early process of apoptosis, cell shrinkage and psychosis (is result of chromatin condensation and this is the most characteristic feature of apoptosis). With cell shrinkage, the cells are smaller in size, the cytoplasm is dense and the organelles are more tightly packed. The apoptotic cell appears as a round or oval mass with dark eosinophilic cytoplasm and dense purple nuclear chromatin fragments, all these changes found to be irreversible. To date, research indicates that there are three main apoptotic pathways: the extrinsic or death receptor pathway, the intrinsic or mitochondrial pathway and Performing/granzyme pathway [3]. Cancer is an example where the normal mechanisms of cell cycle regulation are dysfunctional. In fact, suppression of apoptosis during carcinogenesis is thought to play a central role in the development and progression of some cancers [4]. Searching previous literatures, we found that aurones [5,6] (Z)-2-benzylidenebenzofuran-3-(2H)-ones and flavopridol [7] constitute two subtypes of flavonoid, both of them have remarkable antiproliferative activity. Flavopridol is active against leukemia L1210 (IC_{50} = 0.15 µm) causing cell cycle arrest at G_2 phase and breast cancer MCF-7 (IC50 = 0.03µm). Aurones inhibit both cyclin dependent kinases (CDK's) [8] and tyrosine kinases [9] (Figure 1). Although biological activity of thioaurones [10] was not explored like aurones, they proved to have a similar anticancer activity. 2-(2-

Hydroxy-5-nitro-benzylidene)benzo[b]thiophen-3-one [11] was found to inhibit DHHC-mediated palmitoylation *in vitro* which is necessary for the proper activity of oncoproteins such as H-Ras (involved in regulating cell division in response to growth factors stimulation [12]. Also we found that 2-amino-4-aryl-9-flouro-4,5dihydrobenzo[b]pyrano-[4,3-b]pyran-3-carbonitriles were special active agents against lung, breast and CNS cancer (NCI-H460, MCF-7 and SF-268 cell lines) [13]. Bcl-2 protein [regulate apoptosis by inducing pro-apoptotic) or inhibiting (anti-apoptotic) apoptosis] binding compounds also provides a satisfactory lead compound for the development of potential anticancer agent. Ethyl 2-amino-6-bromo-4-(1-cyano-2- ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (HA14-1) an antagonist for ant apoptotic Bcl-2 proteins was used to overcome drug resistance in cancer [14]. Recently, screening of 2-amino-6-bromo-4-(4-nitrophenyl)-4H-[1]benzothieno[3,2-b]pyran-3-carbonitrile against

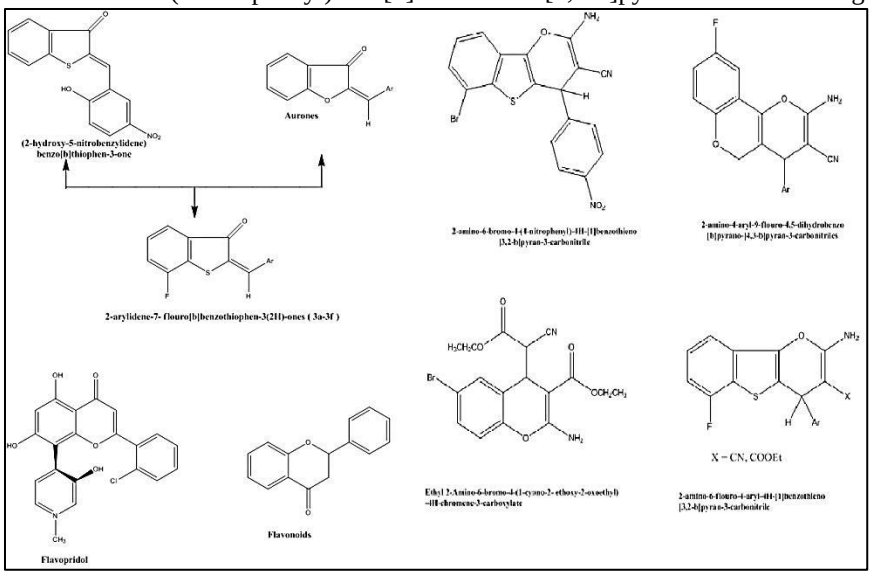


Figure 1: Design of target compounds (3a-f), (4a-f) and (5a-f)

HCT-116 cell line led to outstanding result as anticancer agent and also proved to induce apoptosis at G_0 /G_1 phase [14]. The present work is aimed towards construction of novel heterocyclic compounds of anticipated anticancer activity and to compare anticancer screening result of 2-amino-6-bromo-4-(4-nitrophenyl)-4H-[1]benzothieno[3,2-b]pyran-3-carbonitrile with our target compounds by replacing bromine atom at position 6 with fluorine atom which possess higher electro negativity, higher thermal stability and higher lipophilicty than bromine atom [13]. Moreover, comparing activity of aminocyano containing compounds (4a-f) with aminoester containing compounds (5a-f). By doing this, we found that our target compound (4f) (GI_{50} = 0.079 µM, TGI= 1.21 µM, LC_{50} = 3.48 µM) has higher anticancer activity compared to 3e (GI_{50} = 0.11 µM, TGI= 7.94 µM, LC50 = 42.66 µM) at the same cell line [15]. Compounds (3a-f) have limited anticancer activity where growth percentage was between (80% - 97%). Second series (4a-f) have Avery high activity (-29 to 86.5%) from which NCI selected 4c, 4f for five dose screening. By replacing CN at C-3 with COOEt (5a-f), we found that they have anticancer activity but still less than (4a-f) and finally replacing NH_2 at C-2 with imine group (N=CH) led to unexpected result where IC_{50} was = 0.13 µM which means that replacing amino group with imine can maintain the high antiproliferative activity.

EXPERIMENTAL SECTION

General

Melting points were determined using a Griffin apparatus and were uncorrected. All chemicals and reagents were obtained from Aldrich (Sigma-Aldrich), and were used without further purification. Reactions were monitored by TLC, performed on silica -gel glass plates containing 60 GF-254, and visualization on TLC was achieved by UV light or iodine indicator. IR spectra were determined on Shimadzu IR 435 spectrophotometer (KBr, cm^{-1}). ^{1}HNMR and ^{13}CNMR spectra were carried out using Bruker 400 MHZ and 100 MHZ spectrophotometer using TMS as internal standard faculty of pharmacy Cairo University, Egypt. Chemical shifts (δ) were recorded in ppm on δ scale. Mass spectra and Elemental analysis were carried out at the regional center for mycology and biotechnology, Al-Azhar University, Cairo, Egypt.

General Procedure for the Synthesis of 2-Arylidene-7- Flouro-1-Benzothiophen-3(2H)-ones (3a-f)
7-flouro [1] benzothiophen-3(2H)-one (2) (1.68 gm, 10 mmol) was added to an appropriate amount of aromatic aldhyde (10 mmol) in 50 ml glacial acetic acid and the mixture was refluxed for 5 hr. The product was obtained after evaporation of half amount of glacial acetic acid, dried and crystallized from isopropanol.

7-Flouro-2-(4-Bromobenzylidene)-1-Benzothiophen-3(2H)-one (3a):
Yield: 78%; m.p 180°C; IR (KBr) cm^{-1}: 1685 (C=O), 1589 (C=C);
^{1}H NMR (400 MHz, $CDCl_3$) ppm δ: 7.65-7.87 (m, 7H, CH aromatic), 7.93(s, 1H =CH methine); ^{13}CNMR(100 MHz, $CDCl_3$) ppm: 121.25, 122.75, 125.08, 127.25, 130.11, 132.03, 132.23, 132.27, 132.46, 132.84, 133.12, 133.15, 133.40, 158.98 (CH aromatic,benzylic), 187.51(C=O); MS: m/z (% rel. Abundance): 334 (M^+) (100%), 336 (M+2) (78.48%); Anal. Calcd for $C_{15}H_8BrFOS$: C, 53.75; H, 2.41; S, 9.57; Found C, 53.94; H, 2.39; S, 9.58.

7-Flouro-2-(4-Chlorobenzylidene)-1-Benzothiophen-3(2H)-one (3b):
Yield: 72%; m.p 180°C; IR (KBr) cm^{-1}: 1687 (C=O), 1583 (C=C);
^{1}H NMR (400 MHz, DMSO-d_6) ppm δ: 7.40- 7.80 (m, 7H, CH aromatic), 8.03 (s, 1H, =CH methine); ^{13}CNMR(100 MHz, DMSO) ppm: 122.48, 123.33,124.02, 125.07, 125.57, 126, 126.80, 127.65, 130.01, 132.70, 132.91, 133, 133.05, 133.78, 136.05 (aromatic C, benzylic C , 187.05 (C=O); MS: m/z (% rel. Abundance): 290 (M^+) (60.41%), 292 (M+2) (23.58%); Anal. Calcd for $C_{15}H_8ClFOS$: C, 61.97; H, 2.72; S, 11.03; Found C, 62.23; H, 2.82 S, 11.01.

7-Flouro-2-(4-Flourobenzylidene)-1-Benzothiophen-3(2H)-one (3c):
Yield: 60%; m.p 180°C; IR (KBr) cm^{-1}: 1693 (C=O) , 1581 (C=C);
^{1}H NMR (400 MHz, DMSO- d_6) ppm δ: 7.40-7.90 (m, 7H, CH aromatic), 8.02 (s, 1H, =CH methine); ^{13}CNMR(100 MHz, DMSO) ppm: l117.02,117.23, 122.34, 122.53, 123.24,124.05, 128.86, 130.45, 131.20, 132.43, 133.33, 133.60, 134.01, 134.11(CH aromatic,benzylic), 186.89 (C=O); MS: m/z (% rel. Abundance): 274 (M^+) (100%); Anal. Calcd for $C_{15}H_8F_2OS$: C, 65.68; H, 2.94; S, 11.69 Found: C, 65.89; H, 2.98; S, 11.69.

7-Flouro-2-(4-Hydroxybenzylidene)-1-Benzothiophen-3(2H)-one (3d):
Yield: 70%; m.p 249°C; IR (KBr) cm^{-1}: 3336.85 (OH), 1655 (C=O) , 1604 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) ppm δ: 6.91-7.70 (m, 7H, CH aromatic), 7.90 (s, 1H, =CH methine), 10.49(S, 1H, OH, D_2O exchangeable); MS: m/z (% rel. Abundance): 272 (M^+) (100%); Anal. Calcd for $C_{15}H_9FO_2S$: C, 66.16; H, 3.33; S, 11.78; Found C, 66.42; H 3.41; S, 11.75.

7-Flouro-2-(4-Methoxybenzylidene)-1-Benzothiophen-3(2H)-one (3e):
Yield: 78%; m.p 165°C; IR (KBr) cm^{-1}: 1672 (C=O), 1583 (C=C), 2920-2846 (CH aliphatic); ^{1}HNMR (400 MHz, DMSO-d_6) ppm δ: 3.80(s, 3H, OCH_3), 7.10-7.80 (m, 7H, CH aromatic), 7.90 (s, 1H, =CH methine); MS: m/z (% rel. Abundance): 286 (M^+) (100%); Anal. Calcd for $C_{16}H_{11}FO_2S$: C, 67.16; H, 3.87; S, 11.20; Found C, 67.41; H, 3.93; S, 11.22.

7-Flouro-2-(4-Nitrobenzylidene)-1-Benzothiophen-3(2H)-one (3f):
Yield: 82%; m.p 230°C; IR (KBr) cm^{-1}: 1686 (C=O) , 1523 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) ppm: 7.50-8.30 (m, 7H, CH aromatic), 8.40 (s, 1H, =CH methine); MS: m/z (% rel. Abundance): 301(M^+) (46.24%); Anal. Calcd for $C_{15}H_8FNO_3S$: C, 59.8; H, 2.68; N, 4.65; S, 10.64; Found C, 59.97; H, 2.65; N, 4.77; S, 10.65.

General Procedure for the Synthesis of 2-Amino-4-Aryl-6-Flouro-4H-[1] Benzothieno [3, 2-*b*] Pyran-3-Carbonitrile (4a-f)
A mixture of 7-flouro-2-arylidene-benzothiophen-3-ones 3a-f (1 mmol), malononitrile (0.07 g, 1 mmol) and piperidine (4 drops) in absolute ethanol (50 mL) was heated under reflux for 5 h. The reaction was cooled, and the separated solid was filtered, dried and crystallized from isopropanol.

2-Amino-6-Flouro-4-(4-Bromophenyl)-4H-[1] Benzo thieno [3, 2-*b*] Pyran-3-Carbonitrile (4a)
Yield: 50%; mp: 201°C; IR (KBr) cm^{-1}: 3468, 3321 forked peak of (NH_2), 2886 (CH-aliphatic), 2198 (CN); ^{1}HNMR (400 MHz, DMSO-d_6) ppm: 5.12 (s, 1H, pyran H-4), 7.31-7.32 (s, 2H, NH_2, D_2O exchangeable), 7.33–7.35 (d, 2H, *J = 8 HZ)*, 7.42-7.44 (d, 2H, *J = 8HZ)*, 7.51-7.57 (m, 3H, CH aromatic); ^{13}CNMR(100 MHz, DMSO) ppm: 55.94 (aliphatic C), 111.58, 111.76, 116.43, 116.46, 119, 120.27, 121.25, 122.38, 122.59, 127.84, 127.91, 130.2, 132.24, 132.37, 132.42, 139.40, 143.54, (aromatic C), 160.72 (CN); MS: m/z (% rel. Abundance): 400 (M^+) (51.34%) , 402

(M+2) (7.65%); Anal. Calcd for $C_{18}H_{10}BrFN_2OS$: C, 53.88; H, 2.51; N, 6.98; S, 7.99; Found C, 54.12; H, 2.50; N, 7.21; S, 8.00.

2-Amino-6-Flouro-4-(4-Chlorophenyl)-4H-[1] Benzothieno [3, 2-*b*] Pyran-3-carbonitrile (4b):
Yield: 70%; mp: 203-205°C; IR (KBr) cm^{-1}: 3456,4329 forked peak of (NH_2), 2924 (CH-aliphatic), 2194 (CN); ^{1}HNMR (400 MHz, DMSO-d_6) ppm: 5.10 (s, 1H, H-4), 7.26-7.27 (d, 2H, *J = 4 HZ)*, 7.29-7.30 (d, 2H, *J = 4 HZ)*, 7.32 (s, 2H, NH_2, D_2O exchangeable),7.53-7.58 (m, 3H, CH aromatic); ^{13}CNMR(100 MHz, DMSO) ppm: 56.02 (pyran C-4), 111.56, 111.74, 116.42, 116.45, 119.08, 120.27, 122.38, 127.92, 128.77, 129.31, 129.85, 132.38, 132.42, 132.70, 139.13, 139.18 (aromatic CH), 160.72 (CN); MS: m/z (% rel. Abundance): 356 (M^+) (3.45%), 358 (M+2) (1.20%); Anal. Calcd for $C_{18}H_{10}ClFN_2OS$: C, 60.59; H, 2.82; N, 7.85; S, 8.99 Found: C, 60.81; H, 2.84; N, 8.01; S, 9.00.

2-Amino-6-Flouro-4-(4-Fluorophenyl)-4H-[1] Benzothieno [3, 2-*b*] Pyran-3-Carbonitrile (4c):
Yield: 80%; mp: 180°C; IR (KBr) cm^{-1}: 3471, 3333 forked peak of (NH_2), 2854 (CH-aliphatic), 2194 (CN); ^{1}HNMR (400 MHz, DMSO-d_6) ppm: 5.13(s, 1H, pyran H-4), 7.32 (s, 2H, NH_2, D_2O exchangeable), 7.35-7.36 (d, 2H, *J = 2 HZ*), 7.37-7.38 (d, 2H, *J =2* HZ), 7.50 -7.56 (m, 3H, CH aromatic); MS: m/z (% rel. Abundance): 340 (M^+) (1.19%); Anal. Calcd for $C_{18}H_{10}F_2N_2OS$: C, 63.52; H, 2.96; N, 8.23; S, 9.42; Found: C, 63.78; H, 2.99; N, 8.42; S, 9.44.

2-Amino-6-Flouro-4-(Hydroxy)-4H-[1] Benzothieno [3, 2-*b*] Pyran-3-Carbonitrile (4d):
Yield: 80%; mp: 240°C; IR (KBr) cm^{-1}: 3471, 3329 forked peak (NH_2), 3210 (OH), 2854 (CH-aliphatic), 2191 (CN); ^{1}HNMR (400 MHz, DMSO-d_6) ppm: 4.96(s, 1H, pyran H-4), 6.70 (s, 2H, NH_2, D_2O exchangeable), 7.08-7.54 (m, 7H, CH aromatic), 9.43(s, 1H, OH , D_2O exchangeable); MS: m/z (% rel. Abundance): 338 (M^+) (43.45%);
Anal. Calcd for $C_{18}H_{11}FN_2O_2S$: C, 63.42; H, 3.28; N, 8.28; S, 9.48 Found: C, 63.42; H, 3.41; N, 8.17; S, 9.50.

2.3.5. 2-Amino-6-Flouro-4-(4-Methoxyphenyl)-4H-[1]Benzothieno[3,2-*b*]Pyran-3-Carbonitrile (4e):
Yield: 80%; mp: 210°C; IR (KBr) cm^{-1}: 3460, 3345 forked peak of (NH_2), 2960 (CH-aliphatic), 2189 (CN); ^{1} H NMR (400 MHz, DMSO-d_6) ppm: 3.71 (S, 3H, OCH_3), 5.03(S, 1H, pyran H-4), 7.15-7.17 (d, 2H, *J = 8* HZ), 7.23-7.21 (d, 2H, *J = 8* HZ), 7.32 (s, 2H, NH_2, D_2O exchangeable), 7.33-7.51 (m, 3H, CH aromatic) ; ^{13}CNMR(100 MHz, DMSO) ppm: 55.55 (OCH_3), 56.73 (pyran C-4), 111.4, 111.58, 114.62, 116.35, 120.24, 120.43, 122.3, 122.5, 127.74, 127.81, 129.04, 132.49, 132.55, 136.24, 138.79, 138.83 (aromatic CH), 160.55 (CN); MS: m/z (% rel. Abundance): 352 (M^+) (48.78%); Anal. Calcd for $C_{19}H_{13}FN_2O_2S$: C, 64.76; H, 3.72; N, 7.95; S, 9.10; found: C, 64.95; H, 3.70; N, 8.12; S, 9.08.

2-Amino-6-Flouro-4-(4-Nitrophenyl)-4*H*-[1]Benzothieno[3,2-*b*]Pyran-3-Carbonitrile (4f):
Yield: 95%; mp: 235°C; IR (KBr) cm^{-1}: 3429,4329 forked peak of (NH_2), 2924 (CH-aliphatic), 2198 (CN); ^{1}HNMR (400 MHz, DMSO-d_6) ppm: 5.34 (s, 1H, H-4), 7.30 (s, 2H, NH_2, D_2O exchangeable), 7.58-7.6- (d, 2H, *J = 8* HZ), 7.61-7.63 (d, 2H, *J = 8* HZ), 8.23-8.26 (m, 3H, CH aromatic); MS: m/z (% rel. Abundance): 367 (M^+) (11.26%); Anal. Calcd for $C_{18}H_{10}FN_3O_3S$: C, 58.85; H, 2.74; N, 11.44; S, 8.73; Found: C, 59.17; H, 2.86; N, 11.73; S, 8.74.

General Procedure for the Synthesis of Ethyl 2-Amino-4-Aryl-6-Flouro-4H-Benzo [1] Thieno [3, 2-*b*] Pyran-3-Carboxlates (5a-f)
A mixture of 7-flouro-2-arylidene-benzothiophen-3-ones (3a-f) (1 mmol), ethylcyanoacetate (0.113 g, 1 mmol) and piperidine (4 drops) in absolute ethanol (50 mL) was heated under reflux for 7 hours. The reaction was cooled, and the separated solid was filtered, dried and crystallized from isopropanol.

Ethyl 2-Amino-6-Flouro-4-(Bromo)-4H-[1] Benzo Thieno [3, 2-*b*] Pyran-3-Carboxylate (5a):
Yield: 35%; mp: 115°C; IR (KBr) cm^{-1}: 3468 , 3317 forked peak of (NH_2), 2974-2908 (CH-aliphatic), 1685 (C=O); ^{1}HNMR (400 MHz, DMSO-d_6) ppm: 0.97-1.01 (t, 3H, CH_3, *J = 4HZ*), 3.92-3.95 (q, 2H, OCH_2, *J = 4 HZ*), 5.1 (s, 1H, H-4), 7.45-7.47 (d, 2H, *J = 8* HZ), 7.51-7.52 (d, 2H, *J = 8* HZ), 7.53-7.81 (m, 3H, CH aromatic), 7.91 (s, 2H, NH_2, D_2O exchangeable); MS: m/z (% rel. Abundance): 448 (M^+) (48.27%), 450 (M+2) (3.79%); Anal. Calcd for $C_{20}H_{15}BrFNO_3S$: C, 53.58; H, 3.37; N, 3.12; S, 7.15; found: C, 53.72; H, 3.33; N, 3.19; S, 7.13.

Ethyl 2-Amino-6-Flouro-4-(Chloro)-4H-[1] Benzothieno [3, 2-*b*] Pyran-3-Carboxylate (5b):
Yield: 44%; mp: 125°C; IR (KBr) cm^{-1}: 3363, 3271 forked peak of (NH_2), 2958(CH-aliphatic), 1680 (C=O); ^{1}HNMR (400 MHz, DMSO-d_6) ppm: 0.99- 1.01 (t, 3H, CH_3, *J = 4 HZ*), 3.92- 3.95 (q, 2H, OCH_2, J = 4 HZ), 5 .17(s, 1H, H-4),7.28-7.26 (d, 2H, *J = 8 HZ*), 7.32-7.34 (*d, 2H, J = 8 HZ*), 7.52 -7.90 (m, 3H, CH aromatic), 7.90 (s, 2H,

NH_2, D_2O exchangeable) ; MS: m/z (% rel. Abundance): 403 (M^+), (7.72%), 405 (M+2) (2.93%); Anal. Calcd for $C_{20}H_{15}ClFNO_3S$: C, 59.48; H, 3.74; N, 3.47; S, 7.94; Found: C, 59.76; H, 3.70; N, 3.56; S, 7.95.

Ethyl 2-Amino-6-Flouro-4-(Flour)-4H-[1] Benzothieno [3, 2-*b*] Pyran-3-Carboxylate (5c):
Yield: 40%; mp: 150°C; IR (KBr) cm^{-1}: 3368 , 3270 forked peak (NH_2), 2927 (CH-aliphatic), 1665 (C=O); ^{1}HNMR (400 MHz, DMSO-d_6) ppm: 0.97-1.006 (t, 3H, CH_3, *J = 4 HZ*), 3.89- 3.96 (q, 2H, OCH_2, *J = 4 HZ*), 5.17 (s, 1H, H-4), 7.07-7.06 (d, 2H, *J = 8* HZ), 7.57-7.55 (d, 2H, *J 8* HZ), 7.26- 7.31 (m, 3H, CH aromatic), 7.89 (s, 2H, NH_2, D_2O exchangeable); ^{13}CNMR(100 MHz, DMSO) ppm: 14.52 (CH_3), 59.31(OCH_2), 75.46(CH-4), 111.19, 111.37, 115.57, 116.3, 122.42, 122.63, 122.67, 127. 67, 129.3, 129.38, 132.52, 132.57, 138.39, 138.44, 143.22, 143.25 (aromatic C), 168.70 (C=O); MS: m/z (% rel. Abundance): 387 (M^+) (17.5%); Anal. Calcd for $C_{20}H_{15}F_2NO_3S$: C, 62.01; H, 3.9; N, 3.62; S, 8.28; Found: C, 62.34; H, 3.85; N, 3.80; S, 8.30.

Ethyl 2-Amino-6-Flouro-4-(hydroxy)-4H-[1] Benzothieno [3, 2-*b*] Pyran-3-Carboxylate (5d):
Yield: 30%; mp: 142°C; IR (KBr) cm^{-1}: 3340, 3309 forked peak of (NH_2), 3200 OH, 2924-2854 (CH-aliphatic), 1712 (C=O); ^{1}HNMR (400 MHz, DMSO-d_6) ppm: 1.28-1.31 (t, 3H, CH_3, *J = 4 HZ*), 4.26-4.32 (q, 2H, OCH_2, J = 4 HZ), 5.02 (s, 1H, H-4), 6.94-6.96 (d, 2H, *J = 8 HZ*), 6.99- 7.96 (m, 3H, CH aromatic), 7.99-8.01 (d, 2H, *J = 8 HZ), 8*.24 (s, 2H, NH_2, D_2O exchangeable)10.81(s, 1H, OH, D_2O exchangeable); MS: m/z (% rel. Abundance): 385 (M^+) (72.07%); Anal. Calcd for $C_{20}H_{16}FNO_4S$: C, 62.33; H, 4.18; N, 3.63; S, 8.32; found: C, 62.59; H, 4.20; N, 3.81; S, 8.30.

Ethyl 2-Amino-6-Flouro-4-(Methoxy)-4H-[1] Benzothieno [3, 2-*b*] Pyran-3-Carboxylate (5e):
Yield: 40%; mp: 150°C; IR (KBr) cm^{-1}: 3421, 3298 forked peak of (NH_2), 2924 (CH-aliphatic), 1670 (C=O); ^{1}HNMR (400 MHz, DMSO-d_6) ppm: 1.05- 1-07 (t, 3H, CH_3, *J = 4 HZ*), 3.6(s, 3H, OCH_3) 3.95- 3.97 (q, 2H, OCH_2 , *J = 4 HZ*), 5 .09 (s, 1H, H-4), 7.13-7.14 (d, 2H, *J = 4* HZ), 7.15-7.17 (d, 2H, *J = 4* HZ), 7.47-7.82 (m, 3H, CH aromatic), 8.00(s, 2H, NH_2, D_2O exchangeable) ; MS: m/z (% rel. Abundance): 399 (M^+) (13.38%); Anal. Calcd for $C_{21}H_{18}FNO_4S$: C, 63.15; H, 4.54; N, 3.51; S, 8.03; Found: C, 63.49; H, 4.62; N, 3.80; S, 8.00.

Ethyl 2-Amino-6-Flouro-4-(Nitro)-4H-[1] Benzothieno [3, 2-*b*] Pyran-3-Carboxylate (5f):
Yield: 44%; mp: 125°C; IR (KBr) cm^{-1}: 3460, 3309 forked peak (NH_2), 2981-2920 (CH-aliphatic), 1739 (C=O); ^{1}HNMR (400 MHz, DMSO-d_6) ppm: 0.96-0.997 (t, 3H, CH_3 , *J = 4 HZ*), 3.9-3.96 (q, 2H, OCH_2, *J = 4HZ*), 5.36 (s, 1H, H-4), 7.55-7.57 (d, 2H, *J* = 8 *HZ)*, 8.15-8.17 (d, 2H, *J* = 8 HZ), 7.29- 7.59 (m, 3H, CH aromatic), 8.39 (s, 2H, NH_2, D_2O exchangeable) ; MS: m/z (% rel. Abundance): 414 (M^+) (9.19%); Anal. Calcd for $C_{20}H_{15}FN_2O_5S$: C, 57.97; H, 3.65; N, 6.76; S, 7.74; Found: C, 58.21; H, 3.80; N, 6.89; S.7.70.

2-((4-Chlorobenzylidene)Amino)-4-(4-Chlorophenyl) -6-Flouro-4H-Benzo [4,5] Thieno [3, 2-b] Pyran-3-Carbonitrile (6)
Yield: 80%; m.p: 220°C; IR (KBr) cm^{-1}: 3066 (=CH), 2924 (CH), 2224 (CN), 1630 (N=CH), 1550 (C=C); ^{1}HNMR (400 MHz, DMSO-d_6) ppm: 5.58 (s, 1H, pyran H), 7.34-7.36 (d, 2H, *J = 8* HZ, schiff's base H), 7.68-7.70 (d, 2H, *J = 8* HZ, schiff's base H), 7.98-8.00 (d, 2H, *J = 8* HZ), 8.09-8.11 (d, 2H, *J* = 8 HZ), 7.38-7.70 (m, 3H, aromatic H), 9.3 (s, 1H, CH methine); MS: m/z (% rel. Abundance): 478 (M^+) (20.78%), 480)(M+2) (22.37%); Anal.calc for $C_{25}H_{13}Cl_2FN_2OS$: C, 62.64; H, 2.73; N, 5.84; S, 9.69; Found C, 62.89; H, 2.80; N, 6.02; S, 9.70.

MATERIALS AND METHODS

Antiproliferative Activity
Anticancer activity of the newly synthesized compounds was measured *in vitro* utilizing 60 different human tumor cell lines representing lung, colon, CNS, ovarian, brain, prostate, kidney cancer, leukemia and melanoma at US National Cancer Institute according to the following standard procedures [15]. The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well micro titer plates in 100 μL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the micro titer plates are incubated at 37°C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line are fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addiction (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug growth, (C), and test growth in the presence of drug at the five addition, an aliquot of frozen

concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 µl of these different drug dilutions are added to the appropriate micro titer wells already containing 100 µl of medium, resulting in the required final drug concentrations. Following drug addition, the plates are incubated for an additional 48 h at 37°C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed *in situ* by the gentle addition of 50 µl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4°C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

$[(Ti-Tz)/(C-Tz)] \times 100$ for concentrations for which $Ti \geq Tz$

$[(Ti-Tz)/Tz] \times 100$ for concentrations for which $Ti<Tz$

Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50% (GI50) is calculated from $[(Ti-Tz)/(C-Tz)] \times 100=50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from Ti=Tz. The LC50 (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from $[(Ti-Tz)/Tz] \times 100=-50$. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested.

Cell Line

HT-29 (ATCC HTB-38) is a human coloncancer cell line, and was purchased from American Type Culture Collection (ATCC, USA). The cells were grown as a monolayer sheet in 75 cm^2 culture flask in Dulbecco's Modified Eagle Medium (DMEM)supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, and 10 ug/ml of insulin; all were purchased from (ThermoFisher Scientific®, USA). The cells were incubated at 37°C and 5% CO_2 in air atmosphere until they are ready to be tested.

Cell Cycle Analysis

To clarify the cytotoxic effect of the newly synthesized compounds, the effects of compounds 4C and 4F on the cell cycle progression [16] were examined against HT-29 cells. At a density of 4×10^6 cell/T 25 flask, HT-29 cells were exposed to compound 4C and 4F at their GI_{50} concentration (0.05 µM) for 24 and 48 h. The cells then were harvested by trypsinization, washed with phosphate buffered saline (PBS), and fixed in ice cold absolute alcohol. Thereafter, the cells were stained, using Cycle test TM plus DNA Reagent Kit (BD Biosciences, USA), according to the manufacturer's instructions. Cell cycle analysis was determined using a FACS Calibur flow cytometer (BD Biosciences, USA).

Detection of Apoptosis

Apoptosis of the cells was determined by fluorescein isothiocyanate (FITC) Annexin V apoptosis detection kit purchased from BioVision (USA) using flow cytometry (BD Biosciences, USA). FITC Annexin V is used to quantitatively determine the cells undergoing apoptosis [17]. It relies on the high affinity of Annexin V to bind to phospholipid phosphatidylserine (PS), which translocated from the inner leaflet of the plasma membrane to the outer surface in the cells undergoing apoptosis. Briefly, 4×10^6 cell/T 25 flasks were exposed to compound 4C and 4F at theirGI_{50} concentration (0.05 mM) for 24 and 48 h. After incubation time, cells were harvested by trypsinization; centrifuged; and washed with phosphate buffered saline. Finally, cells were stained according to the manufacturer's protocol.

ELISA Assay for Cyclin-Dependent Kinase 2 (CDK2)

CDK2 content [18] of HT-29 cells was assessed by ELISA kit purchased from Cloud-Clone Crop. (USA) according to the manufacturer's protocol, and the enzyme concentration was expressed as ng/mL.

Erlotinib

Erlotinib [19,20] is a small-molecule inhibitor of the EGFR tyrosine kinase that is approved by the US Food and Drug Administration and the European Medicines Agency for the treatment of NSCLC (Non-Small Cell Lung Cancer) [7]. Retrospective and prospective analysis of large clinical trials identified subgroups of patients, e.g. with activating mutations in the EGFR (Epidermal Growth Factor Receptor) that respond to tyrosine kinase inhibitors like erlotinib or gefitinib.

RESULTS AND DISCUSSION

Chemistry

As shown in scheme (1), the first target compounds 2-arylidene-7-flouro-1-benzothiophen-3(2*H*)-ones (3a-3f) were synthesized by condensation of 7-flourobenzo[b]hiophen-3-one (2) and different aromatic aldehydes in glacial acetic acid for 5 hr which itself were synthesized according to reported methods [21]. The synthetic pathway of the second target compounds (4a-f) were outlined in scheme (2). The activated methylene compounds, containing electron-withdrawing groups, such as malononitrile and ethyl cyanoacetate reacted with α, β-unsaturated ketones by 1, 4-Michael addition reaction followed by intramolecular cyclization to produce novel compounds (4a-f) and (5a-f). Compound (6) was synthesized by refluxing (4b) with p-chlorobenzaldhyde in dry toluene for 24 hr resulting in formation of 2-((4-chlorobenzylidene) amino)-4-(4-chlorophenyl)-6-flouro-4H-benzo [4,5] thieno[3,2-b]pyran-3-carbonitrile. The structures of newely synthesized compounds (3a-f), (4a-f), (5a-f) and 6 (Figure 2) were confirmed by spectral and elemental analysis. IR spectra of (3a-f) revealed the appearance of the conjugated C=O band at arrange of 1673-1680 cm^{-1}. ^{1}HNMR revealed the appearance of methine proton (=CH) at δ 8-8.09 ppm together with the introduced aromatic protons, ^{13}CNMR of this series showed C=O signal at δ 185 ppm. Further, IR spectra of (4a-f) revealed forked peak of NH2 at 3345-3460 cm^{-1} and CN group at 2190 cm^{-1}. ^{1}HNMR spectra of (4a-f) revealed the appearance of the CH aliphatic characteristic signal of the pyran ring at δ 5.03-5.10 ppm as well as an exchangeable singlet signal at δ 7.10-7.35 ppm corresponding to the NH_2 protons. ^{13}CNMR spectra indicated the disappearance of C=O carbon signal around δ 188 ppm and the appearance of CH aliphatic of the pyran ring at δ 58-60 ppm and CN at δ 170 ppm. In addition, IR spectral analysis of Structures (5a-f) showed CH aliphatic at 2930-2890 cm^{-1} and carbonyl group of ester at 1850 cm^{-1} beside forked peak of NH2 at 3300-3400 cm^{-1}. ^{1}HNMR of (5a-f) showed triplet signal for CH_3 at range (1.10-1.20) ppm and quartet signal for CH_2 group at range (4.01 - 4.20) ppm beside pyran C-4 at 5.56 ppm.^{13}CNMR spectra showed CH_3, CH_2 in aliphatic area at 14.51 and 25.92 ppm and carbonyl of ester at (168.70 - 170 ppm). IR of compound 6 showed disappearance of forked NH_2 and instead, appearance of N=CH at 1630 cm^{-1} beside CH aliphatic at 1920 cm^{-1} and =CH at 3100 cm^{-1}. ^{1}HNMR showed N=CH singal at 9.30 ppm beside pyran proton at 5.58 ppm and aromatic protons at 7.10-7.95 ppm. Mass spectra of all synthesized compounds showed molecular ion peaks (M^+) in addition compounds 3a, 3b, 4a, 4b, 5a, 5b and 6 showed M+2 peaks confirming presence of bromine chlorine atom respectively.

Biological Screening Result

As shown in Table 1, most of the synthesized compounds were exposed to one dose screening using sulforhoodamine B (SRB) assay at concentration (10^{-5} M) in NCI- America against different 60 cell line (breast, colon, prostate, ovarian, renal, CNS, Non-small cell cancer, leukemia and melanoma. Series 2-arylidene-7-flouro[1]benzothiophen-3(2*H*)-ones (3a-f), showed no activity with a mean growth percentage (80 to 95%) while tricyclic series 2-amino-4-aryl-6-flouro-4*H*-benzo[1]thieno[3,2-*b*]pyran-3-carbonitrile (4a-f), enhanced the activity with a mean growth (-29 to 86.5%) and series ethyl 2-amino-4-aryl-6-flouro-4*H*-benzo[1]thieno[3,2-*b*]pyran-3-carboxlates (5a-f), showed low activity except for 5d showed high activity (38.3 to 96.7%). Depending on the previous results compound 4f and 4c were selected for five dose screening at concentration (0.01, 0.1, 1, 10 and 100 µM). An outstanding result of the two compounds expressed in three parameters, median growth inhibition (GI_{50}, concentration of compound that cause 50% inhibition in growth of cells), total growth inhibition (TGI, concentration of compound that cause 100% inhibition in growth of cells) and median lethal concentration (LC_{50}, concentration of compound that cause 50% loss of intact cells at the end of growth period) showing a promising anticancer motifs as shown in Table 2 and also summarized in (Figures 3 and 4). IC_{50} of compound 5d was found 0.3 ± 0.05 µM and IC_{50} of compound 6 was found 0.15 ± 0.01µM while IC_{50} of Erlotinib reference was found 0.3 ± 0.02 µM when

measured in VACERA Egypt expecting that compound 6 as a more promising anticancer dervative than 4f and 4c (Figure 5).

Table 1: Single dose results of all compounds

Compound No.	Ar	%mean of growth	GI_{50}	TGI	LC_{50}
3b	4-ClC_6H_4	95.95	n.t	n.t	n.t
3c	4-FC_6H_4	94.86	n.t	n.t	n.t
3d	4-OHC_6H_4	94.95	n.t	n.t	n.t
3e	4-$CH_3OC_6H_4$	80.73	n.t	n.t	n.t
4b	4-ClC_6H_4	28.33	n.t	n.t	n.t
4c	4-FC_6H_4	26.18	1.09	10.19	100
4d	4-OHC_6H_4	86.5	n.t	n.t	n.t
4e	4-$CH_3OC_6H_4$	27.15	n.t	n.t	n.t
4f	4-$NO_2C_6H_4$	-29.85	0.15	1.14	0
5b	4-ClC_6H_4	96.7	n.t	n.t	n.t
5d	4-OHC_6H_4	38.36	0.3	n.t	n.t
5e	4-$CH_3OC_6H_4$	92.2	n.t	n.t	n.t
5f	4-$NO_2C_6H_4$	95.25	n.t	n.t	n.t
6	4-ClC_6H_4	n.t	0.15	n.t	n.t

GI_{50}, TGI and LC_{50} are calculated in µM unit

1- Median growth inhibition (GI50**,** concentration of compound that causes 50% inhibition in growth of cells). 2- Total growth inhibition (**TGI**, concentration of compound that causes 100% inhibition in growth of cells). 3- Median lethal concentration (LC50, concentration of compound that causes 50% loss of intact cells at the end of growth period)

Table 2: Five dose results of selected NCI compounds

Cell line	4f			4c		
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
Leukemia						
CCRF-CEM	0.119	1.02	100	0.42	12.4	100
HL-60(TB)	0.167	0.74	52.8	0.25	0.67	100
K-562	0.0049	1.79	100	0.35	10.4	100
MOLT-4	0.0926	1.96	100	0.58	10.3	100
RPMI-8226	0.206	1.63	100	1.36	10.3	100
SR	0.0054	10.1	100	0.52	20.4	100
Non- Small Cell Lung Cancer						
A549/ATCC	0.112	1.78	8.51	0.89	10.7	67.8
EKVX	0.386	2.26	7.12	4.26	20.1	40.9
HOP-62	0.155	1.58	5.83	0.64	10.7	48.8
HOP-92	0.0717	0.7	4.65	2.02	6.96	30.5
NCI-H226	0.195	1.51	.	2.41	10.8	60.2
NCI-H23	0.276	1.5	6.55	1.5	15.3	46.9
NCI-H322M	0.576	2.1	5.79	2.44	1.74	40.7
NCI-H460	0.0788	.	.	0.41	50.4	33.9
NCI-H522	0.017	0.54	4.93	0.37	8.65	80.4
Colon Cancer						
COLO 205	0.217	0.72	4.04	2.33	10.6	48.1
HCC-2998	0.335	1.59	4.72	2.05	10.1	35.5
HCT-116	0.0792	1.21	3.48	0.42	12	46.1
HCT-15	0.0682	1.41	5.23	0.41	11.2	37.6
HT-29	0.155	1.14	.	1.09	11.9	100
KM12	0.094	1.48	.	0.44	12.6	46.8
SW-620	0.047	1.68	.	0.55	16.2	48.3
CNS Cancer						
SF-268	0.292	1.75	8.29	0.92	13.5	43.6
SF-295	0.173	1.12	6.43	0.37	11.3	38.3
SF-539	0.172	0.54	2.22	0.44	2.46	15.1
SNB-19	0.191	1.3	5.21	0.58	14.1	40.3
SNB-75	0.052	0.72	.	0.34	14.9	48.6
U251	0.15	1.21	3.9	0.55	13.9	39.8

Melanoma						
LOX IMVI	0.132	1.14	5.83	0.85	12.1	38.8
MALME-3M	0.36	2.17	5.62	0.6	22.2	52
M14	0.11	0.64	3.48	0.38	3.37	28.2
MDA-MB435	0.025	0.08	2.77	0.22	0.74	5.19
SK-MEL-2	0.097	1.01	5.55	0.55	17.7	54.8
SK-MEL-28	0.249	2.02	5.84	1.81	19.8	51.3
SK-MEL-5	0.085	1.16	3.41	0.65	14.4	39.5
UACC-257	0.87	2.84	8.34	17.3	39.1	88.4
UACC-62	0.058	1.39	4.06	5.79	14.6	42
Ovarian cancer						
IGROV1	0.172	1.56	9.43	0.68	15.4	47.7
OVCAR-3	0.142	1.28	4.85	0.38	1.94	20.3
OVCAR-4	0.237	1.9	100	1	14.2	45.3
OVCAR-5	0.56	2.42	7.44	2.87	15.5	41.7
OVCAR-8	0.19	1.24	5.52	1.52	14.2	68.7
NCI/ADRRES	0.04	0.58	100	0.35	7.5	66.5
SK-OV-3	0.29	3.04	27.4	1.37	17.5	49.1
Renal Cancer						
786-0	0.26	1.11	4.13	0.53	10.2	33.4
ACHN	0.37	2.42	17.4	0.87	16.2	40.3
CAKI-1	0.25	1.78	6.07	0.5	16.3	43.3
RXF 393	0.15	0.62	3.16	1.1	5.82	27.1
SN 12C	0.27	1.31	4.83	0.88	15.7	41.2
TK-10	0.5	2.71	30.1	6.54	24.4	69.1
UO-31	0.23	1.44	5.34	1.49	15.6	40.5
Prostate Cancer						
PC-3	0.16	1.29	7.49	0.85	14.8	46.2
DU-145	0.35	1.45	4.84	1.74	12.5	36.5
Breast Cancer						
MCF7	0.031	1.47	45.1	0.37	11	53.8
MDA-MB 231/ATCC	0.16	1.12	4.97	1.54	12.8	41.2
HS 578T	0.18	0.93	100	0.48	12	100
BT-549	0.18	1.05	3.55	0.44	10.7	34.6
T-47D	0.18	2.52	64	0.51	22.6	100
MDA-MB468	0.04	0.08	4.24	0.51	17.7	52.6

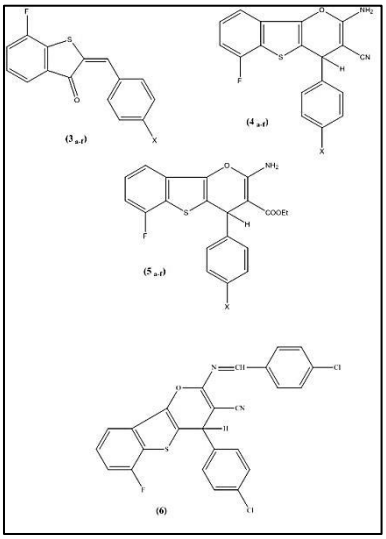


Figure 2: Schematic representation of the synthesized compounds

SAR Result According to NCI

Compounds containing aminocyano group (4a-f) are more active than compounds containing aminoester group (5a-f) using the same cell line.
Presence of fluorine atom instead of bromine at positions 6 (4a-f) results in higher anticancer activity.
Presence of eletron withdrawing group as nitro group at position 4 in phenyl ring give higher activity compared with electron donating group as methoxy group.
4-Subsituted Phenyl ring more active than heterocyclic ring as naphthyl ring [14].
Amino group and imine group at position 2 in compounds (4a-f), 6 respectively give a comparable activity.

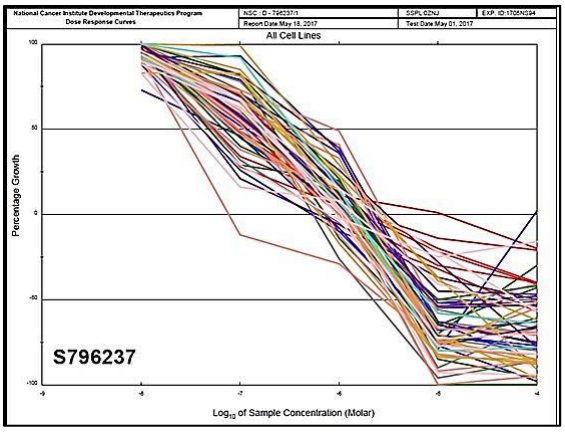


Figure 3: Dose reponse curve of 4f against 60 cell line

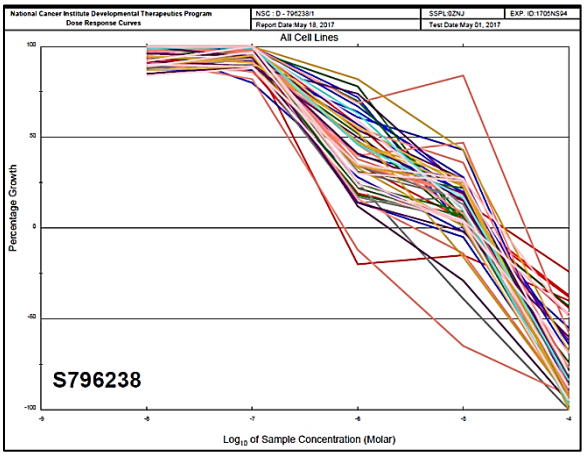


Figure 4: Dose reponse curve of 4c against 60 cell line

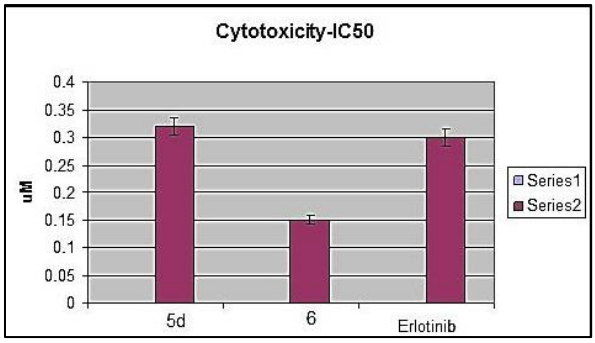
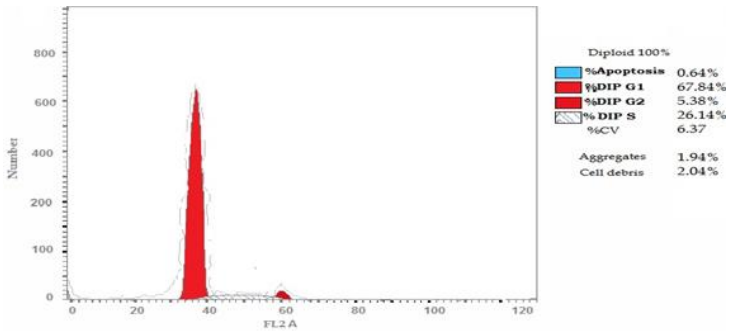
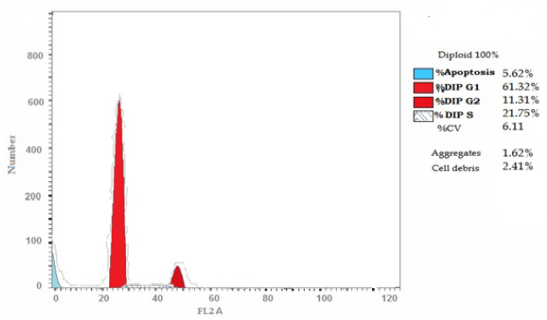
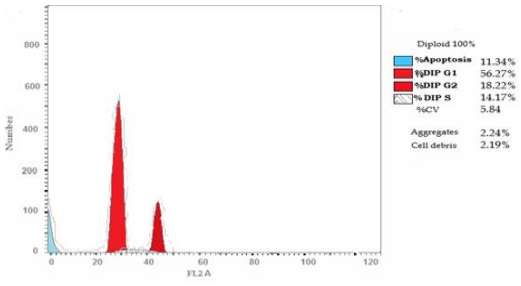


Figure 5: IC50 of compounds 5d and 6 compared with erlotinib reference

Cell Cycle Analysis

The cell cycle is comprised of four ordered, strictly regulated phases referred to as G_1, S (DNA synthesis), G_2, and M (mitosis). Normal cells grown in culture will stop proliferating and enter aquiescent state called G_0 once they become confluentor are deprived of serum or growth factors [22]. The first gap phase (G_1) prior to the initiation of DNA synthesis represents the period of commitment that separates M and S phases as cells prepare for DNA duplication. Cells in G_0 and G1 are receptive to growth signals, but once they have passed a restriction point, they are committed to enter DNA synthesis. Cells demonstrate arrest at different points in G1 in response to different inhibitory growth signals [23]. Therefore, the effect of 4F and 4C on cell cycle was studied in order to elucidate their mechanism of action. Figure 6 and Table 3 displayed that exposure of HT-29 cells to compound 4f and 4c for 24 and 48 h, induced a significant disruption in the cell cycle profile at pre- G_1 phase. This might indicate an ability of compounds 4f and 4c to reduce the cellular proliferation and to induce cell cycle arrest at G_2/M phase and induce DNA fragmentation, the hallmark of cell death, preventing the cells from further replication and proliferation. As indicated in Table 4 Compound 4f showed decreased apoptotic cells by 50% for 24 hour exposure compared to reference drug. The early apoptotic cells declined by 26.5% for 48 hour exposure period compared to erlotinib. When the cells treated with 4c compound for 24 hour, apoptotic cells decreased by 39% whereas the cells declined by 17.6% for 48 hour exposure level compared to reference drug.

Table 3: Effect of 4f and 4c compounds on cell cycle profile of HT-29 cells

	24 hr	**48 hr**
Control		
		

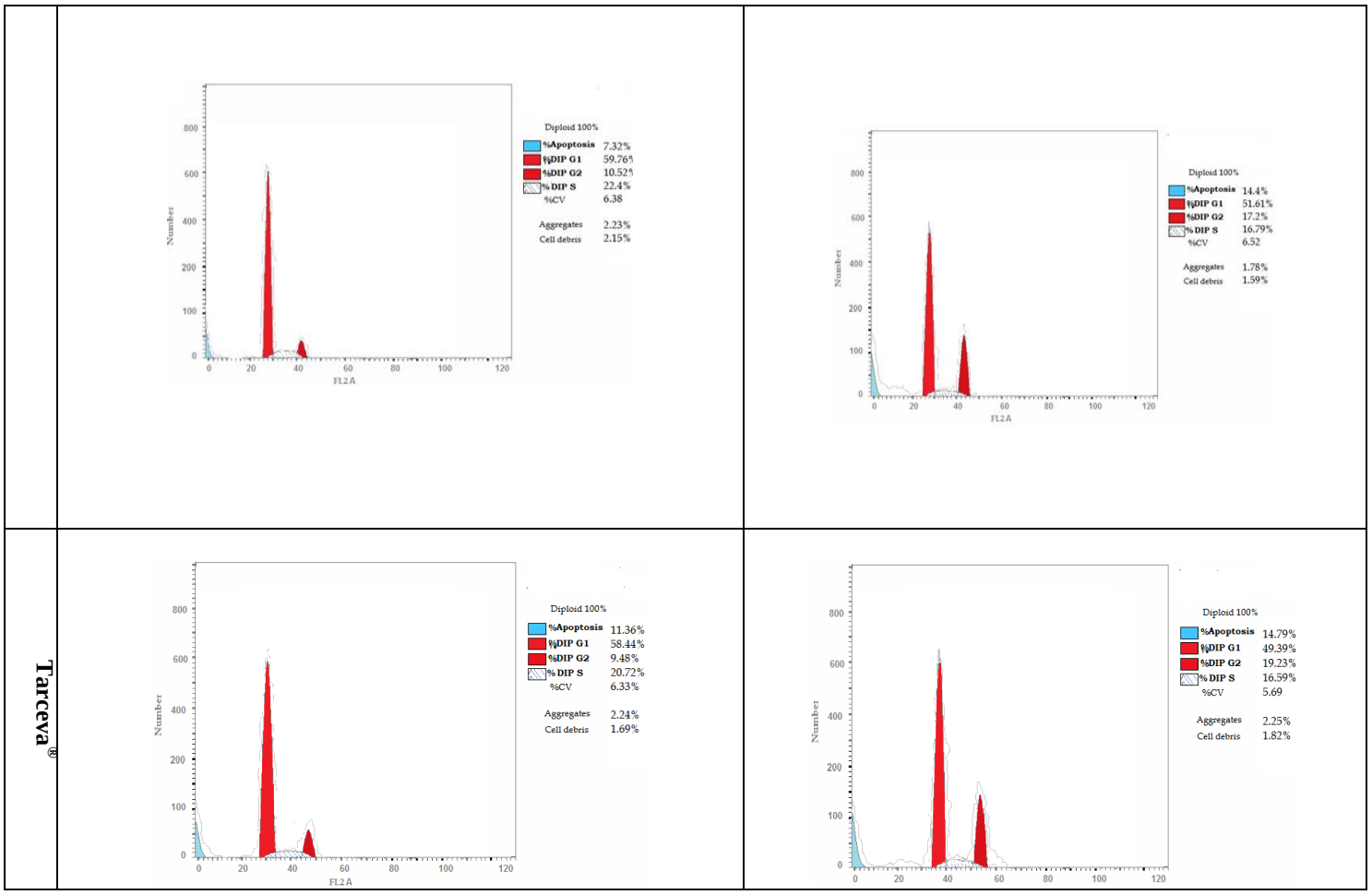
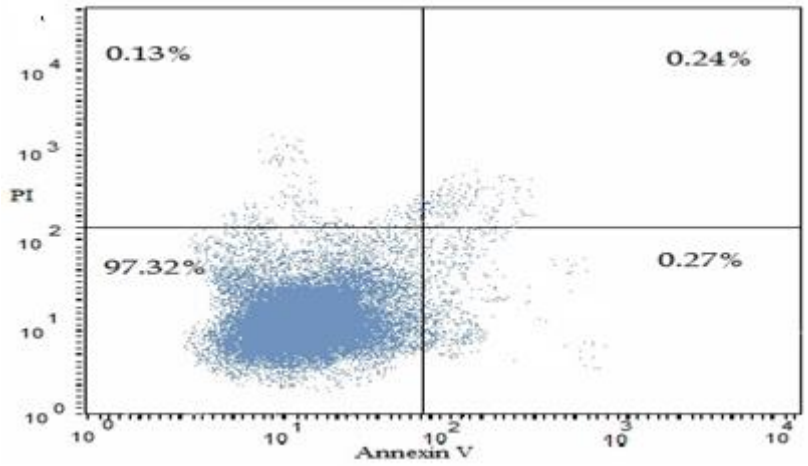
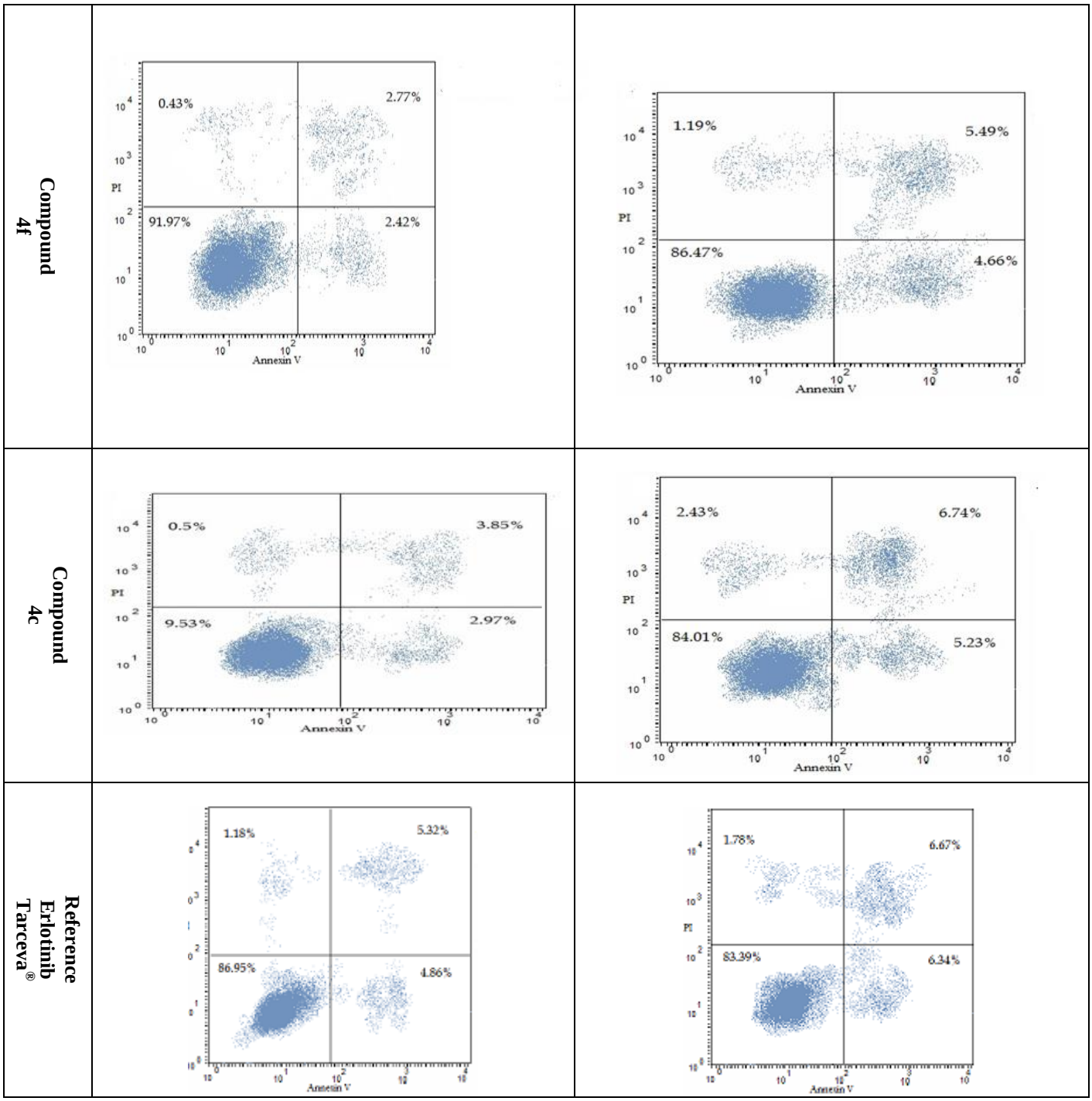


Table 4: Effect of 4f and 4c compounds on apoptosis of HT-29 cells

	24 hr	48 hr
Control		



Effect on CDK2 Level

Compound 4f increased CDK level by 24% for 24 h exposure compared to reference drug (erlotinib).When the cells treated with 4f for 48h, the enzyme concentration decreased by 14.5% compared to the reference. Regarding to 4c compound, enzyme concentration declined by 2% for 24 hour exposure whereas the enzyme level increased by 6.7% for 48 hour exposure compared to reference drug (Figure 7).

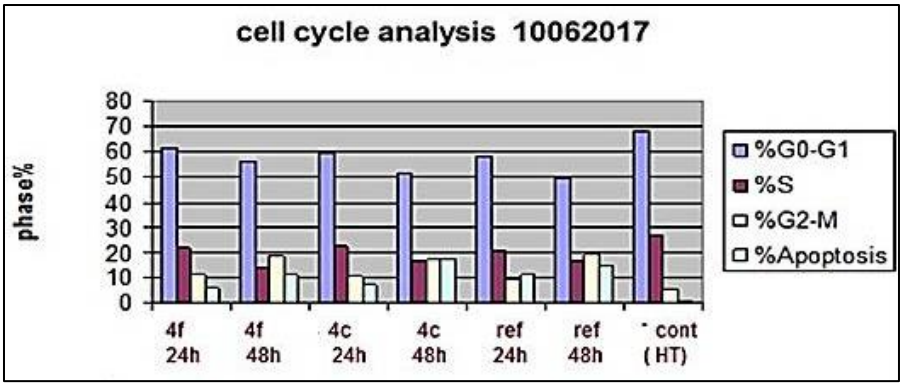


Figure 6: Cell cycle analysis for HT-29 cells treated with 4f, 4c and erlotinib

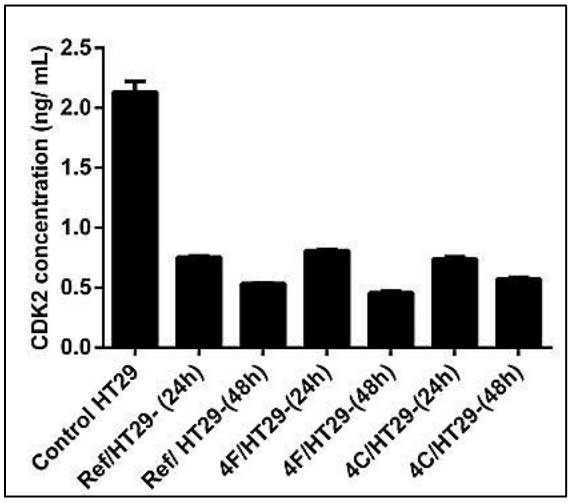


Figure 7: Effect of 4f, 4c and erlotinib on CDK2 of HT-29 cells

CONCLUSION

This study represents the synthesis of novel benzo [1] thiophene, benzo [1] thiophen [3, 2-d] pyran derivatives. All studied compounds exhibited a strong growth inhibitory effect, especially compounds 4f, 4c, 5d, and 6, which were active in the low micro molar range, and therefore, they were submitted to further evaluations. Spectroscopic analysis of the compounds confirmed their structures. Cell cycle study showed that exposure of HT-29 cells to compound 4f and 4c for 24 and 48 h, induced a significant disruption in the cell cycle profile at pre-G1 phase and arrest at G2/M phase, which could be a consequence of several stresses, such as CDK-2 inhibition. In conclusion, the here-presented novel compounds possess high potential as novel leads with anticancer potentials; however, the most intriguing result is the observation that exchanging amino group in compound 4f with imines group in 6 led to outstanding increase in cytotoxic activity which need further investigation in future research .

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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