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Synthesis and pharmacological evaluation of new fluorine substituted pyrimido[1,2-b][1,2,4]triazines and [1,3,5]-triazino-[1,2-b][1,2,4]triazines derived as CDK2 potential inhibitors

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RESEARCH ARTICLE

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CDK2 enzymatic inhibitors evaluation
Fluorinated fused heterocyclic systems

ABSTRACT

New fluorine-substituted polyfunctional pyrimido-[1,2-b]-[1,2,4]triazines and [1,3,5]-triazino[1,2-b]-[1,2,4]triazines were synthesized via the reaction between 3-amino-6-(2-aminophenyl)-1,2,4-triazin-5(2*H*)-one with polyfunctional oxygen/sulfur/nitrogen reagents under different conditions. Structures of the target compounds were deduced by elemental analysis and spectral measurements (IR, $^{1}H/^{13}C$ NMR, and mass spectra). According to the obtained inhibitor assay results, the inhibition activity of the new fluorine-substituted 1,2,4-triazines toward CDK2 decreased in the order of compounds 3 > 8 > 9 > 6 > 13 > 15.

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1. Introduction

Recently, some research groups focused on the synthesis of fluorine-containing heterocyclic nitrogen systems due to their biological, pharmacological, and medicinal properties, including antimetabolite [1], molluscicidal [2,3], anti-inflammatory [4,5], antimicrobial [6], antioxidant [7], cyclin-dependent kinase 2 (CDK2) [8], and anti-HIV activities [9]. On the other hand, fluorine-substituted pyrimido/pyrazolo-1,2,4-triazines not only exhibit antibacterial activities [10] and a protective effect against some plant pathogenic fungi [11], but also serve as a photochemical probe for the inhibition of vitiligo disease [12]. Furthermore, these triazines also exhibit antiviral [13] and anticancer properties [14]. Furthermore, 3-amino-1,2,4-triazine derivatives have been used as starting materials to obtain isolated and/or fused heteropolycyclic nitrogen systems, which exhibit anti-HIV, anticancer, and therapeutic activities [15-17]. These important results and observations motivated our group to synthesize some new fluorine-substituted pyrimido[1,2-b][1,2,4]triazine and [1,3,5]-triazino-[1,2-b] [1,2,4]triazine derivatives due to their potential inhibition of CDK2 activity. The main aim of this study is to obtain some new fluorine substituted heterocyclic compounds derived from [1,2,4]-triazin-5(2*H*)-one bearing an activated amino group.

2. Experimental

2.1. Instrumentation

All analytical-grade chemicals were used without further purification. Melting points, which were determined in capillary tubes using a Stuart Scientific SMP3 melting point apparatus, were uncorrected. A double-beam spectrophotometer (Perkin-Elmer Lambda EZ-2101) was employed for absorption measurements (190-1100 nm). FT-IR spectra of the prepared compounds were recorded on a Perkin Elmer-Spectrum RxI spectrometer. ^{1}H NMR spectra were recorded on a Bruker Avance DPX 400 MHz instrument using tetramethylsilane as the internal standard. Multiplicities were indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet).

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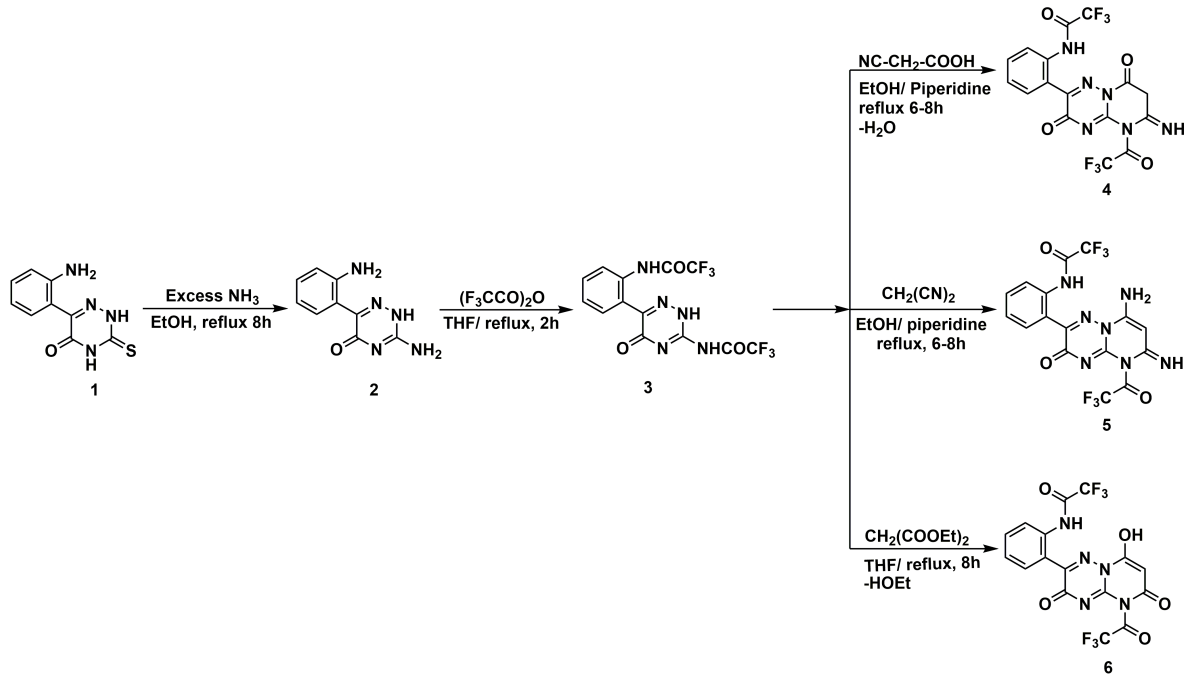


Scheme 1

^{13}C NMR spectra were recorded at 100 MHz and referenced to the internal solvent signals. ^{19}F NMR spectra (376.45 MHz) were recorded on a Bruker Avance DPX 400 MHz spectrometer ($CFCl_3$ as the external reference (0 ppm)). High-resolution mass spectra were recorded on a Shimadzu GC MS-QP 1000 EX spectrometer. Elemental analysis was performed at the Micro Analytical Center of National Reaches Center-Dokki, Cairo, Egypt.

2.2. Synthesis

2.2.1. Synthesis of 6-(2-aminophenyl)-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one

Compound **1** was obtained by refluxing isatin with thiosemicarbazide in aq. NaOH [14,15].

2.2.2. Synthesis of 3-amino-6-(2-aminophenyl)-1,2,4-triazin-5(2H)-one (2)

A mixture of compound **1** (10 g) with ammonia (20 mL, 37%) in ethanol (100 mL) was refluxed for 6-8 h, cooled, and then poured onto ice with drops conc. HCl. The obtained solid was filtered and crystallized using ethanol to afford compound **2** (Scheme 1). Yield: 70%. M.p.: 263-265 °C. FT-IR (KBr, ν, cm^{-1}): 3370, 3280, 3150 (NH), 3062 (Ar-H), 1697 (C=O), 1620 (Def. NH_2), 1597 (C=N), 1346 (NCN). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.33 (s, 2H, NH_2), 6.8-7.2 (m, 4H, Ar-H), 11.2 (s, 2H, NH_2), 12.0 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 163 (C=O), 142, 140 (C4=N, C3-N), 132-128 (Ar-C), 118, 116 (C5 and C6 of 1,2,4-triazine). Anal. calcd. for $C_9H_9N_5O$: C, 53.20; H, 4.46; N, 34.47. Found: C, 53.00; H, 4.30; N, 34.01%.

2.2.3. Synthesis of 2,2,2-trifluoro-N-(2-(5-oxo-3-(2,2,2-trifluoroacetamido)-2,5-dihydro-1,2,4-triazin-6-yl)phenyl) acetamide (3)

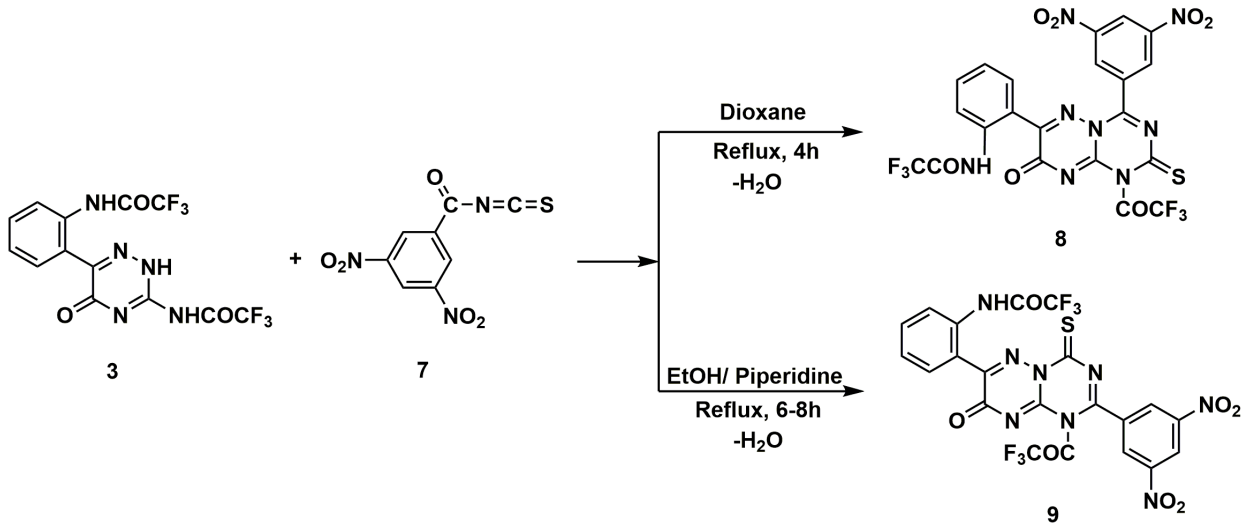
A mixture of compound **2** (0.01 mol) and trifluoroacetic anhydride (0.03 mol) in THF (100 mL) was refluxed for 2 h, cooled, and then poured onto ice water. The resultant solid was filtered and crystallized using dioxane to afford compound **3** (Scheme 1). Yield: 75%. M.p.: 279-280 °C. FT-IR (KBr, ν, cm^{-1}): 3300, 3280, 3165 (NH), 3050 (Ar-H), 1695 (C=O), 1673 (C=C), 1622 (C=N), 1597 (C=N), 1230 (F-C), 708 (C-F). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 6.9-7.3 (t, 1H, Ar-H), 7.4-7.5 (d, 1H, Ar-H), 7.6-7.9 (t, 1H, Ar-H), 8.1-8.7 (d, 1H, Ar-H), 12.4 (s, 1H, NH), 14.5 (s, 2H, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 170, 163, 155 (3C=O), 145 (C-F), 142, 141 (C=N, C-N), 130-124 (Ar-C). ^{19}F NMR (376.45 MHz, DMSO-d_6, δ, ppm): -78 (CF_3). MS (EI, *m/z* (%)): 398 (M+3, 105), 188 (5.11), 152 (18.5), 112 (100), 97 (27.55), 91 (58.1), 68 (3.55). Anal. calcd. for $C_{13}H_7F_6N_5O_3$: C, 39.51; H, 1.79; N, 17.72. Found: C, 39.41; H, 1.55; N, 17.49%.

2.2.4. Synthesis of 2,2,2-trifluoro-N-(2-(6-imino-3,8-dioxo-5-(2,2,2-trifluoroacetyl)-5,6,7,8-tetrahydro-3H-pyrimido [1,2-b][1,2,4]triazin-2-yl)phenyl)acetamide (4)

Equimolar amounts of compound **3** and cyanoacetic acid in ethanol (50 mL) with a few drops of piperidine were refluxed for 6-8 h, cooled, and then poured onto ice-cold saturated Na_2CO_3. The obtained solid was filtered and crystallized using methanol to afford compound **4** (Scheme 1). Yield: 55%. M.p.: >300 °C. FT-IR (KBr, ν, cm^{-1}): 3320, 3280 (NH, HN=), 2915 (CH_2), 1690 (C=O), 1667 (C=O), 1620 (C=N), 1598 (C=N), 1448, 1418 (Def. CH_2), 1225 (C-F), 853, 830 (Ring), 706 (C-F). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.3 (s, 2H, CH_2), 7.3-8.1 (m, 4H, Ar-H), 12.5 (s, 1H, NH), 14.5 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 171, 165, 161, 153 (C=O), 145 (C-F), 142-141 (C=N, C-N), 130-126 (Ar-C), 117, 118 (C5 and C6-1,2,4-triazine), 71 (CH_2). ^{19}F NMR (376.45 MHz, DMSO-d_6, δ, ppm): -78 ppm (CF_3). Anal. calcd. for $C_{16}H_8F_6N_6O_4$: C, 41.57; H, 1.74; N, 18.18. Found: C, 41.39; H, 1.59; N, 18.60%.

2.2.5. Synthesis of N-(2-(8-amino-6-imino-3-oxo-5-(2,2,2-trifluoroacetyl)-5,6-dihydro-3H-pyrimido[1,2-b][1,2,4] triazin-2-yl)phenyl)-2,2,2-trifluoroacetamide (5)

A mixture of compound **3** (0.01 mol) and malononitrile (0.01 mol) in ethanol (100 mL) with a few drops of piperidine was refluxed for 6-8 h, cooled, and then poured onto ice-drops AcOH.



Scheme 2

The obtained solid was filtered and crystallized using dioxane to afford compound **5** (Scheme 1). Yield: 61%. M.p.: 297-298 °C. FT-IR (KBr, ν, cm^{-1}): 3360, 3280, 3163 (NH), 3020 (Ar-H), 1688, 1672 (C=O), 1593 (C=N), 1463, 1410 (Def. CH), 1345 (Cyclic NCN), 1220 (C-F), 702 (C-F). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.4 (s, 2H, NH_2), 4.2 (s, 1H, cyclic CH), 7.3-8.1 (m, 4H, Ar-H), 14.7 (s, 1H, NH), 11.5 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 172, 165, 157, 150 (3C=O), 145 (C-F), 142, 140 (C=N, C-N), 130-128 (Ar-C), 118, 117 (C5 and C6 1,2,4-triazine), 71 (CH_2). ^{19}F NMR (376.45 MHz, DMSO-d_6, δ, ppm): -78 (CF_3). Anal. calcd. for $C_{16}H_9F_6N_7O_3$: C, 41.66; H, 1.97; N, 21.26. Found: C, 41.51; H, 1.77; N, 21.01%.

2.2.6. Synthesis of 2,2,2-trifluoro-N-(2-(8-hydroxy-3,6-dioxo-5-(2,2,2-trifluoroacetyl)-5,6-dihydro-3H-pyrimido[1,2-b][1,2,4]triazin-2-yl)phenyl)acetamide (6)

A mixture of compound **3** (0.01 mol) and diethyl malonate (0.01 mol) was refluxed for 8 h and then cooled. The obtained solid was filtered and crystallized from dioxane to afford compound **6** (Scheme 1). Yield: 62%. M.p.: >300 °C. FT-IR (KBr, ν, cm^{-1}): 3387-3450 (b, OH), 3107 (NH), 3066 (Ar-CH), 2973 (Aliphatic CH), 1800, 1711, 1700 (C=O), 1610 (C=N), 1525 (C=N), 1461 (Def. CH), 1339 (NCN), 1249 (C-F). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.2 (s, 1H, CH), 7.40-8.40 (m, 4H, Ar-H), 12.4 (s, 1H, OH), 14.6 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 171, 162, 157 (3C=O), 146 (C-F), 142, 141 (C=N, C-N), 132-122 (Ar-C), 71 (Cyclic CH). ^{19}F NMR (376.45 MHz, DMSO-d_6, δ, ppm): -78 (CF_3). MS (EI, *m/z* (%)): 464 (M+1, 12.1), 215 (2.5), 187 (13.55), 152 (28.0), 137 (3.15), 112 (100), 70 (1.3). Anal. calcd. for $C_{16}H_7F_6N_5O_5$: C, 41.48; H, 1.52; N, 15.12. Found: C, 41.31; H, 1.42; N, 14.88%.

2.2.7. Synthesis of N-(2-(4-(3,5-dinitrophenyl)-8-oxo-2-thioxo-1-(2,2,2-trifluoroacetyl)-1,8-dihydro-2H-[1,3,5] triazino[1,2-b][1,2,4]triazin-7-yl)phenyl)-2,2,2-trifluoro acetamide (8)

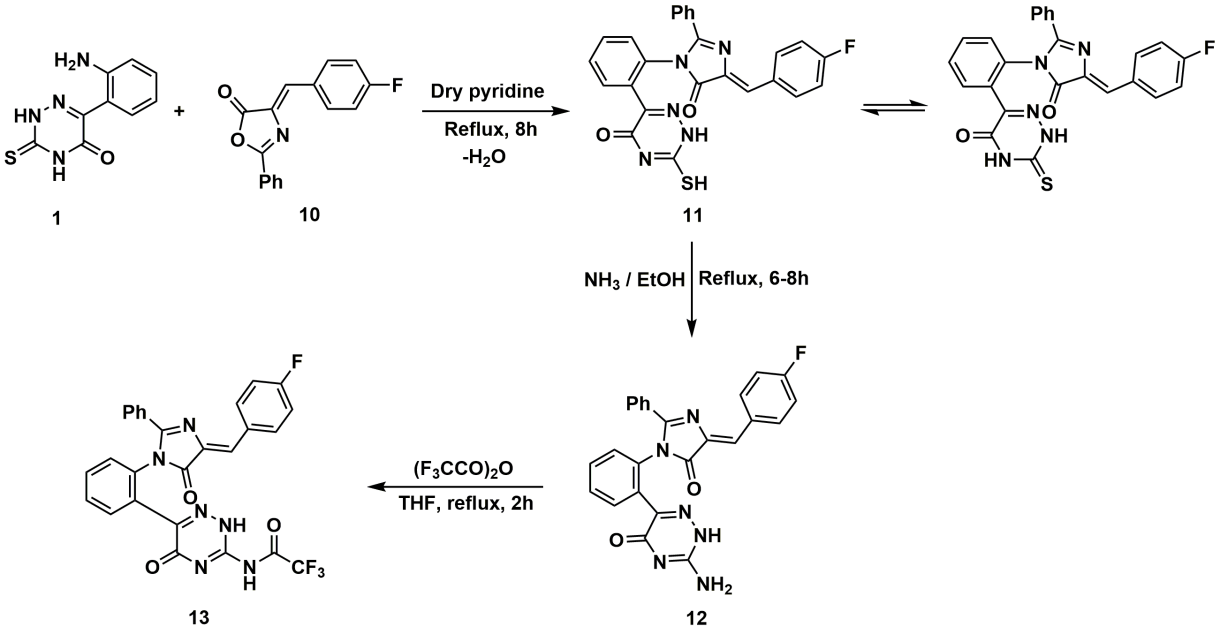
Equimolar amounts of compound **3** and 3,5-dinitrobenzoyl isothiocyanate (**7**) in dioxane (50 mL) were refluxed for 4 h and then cooled. The obtained solid was filtered and crystallized from ethanol to afford compound **8** (Scheme 2). Yield: 72%. M.p.: >300 °C. FT-IR (KBr, ν, cm^{-1}): 3180 (NH), 1699, 1672 (C=O), 1608 (C=N), 1530-1340 (Asym. and sym. NO_2), 1227 (C-F), 1129 (C-S), 935, 887 (Rings). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.0-7.9 (m, 4H, Ar-H), 8.3-8.4 (s, 2H, Ar-H Adjacent NO_2), 9.52 (s, 1H, NO_2-CH-NO_2), 12.8 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 188 (C=S), 171, 167, 155, (C=O), 145 (C-F), 142, 141 (C=N, C-N), 132-122 (Ar-C). ^{19}F NMR (376.45 MHz, DMSO-d_6, δ, ppm): -78 (CF_3).

2.2.8. Synthesis of N-(2-(2-(3,5-dinitrophenyl)-8-oxo-4-thioxo-1-(2,2,2-trifluoroacetyl)-1,8-dihydro-4H-[1,3,5] triazino[1,2-b][1,2,4]triazin-7-yl)phenyl)-2,2,2-trifluoro acetamide (9)

A mixture of compound **3** (0.01 mol) and compound **7** in EtOH (100 mL) with a few drops of piperidine was refluxed for 6-8 h, cooled, and then poured onto ice water. The produced solid was filtered and crystallized using methanol to afford compound **9** (Scheme 2). Yield: 60%. M.p.: >300 °C. FT-IR (KBr, ν, cm^{-1}): 3362 (NH), 1698, 1680 (C=O), 1608 (C=N), 1492-1381 (Asym. and sym. NO_2), 1205 (C-F), 1131 (C-S), 935, 886, 852 (Rings). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 6.92-8.35 (m, 4H, Ar-H), 8.7-8.9 (s, 2H, Ar-H Adjacent NO_2), 9.07 (s, 1H, NO_2-CH-NO_2), 12.8 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 188 (C=S), 171, 165, 153 (C=O), 145 (C-F), 142, 140 (C=N, C-N), 131-124 (Ar-C). ^{19}F NMR (376.45 MHz, DMSO-d_6, δ, ppm): -78 (CF_3). Anal. calcd. for $C_{21}H_8F_6N_8O_7S$: C, 40.01; H, 1.28; N, 17.77. Found; C, 39.78; H, 1.05; N, 17.59%.

2.2.9. Synthesis of (Z)-6-(2-(4-(4-fluorobenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl)phenyl)-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one (11)

Equimolar amounts of compound **1** and oxazolone **10** in dry pyridine (50 mL) was refluxed for 8 h, cooled, and then poured onto ice with drops from Conc.HCl. The obtained solid was filtered and crystallized from methanol to afford compound **11** (Scheme 3). Yield: 60%. M.p.: 217-218 °C. FT-IR (KBr, ν, cm^{-1}): 3362, 3200 (NH), 3061 (Ar-CH), 2887 (Aliph-CH), 1693 (C=O), 1582 (C=N), 1489, 1446 (Def. CH), 1241 (C-F), 1163 (C-S), 981, 926, 879, 848. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 6.42-7.43 (m, 9H, Ar-H), 7.95 (s, 1H, Z/CH=C), 8.43-8.60 (m, 4H, Ar-H), 8.69 (s, 1H, NH), 10.1 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 178.0 (C=S), 159 (C=O), 150 (C-F), 148, 143 (C=N, C-N), 133.87-128.78 (Ar-C), 125 (exo, HC=C), 118.8, 118.7 (C5-C6 1,2,4-triazine), 117, 116 (C=N of imidazole). Anal. calcd. for $C_{25}H_{16}FN_5O_2S$: C, 63.96; H, 3.44; N, 14.92. Found: C, 63.75; H, 3.35; N, 14.71%.



Scheme 3

2.2.10. Synthesis of (Z)-3-amino-6-(2-(4-(4-fluorobenzyli dene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl) phenyl)-1,2,4-triazin-5(2H)-one (12)

A mixture of compound **11** (10 g) and NH_3 (20 mL, 37%) in ethanol (100 mL) was refluxed for 8 h, cooled, and then poured onto ice with drops from acetic acid. The obtained solid was filtered and crystallized using ethanol to afford compound **12** (Scheme 3). Yield: 66%. M.p.: 190-191 °C. FT-IR (KBr, ν, cm^{-1}): 3300 (NH), 3095 (Ar-CH), 2884 (Aliph-CH), 1689 (C=O), 1609 (C=N), 1581 (C=N), 1481, 1454 (Def. CH=), 1309 (NCN), 1223 (C-F), 964, 875, 837. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 6.93-7.70 (m, 9H, Ar-H), 7.8 (s, 2H, NH_2), 7.97 (s, 1H, CH=C), 8.32-9.03 (m, 4H, Ar-H), 11.20 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 169, 163 (C=O), 143 (C-F), 135 (C=N, C-N), 132.7-127 (Ar-C), 125, 124 (C=N imidazol), 120 (C-N), 116.6, 116.4 (C5-C6 1,2,4-triazine), 112.57 (-HC=C). Anal. calcd. for $C_{25}H_{17}FN_6O_2$: C, 66.37; H, 3.79; N, 18.57. Found: C, 66.12; H, 3.39; N, 18.29%.

2.2.11. Synthesis of (Z)-2,2,2-trifluoro-N-(6-(2-(4-(4-fluoro benzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl)phenyl)-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)acetamide (13)

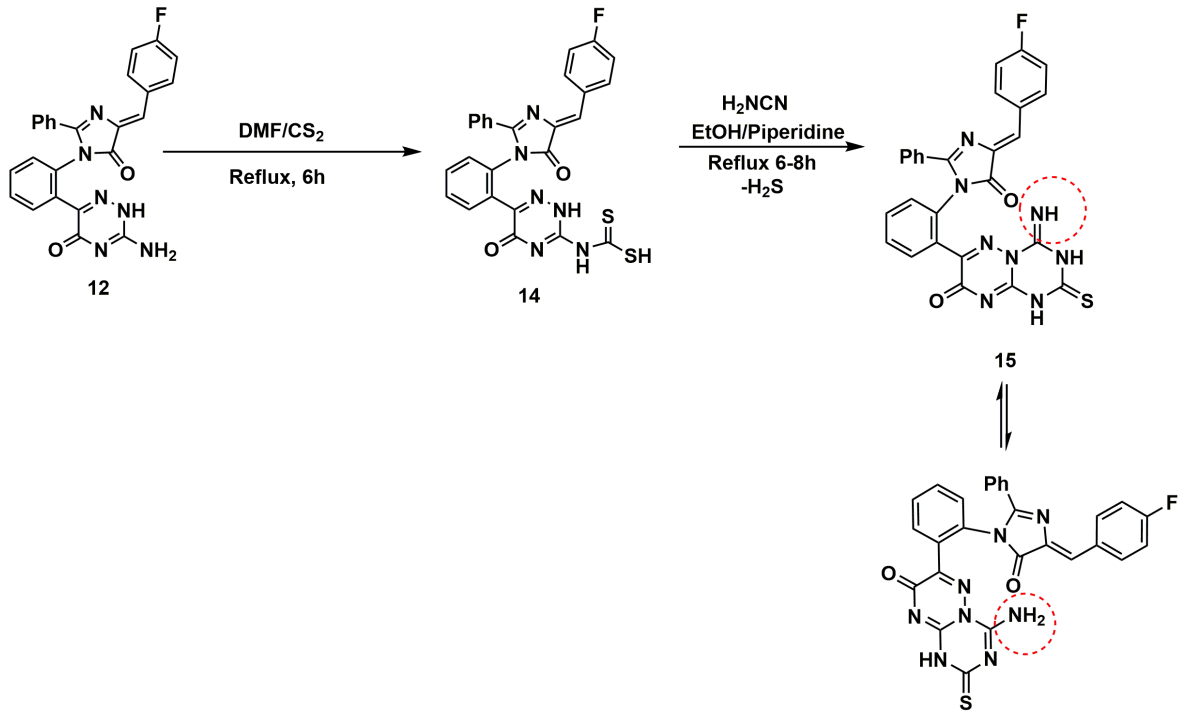
A mixture of compound **12** (0.01 mol) and hexafluoroacetic anhydride (0.02 mol) in THF (100 mL) was refluxed for 2 h, cooled, and then poured onto ice water. The obtained solid was filtered and crystallized using dioxane to afford compound **13** (Scheme 3). Yield: 60%. M.p.: 175-176 °C. FT-IR (KBr, ν, cm^{-1}): 3425 (OH, 5-hydroxy-1,2,4-triazine), 3332 (NH), 1693 (C=O), 1612 (C=N), 1539 (C=N), 1219 (C-F), 944, 853, 801. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 6.75-7.66 (m, 9H, Ar-H), 7.9 (s, 1H, CH=C), 8.13-8.68 (m, 4H, Ar-H), 9.03 (s, 1H, NH), 11.2 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 179.7, 164.75, 163.31 (C=O), 143.0 (C-F), 136.32-133.99 (C=N, C-N), 132.78-127.71 (Ar-C), 124.78 (HC=C), 118.42, 116 (C5 -C6 1,2,4-triazine), 111.71 (CF_3). ^{19}F NMR (376.45 MHz, DMSO-d_6, δ, ppm): -78 (CF_3), -121 (Ar-F). Anal. calcd. for $C_{27}H_{16}F_4N_6O_3$: C, 59.13; H, 2.94; N, 15.32. Found: C, 59.0; H, 2.80; N, 15.21%.

2.2.12. Synthesis of (Z)-(6-(2-(4-(4-fluorobenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl)phenyl)-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)carbamodithioic acid (14)

To compound **12** (0.01 mol), CS_2 (0.02 mol) was added, followed by DMF (50 mL), and warmed for 6 h. After cooling the mixture, it was poured onto ice water. The obtained solid was filtered and crystallized using dioxane to afford compound **14** (Scheme 4). Yield: 45%. M.p.: 198-200 °C. FT-IR (KBr, ν, cm^{-1}): 3091 (NH), 1680 (C=O), 1641 (HN-CS), 1537 (C=N), 1346 (NCSN), 1221 (C-F), 1169 (C-S), 921, 844, 759. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 6.45-7.66 (m, 9H, Ar-H), 7.67 (d, 1H, HC=C-), 9.6-9.7 (d, 2H, Ar-H), 10.0-10.1 (d, 2H, Ar-H), 11.11 (s, 1H, SH), 12.1 (s, 1H, NH), 13.6 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 183 (C=S), 163.4, 163.28 (2 C=O), 153 (C-S), 148.0 (C-F), 143, 137 (C=N, C-N), 133-127 (Ar-C), 125, 124 (C of imidazole), 120 (HC=C), 116.47, 116.13 (C5-C6 1,2,4-triazine), 112.5-111.72 (N=CS_2). ^{19}F NMR (376.45 MHz, DMSO-d_6, δ, ppm): -121 (Ar-F). Anal. calcd. for $C_{26}H_{17}FN_6OS_2$: C, 59.08; H, 3.24; N, 15.90. Found: C, 58.89; H, 3.11; N, 15.79%.

2.2.13. Synthesis of (Z)-7-(2-(4-(4-fluorobenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl)phenyl)-4-imino-2-thioxo-1,2,3,4-tetrahydro-8H-[1,3,5]triazino[1,2-b] [1,2,4] triazin-8-one (15)

Equimolar amounts of compound **14** and cyanamide in ethanol (100 mL) with a few drops of piperidine were refluxed for 6-8 h, cooled, and then poured onto ice. The resultant solid was filtered and crystallized from ethanol to afford compound **15** (Scheme 4). Yield: 68%. M.p.: >300 °C. FT-IR (KBr, ν, cm^{-1}): 3230 (NH), 3169 (NH_2), 2900 (C=CH), 1760, 1674 (C=O), 1594 (C=N), 1507 (C=C), 1221 (C-F), 889, 856, 758. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 6.57 (s, 1H, C=CH), 7.16-7.96 (m, 9H, Ar-H), 8.00-8.42 (d, 2H, Ar-H), 8.67 (d, 2H, Ar-H), 9.1 (s, 1H, NH), 11.19 (s, 1H, NH), 12.47 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 170.3 (C=S), 163.30 (C=O), 143.03 (N-CH=N), 132.7-131.9 (C-aryl), 120.66-123.05 (Ar-C), 111.71 (C=N), 44.44 (CH=C). ^{19}F NMR (376.45 MHz, DMSO-d_6, δ, ppm): -121 (Ar-F). MS (EI, *m/z* (%)): 537 (M+1, 2.55), 271 (13.8), 265(100), 131 (8.15), 103 (15.18), 119 (75), 95 (90.11), 90 (5.11).



Scheme 4

Anal. calcd. for $C_{27}H_{17}FN_8O_2S$: C, 60.44; H, 3.19; N, 20.88. Found: C, 60.29; H, 3.10; N, 20.70%.

2.3. Cyclin-dependent kinase (CDK) inhibitors as anticancer drugs

All the synthesized compounds were evaluated for cyclin-dependent kinase 2 (CDK2) inhibition activity according to previously reported methods [18,19]. Kinase activity was described as a percentage of the maximum activity. The concentration of the target compounds required to decrease by 50% (IC_{50}) was determined from dose-response curves. CDK2 also can mediate UV-induced DNA damage response via the product of mutated genes. Notably, the DNA repair ability significantly decreased after UV-light treatment, attaining the values of the control cell [20,21].

3. Results and discussion

3.1. Chemistry

An amino group bearing a 1,2,4-triazine nucleus was used as the starting material to produce various fused heterobicyclic nitrogen systems. Thus, the aminolysis of 6-(2-aminophenyl)-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2*H*)-one (**1**) [1] is performed by refluxing with excess NH_3/EtOH to afford 3-amino-6-(2-aminophenyl)-1,2,4-triazin-5(2*H*)-one (**2**) (Scheme 1). Notably, the fluoro-acylation of compound **2** by warming with trifluoroacetic anhydride in THF afforded 2,2,2-trifluoro-*N*-(2-(5-oxo-3-(2,2,2-trifluoroacetamido)-2,5-dihydro-1,2,4-triazin-6-yl)phenyl) acetamide (**3**) (Scheme 1).

The introduction of the trifluoroacetyl group into an amino group enhances chemical and biological activities [22,23]. Hence, the heterocyclization of compound **3** by refluxing with cyanoacetic acid (EtOH), malononitrile (EtOH/piperidine), and diethyl malonate in THF afforded compounds **4**-**6**, respectively (Scheme 1). As-synthesized compounds **4**-**6** exhibited tautomeric keto ⇌ enol and imino ⇌ amino isomeric structures and/or type of H-bonds (Figure 1).

The structure of the new fluorinated fused heterobicyclic nitrogen systems containing 1,2,4-triazines moieties (**2**-**6**) was confirmed by their accurate elemental analysis and various spectral measurements. The SH group was not observed, while NH and NH_2 groups were observed at 3370-3150 cm^{-1}, respectively, in the IR spectrum of compound **2**. Compounds **3**-**6** exhibited absorption bands at ν ~3300-3180, 1800-1680, and 1250-1220 cm^{-1}, corresponding to NH, CO, and CF groups, respectively. In the IR spectra of only compounds **4**-**6**, peaks were observed at 2980-2890 and 1480-1440 cm^{-1}, corresponding to active CH_2 groups. ^1H NMR spectra of compounds **3**-**6** serve as a good indicator of the type of hydrogen present compounds **4**-**6** exhibited resonance at δ 14.6, 12.4, and 11.2 ppm for NH and δ 3.5-3.36 ppm, corresponding to the presence of active methylene protons. In addition, ^{13}C NMR spectra of compounds **3**-**6** revealed signals at δ 170-160, 146-145 and 142-140 ppm, corresponding to the presence of C=O, C-F, C=N; δ 130-122 ppm, corresponding to aromatic carbons; and δ 70 ppm, corresponding to aliphatic carbons. Abdel-Monem *et al.* reported the behavior of 3-amino-1,2,4-triazines toward the π-electron acceptor of unsaturated aroyl isothiocyanate in solvents with different polarities and isolated the isomeric structures [24,25]. Similarly, the interaction between compound **3** and aroyl isothiocyanate **7** (obtained by refluxing aroyl chloride with ammonium thiocyanate in dry acetone) [25] in a non-polar solvent such as dioxane and a polar solvent such as ethanol in a few drops of piperidine afforded an isomeric structure of *N*-(2-(4-(3,5-dinitrophenyl)-8-oxo-2-thioxo-1-(2, 2, 2-trifluoroacetyl)-1, 8-dihydro-2*H*-[1,3,5]triazino[1,2-b][1,2,4]triazin-7-yl)phenyl)-2, 2, 2-trifluoroacetamide (**8**) and *N*-(2-(2-(3,5-dinitrophenyl)-8-oxo-4-thioxo-1-(2, 2, 2-trifluoroacetyl)-1,8-dihydro-4*H*-[1, 3, 5] triazino[1, 2-b][1, 2, 4]triazin-7-yl)phenyl)-2, 2, 2-trifluoroacetamide (**9**), respectively (Scheme 2). Structures of compounds **8** and **9** were deduced from element analysis and spectral measurements. An absorption band was observed at 1120-1131 cm^{-1} in the IR spectra of compounds **8** and **9**, corresponding to the C=S group.

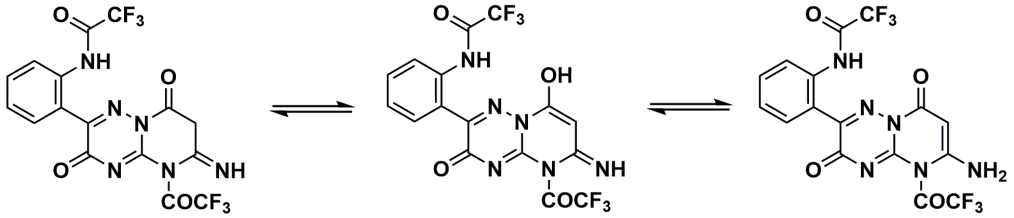


Figure 1. Isomeric structures of compound **4**.

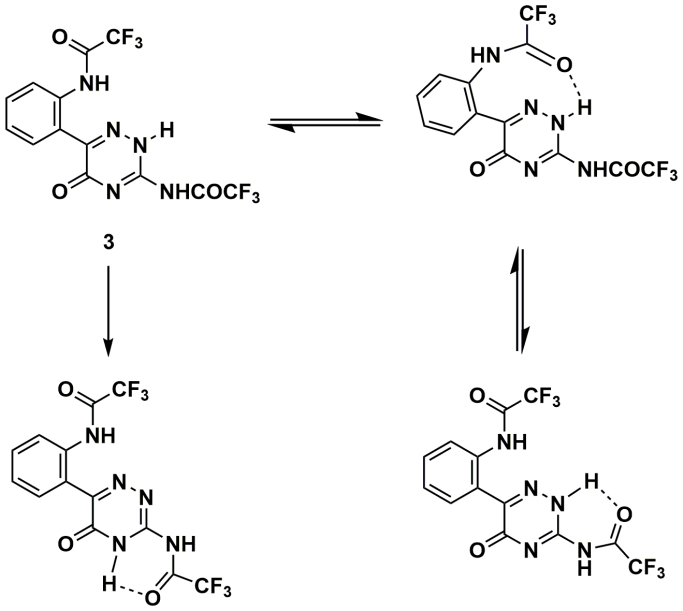


Figure 2. More stable formula and H-bonding present in various tautomeric formula of compound **3**.

Three singlets at δ ~8.7-9.5 ppm were observed in the ^{1}H NMR spectra of compounds **8** and **9** (s, 3H, CH aromatic nitro). On the other hand, imidazoles are one of the most important compounds found in a large number of natural products and pharmacologically targets. For example, histidine, histamine, and biotin are present in several drugs [26,27]. In addition, imidazole is used as a proton pump inhibitor, e.g., omeprazole [28], and as a benzodiazepine antagonist, e.g., flumazenil [29]. Furthermore, a tetra-substituted imidazole scaffold is a core constituent in several biological systems such as olmesartan [30]. Based on these observations, new substituted imidazole **11** was obtained by the reflux of 6-(2-aminophenyl)-3-thioxo-3, 4-dihydro-1, 2, 4-triazin-5(2*H*)-one (**1**) with fluorinated oxazol-5-one (**10**) in dry pyridine (Scheme 3) [31]. In addition, the aminolysis of compound **11** by refluxing with excess NH_3/EtOH afforded 3-amino-6-(2-(4-(4-fluorobenzylidene)-5-oxo-2-phenyl-4, 5-dihydro-1*H*-imidazol-1-yl)phenyl)-1, 2, 4-triazin-5(2*H*)-one (**12**) (Scheme 3). Compound **12** was used as the starting material to prepare newly heterocyclic nitrogen systems (**13**-**15**). Thus, the fluoroacylation of compound **12** by refluxing with trifluoroacetic anhydride in THF afforded 2,2,2-trifluoro-*N*-(6-(2-(4-(4-fluoro benzylidene)-5-oxo-2-phenyl-4, 5-dihydro-1*H*-imidazol-1-yl)phenyl)-5-oxo-2,5-dihydro-1, 2, 4-triazin-3-yl)acetamide (**13**) (Scheme 3), while the thioacylation of compound **12** by refluxing with CS_2 in DMF afforded compound **14** (Scheme 4). Ring-closure of compound **14** via cycloaddition with cyanamide in refluxing EtOH/piperidine led to the direct formation of 7-(2-(4-(4-fluorobenzylidene)-5-oxo-2-phenyl-4, 5-dihydro-1*H*-imidazol-1-yl)phenyl)-4-imino-2-thioxo-1, 2, 3, 4-tetrahydro-8*H*-[1, 3, 5]triazino[1, 2-b][1, 2, 4] triazin-8-one (**15**) (Scheme 4). Formation of compound **15** may occur by the nucleophilic attack of NH (1,2,4-triazine) to the more electrophilic carbon of cyanamide, followed by cyclization and a subsequent tautomeric process (Scheme 4). In the IR spectra of compounds **11**, **14** and **15**, the peaks were observed at ν 1169-1160 cm^{-1}, corresponding to the presence of CS groups. In the ^{1}H NMR spectra of compounds **11**-**15**, a resonance was observed at δ ~7.9 ppm, corresponding to the olefinic (=CH) proton. In the ^{13}C NMR spectrum of compound **14**, resonances were observed at δ 188 and 153 ppm, confirming the presence of C=S and C-S carbons, while in those of compounds **8**, **9**, and **15**, only one peak was observed at δ 180-188 ppm, corresponding to C=S carbons.

In an initial study of mass spectroscopy of the new fluorinated compounds, the molecular ion peak at a moderate percentage with a base peak at *m*/*z* 112 corresponded to F_3CCONH^+ for compound **3**, while in that of compound **15**, peaks were observed at *m*/*z* 265, corresponding to fluoro-imidazole ions $C_{16}H_{10}FN_2O^+$ (265). To completely deduce the former structure, ^{19}F NMR spectral measurements revealed peaks at δ -78 ppm for CF_3 and at δ -121 ppm for Ar-F.

3.2. Cyclin-dependent kinase (CDK) inhibitors as anticancer drugs

In cancer diagnostic research, drug resistance is one of the major obstacles that cause failure in tumor treatment, recurrence of the disease, or even the death of a patient. Thus, resistance to drugs can be observed during diagnosis, or it can develop after the treatment of the tumor. Indeed, the development of new medication continues to offer hope to prevent resistance capabilities of tumor cell lines [32-35]. Some reviews reported an important study of CDKs, which prompted us to research new potential inhibitors for tumor cells [36,37].

Table 1. CDK2 inhibition tests (IC_{50}) for new fluorinated systems.

Compound	IC_{50} CDK2 ± SD (µmol/L) *
3	4.88±1.0
4	>20
5	15.11±1.5
6	5.49±1.5
8	4.22±1.0
9	4.55±1.7
11	>20
12	18.55±1.0
13	12.22±1.0
14	16.11±2.1
15	14.45±2.8
Olomoucine (Control)	5±1.0

* SD = Standard deviation.

Recently, Liu *et al.* investigated the control of dual roles of CDK2 in DNA damage and DNA damage response [38].

Therefore, this study aimed to prepare new fluorosubstituted 1,2,4-triazinone bearing heterobicyclic nitrogen systems as potential inhibitors for the CDK2 activity by a biochemical assay [18,38]. The inhibitory concentration (IC_{50}) values are reported according to assayed methods [19,39]. Olomoucine is used as the standard [40,41], and Table 1 summarizes the obtained results. Compared to Olomoucine, all of the fluorinated 1,2,4-triazines exhibited a degree of activity; this result is in agreement with previous studies [8,42].

In view of the obtained results, the maximum activity may be related to the role of fluorine atoms and the direction of the total charge over centers of new fused heterobicyclic systems via the presence of heterobiconjugation. All compounds containing trifluoroacetamido moieties exhibited more active control on the tumor cells. In addition, the introduction of sulfur atoms (thiol ⇌ thione) in these fluorinated systems with trifluoroacetamido moieties led to enhanced activities. The bioactivity decreased in the order of compounds **3** > **8** > **9** > **6** > **13** > **15** (Table 1). A higher potential inhibitory activity of compound **3** may be related to the presence of F_3CCO-NH at the terminal of asymmetric 1,2,4-triazin-5-one (Figure 2), while the lower activity of compound **15** may be related to the presence of NH_2 bonded to the 1,3,5-triazinothione part, which enhanced the action of enzymes in tumor cell formation.

4. Conclusion

In conclusion, novel fluorine compounds bearing fused heterobicyclic nitrogen systems containing the 1,2,4-triazin-5-one moiety derived from trifluoroacetamide derivatives were prepared by a simple and an effective methodology, which involved the ring-closing reaction with polyfunctional sulfur, oxygen, and nitrogen agents for the purpose of producing new anti-cancer targets. The synthesized new systems were primarily evaluated as potential inhibitors for the CDK2 activity in terms of cell tumor division in comparison with olomoucine as the standard. The results obtained in this article considered next steps to control CDK2 in DNA damage as well as DNA damage response. Some of the examined perfluoro derivatives may be used as anticancer drugs or protect from DNA damage.

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Disclosure statement

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