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Synthesis of Some New Fluorine Substituted Thiobarbituricacid Derivatives as Anti HIV1 and Cyclin-Dependent Kinase 2 (CDK2) for Celltumer Division-Part II

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Abstract

In search for new potential inhibitors some new fluorine substituted thiobarbituric acid derivatives (2-4, 7, 8) and their fused/isolated heterobicyclic nitrogen systems (5, 6, 9, 10, 11, 12) have been synthesized from heterocyclization of fluorinated 1,3-diketoamine (1) with CS_2 followed by ring closure reactions with primary nitrogen reagents. Structures of the targets have been established from elemental analysis and spectral data. Some synthesized systems have been evaluated as anti-HIV-1 and of cyclin-dependent kinase 2 (CDK2) for cell tumor division.

Keywords

Fluorinated Thiobarbituric, Heterobicyclic, Potential Inhibitors

1. Introduction

1,3-Diketoamine structural analogues have attracted special interest by virtue of their varied and pharmaceutically useful biological actions as anticonvulsant [1], antianaesthetic [2], anti HIV agents [3]. Also, thiobarbituric acids are used to measure the autoxidation of brain homogenates for various animals [4], treate non-alcoholic fatty liver disease [5], and determine formaldehyde and acetaldehyde in the food [6] as well as they form a type of bioactive complex with triphenyltin (IV) [7]. The introduction of fluorine atoms to heterocyclic systems often improves their biological properties, due to the stability of C-F formed [8]-[10]. Based on these facts, the

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present work deals with synthetic strategy of some new fluorinated 1,3-diketoamine for building a type of fluorinated thiobarbituric acid derivatives and their fused/isolated heterobicyclic system as anti-HIV agent.

2. Experimental

Melting points were determined with an electrothermal Bibly Stuart Scientific melting point sample (UK). A Perkins Elmer Model RXI-FT IR system 55529 was used for recording IR spectra of the prepared compounds. A Brucker advance DPX 400 MHz model using TMS as internal standard was used for recording the ^{1}H and ^{13}C NMR spectra of the compounds on deuterated $CDCl_3$. A GC-MS-GP 1000 Ex model was used for recording the mass spectra of the compounds. Electronic spectra were recorded in ethanol on Shimadzu UV and visible 310 IPC Spectro-photometer. Microanalysis was performed by the microanalytical Center of Cairo University, Egypt. Hexafluorobenzene was used as external standard for ^{19}F NMR at 8425 MHz (Chemical shift in δ, ppm).

1,3-Di(4'-fluorophenylamino)-propandione (1)

A mixture of 4-fluoroaniline (0.002 mol) added to preheated diethyl malonate (0.001 mol) then warmed for 10 min and cooled. The solid thus obtained washed with ether then crystallized from THF to give **1** as greenish crystals. Yield 78%, m.p. 190˚C - 191˚C. Analytical data; Found: C, 61.85; H, 4.01; N, 9.35; F, 13.00%. Calculated for $C_{15}H_{12}N_2F_2O_2$ (290) C, 62.06; H, 4.13; N, 9.65; F, 13.10%. IR (DMF) ν cm^{-1}: 3542 (OH), 3180 (NH), 2880 (str. CH_2), 1650 (NHCO), 1255 (C-F), 820 (aryl CH). ^{1}H NMR (CD_3Cl) δ: 10.25 (s, 1H, OH), 7.60 - 7.581, 7.53 - 6.98 (each s, 8H, aromatic protons) 2.60 - 2.58 (s, 2H, CH_2), ^{13}C NMR ($CDCl_3$) δ: 165.71, 159.82, 134.34, 134.32, 121.77, 121.72, 121.68, 115.35, 115.20, 77.81, 77.59, 75.38, 66.90, 61.32, 44.39, 43.33, 40.44 - 39.49, 14.08.

1,3-Di(4'-fluorophenyl)thiobarbituric acid (2)

A mixture of **1** (0.001 mol) and CS_2 (0.0015 mol) inethanolic KOH (25 ml, 5%) warmed for 2 h, then poured onto ice-cooled HCl. The solid produced filtered off and crystallized from dioxan to give **2** as yellowish crystals. Yield 68%, m.p. 152˚C - 154˚C. Analytical data; Found: C, 57.53; H, 2.88; N, 8.21; S, 9.33; F, 11.24%. Calculated for $C_{16}H_{10}N_2SF_2O_2$ (332) C, 57.83; H, 3.01; N, 8.43; S, 9.63; F, 11.44%. UV (EtOH) λ_{max} (ϵ): 240 (0.496) nm. IR (DMF) ν cm^{-1}: 3538 (OH), 1660 (C=O), 1480 (deform. CH_2), 1385 (NCSN), 1255 (C-F), 1210 (C=S), and 678 (C-F). ^{1}H NMR (CD_3Cl) δ: 10.04 (s, 1H, OH), 7.60 - 7.44, 7.06 - 6.98 (each m, 8H, aromatic protons) 3.52, 2.59 - 2.58 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 181.15, 165.72, 161.00, 159.38, 138.22, 134.85, 134.32, 126.91, 126.85, 121.74, 121.69, 115.45, 115.21, 77.79, 77.57, 77.36, 44.36, 40.43 - 39.48.

1,3-Di(4'-fluorophenyl)-5-trifluoroacetyl-thiobarbituric acid (3)

A mixture of **2** (0.001 mol) and trifluoroacetic anhydride (0.001 mol) with trifluoroacetic acid (10 m) refluxed for 2 h, then cooled and poured onto ice cooled ethanol. The solid produced filtered off and crystallized from THF to give **3** as deep brown crystals. Yield 55% m.p. 83˚C - 85˚C. Analytical data; Found: C, 50.35; H, 1.88; N, 6.41; S, 7.39; F, 22.01%. Calculated for $C_{18}H_9N_2SF_5O_3$ (428) C, 50.46; H, 2.10; N, 6.54; S, 7.47; F, 22.9%. IR (DMF) ν cm^{-1}: 3489 (OH), 1688, 1660 (C=O), 1386 (NCSN), 1255 (C-F), 1182 (C=S), 880, 838 (aryl CH). M/S (M-19) (372, 100).

1,3-Di(4'-fluorophenyl)-5-(4'-fluorobenzoyl)-thiobarbituric acid (4)

A mixture of **2** (0.001 mol) and 4-fluorobenzoyl chloride (0.001 mol) in DMF (10 ml) refluxed for 1 h, cooled then poured onto ice. The solid produced filtered off and crystallized from dioxan to give **4** as brown crystals. Yield 65% m.p. 102˚C - 104˚C. Analytical data; Found: C, 60.39; H, .2.66; N, 6.01; S, 6.88; F, 12.33%. Calculated for $C_{23}H_{13}N_2SF_3O_3$ (454) C, 60.79; H, 2.86; N, 6.16; S, 7.04; F, 12.55%. IR (DMF) ν cm^{-1}: 3487 (OH), 1688, 1660 (C=O), 1386 (NCSN), 1255 (C-F), 1310 (C=S), 838, 810 (aryl CH), 687 (C-F). ^{1}H NMR ($CDCl_3$) δ: 9.71 (s, 1H, OH), 8.71, (s, 1H, $CHCOCF_3$), 7.75 - 7.16, 7.15 - 7.07 (each m, 12H, aromatic protons). ^{13}C NMR ($CDCl_3$) δ: 172.04, 167.45, 165.17, 164.19, 134.90, 134.88, 132.26, 132.20, 131.84, 130.47, 130.211, 29.54, 129.48, 127.23, 127.22, 122.54, 122.49, 116.76, 115.95, 115.81, 115.47, 115.32, 115.17, 115.14, 77.64, 77.42, 77.21, 40.4 - 39.51. M/S: 412 (M-44, 85%).

5-Trifluoromethyl-1,3-di-(4'-fluorophenyl)-2,7-dithioxo-3H-pyrimido[4,5-d]pyrimidin-4'-one (5); 1,3,5-tri-(4'-fluorophenyl)-2,7-dithioxo-pyrimido[4,5-d]pyrimidin-4-one (6)

A mixture of compound **3** or **4** (0.001 mol) and thiourea (0.001 mol) in ethanol (20 ml) with a few drops of piperidine refluxed for 4 h, cooled and poured onto ice-acetic acid. The solid thus obtained filtered off and crystallized from ethanol to give **5** and/or **6** as brown crystals.

5: Yield 72% m.p. 193˚C - 195˚C. Analytical data; Found: C, 48.35; H, 1.81; N, 11.69; S, 13.55; F, 20.00%.

Calculated for $C_{19}H_9N_4S_2F_5O$ (468) C, 48.71; H, 1.92; N, 11.96; S, 13.67; F, 20.25%.

6: Yield 70% m.p. 126˚C - 128˚C. Analytical data; Found: C, 58.00; H, 2.35; N, 11.01; S, 12.55; F, 11.23%. Calculated for $C_{24}H_{13}N_4S_2F_3$ (561) C, 58.29; H, 2.63; N, 11.33; S, 12.95; F, 11.53%.

6: IR (DMF) ν cm^{-1}: 3130 (NH), 1710 (C=O), 1210 (C=S), 1255, 685 (C-F), (NCSN), 1255 (C-F), 1580 (C=N). ^{1}H NMR (CD_3Cl) δ: 9.55 (s, 1H, NH), 7.8 - 7.6, 7.3 - 6.9 (each m, aromatic protons). ^{13}C NMR (CD_3Cl) δ: 181.1, 171.8, 166.35, 130 - 127.54, 122.54 - 122.11. M/S: M^+ (Int.), 563 (M^{+2} 12.15%), 95 (100).

1,2-Di[1',3'-di(4"-fluorophenyl)-thiobarbituric acid-5-yl] ethane (7)

A mixture of compound **2** (0.002 mol) and 1,2-dichloroethane (0.001 mol) in pyridine (20 ml) refluxed for 1 h, cooled, then poured onto ice cooled-HCl and extraction with diethyl ether and evaporated on water-bath. The solid obtained crystallized from dioxan to give **7** as yellowish crystals. Yield 55% m.p. 142˚C - 144˚C. Analytical data; Found: C, 58.88; H, 3.00; N, 7.87; S, 9.11; F, 10.78%. Calculated for $C_{34}H_{22}N_4S_2F_4O_4$ (690) C, 59.12; H, 3.18; N, 8.11; S, 9.27; F, 10.01%. UV (EtOH) λ_{max} (ϵ): 251 (1.0249) nm. IR (DMF) ν cm^{-1}: 3545 (OH), 2880 (str. CH2), 1662 (C=O), 1255 (C-F), 1188 (C=S), 885 (aryl CH), 683 (C-F).

1,3-Di(4'-fluorophenyl)-5-arylidene-thiobarbituric acid (8)

Equimolar amounts of **2** and thiophene-2-carboxaldehyde (0.001 mol) in absolute ethanol (25 ml) with a few drops of piperidine refluxed for 6 h, cooled then concentration. The solid produced filtered off and crystallized from ethanol to give **8** as orange crystals. Yield 70% m.p. 138˚C - 140˚C. Analytical data; Found: C, 58.88; H, 2.55; N, 6.41; S, 14.83; F, 8.81%. Calculated for $C_{21}H_{12}N_2S_2F_2O_2$ (426) C, 59.15; H, 2.81; N, 6.57; S, 15.02; F, 8.92%. UV (EtOH) λ_{max} (ϵ): 263 (1.026) nm. IR (DMF) ν cm^{-1}: 3010 (aryl CH), 1680, 1662 (C=O), 1611 (CH=C), 1358 (NCSN), 1255 (C-F), 1185 C=S), 890, 853, 780 (aryl CH), 687 (C-F). ^{1}H NMR ($CDCl_3$) δ: 9.23 (s, 1H, CH=C proton), 7.6 - 7.56, 7.42 - 7.44, 7.05 - 6.98 (each m, 11H, thiophene and aryl protons). ^{13}C NMR ($CDCl_3$) δ: 181.10, 165.67, 159.36, 158.15, 134.40, 134.38 126.84, 121.85, 121.67, 121.62, 115.37, 115.32, 115.22, 115.17, 77.88, 77.66, 77.45, 44.52, 40.43 - 39.48.

1,3-Di(4'-fluorophenyl)-5-(thiophene-2-yl)-2,7-dithioxo-8-phenylpyrimido[4,5-d]pyrimidin-4one(9a) and/or 1,3-Di(4'-fluorophenyl)-5-(thiophene-2-yl)-2,7-dithioxo-6-phenylpyrimido[4,5-d]pyrimidin-4-one (9b)

Equimolar mixture of **8** and N-phenyl thiourea (0.001 mol) in absolute ethanol (25 ml) in a few drops of piperidine refluxed for 4 h. the solid produced on warming isolated to give faint yellow crystals. Yield 31% m.p. 132˚C - 134˚C. After cooled, another solid produced which on crystallization give a deep-yellowish crystals. Yield 26% m.p. 124˚C - 126˚C.

9a: analytical data; Found: C, 63.55; H, 2.88; N, 10.41; S, 11.95; F, 7.00%. Calculated for $C_{28}H_{16}N_4S_2F_2O$ (526); C, 63.87; H, 3.04; N, 10.64; S, 12.16; F, 7.22%.

9b: analytical data; Found: C, 63.41; H, 2.81; N, 10.48; S, 11.81; F, 6.89%. Calculated for $C_{28}H_{16}N_4S_2F_2O$ (526); C, 63.87; H, 3.04; N, 10.64; S, 12.16; F, 7.22%.

9a: UV (EtOH) λ_{max} (ϵ): 280 (0.172) nm.

9b: UV (EtOH) λ_{max} (ϵ): 286 (0.0169) nm. IR (DMF) ν cm^{-1}: 3010 (aryl CH), 1668 (C=O), 1385 (NCSN), 1256 (C-F), 1201 (C=S), 886, 838) aryl (CH), 687 (C-F).

9a: ^{1}H NMR ($CDCl_3$) δ: 7.6 - 7.34, 7.29 - 7.026, 7.021 - 6.855 (each m, 17H, thiophene and aryl protons).

9a: ^{13}C NMR ($CDCl_3$) δ: 181.63, 168.15, 137.76, 129.50, 124.44, 77.70, 77.48, 77.27, 40.43, 39.48.

9b: ^{13}C NMR ($CDCl_3$) δ: 181.54, 181.07, 165.711, 159.78, 158.16, 138.53, 138.21, 134.88, 134.33, 129.83, 129.41, 129.13, 128.79, 126.87, 125.76, 124.99, 124.09, 122.54, 121.93, 121.87, 121.71, 121.66, 115.32, 115.24, 115.20, 115.17, 77.81, 77.59, 77.38, 40.38 - 39.43.

M/S 428 (M^{+2}, 88%), 401 (100).

7-Amino-5-(thiophene-5-y)-1,3-di(4'-fluorophenyl)-2-thioxo-pyrimido[4,5-d]pyrimidin-4-one (10)

A mixture of **8** (0.001 mol) and guanidine bicarbonate (0.001 mol, in drops HCl) in ethanol (50 ml) with a few drops of piperidine refluxed for 4 h, cooled then poured onto ice. The yielded solid filtered off and crystallized from ethanol to give **10** as yellowish crystals. Yield 66% m.p. 180˚C - 182˚C. Analytical data; Found: C, 56.43; H, 2.69; N, 14.81; S, 13.51; F, 7.91%. Calculated for $C_{22}H_{13}N_5S_2F_2O$ (465) C, 56.77; H, 2.79; N, 15.05; S, 13.76; F, 8.17%. IR (DMF) ν cm^{-1}: 3310, 3150 (NH_2, NH), 3005 (aryl CH), 1668 (C=O), 1620 (deform. NH_2), 1590 (C=N), 1385 (NCSN), 1255 (C-F), 1201 (C=S), 910, 880, 785 (aryl CH), 687 (C-F). ^{1}H NMR ($CDCl_3$) δ: 4.57 (s, 2H, NH_2), 7.65 - 7.59, 7.01 - 6.98, (each m, 11H, thiophene and aryl protons). ^{13}C NMR ($CDCl_3$) δ: 165.65, 159.63, 158.02, 134.52, 134.31, 121.59, 121.54, 115.26, 115.11, 78.06, 77.84, 77.63, 44.77, 40.31 - 39.36, 30.83.

1,4-Di[1',3'-di(4"-fluorophenyl)-thiobarbituric acid-5-thiophene-2-methyl]piprazine (11)

A mixture of **8** (0.002 mol) and piperazine (0.001 mol) in absolute ethanol (30 ml), with few drops of piperidine refluxed for 4 h, cooled. The solid thus obtained filtered off and crystallized from ethanol to give **11** as yellowish crystals. Yield 71% m.p. 138˚C - 140˚C. M. m.p. with **8** gave m.p. 120˚C - 125˚C. Analytical data; Found: C, 58.41; H, 3.25; N, 8.61; S, 13.33; F, 7.88%. Calculated for $C_{46}H_{34}N_6S_4F_4O_4$ (938) C, 58.84; H, 3.62; N, 8.95; S, 13.64; F, 8.10%. UV (EtOH) λ_{max} (ϵ): 256 (1.019) nm. IR (DMF) ν cm^{-1}: 3420 (OH), 3006 (aryl CH), 2895 (aliphatic CH), 1662 (C=O), 1438 (deform. CH_2), 1385 (NCSN), 1255 (C-F), 1201 (C=S), 864 (aryl CH), 687 (C-F). M/S: (Int.%); 720 (M^{+2}, 25.11) 511 (12.15), 427 (55.15), 108 (5.68); 95 (100), 85 (18.23), 83 (213).

1,2-Di[1',3'-di(4"-fluorophenyl)-thiobarbituric acid-5-yl]bicarbonyl (12)

To a mixture of **2** (0.002 mol) in dry C_6H_6, oxalyl dichloride (0.001 mol) added dropwise the added of a few drops of piperidine the warmed slowly for 20 min. the solid obtained was filtered off and crystallized from dioxan to give **12** as yellowish crystals. Yield 88% m.p. 232˚C - 234˚C. Analytical data; Found: C, 56.55; H, 2.31; N, 7.60; S, 8.81; F, 10.35%. Calculated for $C_{34}H_{18}N_4S_2F_4O_6$ (718) C, 56.82; H, 2.80; N, 7.79; S, 8.91; F, 10.58%. UV (EtOH) λ_{max} (ϵ): 271 (0.14912) nm. IR (DMF) ν cm^{-1}: 3010 (aryl CH), 1785 (C=O), 1662 (C=O), 1384 (NCSN), 1255 (C-F), 1155 (C=S), 864, 841, 786, 749 (aryl CH), 658 (C-F). ^{1}H NMR ($CDCl_3$) δ: 9.18 (s, 2H, OH), 7.43 - 7.23 (m, 16H, aryl protons), 6.65 (s, 2H, CH=C). ^{13}C NMR ($CDCl_3$) δ: 181.631, 168.11, 166.01, 162.13, 137.67, 129.50, 126.40, 124.44, 77.45, 77.24, 77.03, 40.49 - 39.55. M/S: (Int.%): 362 (M^{+1}) 100%, 317 (8), 306 (85), 306 (14), 97 (2), 98 (12).

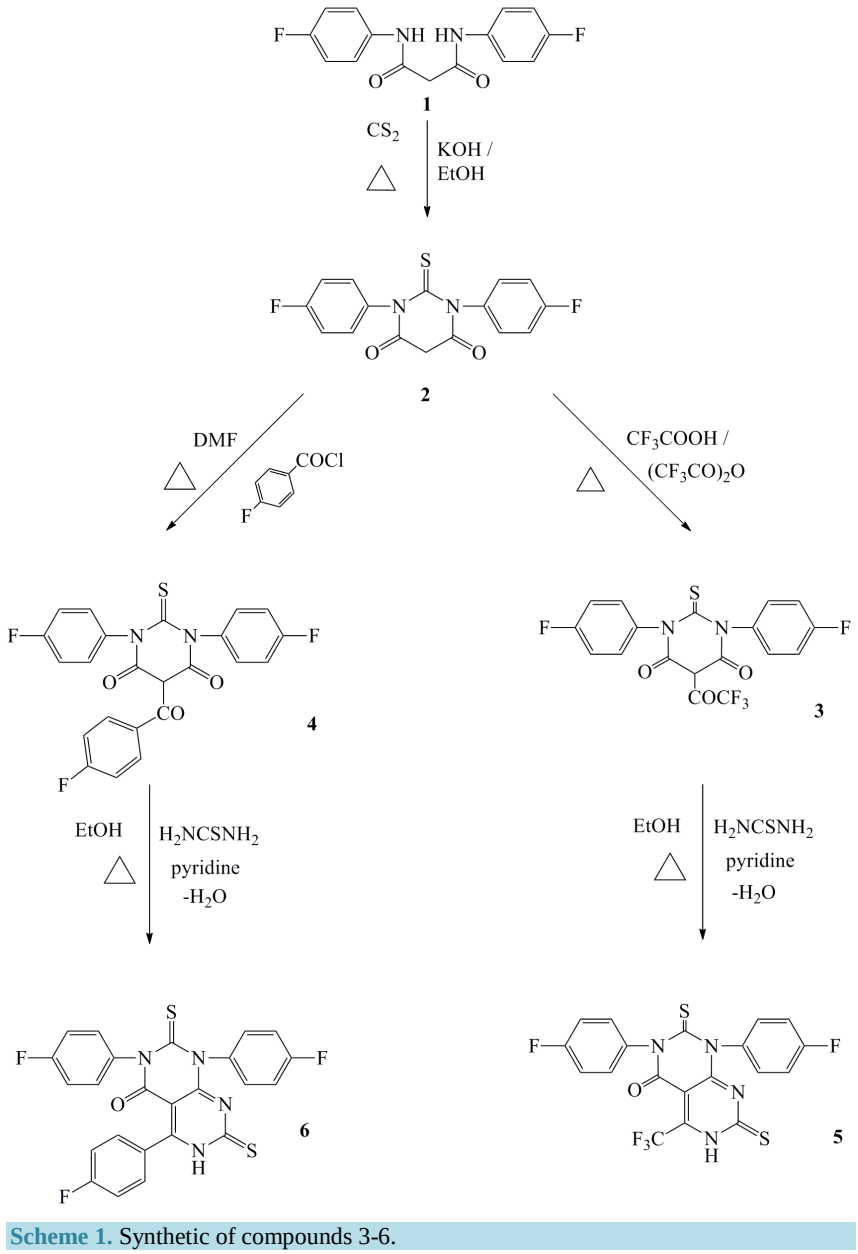
3. Results and Discussion

1,3-Diketoamines obtianed from 1,3-biester with aromatic amine [11]. Similary, diethylmalonate and 4-fluoroaniline added then warmed for few min to obtain the fluorinated 1,3-diketoamine (**1**) (**Scheme 1**). Refluxing of compound **1** with CS_2 in alcoholic KOH [12] yielded 1,3-di(4'-fluorophenyl)thiobarbituric acid (**2**) (**Scheme 1**). UV absorption spectrum of **1** recorded λ_{max} at 230 nm while that of **2** showed λ_{max} at 240 nm. A higher λ_{max} of **2** than **1** confirm that cyclic resonated structure with both bathochromic and hypthochromic factors. IR absorption spectrum of **1** showed ν at 3542 & 3180 attributed to two isolated OH & NH functional groups while that of **2** recorded anenolic $\rightleftharpoons$ ketonic functional groups at ν 3538 cm^{-1} with lacks of NH bands. In addition ν 1385, 1255, 1210 cm^{-1} for cyclic NCSN, C-F and C=S functional groups.

^{1}H NMR spectrum of **2** exhibited a resonated signals at 2.59 and 3.52 ppm for active CH_2 coupling and enolic protons, with a signals at δ 7.65 - 6.98 ppm for aryl protons ^{13}C NMR spectrum of **2** showed resonated signals at δ 165.65, 159.63, and 158.02 ppm C=S, 2C=O carbon and δ at 134.52, 134.51 ppm for the C-F carbons, in addition of aromatic carbons. Mass spectrum **2** exhibited the molecular ion with a base peak at m/e 334 and 95. Treatment of ethanolic solution of **2** with $FeCl_3$ solution gave the deep violet colour which confirms that phenolic formula. Presence of α-active methylene at position-5 of the thiobarbituric acid **2**, led to synthesize various fluorinated isolated and/or fused heterobicyclic nitrogen systems.

Fluorinated compound **2** underwent acylation using trifluoroacetic anhydride-trifluoroacetic acid [13] produced5-trifluoroacetyl derivatives **3**, while fluorinated aroylation using 4-fluorobenzoyl chloride in warming DMF yielded 5-(4'-fluorobenzoyl) derivative **4** (**Scheme 1**). Structures of compounds **3** and **4** deduced from spectral measurements. A higher λ_{max} of **4** > **3** > **2** confirm that extension conjugation system was as well as adductive electronic density of $COCF_3$ and or COC_6H_4F. IR spectrum of **4** showed absorption bands at ν 3489 and 3304 attributed to OH, and C=O with 1688, 1660 cm^{-1} cyclic NCSN in addition at ν 1255 cm^{-1} for C-F absorption bands. Compounds **3** & **4** showed the lacks of CH_2. ^{1}H NMR spectrum of **4** exhibited a resonated signals at 9.71 and 8.71 ppm for OH and aliphatic CH-$COCF_3$ down field, different aromatic protons at δ 7.75 - 7.166 and 7.156 - 7.07 ppm. ^{13}C NMR spectrum of **4** recorded signals at δ 172.04, 167.45, 165.17, 164.19 ppm attributed to C=S, and 3C=O carbons. In addition, δ 134.9, 132.2 ppm for C-F and C-N with aromatic carbons at δ 131.8 - 122.4 ppm. Morever M/S spectrum of **3** recorded m/z at 372 (M-19) as a base peak, while that of **4** exhibited m/e at 417 (M-44) with a base peak at 379.

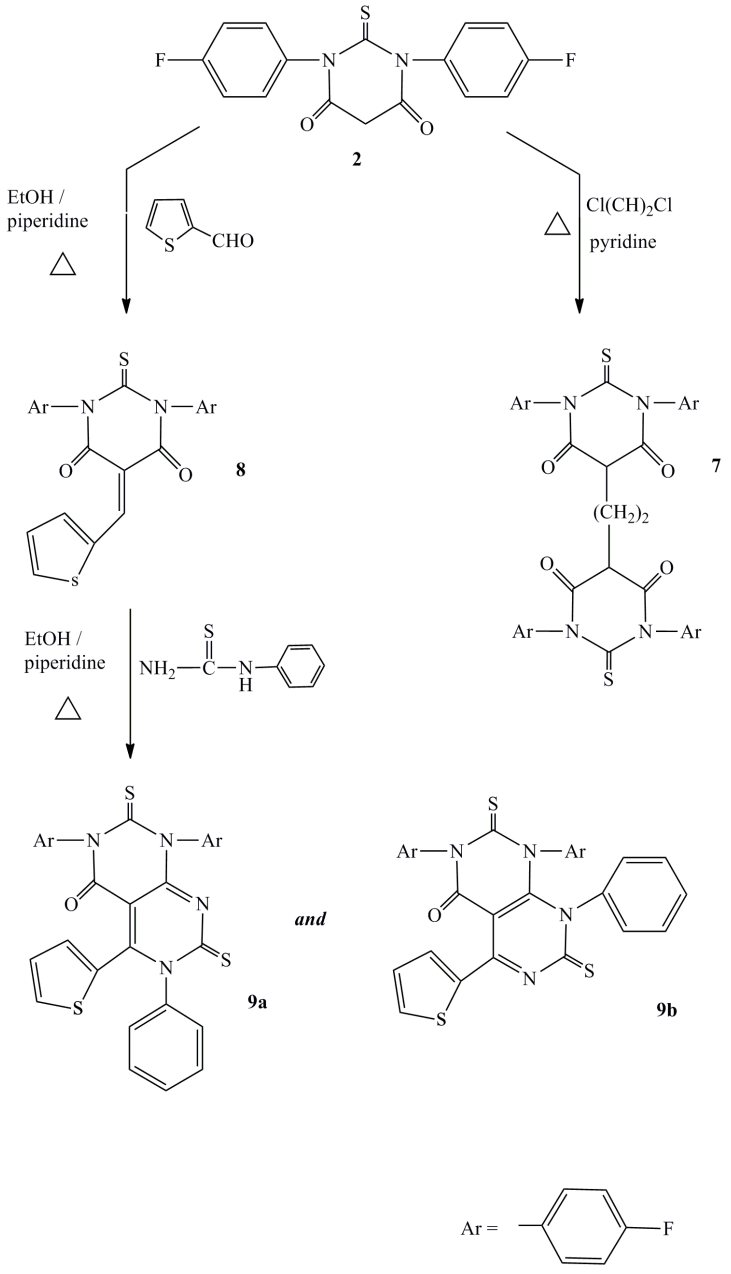
Fluorinated fused heterobicyclic nitrogen systems **5** and **6** obtained from cyclocondensation of **3** and/or **4** with 4-chlorophenyl hydrazine hydrochloride in refluxing absolute ethanol [14] furnished the fluorinated pyrimido [3,4-d]pyrimidine thiones (**5** & **6**) respectively (**Scheme 1**). Structures of compounds **5** and **6** were established from correcteelemental analysis and spectral data. IR Spectrum of **6** recorded an absorption bands at ν 3130, 1710 and 1210 cm^{-1} for the NH, C=O and C=S functional groups with ν at 1255 for C-F with 1580 cm^{-1} for C=N functional group. ^{1}H NMR spectrum of **6** showed a resonated signals at δ 9.55 and 7.8 - 7.6 & 7.3 - 6.9



Scheme 1. Synthetic of compounds 3-6.

ppm for NH and aromatic protons. ^{13}C NMR spectrum of **6** exhibiteda resonated signals at δ 181.1, 171.8, 166.35 ppm for 2 C=S and C=O carbons with aromatic carbons at δ 130 - 127.54, 122.54 - 122.11 ppm. Mass spectrum of **6** showed the molecular ion and a base peak at *m/z* 563 (M + 2), 95 as 4-fluorophenyl radical. A simple alkylation for active methylene at position-5 in the compound **2** deduced from treatment with 1,2-dichloroethane (2:1 by mole) in refluxing DMF to produce 1,2-di(thiobarbituric acid-5-yl)ethane (7) (**Scheme 2**). Fine structure of compound **7** deduced from that elemental analysis and spectral measurements. UV absorption of **7** recorded λ_{max} at 251 nm in compare with **2** at 240 nm. IR absorption spectrum showed an aliphatic bands at ν 3545, 2880, 1662 cm^{-1} for stretching OH, CH_2 & C=O functional groups with 1255 cm^{-1} for C-F. It is interesting that condensation of compound **8** with thiophene-2-carboxaldehyde in warming ethanol with a few drops of piperidine according to Knoevenagel reaction [15] yielded 1,3-di(4'-fluorophenyl)-5-arylidene-thiobarbituric acid (**8**) (**Scheme 2**).

Cycloaddition Compound **8** with N-phenyl thiourea in refluxing ethanol-piperidine furnished the pyrimido [4,5-d]pyrimidindithiones (**9a**, **b**) (**Scheme 2**). Formation of compounds **9** may be take place via cycloaddition



Scheme 2. Synthetic of compounds 7-9.

reaction through two possible routes (**Figure 1**). Structure of **8** established mainly from spectral data. UV absorption spectrum showed λ_{max} at 263 nm higher than **2** which is due to new α, β-unsaturated ketonic system formed of **8**. ^{1}H NMR spectrum recorded a resonated signals at δ 9.23 ppm for the arylidene proton. Also, ^{13}C NMR spectrum of exhibited a different types of carbons at δ 181.10, 165.67, 160.91, 159.76, 159.29, 158.15, and 134.40 - 115.17 ppm attributed to C=S, C=O, C=N, C-S, C-N and C=C carbons. IR spectrum recorded lacks of OH, NH groups and CH_2 with addition a new group at 1610 cm^{-1} for CH=C. M/S of **8** showed a molecular ion at *m/z* 428 (M^{+2}, 88%), with a base peak at 401. The higher reactivity of compound **8** towards cycloaddition with N-phenyl thiourea to electrophilic carbon at position-3 of thiobarbituric acid followed by addition reaction of H_2-N-CS group to electrophilic carbon arylidene at posiyion-5 to give **9a**. A possible other route is a nucleophilic reaction CS-NH_2 group to a electrophilic carbon at position-3 of thiobarbituric acid followed by addition reaction of HN-Ph group to electrophilic carbons of arylidene at position-5 to give **9a**.

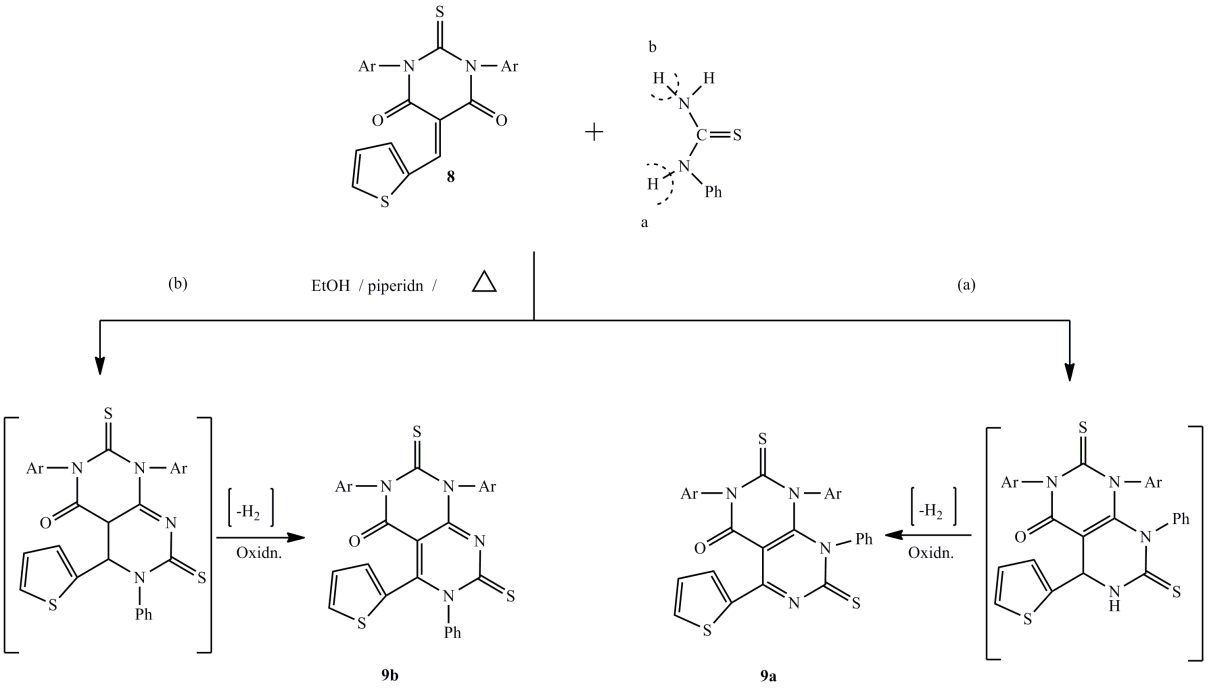
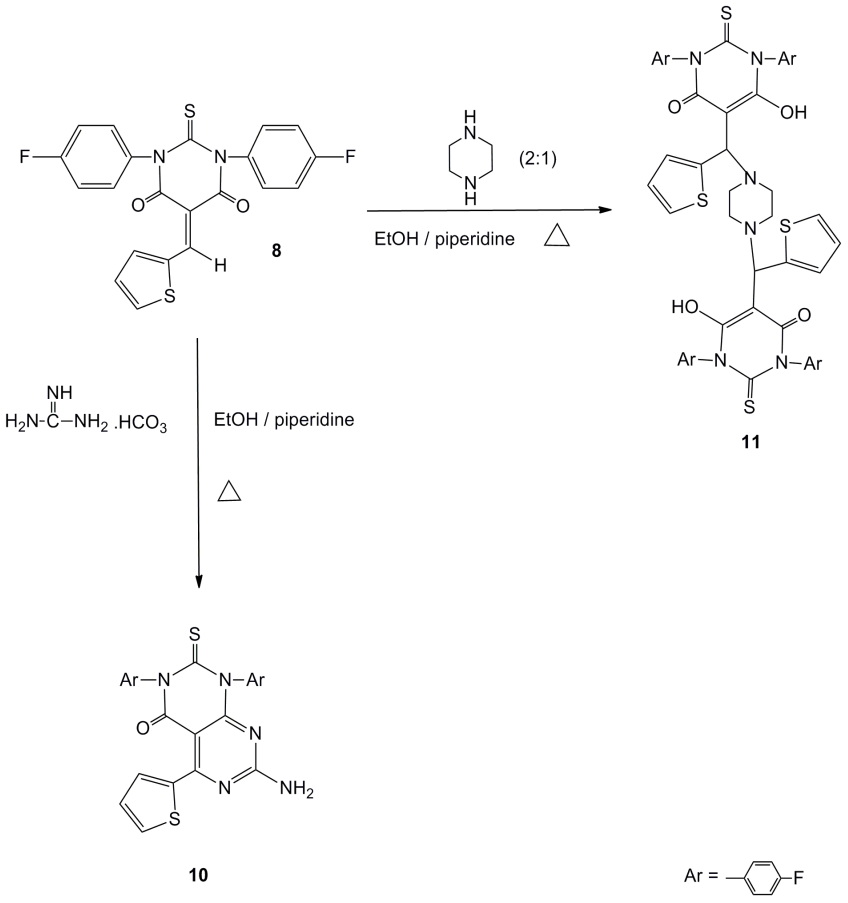


Figure 1. Possible formation of 9a and 9b from 8.

A possible other route is a nucleophilic reaction CS-NH_2 group to a electrophilic carbon at position-3 of thiobarbituric acid followed by addition reaction of HN-Ph group to electrophilic carbons of arylidene at position-5 to give **9a**. The kinetics and mechanism of this reaction were controlled by the highly acidic proton of Ph-NH than H_2N-CS proton (as thioamide). A higher yield of **9a** is due to a high withdrawing of Ph group than amidic $CONH_2$. Also, a higher melting point of **9a** than **9b** is due to a higher stability of **9a** than **9b** which characterized by a repulsion between phenyl and thiophenegroups. Thus, compounds **9a** and **9b** are isomeric structure. Structure of compounds **9a** & **9b** determined from: 1) UV absorption of compounds **9a** recorded λ_{max} at 280 nm, and that of **9b** at 286 nm; 2) IR absorption spectra of **9a** & **9b** showed a lacks of OH, NH functional groups and CH_2; 3) ^{1}H NMR spectra of **9a** and **9b** recorded a resonated signals at δ 7.60 - 7.34, 7.29 - 7.026 and 7.021 - 6.855 ppm for different thiophene and aryl protons; 4) ^{13}C NMR for compound **9a**, recorded the resonated signals at δ 181.63, 168.15 ppm for C=S and C=O with 137.67, 129.50 - 124.44 ppm for aromatic carbons, while that of **9b** showed signals at δ 181.54, 181.07, 165.711 ppm for 2C=S and C=O with other δ at 138.166, 134.33 - 124.99 and 122.54 - 121.66 ppm for aromatic carbons.

Cycloaddition of compound **8** with gaunidine bicarbonate in refluxing ethanol-piperidine afforded the aminopyrimido[4,5-d]pyrimidinthione (**10**) (**Scheme 3**). Structure of compound **10** characterized by spectral data. IR absorption spectrum gave a good indication by exhibited the absorption band at ν 3350 cm^{-1} for NH_2 group with other at ν 1620, 1580 cm^{-1} for bending NH_2 and C=N functional groups. ^{1}H NMR spectrum showed a resonated signals at δ 4.57 ppm for the NH_2 protons with thiophene and aryl protons at δ 7.65 - 7.59 and 7.01 - 6.98 ppm with lacks of CH=C protons. ^{13}C NMR spectrum recorded the resonated signals of different carbons at δ 165.65, 159.63 and 158.02 ppm for C=S, C=O and C=NH. The interaction between compound **8** and piperazine (2:1 by mole) in refluxing ethanol-piperidine [16]-[18] furnished 1,4-disubstituted piperazine (**11**) (**Scheme 3**). Former structure of compound **11** deduced for spectral measurements. A good characterized obtained from that UV spectrum which showed λ_{max} at 256 nm higher than that of **2** (240 nm). IR spectrum recorded a weak band at 3550 cm^{-1} for OH with 2928, 2858 cm^{-1} for CH, CH_2. In addition to 1662 cm^{-1} attributed to C=O, with ν at 1504, 1438 cm^{-1} for deformation of CH_2. Mass spectrum of **11** recorded the molecular ion and a base peak at m/e 720 (M + 25.11), 95 (100). Formation of **12** was deduced from treatment of **2** with piperidine as base to produce the carbanion which attacks the first electrophilic carbon of oxalyl followed by a second attack of other nucleophiliccarbanion of thiobarbituric acid to the second electrophilic carbon of oxalyl chloride (**Scheme 4**). A possible tautomerism of compound **12** gives us a good indication about the acidic character of two protons within



Scheme 3. Synthetic of compounds 10 & 11.

the former structure which facilitated the oxidation $\rightleftharpoons$ reduction process.

Finally structure of compound **12** established spectral data. UV absorption spectrum showed λ_{max} at 271 nm which is higher than starting material **2**. These highly absorption is confirmed that larger extension of hetero-conjugation system bearing both bathochromic and hypsochromic moieties. IR spectrum showed a lacks of bands for OH, NH functional groups and CH_2 with the additional bands at 1785, 1680 and 1662 cm^{-1} for 3C=O groups. A functional groups at ν 1380, 1255 and 1210 cm^{-1} attributed to NCSN, C=S and C-F functional groups.

^{1}H NMR spectrum exhibited two resonated signals at δ 9.18 ppm attributed to OH proton from the free solubility of **12** in sodium hydroxide solution. ^{13}C NMR spectrum of **12** recorded the resonated signals at δ 181.63, and 168, 166, 162 ppm C=S and 3C=O carbons. Mass spectrum of **12** recorded the m/e at 362 (M^{+2}) as a base peak. Finally, synthesized single fluorine which attributed to phenyl ring in all fluorinates systems appeared in the region at δ −125 to −128 ppm.

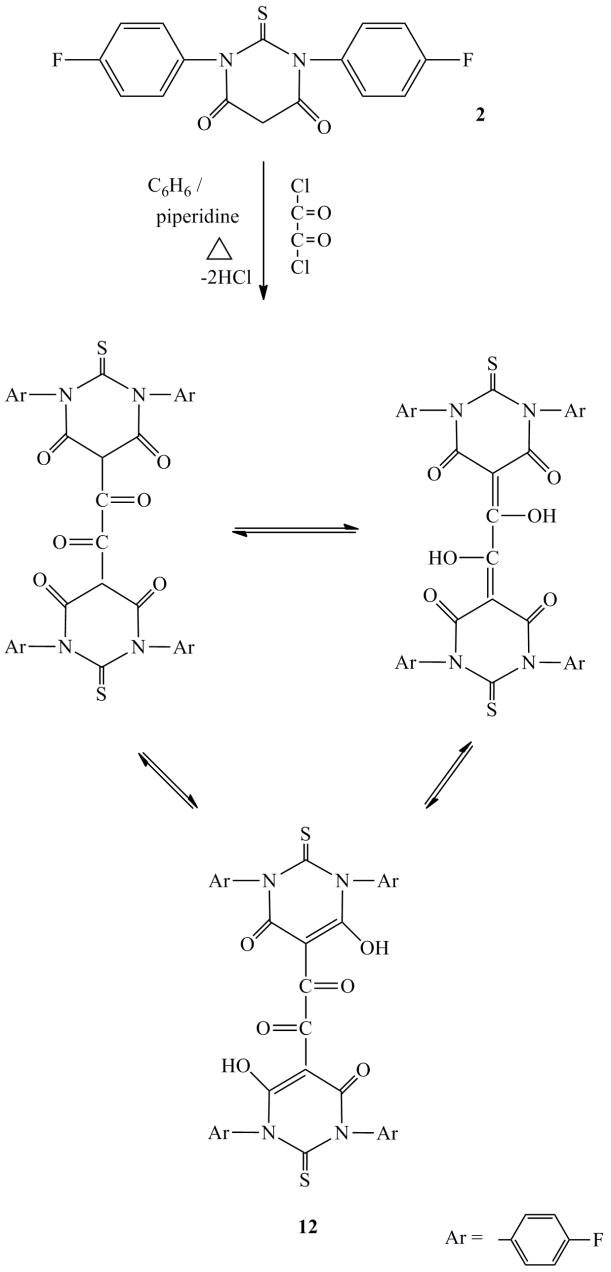
4. Conclusion

Novel fluorinated thiobarbituric acid derivatives have been synthesized and then evaluated as medicinal agents, Among these compounds **9a** > **12** > **2** > **11** exhibited a higher activity as anti HIV agents ,while compounds **12** > **7** > **2** > **3** > **4** have a good activity toward enzymatic inhibition as cyclin-dependent kinase 2 (CDK2) for cell tumer division

5. Biology

5.1. Anti-HIV Evaluation

The research for a more effective and less toxic agents has brought into focus potent yet structurally different



Scheme 4. Synthetic of compounds 12.

non-nucleoside HIV-1 reverse transcriptase inhibitors (NNRTIs) [19]. Abdel-Rahman [20]-[23] synthesized a pool of new fluorinate heterocyclic nitrogen systems as HIV inhibitor agents. Thus, the main aim of the present work tends to search for new fluorinated thiobarbituric acid derivatives and their related fused heterobicyclic nitrogen systems as inhibitors of HIV cases. Some of the synthesized targets have been evaluated as anti-HIV agents. The procedure used in the National Cancer Institute test for agents active against Human Immunodeficiency Virus (HIV) is designed to detect agent acting at any stage of the virus reproductive cycle. The assay basically involves the killing of T_4 lymphocytes by HIV and used the tetrazoliumsalt XTT, as indicator [24]. From the results obtained in (**Table 1**) we showed that, all the fluorinated thiobarbituric acid derivatives exhibited maximum protection <10% for the infected cell and >50% for the uninfected cell. It is interested that, the full fluorinated thiobarbituric acids obtained exhibited maximum protection >20% for the infected cell and >50% for the uninfected cell. Only, the compound **9a** recorded a highly percent of protection at lower dose (molar)

Table 1. The anti-HIV-IC50 values of the fluorinated compounds.

Compoud	IC_{50} (μg ml)	Dose (molar)	Percent of protection	Percent of Infected	Control uninfected
2	1.63×10^{-4}	2.00×10^{-4}	7.74	10.51	40.16
		6.35×10^{-8}	6.30	9.11	99.62
3	6.55×10^{-5}	3.17×10^{-8}	6.68	9.48	99.74
4	$>1.60 \times 10^{-4}$	3.17×10^{-8}	5.08	6.98	98.57
5	1.07×10^{-4}	2.00×10^{-4}	1.94	3.90	5.32
6	$>1.00 \times 10^{-4}$	1.00×10^{-7}	7.55	10.32	88.33
7	$>100 \times 10^{-4}$	3.17×10^{-8}	4.9	7.75	44.93
		1.00×10^{-4}	3.33	9.13	53.27
9a	1.05×10^{-4}	2.00×10^{-7}	12.31	14.94	86.39
		6.32×10^{-5}	14.84	13.39	90.04
11	$>1.00 \times 10^{-4}$	3.17×10^{-8}	0.38	2.37	101.10
		1.00×10^{-4}	7.86	9.11	95.52
12	9.55×10^{-5}	2.00×10^{-7}	5.06	6.96	90.71
		6.33×10^{-6}	23.03	24.57	94.28
		2.00×10^{-5}	6.30	8.17	92.17
		6.32×10^{-5}	19.28	20.89	80.00

The indication > specific only partial production of the infected cells at the indicated highest concentration tested.

than other tested compounds. Also, the order reactivities as **9a** > **12** > **2** > **11**. Based on the resulted data and in compares with results obtained from thiobarbituric acids based HIV-1 integrase inhibitors [3] we can be additional that, the novel fluorinated thiobarbituric acid derivatives in the treatment of HIV infection: 1) nucleoside reverse transcriptase inhibitors; 2) non nucleoside reverse transcriptase inhibitors; 3) protease inhibitors and and fusion inhibitors which led to development of new therapies against the virus especially HIV-1 inhibitors.

5.2. Induction of Apoptosis in Human Leukemia Cells

Cancer cells differ from the normal cells in a biochemical processes particularly during the control of cell growth and division. Leukemia is one of the major types of cancer affecting significant segment of the population. An important application of small molecule libraries is the preparation of a directed of focused combinatorial library for assay against specific biological target, fluorine substituted thiobarbituric acids were proven to be biologically very potent and selective. In this study, using the fluorine substituted thiobarbituric acid derivatives as anticancer activity via inhibition of cyclin-dependent kinase 1 (CDK2) especially as inhibitors towards human leukemia cells. These evaluations carried out by applied of standard method [25] in biochemical assay with IC_{50} values comparable to olomoucine as control. The result obtained recorded in (**Table 2**).

From the results obtained in (**Table 2**) we showed that: detailed anti-cancer (CDK2 for leukemia cells) activity of the fluorinated thiobarbituric acids are very important used as kinase inhibitor activityin the order **12** > **7** > **2** > **3** > **4** > Olomoucine. Preliminary results indicate that many of compounds exhibit an strong effects in compare with the standard used (Olomoucine). The accepting properties of these fluorothiobarbituric acid derivatives are associated with nucleophilicintramolecular substitutions of fluorine atoms, towards the acceptors with cell-cancer. The prominent role of fluorine substituent effects on bioactivity is mainly due to the effect of fluorination of C-H acidity which is predictable and depends on some factors, including the number site of fluorine and the geometry of the conjugate carbanion, which is called bio-conjugation effects.

Table 2. Results of CDK2 inhibtion evaluation (IC_{50} in μmol/Dm3).

Compound	IC_{50} CDK2 ± SD (μM)*
2	4.6 ± 1.80
3	4.7 ± 1.00
4	4.8 ± 1.70
5	6.2 ± 1.20
6	6.4 ± 1.97
7	4.5 ± 1.20
9a	6.8 ± 1.70
11	4.7 ± 1.90
12	4 ± 1.40
Olomoucine	5.0 ± 1.0

*The presented data represent mean values from three independent experiments plus the standard deviation (SD).

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