Research Article

Fluorine Substituted 1,2,4-Triazinones as Potential Anti-HIV-1 and CDK2 Inhibitors

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Fluorine substituted 1,2,4-triazinones have been synthesized via alkylation, amination, and/or oxidation of 6-(2-amino-5-fluorophenyl)-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one **1** and 4-fluoro-N-(4-fluoro-2-(5-oxo-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)phenyl)benzamide **5** as possible anti-HIV-1 and CDK2 inhibitors. Alkylation on positions 2 and 4 in 1,2,4-triazinone gave compounds **6–8**. Further modification was performed by selective alkylation and amination on position 3 to form compounds **9–15**. However oxidation of **5** yielded compounds **16–18**. Structures of the target compounds have been established by spectral analysis data. Five compounds (**5**, **11**, **14**, **16**, and **17**) have shown very good anti-HIV activity in MT-4 cells. Similarly, five compounds (**1**, **3**, and **14–16**) have exhibited very significant CDK2 inhibition activity. Compounds **14** and **16** were found to have dual anti-HIV and anticancer activities.

1. Introduction

Human immunodeficiency virus (HIV) type-1 is the causative agent of acquired immunodeficiency syndrome (AIDS), which is one of the serious global health problems [1]. The currently approved anti-HIV drugs can be divided into five groups: reverse transcriptase inhibitors (RTIs), protease inhibitors [2] (PIs), fusion inhibitors (FIs), coreceptor inhibitors (CRIs), and integrase inhibitors (INIs). This arsenal of drugs, which is used in combinations, has moved the prognosis of HIV patients from that of high morbidity and mortality to, for many at least, a chronic, manageable but still complex disease [3–5]. However, the use of these drugs has been relatively limited by their toxicity [6], drug resistance development [7], and, more worryingly, the fact that some newly HIV-infected patients carry viruses that are already resistant to the currently approved AIDS treatments [8]. These issues along with drug-related side effects make it apparent that new anti-HIV drugs with novel mechanisms of action are clearly needed.

On the other hand, during the past 30 years, a variety of approaches have been taken for cancer chemotherapy and many antitumor drugs have been developed for clinical use. In the treatment of solid tumors, however, the conventional

approaches have met with only limited success and cancer still remains as one of the leading causes of human mortality [9].

Chemotherapy drugs are sometimes feared because of a patients' concern about its toxic effects. There are three goals associated with the use of the most commonly used anticancer agents: (a) damage the DNA of the affected cancer cells, (b) inhibit the synthesis of new DNA strands to stop the cells from replicating, and (c) stop the mitosis or the actual splitting of the original cell into two new daughter cell [10].

In the past few years fluorine substituted heterocyclic nitrogen systems have been incorporated into drug discovery research [11–23] to improve the physicochemical properties of drugs. Organic fluorine is a prominent tool in the design and improvement of pharmacokinetic properties of drug molecules. Replacing hydrogen and other functional groups with fluorine can have a dramatic effect on the modulation of electronic, lipophilic, and steric parameters, all of which can critically influence both the pharmacodynamic and pharmacokinetic properties of drugs. Substitution of fluorine into a potential drug molecule not only alters the electronic environment, but it also influences the properties of neighboring functional groups. Fluorine can have significant effects on the binding affinity in protein-ligand complexes. The DNA polymerase inhibitors fludarabine (I) (F-ara-A),

FIGURE 1

clofarabine (II), and tezacitabine (III) are used as cancer chemotherapeutic agents [24], while Gleevec (IV) is used as catalytic inhibitor of imatinib mesylate [25]. In addition, BX-1382BS (V) showed a significant activity as protein kinase inhibitor [26] and a cyanopyrimidine scaffold JNJ-17029259 (VI) is an oral inhibitor of VEGF-mediated signal transduction [27] (Figure 1). Recently a great deal of synthetic efforts has been spent on fluorinated uncondensed 1,2,4-triazines by our group searching for new anti-HIV and anticancer agents [28–38] (compounds A–F; Figure 2).

Fluorine incorporation on key positions plays a significant role to alter the physicochemical and biological characteristics of organic compounds. Frequently, it is found that a fluorine substituent leads to an enhancement of the binding affinity of a molecule with proteins through a noncovalent bond formation. Fluorine increases binding affinity, reduces plasma protein binding leading to a higher free fraction of the drug, and increases cell penetration. The combination of these effects results in a dramatically improved biological activity. Based on these valuable observations and in part of our continuing efforts in drug development, the present work describes an attempt towards the synthesis of fluorine substituted 1,2,4-triazinones. The purpose of the work is to extend the scope of our previous studies [29, 30] and to substitute fluorine on various positions in 1,2,4-triazines in order to obtain effective anti HIV-1 and CDK2 inhibiting agents.

2. Experimental

2.1. Chemistry. Melting points were determined on an electrothermal Bibby Stuart Scientific melting point apparatus

and are uncorrected. The infrared (IR) spectra were recorded on PerkinElmer RXI FT-IR infrared spectrophotometer using the KBr pellet technique. Electronic absorption spectra were recorded in DMF on Shimadzu UV-Visible 3101 PC spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FT NMR spectrometer using tetramethylsilane as the internal standard DMSO- d_6 as a solvent (chemical shifts in δ , ppm). ^{19}F NMR spectra were determined at 84.25 MHz using hexafluorobenzene as an internal standard. Splitting patterns were designated as follows: *s*: singlet; *m*: multiplet. Mass spectra were measured on a GCMS-Q 1000 Ex spectrometer. Elemental analyses were performed on a 2400 PerkinElmer Series 2 analyzer. Follow-up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254.

The anti-HIV and anticancer activity were evaluated in DTP, DCT, National Cancer Institute, Bethesda, MD 20892, USA.

2.1.1. 6-(2-Amino-5-fluorophenyl)-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one (I). A mixture of 5-fluoroisatin (0.001 mol in warm 5% aqueous NaOH) and thiosemicarbazide (0.001 mol, in hot water 10 mL) was refluxed for 2 h. The reaction mixture was then poured onto ice and neutralized with dil. HCl. The solid thus obtained was filtered off and crystallized from ethanol to give **1** as pale yellow crystals. Yield 80%, m.p. 285–287°C. UV: λ_{max} (ϵ): 282.8 (2.06) nm. IR (ν , cm^{-1}): 3528, 3300 (NH, NH₂), 1661 (C=O), 1385 (NCSN), 1255 (C–F), 865 (aryl C–H), 658 (C–F). ^1H NMR (δ , ppm):

FIGURE 2

13.47, (s, 1H, NH), 7.52–7.42, 6.93–6.87 (3H, aromatic H), 3.2 (s, 2H, NH₂). ¹³C NMR (δ, ppm): 172.74 (C=S), 156–152 (C=O), 144.53 (C–F), 138.73 (C=N), 119.68, 117.60, 117.45, 116.50, 116.30 (aromatic carbons), 77.27–76.84, 39.91–39.07 (C–C, C–N carbons). MS (relative intensity): 238 (M + H₂O, 254, 5%), 206 (207, 36), 182 (10), 108 (1.1), 103 (100), 75 (35). Anal. calcd. C₉H₇N₄SOF (238): C, 45.38; H, 2.93; N, 23.52; S, 13.0; F, 7.98. Found: C, 44.88; H, 2.55; N, 23.41; S, 12.85; F, 7.77. ¹⁹F NMR: δ –127 ppm.

2.1.2. 2-((6-(2-((Carboxymethyl)amino)-5-fluorophenyl)-5-oxo-4,5-dihydro-1,2,4-triazin-3-yl)thio)acetic acid (**2**). A mixture of **1** (0.001 mol) and monochloroacetic acid (0.002 mol) in ethanolic NaOH (5%, 50 mL) was warmed for 30 min and then cooled and poured onto ice-cold dil. acetic acid. The product is precipitated as pale solid which was then filtered and crystallized from acetic acid to give yellow crystals of compound **2**. Yield 70%, m.p. 262–264°C. UV: λ_{max} (ε): 283.3 (1.93) nm. IR (ν, cm^{−1}): 3531 (OH), 3300 (NH), 2929, 2861 (2 CH₂), 1660 (C=O), 1499, 1439 (deformation CH₂), 1385 (NCSN), 1255 (C–F), 865 (aryl C–H), 658 (C–F). ¹H NMR (δ, ppm): 13.38, (s, 1H, OH), 11.82 (s, NH), 7.55–7.37, 6.85–6.71 (aromatic H), 3.55–3.54, 3.53–3.51 (each s, 2 CH₂). ¹³C NMR (δ, ppm): 172.67, 152.42 (2 C=O), 145 (C–F), 117.64, 117.50, 116.41, (aromatic carbons), 77.15, 76.94, 76.73 39.91–39.07 (C–S, C–O, and C–N carbons), 39.91, 39.07 (2 CH₂). MS (relative intensity): 266 (M–2 (CO₂), 100. Anal. calcd. C₁₃H₁₁N₄SOF (266): C, 44.07; H, 3.10; N, 16.0; S, 9.03; F, 5.35. Found: C, 43.78; H, 2.85; N, 15.69; S, 8.81; F, 5.21. ¹⁹F NMR: δ –130 ppm.

2.1.3. 6-(5-Fluoro-2-(methylamino)phenyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one (**3**)

Procedure A. A mixture of compound **2** (0.050 mol) and 10% K₂CO₃ (20 mL) was refluxed for 10 min and then left to acquire room temperature. The reaction mixture was now

poured in an ice-cold dil. HCl. The precipitated solid was filtered and crystallized as faint yellow crystals. Yield 50%, m.p. 163–164°C.

Procedure B. A mixture of compound **1** (0.001), methyl iodide (0.002 mol), and 1% alcoholic KOH (20 mL) was stirred at room temperature for 4 h. The reaction mixture was then neutralized with dil. HCl. The resulting light brown solid was filtered and crystallized from ethanol to form yellowish crystals of compound **3**. Yield 65%, m.p. 162–163°C. UV: λ_{max} (ε): 315.4 (1.154) nm. IR (ν, cm^{−1}): 3310 (NH), 2929, 2862 (2 CH₂), 1661 (C=O), 1499, 1438 (deformation CH₂), 1385 (NCSN), 1255 (C–F), 865 (aryl C–H), 658 (C–F). MS (relative intensity): 266 (M+H₂O, 284, 5%), 110 (5.5), 104 (100), 95 (1.18), 88 (100), 47 (65.0). Anal. calcd. C₁₁H₁₁N₄SOF (266): C, 49.95; H, 4.13; N, 21.05; S, 12.03; F, 7.14. Found: C, 49.71; H, 4.01; N, 20.99; S, 11.88; F, 6.91. ¹⁹F NMR: δ –126 ppm.

2.1.4. 2,2'-(((3,3'-((Carboxymethylene)bis(sulfanediyl))bis(5-oxo-4,5-dihydro-1,2,4-triazine-6,3-diyl))bis(4-fluoro-2,1-phenylene))bis(azanylylidene)diacetic acid (**4**). A mixture of compound **1** (0.001 mol) and 1,1-dichloroacetic acid (0.003 mol) in DMF (20 mL) was refluxed for 10 min, cooled, and then poured on ice. The solid thus separated was filtered and crystallized from THF to give deep orange crystals. Yield 66%, m.p. 280–282°C. UV: λ_{max} (ε): 266.5 (1.51) nm. IR (ν, cm^{−1}): 3534, 3480 (OH), 3350 (NH), 2929, 2861 (aliphatic C–H), 1680, 1660 (C=O), 1499, 1439 (deformation C–H), 1386 (NCSN), 1255 (C–F), 865 (aryl C–H), 658 (C–F). MS (relative intensity): 644 (M–208, 3%), 298 (100), 167 (31.01), 149 (100), 85 (5.00). Anal. calcd. C₂₄H₁₄N₈S₂O₈F₂ (266): C, 44.72; H, 2.17; N, 17.36; S, 9.9; F, 5.95. Found: C, 44.51; H, 2.11; N, 17.12; S, 9.55; F, 5.71. ¹⁹F NMR: δ –128 ppm.

2.1.5. 4-Fluoro-N-(4-fluoro-2-(5-oxo-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)phenyl)benzamide (**5**). A mixture of compound **1** (0.001 mol) and 4-fluorobenzoyl chloride

(0.001 mol) in dry pyridine (10 mL) was warmed for 10 min, cooled, and then poured on ice. The precipitated solid was filtered and crystallized from dioxane to give faint yellow crystals of **5**. Yield 85%, m.p. 238–240°C. UV: $\lambda_{\max}$ (ϵ): 288.4 (1.28) nm. IR (ν , cm^{-1}): 3370 (NH), 3320 (NH), 1663, 1610 (C=O, CONH), 1385 (NCSN), 1255 (C–F), 1190 (C–S), 864 (aryl C–H), 658 (C–F). ^1H NMR (δ , ppm): 13.75, (NH), 10.07 (NH), 8.60 (NH), 7.98–7.82, 7.53–7.41, 7.10–7.05 (aromatic H). ^{13}C NMR (δ , ppm): 173.15 (C=S), 164.97–163.30, 158.87–158.57 (C=O), 146.91 (C–F), 144.70 (C–F), 138.07 (C–N), 132.21, 130.16, 129.57, 129.51, 125.5, 124.26 (aromatic carbons), 116.63, 115.04, 114.90 (C–S, C–O, C–N) 77.50–77.07, 40.03, 39.08. MS (relative intensity): 360 (M+4, 6%), 265 (78.0), 123 (12.05), 96 (2.0), 89 (100), 76 (75.0). Anal. calcd. $\text{C}_{16}\text{H}_{10}\text{N}_4\text{SO}_2\text{F}_2$ (360): C, 53.30; H, 2.78; N, 15.50; S, 9.16; F, 10.55. Found: C, 52.88; H, 2.55; N, 15.02; S, 8.95; F, 10.35. ^{19}F NMR: δ –126 and –129 ppm.

2.1.6. N-(2-(2,4-Disubstituted-5-oxo-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)-4-fluorophenyl)-4-fluorobenzamide (6–8). A mixture of **5** (0.001 mol) methanol or morpholine or 2,2'-dipyridylamine (0.001 mol) in methanol (20 mL) and formaldehyde (0.001 mol) was refluxed for 2 h. The reaction mixture was cooled to acquire room temperature and then poured onto ice-cold water. The solid product precipitated was filtered and crystallized from methanol to form yellow-orange crystals.

6. Yield 72%, m.p. 310–312°C. IR (ν , cm^{-1}): 3527 (OH), 3100 (NH), 2935, 2890 (aliphatic C–H), 1660, 1600 (C=O, CONH), 1498, 1440 (deformation CH_2), 1386 (NCSN), 1255 (C–F), 864 (aryl C–H), 658 (C–F). ^1H NMR (δ , ppm): 12.54 (s, 1H, NH), 10.15, 8.04 (Two s, 2 OH), 7.96–7.94, 7.87–7.83, 7.64–7.63 (aromatic H), 2.49 and 2.48 (Two s, 2 CH_2). ^{13}C NMR (δ , ppm): 164.99 (C=S), 163.32, 157.02 (C=O), 135.99 (C–F), 130.39, 130.20, 129.53, 129.44, 129.36, 123.45, (aromatic carbons), 115.14, 114.99 (C–S, C–O) 77.38–76.95, 39.83, 39.29. Anal. calcd. $\text{C}_{18}\text{H}_{14}\text{N}_4\text{SO}_4\text{F}_2$ (420): C, 51.42; H, 3.36; N, 13.30; S, 7.61; F, 9.04. Found: C, 51.23; H, 3.01; N, 13.11; S, 7.51; F, 8.88. ^{19}F NMR: δ –126 and –129 ppm.

7. Yield 70%, m.p. 260–262°C. Anal. calcd. $\text{C}_{26}\text{H}_{28}\text{N}_6\text{SO}_4\text{F}_2$ (558): C, 55.90; H, 5.02; N, 15.05; S, 5.70; F, 6.81. Found: C, 55.68; H, 4.98; N, 14.79; S, 5.35; F, 6.66.

8. Yield 72%; m.p. 202–204°C. Anal. calcd. $\text{C}_{38}\text{H}_{28}\text{N}_{10}\text{SO}_2\text{F}_2$ (726): C, 62.79; H, 3.88; N, 19.28; S, 4.27; F, 5.23. Found: C, 62.33; H, 3.58; N, 19.11; S, 4.15; F, 4.89.

2.1.7. 4-Fluoro-N-(4-fluoro-2-(3-(substituted-amino)-5-oxo-4,5-dihydro-1,2,4-triazin-6-yl)phenyl)benzamide (9–12). A mixture of **5** (0.001 mol) and sulfathiazole or sulfaoxazole or 4-fluoroaniline or 2,2'-dipyridylamine (0.001 mol) in absolute ethanol (50 mL) was refluxed for 6 h. On cooling a solid is separated which is filtered and crystallized from ethanol to give yellow crystals of **9–12**.

9. Yield 85%, m.p. 172–174°C. IR (ν , cm^{-1}): 3484–3310 (NH), 1658, 1610 (C=O, CONH), 1386 (NCSN), 1255 (C–F), 864

(aryl C–H), 658 (C–F). ^1H NMR (δ , ppm): 13.72, 10.06, 8.00 (each s, 3 NH), 7.94 (s, 1H, NHSO_2), 7.89, 7.84 (each s, 2H, thiazole), 7.56, 7.49, 7.39, 7.38, 7.10, 7.09, 7.78, 7.07, 6.79, 6.78, 6.55, 6.53, 6.36 (aromatic H). ^{13}C NMR (δ , ppm): 173.18 (C– SO_2), 167.90, 157.28 (C=O), 144.81 (C–F), 132.24, 132.22 (C–N), 130.22, 130.21, 129.62, 129.56, 128.93, 127.50, 125.58, 125.53, 124.40, 123.14, 116.78, 116.73, 116.62, 116.58, 115.03, 114.88, 112.82, 106.46 (aromatic carbons), 77.62–77.19. Anal. calcd. $\text{C}_{25}\text{H}_{17}\text{N}_7\text{S}_2\text{O}_4\text{F}_2$ (581): C, 51.63; H, 2.92; N, 16.86; S, 11.01; F, 6.55. Found: C, 51.34; H, 2.85; N, 16.70; S, 10.88; F, 6.35.

10. Yield 82%, m.p. 178–180°C. Anal. calcd. $\text{C}_{25}\text{H}_{17}\text{N}_7\text{SO}_5\text{F}_2$ (565): C, 53.09; H, 3.00; N, 17.16; S, 5.66; F, 6.72. Found: C, 52.88; H, 2.89; N, 17.01; S, 5.41; F, 6.55.

11. Yield 89%, m.p. 247–249°C. UV: $\lambda_{\max}$ (ϵ): 288.7 (1.09) nm. IR (ν , cm^{-1}): 3500, 3310, 3290 (NH), 1661, 1620 (C=O), 1385 (NCSN), 1255 (C–F), 865 (aryl C–H), 658 (C–F). ^1H NMR (δ , ppm): 13.79, 11.72, 10.92 (each s, 3 NH), 7.94, 7.88, 7.86, 7.70, 7.54, 7.53, 7.47, 7.25, 7.09, 7.07, 7.06 (aromatic H). ^{13}C NMR (δ , ppm): 164.95–163.27, 158.86–157.24 (C=O), 146.34, 144.74, 138.74 (C–F), 132.22 (C–N), 129.56, 129.51, 125.48, 124.46, 116.77, 116.61, 115.02, 114.88 (aromatic carbons), 77.49–77.07. MS (relative intensity): 437 (M–45, 392, 1.0%), 166 (32.0), 148 (100), 122 (1.1), 110 (11), 95 (3.0). Anal. calcd. $\text{C}_{22}\text{H}_{14}\text{N}_5\text{S}_2\text{F}_3$ (437): C, 60.41; H, 3.20; N, 16.01; F, 13.04. Found: C, 59.59; H, 3.10; N, 15.66; F, 12.85. ^{19}F NMR: δ –127 and –129 ppm.

12. Yield 80%, m.p. 200–202°C. UV: $\lambda_{\max}$ (ϵ): 271.8 (1.51) nm. IR (ν , cm^{-1}): 3525–3100 (3 NH), 1659, 1610 (C=O, CONH), 1386 (NCSN), 1255 (C–F), 865 (aryl C–H), 658 (C–F). ^1H NMR (δ , ppm): 13.79, 11.72, 10.92 (each s, 3 NH), 9.98–9.74, 8.16–8.15, 7.96–7.95, 7.94 (4H of pyridine), 7.86–7.84, 7.59–7.55, 7.40–7.37, 7.10–7.08, 7.07–7.05, 6.82–6.81, 6.80–6.79 (aromatic H). ^{13}C NMR (δ , ppm): 164.92–163.25, 158.87–157.25 (C=O), 145.44, 144.84 (C–F), 137.71 (C–N), 132.24, 130.23, 129.61, 129.55, 125.58, 125.53, 124.45, 124.39 (aromatic carbons), 116.76, 116.69, 116.54, 115.69, 114.99 (pyridine carbons). Anal. calcd. $\text{C}_{26}\text{H}_{17}\text{N}_7\text{O}_2\text{F}_2$ (497): C, 62.75; H, 3.40; N, 19.71; F, 7.62. Found: C, 62.45; H, 3.30; N, 19.55; F, 7.43.

2.1.8. 4-Fluoro-N-(4-fluoro-2-(3-((4-fluorophenyl)amino)-5-oxo-4,5-dihydro-1,2,4-triazin-6-yl)phenyl)benzamide (II)

Procedure A. Equimolar mixture of compound **14** and 4-fluoroaniline was refluxed for 6 h then cooled. The solid thus obtained was filtered and crystallized from ethanol to give **II** as faint yellow crystals. Yield 78%, m.p. 247–249°C.

Procedure B. An equimolar mixture of compound **5** and 4-fluoroaniline in ethanol (50 mL) was refluxed for 6 h and then cooled. The resulting solid was filtered and crystallized from ethanol to give faint yellow crystals of **II**. Yield 75%, m.p. 247–249°C.

2.1.9. 2-(((5-Fluoro-2-(4-fluorobenzamido)phenyl)-5-oxo-4,5-dihydro-1,2,4-triazin-3-yl)thio)acetic acid (13). A mixture

of **5** (0.001 mol) and monochloroacetic acid (0.001 mol) in pyridine (20 mL) was refluxed for 15 min, cooled, and then poured onto ice-cold dil. HCl. The resulting solid was filtered and crystallized from ethanol to give **13** as yellow crystals. Yield 75%, m.p. 190–192°C. IR (ν , cm^{-1}): 3550–3100 (br, OH and NH), 2919, 2885 (C–H), 1658, 1610 (C=O, CONH), 1489, 1429 (deformation CH_2), 1386 (NCSN), 1255 (C–F), 865 (aryl C–H), 658 (C–F). ^1H NMR (δ , ppm): 14.45 (NH), 12.58 (NH), 10.25–10.08 (OH), 7.88–7.85, 7.84–7.83, 7.65–7.31, 7.15–7.14, 7.13–7.12, 7.10–7.07 (aromatic H), 3.96 (CH_2). ^{13}C NMR (δ , ppm): 168.74 (C=O), 164.99 (C=O), 159.27–157.65 (C=O), 132.87–132.85 (C–S), 130.20, 130.18, 129.61, 129.49, 129.43, 127.19, 126.20, 117.18, 117.01, 116.73, 116.58, 115.17, 115.03, (aromatic carbons), 77.89–77.45, 40.88, 31.84 (CH_2). MS (relative intensity): 418 (M–28, 390, 1.0%), 391 (1.5), 149 (100), 132 (2.8), 91 (1.10), 57 (47.00). Anal. calcd. $\text{C}_{18}\text{H}_{12}\text{N}_4\text{SO}_4\text{F}_2$ (418): C, 53.11; H, 2.87; N, 13.35; S, 7.65; F, 9.09. Found: C, 52.88; H, 2.58; N, 13.05; S, 7.33; F, 8.79.

2.1.10. 4-Fluoro-N-(4-fluoro-2-(3-(methylthio)-5-oxo-4,5-dihydro-1,2,4-triazin-6-yl)phenyl)benzamide (**14**)

Procedure A. A mixture of compound **13** (0.050 mol) and 10% ethanolic K_2CO_3 (10 mL) was warmed for 15 min, cooled, and then poured onto ice-cold dil. HCl. The solid product was filtered and crystallized from dioxane to give deep orange crystals of **14**. Yield 82%, m.p. 228–230°C.

Procedure B. A mixture of compound **5** (0.001 mol) and CH_3I (0.001 mol) in ethanolic KOH (1%, 20 mL) was stirred at room temperature for 12 h. The resulting reaction mixture was acidified with dil. HCl. A brownish orange precipitate was separated which on crystallization with dioxane gave **14** as orange crystals. Yield 80%, m.p. 228–229°C. Mixed m.p. 228°C. IR (ν , cm^{-1}): 3484–3250 (br, NH, NH), 2930, 2864 (C–H), 1657, 1620 (C=O, CONH), 1489, 1449 (deformation CH_2), 1386 (NCSN), 1255 (C–F), 864 (aryl C–H), 658 (C–F). ^1H NMR (δ , ppm): 12.65, 12.37–12.35 (each s, NH, NH), 7.89–7.88, 7.87, 7.40–7.25, 7.14–7.13, 7.12, 7.12–7.10, 7.08–7.04 (aromatic H). ^{13}C NMR (δ , ppm): 163.60, 159.38 (C=O), 157.79, 147.45 (C–F), 132.89 (C–S), 129.85, 129.28, 129.26, 129.22, 129.20, 127.43, 126.28, 126.25, 117.19, 117.03, 116.85, 116.82, 116.70, 116.67, 115.14, 115.12, 115.00, 114.97 (aromatic and heteroaromatic carbons), 77.21–76.78, 39.91–39.07 (CH_3). Anal. calcd. $\text{C}_{17}\text{H}_{12}\text{N}_4\text{SO}_2\text{F}_2$ (374): C, 54.50; H, 3.20; N, 14.89; S, 8.56; F, 10.17. Found: C, 53.89; H, 3.01; N, 14.55; S, 8.19; F, 10.11.

2.1.11. 6-(2-Amino-5-fluorophenyl)-3-((4-fluorophenyl)amino)-1,2,4-triazin-5(4H)-one (**15**). A mixture of **1** (0.001 mol) and 4-fluoroaniline (0.001 mol) in ethanol (50 mL) was refluxed for 6 h. On cooling a solid was precipitated which was filtered and crystallized from ethanol to form yellowish crystals of **15**. Yield 80%, m.p. 308–310°C. IR (ν , cm^{-1}): 3534, 3300 (NH_2 , NH), 1661 (C=O), 1386 (NCSN), 1255 (C–F), 864 (aryl C–H), 658 (C–F). Anal. calcd. $\text{C}_{15}\text{H}_{11}\text{N}_5\text{OF}_2$ (315): C, 58.00; H, 3.49; N, 22.22; F, 12.06. Found: C, 57.72; H, 3.15; N, 21.88; F, 11.78. ^{19}F NMR: δ –127 and –129 ppm.

2.1.12. Aroylation of **15** to Form **11**. An equimolar mixture of **15** and 4-fluorobenzoyl chloride in DMF (20 mL) was warmed for 10 min, cooled, and then poured onto ice. The resultant solid was filtered and crystallized from ethanol to give **11** as yellowish crystals. Yields 82%, m.p. 248°C. Mixed m.p. 247°C.

2.1.13. N,N' -((3,3'-Disulfanediylbis(5-oxo-4,5-dihydro-1,2,4-triazine-6,3-diyl))bis(4-fluoro-2,1-phenylene))bis(4-fluorobenzamide) (**16**). A mixture of compound **5** (0.002 mol) and sulfur (0.002 mol) flowers in dry benzene (100 mL) was refluxed for 4 h. On cooling the reaction mixture a solid appeared which was filtered and crystallized from benzene to give **16** as orange crystals. Yield 88%, m.p. 246–248°C. UV: λ_{max} (ϵ): 288.8 (1.14) nm. IR (ν , cm^{-1}): 3527–3320 (br, NH, NH), 1660, 1608 (C=O, CONH), 1386 (NCSN), 1255 (C–F), 1090 (C–S), 864 (aryl C–H), 658 (C–F). MS (relative intensity): 718 (M–359, $-\text{H}_2\text{O}$, 341, 85%), 309 (55.01), 306 (100), 290 (90.00), 209 (8.11). Anal. calcd. $\text{C}_{32}\text{H}_{18}\text{N}_8\text{S}_2\text{O}_4\text{F}_4$ (718): C, 53.48; H, 2.50; N, 15.60; S, 8.91; F, 10.58. Found: C, 53.15; H, 2.30; N, 15.31; S, 8.56; F, 10.59. ^{19}F NMR: δ –127 and –130 ppm.

2.1.14. 2,2,2-Tris((6-(5-fluoro-2-(4-fluorobenzamido)phenyl)-5-oxo-4,5-dihydro-1,2,4-triazin-3-yl)thio)acetic acid (**17**). A mixture of compounds **1** (0.001 mol) and **5** (0.001 mol) in DMF (50 mL) was refluxed for 1 h. The reaction mixture was left to cool at room temperature. The solid thus generated was filtered and crystallized from acetic acid to give **17** as yellow crystals. Yield 65%, m.p. 230–232°C. UV: λ_{max} (ϵ): 288.2 (1.23) nm. IR (ν , cm^{-1}): 3544 (OH), 3320 (NH), 2990, 2898 (C–H), 1760–1610 (C=O), 1499, 1449 (deformation CH_2), 1386 (NCSN), 1255 (C–F), 1089 (C–S), 864 (aryl C–H), 658 (C–F). ^1H NMR (δ , ppm): 13.72, 13.25, 12.61, 12.17 (4s, 4 NH), 10.09, 10.06 (2s, 2 NH), 7.98, 7.97, 7.96, 7.95, 7.87, 7.86, 7.85, 7.84, 7.83, 7.54, 7.40, 7.39, 7.38, 7.35, 7.10, 7.09–7.05 (aromatic protons), 5.49 (s, 1H, OH). ^{13}C NMR (δ , ppm): 164.98, 163.83, 163.32 (3 C=O), 148, 140 (C–F), 132.11 (C–S), 130.23, 130.19, 130.17, 129.62, 129.56, 129.47, 129.42, 125.51, 125.40 and 116.91, 116.81, 116.77, 116.74, 116.64, 116.62, 116.11, 115.12, 115.06, 114.97, 114.91, 77.59–77.16, 39.91–39.21 (aromatic, heteroaromatic and aliphatic carbons). Anal. calcd. $\text{C}_{50}\text{H}_{28}\text{N}_{12}\text{S}_3\text{O}_8\text{F}_6$ (1134): C, 52.55; H, 2.46; N, 14.81; S, 8.43; F, 10.05. Found: C, 51.98; H, 2.16; N, 14.53; S, 8.19; F, 9.85.

2.1.15. 4-Fluoro-N-(4-fluoro-2-(3-((4-fluoro-2-(5-oxo-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)phenyl)amino)-5-oxo-4,5-dihydro-1,2,4-triazin-6-yl)phenyl)benzamide (**18**). An equimolar mixture of **1** and **5** (0.001 mol) in isopropyl alcohol (50 mL) was refluxed for 6 h. Some of the solvent of reaction mixture was evaporated *in vacuo*. The product was separated as brownish orange solid which was crystallized from ethanol to form orange crystals of compound **18**. Yield 80%, m.p. 246–248°C. UV: λ_{max} (ϵ): 287.8 (2.07) nm. IR (ν , cm^{-1}): 3320, 3300 (NH, NH), 1668, 1620 (C=O, CONH), 1385 (NCSN), 1255 (C–F), 1185 (C=S), 864 (aryl C–H), 657 (C–F). MS (relative intensity): 564 (M–196, 368, 7.0%), 247 (0.01), 204 (3.0), 203 (5.0), 196 (1.0), 100 (1.1), 88 (100), 76 (66.0), 42

SCHEME 1

(0.5). Anal. calcd. $\text{C}_{25}\text{H}_{15}\text{N}_8\text{SO}_3\text{F}_3$ (564): C, 53.34; H, 5.67; N, 19.89; S, 5.67; F, 10.10. Found: C, 52.89; H, 5.55; N, 19.51; S, 5.38; F, 9.88. ^{19}F NMR: δ -126, -128 and -130 ppm.

2.2. Biological Evaluation

2.2.1. Anti-HIV-1 Activity. All the new synthesized compounds have been evaluated for their *in vitro* anti-HIV activity that was performed on T-4 lymphocytes infected and uninfected with HIV-1 using DMSO as solvent. The assay involves the killing of T-4 lymphocytes by HIV. Uninfected cells with the compound serve as a toxicity control, and infected and uninfected cells without the compound serve as basic controls. Cultures are incubated at 37°C in a 5% carbon dioxide atmosphere for 6 days. The tetrazolium salt, XTT, is added to all wells, and cultures are incubated to allow formazan color development by viable cells. Compounds that degenerate or are rapidly metabolized in the culture conditions may not show activity in this screen. Zidovudine (AZT) at $10\ \mu\text{M}$ was used as a control. The viability of the cells was determined spectrophotometrically to quantitate formazan production and in addition is viewed microscopically for detection of viable cells and confirmation of protective activity. Drug-treated virus-infected cells are compared with drug treated noninfected cells and with other appropriate controls (untreated infected and untreated noninfected cells, drug-containing wells without cells) on the same plate.

2.2.2. CDK2 Inhibition Assay. CDK2-cyclin E kinase was expressed and assayed as previously described [39]. Kinase activity was expressed as a percentage of maximum activity. The concentration of the test compounds required to decrease the CDK activity by 50% was determined from dose-response curves and designated IC_{50} .

3. Results and Discussion

3.1. Chemistry. The proposed synthetic strategies to obtain the target compounds are outlined in Schemes 1–5. The structures of target compounds have been established by physicochemical and spectroscopic techniques.

Reaction of compound 1 with monochloroacetic acid (1:2 by mole) in refluxing ethanolic sodium hydroxide afforded the α -amino acid 2. Decarboxylation of 2 by warming with aqueous potassium carbonate gave 6-[5'-fluoro-2-methylaminophenyl]-3-methylthio-1,2,4-triazin-5(4H)-one 3. Compound 3 was also obtained from methylation of 1 by stirring it with methyl iodide in aqueous potassium hydroxide for 12 h (Scheme 1).

The IR of 1 showed strong peaks at 3528 and $3300\ \text{cm}^{-1}$ for NH, NH_2 and at 1661 and $1255\ \text{cm}^{-1}$ for C=O and C–F functional groups, respectively. The mass spectrum showed a peak at m/z 254 ($M + \text{H}_2\text{O}$, 5%) with a base peak at 103. Similarly, compound 3 exhibited in its IR spectrum three peaks at 3310 , 2929 , and $2862\ \text{cm}^{-1}$ for NH and methyl groups. The mass of 3 exhibited a peak at m/z 284 ($M + \text{H}_2\text{O}$, 5%) with a base peak at 88. The ^1H NMR spectra of 3 showed two singlets at δ 3.2 and 3.5 ppm for N– CH_3 and S– CH_3 protons.

El-Gendy et al. [37], obtained 1,1-di[4'-amino-6'-substituted-5-oxo-1,2,4-triazin-3-yl]thio]acetic acid as anti-cancer agent. Similarly refluxing compound 1 with 1,1-dichloroacetic acid (2:1 by mole) in DMF produced the 1,1-di[heteroaryl]-thioacetic acid 4 through nucleophilic displacement of chlorine by thioheterocyclic moieties (Scheme 2). The UV absorption spectra of 4 recorded λ_{max} at $266.5\ \text{nm}$ while that of 1 at $282.8\ \text{nm}$.

Reaction of 1 with 4-fluorobenzoyl chloride in dry pyridine furnished 5 (Scheme 3). IR spectrum of 5 showed two carbonyl groups at 1663 and $1610\ \text{cm}^{-1}$. The ^{13}C NMR spectrum showed peaks at δ 173.15, 164.14, and $158.72\ \text{ppm}$ attributed to C=S, C=O, and CONH carbons. The mass

SCHEME 2

spectrum exhibited a peak at m/z 360 ($M + 4$, 6%) with a base peak at 89.

Compound **5** was used as starting material for building some more N^2 -substituted and/or C^3 -NH substituted 1,2,4-triazinones. Thus hydroxymethylation of **5** using methanol-formaldehyde yielded 2,4-dihydroxymethyl derivative **6** which on its treatment with secondary amine as morpholine and/or 2,2-dipyridylamine under the same reaction conditions Mannich bases of the type **7** and/or **8** were isolated (Scheme 3). IR spectrum of **6** showed absorptions at 3537, 2935, 2890, 1498, and 1440 cm^{-1} mainly attributed to O-H, C-H stretching, and CH_2 bending vibrations. The ^1H NMR spectrum of **6** showed peaks at δ 12.54, 10.15 and 8.04 ppm for NH and OH protons. The ^{13}C NMR spectrum, however, exhibited three resonance signals at δ 164.99, 163.32, and 157 ppm for C=S, C=O, and CONH groups and at δ 39.0 ppm for CH_2 carbon.

3-Substituted amino-1,2,4-triazine derivatives have shown chemotherapeutic [40] activities and have been proven as copper corrosion inhibitors [41]. Thus a simple nucleophilic displacement of SH group of 1,2,4-triazines with NH of different amines and sulfa drugs via reaction of compound **5** with sulfa drugs (sulfathiazole and sulfoxazoles), 4-fluoroaniline, and 2,2-dipyridylamine gave 3-substituted-amino-6-[5'-fluoro-2'-(4''-fluorophenylcarbamidophenyl)-1,2,4-triazin-5(4H)ones **9–12** (Scheme 3). The IR spectrum of compound **11** showed absorptions at 1255 and 658 cm^{-1} for C-F. The ^{13}C NMR spectrum showed absence of C=S carbon. The mass spectra exhibited m/z 437 ($M-45$, 392, 1.0%) with a base peak at 148 for the fragment $\text{C}_7\text{H}_3\text{N}_3\text{F}$.

Alkylation of 3-mercapto-1,2,4-triazinone **5** using monochloroacetic acid in dry pyridine produced the mercaptoacetic acid derivative **13**. Decarboxylation of **13** by warming it with aqueous K_2CO_3 afforded 6-[5'-fluoro-2'-(4''-fluorophenyl-carbamidophenyl)-3-methylthio-1,2,4-triazin-5(4H)-one **14**. Compound **14** was also obtained from

treatment of compound **5** with methyl iodide in ethanolic KOH (Scheme 4).

Structure of **13** was deduced from elemental analysis and spectral data. The IR spectrum showed peaks at 3550–3100, 2919, 2882, 1658, and 1610 cm^{-1} attributed to O-H, N-H, CH_2 , C=O, and CONH groups. In ^1H NMR spectrum signals at δ 14.45, 12.58, and 10.25 ppm for NH, NH, and OH protons. In addition to this, a peak at δ 3.96 appeared for CH_2 protons. Further support was given by its ^{13}C NMR where there are three peaks for three carbonyl groups at δ 168.74, 164.99, and 159.27. The mass spectra recorded a peak at m/z 418 ($M-28$, 390, 1.00%) with a base peak at 149 as $\text{C}_8\text{H}_4\text{NOF}$.

Full fluorine substituted aryl-1,2,4-triazinones were synthesized by refluxing **14** and/or **5** with 4-fluoroaniline in ethanol led to the formation of 4-fluoro-N-(4-fluoro-2-(3-((4-fluorophenyl)amino)-5-oxo-4,5-dihydro-1,2,4-triazin-6-yl)phenyl)benzamide one **11** (Scheme 4). However, compound **15** was also obtained by treating compound **1** with 4-fluoroaniline in boiling ethanol to give 3-(4'-fluorophenyl)-6-(5'-fluoro-2'-aminophenyl)-1,2,4-triazin-5(4H) one **15** which upon warming with 4-fluorobenzoyl chloride in DMF yielded **11** (Scheme 4). The structure of **15** was established by elemental analysis and spectral data. The IR spectra showed absorptions at 3534, 3300, 1661, and 1640 cm^{-1} for NH_2 , NH, and C=O functional groups. The spectrum did show two peaks at 1255 and 658 cm^{-1} for C-F group. The ^{13}C NMR spectrum lacks a signal for C=S carbon.

Oxidation of compound **5** by refluxing with sulfur flowers in dry benzene yielded the disulfide **16** (Scheme 5). Due to higher nucleophilicity of sulfur when compared with oxygen and nitrogen, the removal of H^+ from S-H is easily followed by removal of H^- from other molecules to form a disulfide **16** with evolution of hydrogen (Scheme 5). The IR spectrum showed an absorption band at 1090 cm^{-1} for C-S-S-C group. The mass recorded m/z 341 ($M-18$, $-\text{H}_2\text{O}$, 85%) with a base peak at m/z 306 for $\text{C}_{16}\text{H}_5\text{N}_3\text{SOF}$.

SCHEME 3

Treatment of compound **5** with 1,1,1-trichloroacetic acid in warming DMF generates a thioether **17** (Scheme 5). The IR spectrum showed absorption bands at 1255 and 1089 cm⁻¹ which are attributed to C-F and C-S of the thioether. The ¹³C NMR spectrum lacks a signal for C=S carbon. However, resonances at δ 132.11 ppm for C-S carbon in addition to a signal at δ 164.98 ppm for carbonyl carbon.

Finally synthesis of fluorine substituted 1,2,4-triazinone bearing other amino-1,2,4-triazinone **18** was done by refluxing compounds **1** and **15** in boiling isopropyl alcohol as amination reaction (Scheme 5). The IR spectrum of **18** showed bands at 3320, 3300, 1668, 1620, and 1185 cm⁻¹ for NH, NH, C=O, CONH, and C=S groups. The mass spectrum showed a peak at *m/z* 368 (M-196, 7%) along with a base peak at 89 for CH₂N₃S.

The presence of fluorine was confirmed by ¹⁹F NMR. A single fluorine atom attached to phenyl and/or benzoyl ring appeared in the region δ 120–130 ppm using hexafluorobenzene as internal standard.

The mass fragmentation patterns of some of the target molecules are mentioned in Schemes 6, 7, 8, 9, and 10.

3.2. Biological Evaluation

3.2.1. Anti-HIV-1 Activity. The present work aimed to synthesize fluorine substituted 1,2,4-triazinones as potential anti-HIV-1 compounds as nucleoside, nonnucleoside reverse transcriptase, protease, and fusion inhibitors. The newly synthesized compounds have been evaluated for their *in vitro* anti-HIV activities on T4 lymphocytes, uninfected, or

The results revealed by Table 1 suggest that compounds **5**, **11**, **14**, **16**, and **17** displayed very good anti-HIV-1 activity having a favorable selectivity index between 3 and 7.

SCHEME 6: Mass fragmentation pattern of compound 1.

SCHEME 7: Mass fragmentation pattern of compound 3.

The CC_{50} and EC_{50} data are used to calculate the selectivity index (SI) of each compound as an estimate of a therapeutic window and a mechanism to identify candidates for efficacy studies. The other compounds though are active as anti-HIV-1 agents but do not show very good selectivity index ratio. The remaining compounds exhibited an average to poor activity with selective index <3 (Table 1). In particular, a high activity level was observed for compounds **11** and **14**. A close examination of these two compounds reveals that both the structures have 4-fluoro-N-(4-fluorophenyl)benzamide part connected at position 6 in triazinone nucleus. Similarly, in compound **16** which is an oxidised dimer of the active compound **5**, the selectivity index is almost doubled. The antiviral activity diminishes if the triazinone ring experience crowding at carbon-3 in triazinone part as in case of **15** and **18**. Sulfonamide moieties, however, could not contribute towards improving the antiviral profile; rather it significantly

diminishes the selectivity index ratio as can be seen in compounds **9** and **10**.

The influence of fluorine on the acidity, hydrogen bonding, and lipophilicity of these systems can be envisaged not only a biological activity modulator but also influences the bioavailability of the drug. The active fluorine compounds obtained are capable of forming stronger DNA complexes than their nonfluorinated analogs. These results suggest that the electronic nature of the chain of 3-thioxo-1,2,4-triazines tethering an intercalator that not only influences the DNA-binding process but might also be used to tune the new DNA-drug complex. It can be implied that the fluorine substituted-3-thioxo-1,2,4-triazin-5-ones represent suitable prodrug principle leading to higher bioavailability.

The biological activity depends not only on the site of fluorination and the geometry of the conjugate carbanion

SCHEME 8: Mass fragmentation pattern of compound 5.

SCHEME 9: Mass fragmentation pattern of compound 11.

formed but also on the total electronegativity of new heterocyclic nitrogen systems. In view of these characteristics and the results obtained in Table 1 we can infer that fluorination invariably increases C–H acidity through a combination of inductive and hyperconjugative resonance stabilization of the carbanion and thus influences the biological activity of this series of compounds (Figure 3).

3.2.2. Anticancer Activity. The new fluorine substituted 1,2,4-triazinones (**1–18**) have been evaluated to inhibit activity of CDK2 in a biochemical assay. The inhibitory concentration (IC_{50}) values were obtained according to the reported methods [39, 42]. Olomoucine has been used as a standard. The results are reported in Table 2.

The results from Table 2 reveal that compounds **1**, **3**, and **14–16** show very significant CDK2 inhibitory activity.

TABLE 1: Anti-HIV-1 activities of fluorinated 1,2,4-triazinones **1–18**.

Compounds	EC ₅₀ (μM) ^a	CC ₅₀ (μM) ^b	SI ^c	log P
1	10.8 ± 1.0	25 ± 1.0	2.51	0.64
3	20 ± 1.0	49 ± 1.0	2.50	2.09
5	25 ± 1.0	75 ± 1.0	3.00	2.4
6	30 ± 1.0	60 ± 1.0	2.00	2.39
9	35 ± 1.0	75 ± 1.0	2.20	4.41
10	42 ± 1.0	82 ± 1.0	2.11	3.04
11	6.6 ± 1.0	44 ± 2.0	7.15	4.44
14	5.8 ± 1.0	35 ± 1.0	6.2	3.56
15	14.0 ± 1.0	29 ± 1.0	2.01	2.67
16	6.9 ± 1.0	36 ± 1.1	5.1	7.07
17	25 ± 1.8	76 ± 1.0	3.01	—
18	50 ± 1.1	90 ± 2.1	1.8	3.69

^aEC₅₀: inhibiting p24 production in the MT-4 cells.^bCC₅₀: concentration of compound that reduces the viability of mock-infected cells by 50%, as determined by the MTT method.^cSI: selectivity index; CC₅₀/EC₅₀.

Compound **3** was found to be as active as olomoucine, while compounds **1** and **14–16** were more potent than the standard compound. Once again the same structural features are present in active compounds as is evident in compounds active as anti-HIV-1.

4. Conclusions

According to the data obtained from the biological assay five compounds **5**, **11**, **14**, **16**, and **17** have shown very good

FIGURE 3: Hyperconjugation between atom and C-H.

anti-HIV activity in MT-2 cells. Similarly, five compounds (**1**, **3**, and **14–16**) have exhibited remarkable CDK2 inhibition activity. Compounds **14** and **16** can be considered as a significant matrix for the design and synthesis of novel candidates with dual anti-HIV and anticancer activities. Further investigation into the other aspects of structure activity relationship studies of this series of compounds is required in order to explore the scope and limitation of its biological activities.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

TABLE 2: CDK2 inhibiting activity of fluorinated 1,2,4-triazinones 1–18.

Compounds	IC ₅₀ ± SD (μM) ^a
1	3.8 ± 1.7
3	5.0 ± 1.7
5	12.8 ± 1.7
6	12.2 ± 1.7
9	14.0 ± 1.5
10	15.0 ± 2.5
11	15.0 ± 5.0
14	4.5 ± 2.8
15	4.1 ± 2.8
16	4.0 ± 2.8
17	16.2 ± 5.0
18	18.1 ± 4.0
Olomoucine	5.0 ± 1.0

^aIC₅₀ in μM/d_m⁻¹.

SD: standard deviation.

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References

- [1] E. De Clercq, "New developments in anti-HIV chemotherapy," *Biochimica et Biophysica Acta, Molecular Basis of Disease*, vol. 1587, no. 2-3, pp. 258–275, 2002.
- [2] E. De Clercq, "Antiviral drugs in current clinical use," *Journal of Clinical Virology*, vol. 30, no. 2, pp. 115–133, 2004.
- [3] C. M. Kitchen, S. G. Kitchen, J. A. Dubin, and M. S. Gottlieb, "Initial virological and immunologic response to highly active antiretroviral therapy predicts long-term clinical outcome," *Clinical Infectious Diseases*, vol. 33, no. 4, pp. 466–472, 2001.
- [4] W. M. Valenti, "HAART is cost-effective and improves outcomes," *AIDS Reader*, vol. 11, no. 5, pp. 260–262, 2001.
- [5] J. T. King Jr., A. C. Justice, M. S. Roberts, C.-C. H. Chang, and J. S. Fusco, "Long-term HIV/AIDS survival estimation in the highly active antiretroviral therapy era," *Medical Decision Making*, vol. 23, no. 1, pp. 9–20, 2003.
- [6] A. Carr, "Toxicity of antiretroviral therapy and implications for drug development," *Nature Reviews Drug Discovery*, vol. 2, no. 8, pp. 624–634, 2003.
- [7] J. Martinez-Picado, M. P. DePasquale, N. Kartsonis et al., "Antiretroviral resistance during successful therapy of HIV type 1 infection," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 20, pp. 10948–10953, 2000.
- [8] S. J. Little, S. Holte, J.-P. Routy et al., "Antiretroviral-drug resistance among patients recently infected with HIV," *The New England Journal of Medicine*, vol. 347, no. 6, pp. 385–394, 2002.
- [9] E. Pomarnacka and A. Kornicka, "Synthesis and in vitro anticancer and anti-HIV evaluation of new 2-mercaptobenzenesulfonamides," *Farmaco*, vol. 56, no. 8, pp. 571–577, 2001.
- [10] D. A. Karnofsky, "Mechanism of action of anticancer drugs at a cellular level," *Ca-A Cancer Journal for Clinicians*, vol. 18, no. 4, pp. 232–234, 1968.
- [11] S. T. M. Makki, D. A. Bakhotmah, R. M. Abdel-Rahman, and M. S. El-Shahawy, "Designing and synthesis of new fluorine substituted pyrimidine-thion-5-carbonitriles and the related derivatives as photochemical probe agents for inhibition of vitiligo disease," *International Journal of Organic Chemistry*, vol. 2, no. 3, pp. 311–320, 2012.
- [12] R. M. Abdel-Rahman, M. S. I. T. Makki, and W. A. Bawazir, "Synthesis of some more fluorine heterocyclic nitrogen systems derived from sulfa drugs as photochemical probe agents for inhibition of vitiligo disease-part I," *E-Journal of Chemistry*, vol. 8, no. 1, pp. 405–414, 2011.
- [13] R. M. Abdel-Rahman, M. S. I. T. Makki, and W. A. B. Bawazir, "Synthesis of fluorine heterocyclic nitrogen systems derived from sulfa drugs as photochemical probe agents for inhibition of vitiligo disease-Part II," *E-Journal of Chemistry*, vol. 7, no. 1, pp. S93–S102, 2010.
- [14] D.-J. Wang, L. Fan, C.-Y. Zheng, and Z.-D. Fang, "Synthesis and anti-microbial activity of some new fluorinated 1H-pyrazoles," *Journal of Fluorine Chemistry*, vol. 131, no. 5, pp. 584–586, 2010.
- [15] R. Filler and R. Saha, "Fluorine in medicinal chemistry: a century of progress and a 60-year retrospective of selected highlights," *Future Medicinal Chemistry*, vol. 1, no. 5, pp. 777–791, 2009.
- [16] G. Sandford, "Elemental fluorine in organic chemistry (1997–2006)," *Journal of Fluorine Chemistry*, vol. 128, no. 2, pp. 90–104, 2007.
- [17] C. Isanbor and D. O'Hagan, "Fluorine in medicinal chemistry: a review of anti-cancer agents," *Journal of Fluorine Chemistry*, vol. 127, no. 3, pp. 303–319, 2006.
- [18] B. E. Smart, "Fluorine substituent effects (on bioactivity)," *Journal of Fluorine Chemistry*, vol. 109, no. 1, pp. 3–11, 2001.
- [19] S. Liu, X. Qian, G. Song, J. Chen, and W. Chen, "Fluorine containing heterocyclic compounds: Synthesis of 6-substituted-2-substituted-aryl-1,2,4-triazolo[5,1-b] 1,3,5-thiadiazin-7-one derivatives," *Journal of Fluorine Chemistry*, vol. 105, no. 1, pp. 111–115, 2000.
- [20] R. M. Abdel-Rahman, "Synthesis and chemistry of fluorine containing bioactive 1,2,4-triazines—an overview: chemistry of uncondensed 1,2,4-triazines, part III," *Pharmazie*, vol. 54, no. 11, pp. 791–803, 1999.
- [21] T. Ramadan, R. M. Abdel-Rahman, and M. Seada, "Studies on complexes of Cu(II), Ni(II), Co(II) and Ln(III) with 3-(α-benzoyl)benzylidene hydrazino-5,6-diphenyl-1,2,4-triazine," *Asian Journal of Chemistry*, vol. 4, pp. 569–575, 1992.
- [22] A. A. T. Ramadan, R. M. Abdel-Rahman, M. A. El-Behairy, A. I. Ismail, and M. M. Mahmoud, "The thermodynamics of complexation of transition and lanthanide ions by 3-(α-carboxymethylaminobenzylidenehydrazino)-5,6-diphenyl-1,2,4 triazine (HipHt)," *Thermochimica Acta*, vol. 222, no. 2, pp. 291–303, 1993.
- [23] F. M. Foss, "Nucleoside analogs and antimetabolite therapies for myelodysplastic syndrome," *Best Practice and Research: Clinical Haematology*, vol. 17, no. 4, pp. 573–584, 2004.
- [24] G. S. Cockerill and K. E. Lackey, "Small molecule inhibitors of the class 1 receptor tyrosine kinase family," *Current topics in medicinal chemistry*, vol. 2, no. 9, pp. 1001–1010, 2002.
- [25] E. K. Rowinsky, "Signal Events: cell Signal Transduction and Its Inhibition in Cancer," *Oncologist*, vol. 8, no. 3, pp. 5–17, 2003.

- [26] V. Aparna, G. Rambabu, S. K. Panigrahi, J. A. R. P. Sarma, and G. R. Desiraju, "Virtual screening of 4-anilinoquinazoline analogues as EGFR kinase inhibitors: importance of hydrogen bonds in the evaluation of poses and scoring functions," *Journal of Chemical Information and Modeling*, vol. 45, no. 3, pp. 725–738, 2005.
- [27] S. Emanuel, R. H. Gruninger, A. Fuentes-Pesquera et al., "A vascular endothelial growth factor receptor-2 kinase inhibitor potentiates the activity of the conventional chemotherapeutic agents paclitaxel and doxorubicin in tumor xenograft models," *Molecular Pharmacology*, vol. 66, no. 3, pp. 635–647, 2004.
- [28] R. M. Abdel-Rahman, "Role of uncondensed 1,2,4-triazine compounds and related heterobicyclic systems as therapeutic agents—a review," *Pharmazie*, vol. 56, no. 1, pp. 18–22, 2001.
- [29] R. M. Abdel-Rahman, "Synthesis and anti human immune virus activity of some new fluorine containing substituted-3-thioxo-1,2,4-triazin-5-ones," *Farmaco*, vol. 46, no. 2, pp. 379–389, 1991.
- [30] R. M. Abdel-Rahman, "Synthesis of some new fluorine bearing trisubstituted 3-thioxo-1,2,4-triazin-5-ones as potential anticancer agents," *Farmaco*, vol. 47, no. 3, pp. 319–326, 1992.
- [31] R. M. Abdel-Rahman, M. Seada, M. Fawzy, and I. El-Baz, "Synthesis of some new thioethers of 1,2,4-triazine-3-hydrazones and assays for their anticancer and anti human immune virus activities," *Farmaco*, vol. 48, no. 3, pp. 397–406, 1993.
- [32] R. M. Abdel-Rahman, M. Seada, M. Fawzy, and I. El-Baz, "Synthesis of some new 1,6-dihydro-3-substituted 6-spiro-(9'-fluorene)-1,2,4-triazin-5-(4H)-ones as potential anti HIV and anticancer drugs," *Pharmazie*, vol. 49, no. 10, pp. 729–733, 1994.
- [33] R. M. Abdel-Rahman, M. Seada, M. Fawzy, and I. El-Baz, "Synthesis of some new heterobicyclic compounds containing spiro-1,2,4-triazine moiety as potential anti-HIV and anticancer agents," *Pharmazie*, vol. 49, no. 11, pp. 811–814, 1994.
- [34] A. M. Abdel-Halim, Z. El-Gendy, and R. M. Abdel-Rahman, "Synthesis and biological activity of 1,2,4-triazinotriazinone derivatives," *Pharmazie*, vol. 50, no. 11, pp. 726–729, 1995.
- [35] R. M. Abdel-Rahman, J. M. Morsy, F. Hanafy, and H. A. Amene, "Synthesis of heterobicyclic nitrogen systems bearing the 1,2,4-triazine moiety as anti-HIV and anticancer drugs: part I," *Pharmazie*, vol. 54, no. 5, pp. 347–351, 1999.
- [36] R. M. Abdel-Rahman, J. M. Morsy, S. El-Edfawy, and H. A. Amine, "Synthesis of some new heterobicyclic nitrogen systems bearing the 1,2,4-triazine moiety as anti-HIV and anti-cancer drugs, part II," *Pharmazie*, vol. 54, no. 9, pp. 667–671, 1999.
- [37] Z. El-Gendy, J. M. Morsy, H. A. Allimony, W. R. Abdel-Monem Ali, and R. M. Abdel-Rahman, "Synthesis of heterobicyclic nitrogen systems bearing the 1,2,4-triazine moiety as anti-HIV and anticancer drugs, part III," *Pharmazie*, vol. 56, no. 5, pp. 376–383, 2001.
- [38] Z. El-Gendy, J. M. Morsy, H. A. Allimony, W. R. Abdel-Monem, and R. M. Abdel-Rahman, "Synthesis of heterobicyclic nitrogen systems bearing a 1,2,4-triazine moiety as anticancer drugs: part IV," *Phosphorus, Sulfur and Silicon and the Related Elements*, vol. 178, no. 9, pp. 2055–2071, 2003.
- [39] V. Kryštof, P. Cankař, I. Fryšová et al., "4-Arylazo-3,5-diamino-1H-pyrazole CDK inhibitors: SAR study, crystal structure in complex with CDK2, selectivity, and cellular effects," *Journal of Medicinal Chemistry*, vol. 49, no. 22, pp. 6500–6509, 2006.
- [40] R. M. Abdel-Rahman, "Chemistry of uncondensed 1,2,4-triazines, part iv synthesis and chemistry of bioactive 3-amino-1,2,4-triazines and related compounds—an overview," *Pharmazie*, vol. 56, no. 4, pp. 275–286, 2001.
- [41] K. F. Khaled, "Adsorption and inhibitive properties of a new synthesized guanidine derivative on corrosion of copper in 0.5 M H₂SO₄," *Applied Surface Science*, vol. 255, no. 5, pp. 1811–1818, 2008.
- [42] T. Gucký, E. Řezníčková, P. Džubák, M. Hajdúch, and V. Kryštof, "Synthesis and anticancer activity of some 1,5-diaryl-3-(3,4,5-Džubák trihydroxyphenyl)-1H-pyrazolo[4,3-e][1,2,4]triazines," *Monatshefte für Chemie*, vol. 141, no. 6, pp. 709–714, 2010.