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Design, synthesis and molecular docking of novel pyrazolo[1,5-*a*][1,3,5]triazine derivatives as CDK2 inhibitors

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ABSTRACT

Cyclin Dependent Kinases CDKs unpredictable activity has been accounted for a wide assortment of human malignancies, so it might be conceivable to design pharmacologically relevant ligands that go about as specific and potent inhibitors of CDK2 action. In this respect, a series of novel pyrazolo[1,5-*a*][1,3,5]triazine derivatives were designed, synthesized and evaluated for CDK2 enzyme inhibitory and anticancer activity. Compounds **9f** and **10c** showed best CDK2 inhibition among the newly synthesized compounds, with percent inhibition at 82.38%, and 81.96% against CDK2 and IC_{50} of 1.85 and 2.09 µM, respectively. Additionally, the newly synthesized compounds were tested for their antiproliferative activity against 60 NCI cell lines. Molecular docking revealed the binding mode of these new compounds into the roscovitine binding site of CDK2 enzyme (PDB code: 3ddq). Conclusively, pyrazolotriazine derivatives represent a talented starting point for further study as anticancer drug.

1. Introduction

It is winding up obvious that many sorts of tumors are the consequence of irregular signal-transducing proteins that prompt a persistent generation of the signal for cell division [1]. Protein Kinases are key controllers of cell work, they constitute one of the biggest and most practically differing gene families. They oversee the catalysis of protein phosphorylation through the exchange of phosphate of ATP to certain residues in the amino acid side chain as indicated by the kind of demonstrated kinase [2]. CDKs are individuals from serine/threonine kinase family and key compounds in cell cycle progression and transcription [3] and other major natural procedures including neural differentiation [4] and metabolism [5]. Binding of a cyclin with associated kinase to form CDK/cyclin complexes, activates the enzyme and serves to move the cell from one phase to another e.g. CDK2-cyclin A complex controls the G1 to S-stage checkpoint [6]. So CDK/Cyclin complexes are considered an interesting target for therapeutics designed to recuperate control of the cell cycle in abnormally dividing cells. CDK2 hyper activation in human tumors is frequently connected with intensification or potentially overexpression of its accomplice cyclins A and E in a wide assortment of human cancers, yet in particular in breast cancer, ovarian and endometrial carcinomas, lung, thyroid carcinoma, melanoma and osteosarcoma [7,8,9,10] and clinically, it regularly presents a poor prognosis.

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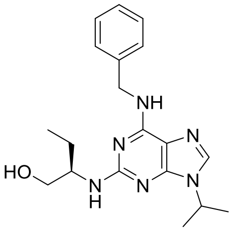
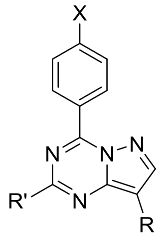
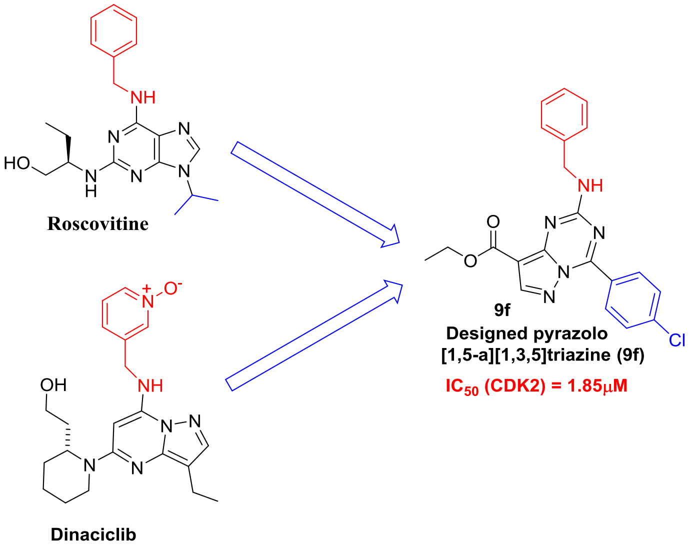


Roscovitine (I)



pyrazolo[1,5-a][1,3,5]triazine derivatives (II)

The strong hereditary connection amongst CDK2 and the molecular pathology of the disease has provided the reason for developing small-molecule inhibitors of these kinases. Knowledge of the structure of CDK2 has been a key in driving the outline and in improvement of an expansive number of ATP-competitive inhibitors [11]. Crystallography has uncovered that the ATP-binding site of CDK2 can suit various assorted of chemical structures, exploiting different sites of interaction. In addition, residues outside the principle ATP-binding cleft have been recognized that could be targeted to expand specificity and potency [12,13]. The most recent decades, purines heterocycles attracted the researchers and turned into the most utilized scaffolds for the advancement of CDK2 inhibitor that prompts the revelation of many molecules that act as CDK2 inhibitors and potent anticancer [14]. Roscovitine **(I)** was among the first CDK2 inhibitors that entered clinical trials. It induces apoptosis from all phases of the cell cycle in cancer cell line. It inhibits CDKs with various IC_{50} values: CDK1 (2.7 µM), CDK2 (0.1 µM), CDK7 (0.5 µM), CDK9 (0.8 µM) [15,16]. Due to the positive feedback of roscovitine and dinaciclib as a potent cytotoxic agents [17], several bioisosteres were synthesized mainly focusing on redistribution of purine heteroatom in order to have similarity with roscovitine in high selectivity and low toxicity and to overcome the disadvantage of its short half-life (i.e. 2–5 h) in human [18]. Thus, the above–mentioned data guided us to synthesize some novel pyrazolo [1,5-*a*][1,3,5]triazine derivatives **(II)** as purine bioisosteres and investigate their inhibitory activity against CDK2 enzyme and anticancer activity.



2. Materials and methods

2.1. Chemistry

2.1.1. General

Starting materials and reagents were purchased from Sigma-Aldrich (USA) or Alfa-Aesar Organics and used without further purification. Melting points were recorded on Stuart Scientific apparatus and were uncorrected. The purities of compounds were monitored by analytical TLC, performed on silica gel 60 F254 packed on Aluminium sheets, purchased from Merck, with visualization under U.V. light (254 nm). ^{1}HNMR and ^{13}CNMR spectra were recorded in δ scale given in ppm (on a Bruker 400 MHz for ^{1}H and 101 MHz for ^{13}C spectrophotometer) and referred to TMS as internal reference at Center of Drug Discovery and Research Development, Ain Shams University. FT-IR spectra were recorded on a Thermo Scientific Nicolet iS10 spectrometer at Ain Shams University. EI-MS spectra and Elemental analyses were performed at the Regional Center for Mycology and Biotechnology, Al-Azhar University.

2.1.2. Synthesis

Compounds **1a-c, 2, 3a, 5** and **6a** were prepared according to the previously reported procedure [19–23].

2.1.2.1. General procedure for preparation of compounds ***(3b and 3c)***. To the respective isothiocyanates **(1b and 1c)** (1 equiv., 10 mmol), 5-amino-1H-pyrazole-4-carbonitrile **(2)** (1 equiv., 10 mmol, 1.08 g) in (10 ml) anhydrous acetone was added, and the reaction mixture was heated under reflux for 3 h to give the thiourea derivatives **(3b and c)**. Ice water was added to the reaction mixture and the resulting solid was collected by filtration, washed with cold water and ethanol, and finally dried at room temperature.

2.1.2.2. 1-(4-Chlorobenzoyl)-3-(4-cyano-1H-pyrazol-5-yl)thiourea ***(3b)***. The titled compound was separated as white solid, crystallization solvent was ethanol (4.50 g, 79.55%); m.p. 166–168 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ14.02 (s,1H,NH D_2O exchangeable), 9.18 (s, 1H,NH D_2O exchangeable), 7.73 (d, J = 8.5 Hz, 2H, Ar-H), 7.63 (s, 1H,pyrazole-H), 7.61 (d, J = 8.5 Hz, 2H,Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.82, 166.70, 158.40, 139.45, 137.60, 135.22, 128.80, 127.14, 113.90, 84.60. FT-IR (ύ max, cm^{-1}): 3250–3120 (NH), 2232 (C≡N nitrile), 1680 (C=O amide). Anal. Calcd for $C_{12}H_8ClN_5OS$: C, 47.14; H, 2.64; N, 22.91; Found: C, 47.24; H, 2.70; N, 22.97.

2.1.2.3. 1-(4-Bromobenzoyl)-3-(4-cyano-1H-pyrazol-5-yl)thiourea ***(3c)***. The titled compound was separated as pale brown solid, reaction time 3 h, crystallization solvent was ethanol (5.20 g, 80.26%); m.p. 156–158 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.04 (s, 1H,NH D_2O exchangeable), 9.28 (s, 1H,NH D_2O exchangeable), 7.78 (d, J = 8.4 Hz, 2H, Ar-H), 7.67 (s, 1H,pyrazole-H), 7.63 (d, J = 8.4 Hz, 2H, Ar-H).^{13}C NMR (101 MHz, $CDCl_3$) δ 179.72, 166.65, 158.45, 139.32, 136.21, 135.26, 129.15, 126.95, 113.75, 85.22. FT-IR (ύ max, cm^{-1}): 3223–3194 (NH), 2225 (C≡N nitrile), 1683 (C=O amide). Anal. Calcd for $C_{12}H_8BrN_5OS$: C, 41.16; H, 2.30; N, 20.0; Found: C, 41.35; H, 2.35; N, 20.15.

2.1.2.4. General procedure for preparation of compounds ***(4a-c)***. A slight excess of sodium hydride (60% suspension in oil, 1 equiv., 0.005 mmol, 0.119 g) was added to a solution of 1-(4-un/substitutedbenzoyl)-3-(4-cyano-1H- pyrazol-5-yl)thiourea **(3a-c)** (1 equiv., 0.005 mmol) in DMF (5 ml). The mixture was stirred for 10 min at room temperature and then ethylbromide (1 equiv., 0.005 mmol) was added drop wise to the mixture. The reaction mixture was stirred for further 30–45 min and monitored using TLC (Hexane/ethyl acetate 4:1) to yield the carbamimidothioate derivatives which were not isolated. The reaction mixture was then heated under reflux for 45–60 min and then diluted with cold water. The formed precipitate was collected by filtration and purified by column chromatography on silica gel (200–400 mesh), using a mixture of hexane/ethyl acetate (4:1) as eluent, to give the titled products **(4a-c).**

*2.1.2.5. 2-(Ethylthio)-4-phenylpyrazolo[1,5-a][1,3,5]triazine-8-carbonitrile **(4a)**.* The product was separated as pale orange crystals, reaction time 45 min, (1.01 g, 71%) m.p.: 160–162 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.67 (d, J = 7.2 Hz, 2H, Ar-H), 8.35 (s, 1H, Pyrazole- H), 7.72–7.59 (m, 3H, Ar-H), 3.32 (q, J = 7.4 Hz, 2H, SCH$_2$), 1.51 (t, J = 7.4 Hz, 3H, CH$_3$). ^{13}C NMR (101 MHz, CDCl$_3$) δ 171.80, 153.32, 152.62, 147.90, 133.06, 132.07, 129.75, 127.39, 112.05, 81.20, 26.09, 13.87. FT-IR (ύ max, cm^{-1}): 3119 (CH aromatic), 2854 (CH aliphatic), 2210 (C≡N nitrile). MS: (Mwt: 281.07) *m/z*, 281 [M^{+}, (34.7%)]. Anal. Calcd for $C_{14}H_{11}N_5S$: C, 59.77; H, 3.94; N, 24.89; Found: C, 59.87; H, 3.94; N, 24.99.

*2.1.2.6. 4-(4-Chlorophenyl)-2-(ethylthio)pyrazolo[1,5-a][1,3,5]triazine-8-carbonitrile **(4b)**.* The product was separated as pale orange crystals, reaction time 60 min, (1.13 g, 71.57%) m.p.: 185–188 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.65 (d, J = 8.4 Hz, 2H, Ar-H), 8.35 (s, 1H, Pyrazole-H), 7.76 (d, J = 8.4 Hz, 2H, Ar-H), 3.34 (q, J = 7.2 Hz, 2H, CH$_2$), 1.52 (t, J = 7.2 Hz, 3H, CH$_3$). ^{13}C NMR (101 MHz, CDCl$_3$) δ 171.80, 153.22, 152.61, 147.90, 133.05, 132.07, 129.38, 127.39, 112.06, 81.22, 26.08, 13.85. FT-IR (ύ max, cm-1): 3110 (CH aromatic), 2964 (CH aliphatic), 2232 (C≡N nitrile). MS: (Mwt: 315.03) *m/z*, 317 [M^{+} + 2, (16.3%)], 315 [M^{+}, (38.2%)]. Anal. Calcd for $C_{14}H_{10}ClN_5S$: C, 53.25; H, 3.19; N, 22.18; Found: C, 53.35; H, 3.19; N, 22.28.

*2.1.2.7. 4-(4-Bromophenyl)-2-(ethylthio)pyrazolo[1,5-a][1,3,5]triazine-8-carbonitrile **(4c)**.* The product was separated as pale orange crystals, reaction time 60 min, (1.25 g, 69.44%) m.p.: 178–180 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.63 (d, J = 8.6 Hz, 2H, Ar-H), 8.35 (s, 1H, Pyrazole-H), 7.74 (d, J = 8.6 Hz, 2H, Ar-H), 3.31 (q, J = 7.4 Hz, 2H, CH$_2$), 1.51 (t, J = 7.4 Hz, 3H, CH$_3$). ^{13}C NMR (101 MHz, CDCl$_3$) δ 171.80, 153.32, 152.62, 147.90, 133.06, 132.07, 129.75, 127.39, 112.05, 81.20, 26.09, 13.87. FT-IR (ύ max, cm^{-1}): 3115 (CH aromatic), 2874 (CH aliphatic), 2239 (C≡N nitrile). MS: (Mwt: 360.98) *m/z*, 362 [M^{+} + 2, (47.2%)], 360 [M^{+}, (52.8%)]. Anal. Calcd for $C_{14}H_{10}BrN_5S$: C, 46.68; H, 2.80; N, 19.44; Found: C, 46.78; H, 2.74; N, 19.67.

*2.1.2.8. General procedure for the preparation of **6b and 6c**.* To a stirred solution of ethyl 5-amino-1H-pyrazole-4-carboxylate (**5**) (1 equiv., 10 mmol, 1.55 g) in acetonitrile (15 ml), the solution of the freshly prepared proper substituted benzoyl isothiocyanate (**1a-c**) (1 equiv., 10 mmol) in acetonitrile (15 ml) was added dropwise. The reaction mixture was then heated under reflux for 1.5–2 h. The mixture was allowed to cool to room temperature. Ice water was added to the reaction mixture and the resulting solid was collected by filtration, washed with cold water and ethanol, recrystallized from ethanol to afford the titled compounds (**6b and c**).

*2.1.2.9. Ethyl 5-(3-(4-chlorobenzoyl)thioureido)-1H-pyrazole-4-carboxylate **(6b)**.* The titled compound was separated as white crystals (2.8 g, 79.37%); m.p. 199–201 ^{0}C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 14.02 (s, 1H, NH D$_2$O exchangeable), 9.18 (s, 1H, NH D$_2$O exchangeable), 7.73 (d, J = 8.6 Hz, 2H, Ar H), 7.63 (s, 1H, pyrazole H), 7.61 (d, J = 8.5 Hz, 2H, Ar H), 4.43 (q, J = 7.1 Hz, 2H, O-CH$_2$CH$_3$), 1.39 (t, J = 7.1 Hz, 3H, CH$_2$CH$_3$); FT-IR (ύ max, cm^{-1}): 3289–3120 (NH), 3110 (CH aromatic), 2984 (CH aliphatic), 1748 (C=O ester), 1672 (C=O amide).

*2.1.2.10. Ethyl 5-(3-(4-bromobenzoyl)thioureido)-1H-pyrazole-4-carboxylate **(6c)**.* The titled compound was separated as faint yellow crystals (3.1 g, 78.04%); m.p. 203–205 ^{0}C**;** ^{1}H NMR (400 MHz, CDCl$_3$) δ 14.04 (s, 1H, NH D$_2$O exchangeable), 9.23 (s, 1H, NH D$_2$O exchangeable), 7.78 (d, J = 8.6 Hz, 2H, Ar H), 7.66 (s, 1H, pyrazole H), 7.62 (d, J = 8.5 Hz, 2H, Ar H), 4.37 (q, J = 7.1 Hz, 2H, O-CH$_2$ CH$_3$), 1.33 (t, J = 7.1 Hz, 3H, CH$_2$CH$_3$); FT-IR (ύ max, cm^{-1}): 3338–3100 (NH), 3051 (CH aromatic), 2981 (CH aliphatic), 1745 (C=O ester), 1667 (C=O amide).

*2.1.2.11. General procedure for the preparation of **7a-c**.* To a solution of the ethyl 5-(3-(4-substituted) benzoylthioureido)-1H-pyrazole-4-carboxylate (**6a-c**) (1 equiv., 3.5 mmol) in DMF (5 ml), sodium hydride (60% suspension in oil,1 equiv., 3.5 mmol, 84 mg) was added. The mixture was stirred for 10 min at room temperature and then a solution of ethyl bromide (1 equiv., 3.5 mmol, 0.26 ml) was added dropwise and the stirring was continued for a further 30 min to afford intermediate carbamimidothioate derivatives which were not isolated (checked by TLC). After that, the reaction mixture was heated under reflux for 45–60 min. till completion of reaction (checked by TLC). Then the reaction mixture diluted with cold water, the precipitate solid was collected by filtration, washed with water and ethanol. The product was purified by flash column chromatography on silica gel, using a mixture of hexane:ethyl acetate (7:3) as eluent to afford the titled compounds **(7a-c).**

*2.1.2.12. Ethyl 2-(ethylthio)-4-phenylpyrazolo[1,5-a][1,3,5]triazine-8-carboxylate **(7a)**.* The titled compound was separated as yellow powder (0.794 mg, 69%); m.p. 121–123 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.59 (d, J = 7.6 Hz, 2H, Ar H), 8.47 (s, 1H, pyrazole H), 7.62–7.50 (m, 3H, Ar H), 4.34 (q, J = 7.2 Hz, 2H, O-CH$_2$), 3.25 (q, J = 7.3 Hz, 2H, S-CH$_2$), 1.45 (t, J = 7.2 Hz, 3H, CH$_3$), 1.36 (t, J = 7.3 Hz, 3H, CH3); ^{13}C NMR (101 MHz, CDCl$_3$) δ 171.32, 163.45, 153.85, 152.29, 147.95, 133.92, 132.23, 129.52, 127.42, 99.67, 60.65, 26.72, 14.76, 13.92; FT-IR (ύ max, cm^{-1}): 3127 (CH aromatic), 2926 (CH aliphatic), 1750 (C=O ester); MS: (Mwt.: 328.39): *m/z* (% rel. Int.), 328.21 [M^{+}, (28.4%)], 91.06 (100%); Anal. Calcd for $C_{16}H_{16}N_4O_2S$: C, 58.52; H, 4.91; N, 17.06; Found: C, 58.72; H, 4.88; N, 17.25.

*2.1.2.13. Ethyl 4-(4-chlorophenyl)-2-(ethylthio)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate **(7b)**.* The titled compound was separated as yellow powder (0.827 g, 65%); m.p. 122–124 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.73 (d, J = 8.7 Hz, 2H, Ar H), 8.55 (s, 1H, pyrazole H), 7.58 (d, J = 8.7 Hz, 2H, Ar H), 4.43 (q, J = 7.1 Hz, 2H, O-CH$_2$), 3.34 (q, J = 7.4 Hz, 2H, S-CH$_2$), 1.53 (t, J = 7.4 Hz, 3H, CH$_3$), 1.45 (t, J = 7.1 Hz, 3H, CH$_3$).; ^{13}C NMR (101 MHz, CDCl$_3$) δ 171.37, 163.47, 153.75, 152.27, 147.89, 133.65, 132.64, 129.65, 127.39, 99.85, 60.53, 26.76, 14.69, 13.87; FT-IR (ύ max, cm^{-1}): 3115 (CH aromatic), 2929 (CH aliphatic), 1745 (C=O ester); MS: (Mwt.: 362.83): *m/z* (% rel. Int.), 364.74 [M^{+} + 2, (13.8%)], 362.43 [M^{+}, (37.2%)], 147.14 (100%); Anal. Calcd for $C_{16}H_{15}ClN_4O_2S$: C, 52.96; H, 4.17; N, 15.44; S, 8.84; Found: C, 52.80; H, 4.35; N, 15.61; S, 8.52.

*2.1.2.14. Ethyl 4-(4-bromophenyl)-2-(ethylthio)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate **(7c)**.* The titled compound was separated as yellow powder (0.87 g, 61%); m.p. 124–126 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.62 (d, J = 8.4 Hz, 2H, Ar H), 8.55 (s, 1H, pyrazole H), 7.74 (d, J = 8.4 Hz, 2H, Ar H), 4.43 (q, J = 7.1 Hz, 2H, O-CH$_2$), 3.33 (q, J = 7.4 Hz, 2H, S-CH$_2$), 1.53 (t, J = 7.4 Hz, 3H, CH$_3$), 1.45 (t, J = 7.1 Hz, 3H, CH$_3$).^{13}C NMR (101 MHz, CDCl$_3$) δ 171.39, 163.42, 153.78, 152.32, 147.92, 133.77, 132.42, 129.57, 127.46, 99.92, 60.59, 26.78, 14.70, 13.95; FT-IR (ύ max, cm^{-1}): 3130 (CH aromatic), 2935 (CH aliphatic), 1750 (C=O ester); MS: (Mwt.: 407.28): *m/z* (% rel. Int.), 409.08 [M^{+} + 2, (36.8%)], 407.06 [M^{+}, (37.2%)], 104.11

(100%); Anal. Calcd for $C_{16}H_{15}BrN_4O_2S$: C, 47.18; H, 3.71; N, 13.76; S, 7.87; Found: C, 47.35; H, 3.60; N, 13.86; S, 7.52.

*2.1.2.15. General procedure for preparation of compounds **(8a-p)**.* A mixture of the appropriate derivative of compounds **(4a-c)** (1 equiv., 2 mmol) and the selected amine (R) (1 equiv., 2 mmol) were fused in an oil bath at 150 °C for 1–1.5 h. The reaction mixture was then triturated with diethyl ether, and the formed precipitate was collected by filtration and purified by column chromatography on slica gel (200–400 mesh), using a mixture of hexane: ethyl acetate (4:1) as an eluent, to give the target compounds **(8a-p).**

*2.1.2.16. 2-(Benzylamino)-4-phenylpyrazolo[1,5-a][1,3,5]triazine-8-carbonitrile **(8a)**.* The product was separated as light yellow crystals, reaction time 1 h, (0.33 g, 50.61%) m.p.: 148–150 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.27 (s,1H,Pyrazole-H), 7.81–7.43 (m, 5H, Ar-H), 7.36–7.28 (m, 5H, Ar-H), 6.66 (s,1H,NH D_2O exchangeable), 4.65 (s, 2H,CH_2).^{13}C NMR (101 MHz, $CDCl_3$) δ 156.64, 156.20, 154.30, 147.75, 133.71, 131.10, 129.29, 128.85, 128.33, 127.59, 126.23, 126.52, 113.90, 101.95, 46.15. FT-IR (ὐ max, cm^{-1}): 3320 (NH), 3115 (CH aromatic), 2947 (CH aliphatic), 2225 ($C\equiv N$ nitrile). MS: (Mwt: 326.13): *m/z* (% rel. Int.), 326 [M^+, (38.9%)]; Anal. Calcd for $C_{19}H_{14}N_6$: C, 69.92; H, 4.32; N, 25.75; Found: C, 69.98; H, 4.30; N, 25.85.

*2.1.2.17. 2-(Phenethylamino)-4-phenylpyrazolo[1,5-a][1,3,5]triazine-8-carbonitrile **(8b)**.* The product was separated as pale yellow powder, reaction time 1 h, (0.35 g, 51.47%) m.p.: 138–140 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.46 (d, $J = 7.6$ Hz, 2H, Ar- H), 8.15 (s, 1H, Pyrazole-H), 7.66–7.57 (m, 3H, Ar-H), 7.34–7.21 (m, 5H, Ar-H), 5.88 (s, 1H, NH D_2O exchangeable), 3.86 (t, $J = 6.5$ Hz, 2H, $NHCH_2$), 3.02 (t, $J = 6.5$ Hz, 2H, CH_2Ar).^{13}C NMR (101 MHz, $CDCl_3$) δ 157.46, 155.02, 154.83, 147.84, 140.17, 138.01, 130.92, 129.45, 128.75, 128.66, 127.33, 126.18, 113.50, 100.95, 42.32, 34.85. FT-IR (ὐ max, cm^{-1}): 3340 (NH), 3095 (CH aromatic), 2935 (CH aliphatic), 2232 ($C\equiv N$ nitrile). MS: (Mwt: 340.14) *m/z* (% rel. Int.), 340 [M^+, (41.2%)]. Anal. Calcd for $C_{20}H_{16}N_6$: C, 70.57; H, 4.74; N, 24.69; Found: C, 70.67; H, 4.74; N, 24.75.

*2.1.2.18. 2-(3-Isopropoxypropylamino)-4-phenylpyrazolo[1,5-a][1,3,5] triazine-8- carbonitrile **(8c)**.* The product was separated as green yellow crystals, reaction time 1 h, (0.35 g, 52.23%) m.p.: 153–157 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.49 (d, $J = 7.6$ Hz, 2H, Ar- H), 8.1–2 (s, 1H, Pyrazole-H), 7.65–7.57 (m, 3H, Ar-H), 6.52 (s, 1H, NH D_2O exchangeable), 3.80–3.67 (septet, 1H, $OCH(CH_3)_2$), 3.66–3.57 (m, 4H, $NH\underline{CH_2}CH_2\underline{CH_2}O$), 2.01–1.93 (m, 2H, $NHCH_2\underline{CH_2}CH_2O$), 1.23 (d, $J = 6.0$ Hz, 6H, CH_3).^{13}C NMR (101 MHz, $CDCl_3$) δ 157.60, 156.20, 154.20, 147.35, 139.25, 132.35, 128.25, 127.30, 113.42, 96.01, 71.76, 67.25, 40.82, 28.85, 22.30. FT-IR (ὐ max, cm^{-1}): 3336 (NH), 3078 (CH aromatic), 2972 (CH aliphatic), 2228 ($C\equiv N$ nitrile). MS: (Mwt: 336.17) *m/z* (% rel. Int.), 336 [M^+, (25.2%)]. Anal. Calcd for $C_{18}H_{20}N_6O$: C, 64.27; H, 5.99; N, 24.98; Found: C, 64.37; H, 5.99; N, 25.2.

*2.1.2.19. 2-(Butylamino)-4-phenylpyrazolo[1,5-a][1,3,5]triazine-8-carbonitrile **(8d)**.* The product was separated as pale yellow powder, reaction time 1 h, (0.29 g, 50.0%) m.p.: 168–170 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H, Pyrazole-H), 7.66–7.42 (m, 5H, Ar-H), 5.82 (s, 1H, NH D_2O exchangeable), 3.62 (t, $J = 3.4$ Hz, 2H, $NHCH_2$), 1.70–1.62 (m, 2H, CH_2), 1.55–1.47 (m, 2H, CH_2), 1.05 (t, $J = 7.3$ Hz, 3H, CH_3).^{13}C NMR (101 MHz, $CDCl_3$) δ 156.46, 156.02, 154.83, 147.89, 133.17, 131.01, 129.92, 128.45, 113.90, 101.76, 42.38, 29.95, 20.24, 14.20. FT-IR (ὐ max, cm^{-1}): 3310 (NH), 3085 (CH aromatic), 2954 (CH aliphatic), 2231 ($C\equiv N$ nitrile). MS: (Mwt: 292.14) *m/z* (% rel. Int.), 292 [M^+, (28.6%)]. Anal. Calcd for $C_{16}H_{16}N_6$: C, 65.75; H, 5.52; N, 28.75; Found: C, 65.84; H, 5.52; N, 28.85.

*2.1.2.20. 2-(Isobutylamino)-4-phenylpyrazolo[1,5-a][1,3,5]triazine-8-carbonitrile **(8e)**.* The product was separated as buff crystals, reaction time 1 h, (0.31 g, 53.44%) m.p.: 175–177 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, $J = 7.4$ Hz, 2H, Ar-H), 8.13 (s, 1H, Pyrazole-H), 7.67–7.58 (m, 3H, Ar-H), 5.80 (s, 1H, NH D_2O exchangeable), 3.43 (d, $J = 6.4$ Hz, 2H, $NHCH_2$), 2.07–1.94 (m, 1H, $CH(CH_3)_2$), 1.05 (d, $J = 6.6$ Hz, 6H, CH_3).^{13}C NMR (101 MHz, $CDCl_3$) δ 156.70, 156.35, 154.72, 147.38, 133.27, 131.10, 129.85, 128.65, 113.20, 100.42, 49.75, 28.15, 20.50. FT-IR (ὐ max, cm^{-1}): 3317 (NH), 3070 (CH aromatic), 2924 (CH aliphatic), 2229 ($C\equiv N$ nitrile). MS: (Mwt: 292.14) *m/z* (% rel. Int.), 292 [M^+, (32.4%)]. Anal. Calcd for $C_{16}H_{16}N_6$: C, 65.74; H, 5.52; N, 28.75; Found: C, 65.84; H, 5.52; N, 28.85.

*2.1.2.21. 2-(Ethylsulfonyl)-4-phenylpyrazolo[1,5-a][1,3,5]triazine-8-carbonitrile **(8f)**.* The product was separated as bright yellow crystals, reaction time 1.5 h, (0.32 g, 51.61%) m.p.: 163–165 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, $J = 8.2$ Hz, 2H Ar-H), 8.11 (s, 1H, pyrazole-H), 7.67–7.57 (m, 3H, Ar-H), 4.01–3.95 (m, 2H, CH_2), 1.75 (t, $J = 7.3$ Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.02, 154.83, 147.84, 145.7, 133.17, 131.01, 129.92, 128.45, 113.90, 98.13, 45.38, 14.53. FT-IR (ὐ max, cm^{-1}): 3140 (CH aromatic), 2942 (CH aliphatic), 2217 ($C\equiv N$ nitrile), 1257 (SO_2). MS: (Mwt: 313.06) *m/z* (% rel. Int.), 313 [M^+, (40.2%)]. Anal. Calcd for $C_{14}H_{11}N_5O_2S$: C, 53.66; H, 3.54; N, 22.35; S, 10.23; Found: C, 53.56; H, 3.44; N, 22.35; S, 10.13.

*2.1.2.22. 2-(Benzylamino)-4-(4-chlorophenyl)pyrazolo[1,5-a][1,3,5] triazine-8- carbonitrile **(8g)**.* The product was separated as light yellow crystals, reaction time 1.5 h, (0.39 g, 54.16%) m.p.: 140–143 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, $J = 8.7$ Hz, 2H, Ar-H), 8.16 (s, 1H, Pyrazole-H), 7.55 (d, $J = 8.7$ Hz, 2H, Ar-H), 7.44–7.36 (m, 5H, Ar-H), 6.02 (s, 1H,NH D_2O exchangeable), 4.76 (s, 2H, CH_2).^{13}C NMR (101 MHz, $CDCl_3$) δ 157.58, 156.20, 154.20, 147.56, 139.90, 132.45, 128.82, 127.79, 128.45, 128.26, 127.57, 126.32, 113.65, 102.40, 46.16. FT-IR (ὐ max, cm^{-1}): 3335 (NH), 3075 (CH aromatic), 2915 (CH aliphatic), 2227 ($C\equiv N$ nitrile). MS: (Mwt: 360.09) *m/z* (% rel. Int.), 362 [$M^+ + 2$, (10.8%)], 360 [M^+, (32.4%)]. Anal. Calcd for $C_{19}H_{13}ClN_6$: C, 63.25; H, 3.63; N, 23.29; Found: C, 63.35; H, 3.63; N, 23.34.

*2.1.2.23. 4-(4-Chlorophenyl)-2-(phenethylamino)pyrazolo[1,5-a][1,3,5] triazine-8- carbonitrile **(8h)**.* The product was separated as light yellow crystals, reaction time 1.5 h, (0.39 g, 52.13%) m.p.: 163–165 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, $J = 8.6$ Hz, 2H, Ar- H), 8.16 (s, 1H, Pyrazole-H), 7.55 (d, $J = 8.6$ Hz, 2H, Ar-H), 7.36–7.24 (m, 5H, Ar-H), 5.73 (s, 1H, NH D_2O exchangeable), 3.88 (t, $J = 6.5$ Hz, 2H, $NHCH_2$), 3.03 (t, $J = 6.6$ Hz, 2H, CH_2Ar). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.86, 155.82, 153.86, 147.92, 140.23, 138.59, 132.93, 129.93, 128.64, 127.67, 126.62, 125.9, 113.37, 100.41, 42.67, 34.68. FT-IR (ὐ max, cm^{-1}): 3331 (NH), 3103 (CH aromatic), 2923 (CH aliphatic), 2223 ($C\equiv N$ nitrile). MS: (Mwt: 374.10) *m/z* (% rel. Int.), 376 [$M^+ + 2$, (12.3%)], 374 [M^+, (35.7%)]. Anal. Calcd for $C_{20}H_{15}ClN_6$: C, 64.09; H, 4.03; N, 22.42; Found: C, 64.19; H, 4.03; N, 22.48.

*2.1.2.24. 4-(4-Chlorophenyl)-2-(3-isopropoxypropylamino)pyrazolo[1,5-a] [1,3,5]triazine-8-carbonitrile **(8i)**.* The product was separated as green yellow crystals, reaction time 1 h, (0.39 g, 52.7%) m.p.: 155–158 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, $J = 8.5$ Hz, 2H, ArH), 8.12 (s,1H, Pyrazole H), 7.55 (d, $J = 8.5$ Hz, 2H, ArH), 6.54 (s, 1H, NH D_2O exchangeable), 3.70 (m, 1H, $OCH(CH_3)_2$), 3.66–3.59 (m, 4H, $NH\underline{CH_2}CH_2\underline{CH_2}O$), 1.95 (m, 2H, CH_2), 1.24 (d, $J = 6.0$ Hz, 6H, CH_3).^{13}C NMR (101 MHz, $CDCl_3$) δ157.58, 156.20, 154.20, 147.56, 139.86, 132.41, 128.82, 127.79, 113.44, 96.0, 71.95, 67.22, 40.91, 28.73, 22.12. FT-IR (ὐ max, cm^{-1}): 3333 (NH), 3102 (CH aromatic), 2969 (CH aliphatic), 2224 ($C\equiv N$ nitrile). MS: (Mwt: 370.13) *m/z* (% rel. Int.), 372 [$M^+ + 2$, (13.4%)], 370 [M^+, (38.9%)]. Anal. Calcd for $C_{18}H_{19}ClN_6O$: C, 58.30; H, 5.16; N, 22.66; Found: C, 58.40; H, 5.16; N, 22.75.

*2.1.2.25. 2-(Butylamino)-4-(4-chlorophenyl)pyrazolo[1,5-a][1,3,5] triazine-8-carbonitrile **(8j)**.* The product was separated as pale yellow crystals, reaction time 1.5 h, (0.42 g, 50.73%) m.p.: 174–177 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.53 (d, J = 8.7 Hz, 2H, Ar- H), 8.12 (s, 1H, Pyrazole-H), 7.54 (d, J = 8.7 Hz, 2H, Ar-H), 5.75 (s, 1H, NH D$_2$O exchangeable), 3.59 (t, J = 3.7 Hz, 2H, NHCH$_2$), 1.72–1.66 (p, 2H, CH$_2$), 1.51–1.45 (m, 2H, CH$_2$), 1.01 (t, J = 7.3 Hz, 3H, CH$_3$). ^{13}C NMR (101 MHz, CDCl$_3$) δ 157.53, 156.25, 154.25, 147.65, 139.68, 132.14, 128.28, 127.97, 113.26, 104.25, 43.23, 31.06, 20.25, 13.95. FT-IR (ύ max, cm^{-1}): 3309 (NH), 3080 (CH aromatic), 2945 (CH aliphatic), 2217 (C≡N nitrile). MS: (Mwt: 326.10) m/z (% rel. Int.), 328 [M^{+} +2, (12.9%)], 326 [M^{+}, (37.7%)]. Anal. Calcd for $C_{16}H_{15}ClN_5$: C, 58.81; H, 4.63; N, 25.72; Found: C, 58.91; H, 4.63; N, 25.82.

*2.1.2.26. 4-(4-Chlorophenyl)-2-(isobutylamino)pyrazolo[1,5-a][1,3,5] triazine-8- carbonitrile **(8k)**.* The product was separated as buff crystals, reaction time 1 h, (0.43 g, 51.94%) m.p.: 176–178 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.12 (s, 1H, Pyrazole-H), 7.72 (d, J = 8.5 Hz, 2H, Ar-H), 7.40 (d, J = 8.5 Hz, 2H, Ar-H), 6.32 (s, 1H, NH D$_2$O exchangeable), 3.28 (d, J = 6.4 Hz, 2H, NHCH$_2$), 1.97–1.82 (m, 1H, CH(CH$_3$)$_2$), 0.99 (d, J = 6.7 Hz, 6H, CH$_3$).^{13}C NMR (101 MHz, CDCl$_3$) δ 156.82, 156.12, 153.56, 147.94, 139.83, 132.94, 128.58, 128.72, 113.35, 102.40, 52.15, 27.20, 20.17. FT-IR (ύ max, cm^{-1}): 3294 (NH), 3070 (CH aromatic), 2950 (CH aliphatic), 2219 (C≡N nitrile). MS: (Mwt: 326.10) m/z (% rel. Int.), 328 [M^{+} +2, (12.9%)], 326 [M^{+}, (39.2%)]. Anal. Calcd for $C_{16}H_{15}ClN_6$: C, 58.81; H, 4.63; N, 25.72 Found: C, 58.91; H, 4.63; N, 25.82.

*2.1.2.27. 4-(4-Chlorophenyl)-2-(ethylsulfonyl)pyrazolo[1,5-a][1,3,5] triazine-8- carbonitrile **(8l)**.* The product was separated as bright yellow crystals, reaction time 1.5 h, (0.35 g, 50.72%) m.p.: 165–167 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.56 (d, J = 8.2 Hz, 2H, Ar-H), 8.10 (s, 1H, pyrazole-H), 7.53 (d, J = 8.2 Hz, 2H, Ar-H), 4.00–3.89 (m, 2H, CH$_2$), 1.77 (t, J = 7.4 Hz, 3H, CH$_3$). ^{13}C NMR (101 MHz, CDCl$_3$) δ 156.01, 153.65, 147.91, 145.80, 139.80, 132.49, 128.80, 128.27, 113.75, 96.04, 45.37, 14.50. FT-IR (ύ max, cm^{-1}): 3145 (CH aromatic), 2941 (CH aliphatic), 2226 (C≡N nitrile), 1301 (SO$_2$). MS: (Mwt: 347.02) m/z (% rel. Int.), 349 [M^{+} +2, (13.6%)], 347 [M^{+}, (39.9%)]. Anal. Calcd for $C_{14}H_{10}ClN_5O_2S$: C, 48.35; H, 2.90; N, 20.14; Found: C, 48.25; H, 2.90; N, 20.24.

*2.1.2.28. 4-(4-Chlorophenyl)-2-(cyclopropylamino)pyrazolo[1,5-a] [1,3,5]triazine-8- carbonitrile **(8m)**.* The product was separated as pale yellow crystals, reaction time 1 h, (0.31 g, 50.0%) m.p.: 178–180 ^{0}C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.54 (d, J = 8.3 Hz, 2H, Ar-H), 8.18 (s, 1H, Pyrazole-H), 7.56 (d, J = 8.3 Hz, 2H, Ar-H), 5.82 (s, 1H ,NH D$_2$O exchangeable), 3.08–2.97 (m, 1H, CH), 0.99 (d, J = 5.8 Hz, 4H, 2CH$_2$). ^{13}C NMR (101 MHz, CDCl$_3$) δ156.25, 155.83, 153.25, 146.25, 139.35, 132.46, 128.38, 127.42, 113.42, 96.35, 28.01, 9.12. FT-IR (ύ max, cm^{-1}): 3324 (NH), 3115 (CH aromatic), 2922 (CH aliphatic), 2225 (C≡N nitrile). MS: (Mwt: 310.07) m/z (% rel. Int.), 312 [M^{+} +2, (12.8%)], 310 [M^{+}, (37.9%)]. Anal. Calcd for $C_{15}H_{11}ClN_6$: C, 57.98; H, 3.57; N, 27.05; Found: C, 58.06; H, 3.57; N, 27.15.

*2.1.2.29. 2-(Benzylamino)-4-(4-bromophenyl)pyrazolo[1,5-a][1,3,5] triazine-8- carbonitrile **(8n)**.* The product was separated as light yellow crystals, reaction time 1.5 h, (0.42 g, 52.5%) m.p.: 163–165 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.52 (d, J = 8.6 Hz, 2H, Ar- H), 8.16 (s, 1H, Pyrazole-H), 7.55 (d, J = 8.6 Hz, 2H, Ar-H), 7.36–7.24 (m, 5H, ArH), 5.73 (s, 1H, NH D$_2$O exchangeable), 3.88 (s, 2H, NHCH$_2$).^{13}C NMR (101 MHz, CDCl$_3$) δ 157.86, 155.82, 153.86, 147.92, 140.23, 138.59, 132.93, 129.93, 128.64, 127.67, 126.62, 113.37, 100.41, 42.67, 34.68. FT-IR (ύ max, cm^{-1}): 3331 (NH), 3103 (CH aromatic), 2923 (CH aliphatic), 2223 (C≡N nitrile). MS: (Mwt: 404.04) m/z (% rel. Int.), 406 [M^{+} +2, (51.8%)], 404 [M^{+}, (48.2%)]. Anal. Calcd for $C_{19}H_{13}BrN_6$: C, 56.31; H, 3.23; N, 20.74; Found: C, 56.40; H, 3.23; N, 20.80.

*2.1.2.30. 4-(4-Bromophenyl)-2-(phenethylamino)pyrazolo[1,5-a][1,3,5] triazine-8- carbonitrile **(8o)**.* The product was separated as light yellow crystals, reaction time 1.5 h, (0.44 g, 53.0%) m.p.: 148–151 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.54 (d, J = 8.5 Hz, 2H, Ar-H), 8.16 (s, 1H, Pyrazole-H), 7.57 (d, J = 8.5 Hz, 2H, Ar-H), 7.38–7.16 (m, 5H, Ar-H), 5.77 (s, 1H, NH D$_2$O exchangeable), 3.87 (t, J = 6.6 Hz, 2H, NHCH$_2$), 3.05 (t, J = 6.6 Hz, 2H, CH$_2$Ar). ^{13}C NMR (101 MHz, CDCl$_3$) δ157.82, 155.25, 153.25, 147.19, 140.50, 138.56, 132.95, 129.72, 128.56, 127.20, 126.23, 126.17, 113.20, 100.15, 43.95, 35.70. FT-IR (ύ max, cm^{-1}): 3328 (NH), 3115 (CH aromatic), 2920 (CH aliphatic), 2222 (C≡N nitrile). MS: (Mwt: 418.05) m/z (% rel. Int.), 420 [M^{+} +2, (32.7%)], 418 [M^{+}, (28.5%)]. Anal. Calcd for $C_{20}H_{15}BrN_6$: C, 57.29; H, 3.61; N, 20.04; Found: C, 57.39; H, 3.61; N, 20.14.

*2.1.2.31. 4-(4-Bromophenyl)-2-(3-isopropoxypropylamino)pyrazolo[1,5-a][1,3,5] triazine-8-carbonitrile **(8p)**.* The product was separated as green yellow crystals, reaction time 1.5 h, (0.44 g, 53.14%) m.p.: 162–165 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.52 (d, J = 8.4 Hz, 2H, Ar-H), 8.12 (s, 1H, Pyrazole-H), 7.53 (d, J = 8.4 Hz, 2H, Ar-H), 6.52 (s, 1H, NH D$_2$O exchangeable), 3.78–3.65 (m, 1H, OCH(CH$_3$)$_2$), 3.64–3.50 (m, 4H, NHCH$_2$CH$_2$CH$_2$O), 2.00–1.92 (m, 2H, CH$_2$), 1.24 (d, J = 6.0 Hz, 6H, CH$_3$). ^{13}C NMR (101 MHz, CDCl$_3$) δ 157.53, 156.35, 154.25, 147.58, 139.85, 132.45, 128.82, 127.82, 113.45, 95.98, 71.96, 67.30, 40.92, 28.94, 22.11. FT-IR (ύ max, cm^{-1}): 3329 (NH), 3069 (CH aromatic), 2965 (CH aliphatic), 2220 (C≡N nitrile). MS: (Mwt: 414.08) m/z (% rel. Int.), 416 [M^{+} +2, (37.2%)], 414 [M^{+}, (31.8%)]. Anal. Calcd for $C_{18}H_{19}BrN_6O$: C, 52.06; H, 4.61; N, 20.24; Found: C, 52.16; H, 4.61; N, 20.34.

*2.1.2.32. General procedure for the preparation of **9a-o**.* Compounds ethyl 2-(ethylthio)-4-substituted phenylpyrazolo[1,5-a][1,3,5]triazine-8-carboxylate **(7a-c)** (1 equiv., 1 mmol) were fused with the appropriate amine (1 equiv., 1 mmol) at 150 °C using oil bath for 1.5–2 h. The reaction mixture was then triturated with diethyl ether and the precipitate was collected by filtration and purified by flash column chromatography on silica gel, using a mixture of hexane/ethyl acetate (8:2) as eluent.

*2.1.2.33. Ethyl 2-(benzylamino)-4-phenylpyrazolo[1,5-a][1,3,5]triazine-8-carboxylate **(9a)**.* The titled compound was separated as yellow powder (0.3 g, 52%); m.p. 143–145 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.49 (d, J = 7.6 Hz, 2H, Ar H), 8.39 (s, 1H, H pyrazole), 7.65–7.30 (m, 8H, Ar H), 6.01 (s, 1H, NH D$_2$O exchangeable), 4.82 (s, 2H, N-CH$_2$), 4.41 (q, J = 7.1 Hz, 2H, $\underline{CH_2}$CH$_3$), 1.44 (t, J = 7.1 Hz, 3H, CH$_2$,$\underline{CH_3}$) ; ^{13}C NMR (101 MHz, CDCl$_3$) δ 163.25, 157.82, 153.78, 147.32, 145.73, 138.69, 136.81, 133.42, 129.56, 128.92, 128.56, 126.92, 126.48, 98.95, 59.95, 42.65, 14.61; FT-IR (ύ max, cm^{-1}): 3409 (NH), 3117 (CH aromatic), 2853 (CH aliphatic), 1740 (C═O ester); MS: (Mwt.: 373.41): m/z (% rel. Int.), 373.25 [M^{+}, (35.8%)]., 309.23 (100%); Anal. Calcd for $C_{21}H_{19}N_5O_2$: C, 67.55; H, 5.13; N, 18.76; Found: C, 67.68; H, 5.26; N, 18.92.

*2.1.2.34. Ethyl 2-(phenethylamino)-4-phenylpyrazolo[1,5-a][1,3,5] triazine-8-carboxylate **(9b)**.* The titled compound was separated as yellow powder (0.31 g, 53%); m.p. 135–137 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.46 (d, J = 7.6 Hz, 2H, Ar H), 8.38 (s, 1H, pyrazole H), 7.70–7.55 (m, 3H, Ar H), 7.35–7.26 (m, 5H, Ar H), 5.73 (s, 1H, NH D$_2$O exchangeable), 4.41 (q, J = 7.5 Hz, 2H, CH$_2$CH$_3$), 3.92 (t, J = 6.3 Hz, 2H, CH$_2$), 3.05 (t, J = 6.4 Hz, 2H, CH$_2$), 1.44 (t, J = 7.5 Hz, 3H, CH$_2$CH$_3$); ^{13}C NMR (101 MHz, CDCl$_3$) δ 168.70, 157.87, 152.32, 148.87, 146.59, 139.62, 137.32, 132.29, 129.35, 128.91, 127.54, 126.76, 126.29, 98.79, 60.42, 42.61, 35.23, 14.65; FT-IR (ύ max, cm^{-1}): 3368 (NH), 3090 (CH aromatic), 2927 (CH aliphatic), 1745 (C═O ester); MS: (Mwt.: 387.43): m/z(% rel. Int.), 387.23, 194.12 (100%); Anal. Calcd for $C_{22}H_{21}N_5O_2$: C, 68.20; H, 5.46; N, 18.08; Found: C, 68.72; H, 5.22; N, 18.13.

*2.1.2.35. Ethyl 4-phenyl-2-(piperidin-1-yl)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate **(9c)***. The titled compound was separated as yellow powder (0.35 g, 65%); m.p. 133–135 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, J = 7.2 Hz, 2H, Ar H), 8.33 (s, 1H, pyrazole H), 7.64–7.54 (m, 3H, Ar H), 4.38 (q, J = 7.1 Hz, 2H, CH_2CH_3), 4.02 (br s, 4H, piperidine H), 1.72 (br s, 6H, piperidine H), 1.42 (t, J = 7.1 Hz, 3H, CH_2CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ163.35, 157.53, 152.35, 149.32, 143.40, 133.39, 130.89, 129.72, 128.36, 99.20, 60.72, 47.18, 29.30, 24.42, 14.60; FT-IR (ύ max, cm^{-1}): 3115 (CH aromatic), 2920 (CH aliphatic), 1750 (C=O ester) MS: (Mwt.: 351.40): m/z(% rel. Int.), 351.35, 103.17 (100%); Anal. Calcd for $C_{19}H_{21}N_5O_2$: C, 64.94; H, 6.02; N, 19.93; Found: C, 64.82; H, 6.26; N, 19.85.

*2.1.2.36. Ethyl 4-phenyl-2-(pyrrolidin-1-yl)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate **(9d)***. The titled compound was separated as yellow powder (0.37 g, 72%); m.p. 155–157 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, J = 7.4 Hz, 2H, Ar H), 8.35 (s, 1H, pyrazole H), 7.66–7.55 (m, 3H, Ar H), 4.39 (q, J = 7.1 Hz, 2H, CH_2CH3)$_,$ 3.82 (br s, 4H, $N(CH_2)_2$), 2.06 (br s, 4H, pyrroldine $(CH_2)_2$), 1.43 (t, J = 7.1 Hz, 3H, CH_2CH3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.95, 155.72, 153.75, 148.80, 144.65, 132.82, 131.65, 129.42, 127.81, 98.05, 60.25, 47.54, 25.45, 14.70; FT-IR (ύ max, cm^{-1}): 3098 (CH aromatic), 2890 (CH aliphatic), 1750 (C=O ester); MS: (Mwt.: 337.38): m/z(% rel. Int.), 337.21, 194.12 (100%); Anal. Calcd for $C_{18}H_{19}N_5O_2$: C, 64.08; H, 5.68; N, 20.76; Found: C, 64.15; H, 5.53; N, 20.82.

*2.1.2.37. Ethyl 2-(isopropylamino)-4-phenylpyrazolo[1,5-a][1,3,5]triazine-8-carboxylate **(9e)***. The titled compound was separated as yellow powder (0.32 g, 64%); m.p. 159–161 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (d, J = 7.3 Hz, 2H, Ar H), 8.35 (s, 1H, pyrazole H), 7.63–7.55 (m, 3H, Ar H), 5.58 (s, 1H, NH D_2O exchangeable), 4.38 (q, J = 7.1 Hz, 2H, CH_2CH_3) 4.1 (m, 1H, $\underline{CH}(CH_3)_2$) 1.41 (t, J = 7.1 Hz, 3H, CH_2CH_3), 1.34 (d, J = 6.5 Hz, 6H, $(CH_3)_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.36, 157.26, 153.04, 148.85, 144.68, 131.66, 130.91, 128.59, 127.81, 98.82, 59.82, 43.03, 22.78, 14.35; FT-IR (ύ max, cm^{-1}): 3317 (NH), 3110 (CH aromatic), 2924 (CH aliphatic), 1750 (C=O ester); MS: (Mwt.: 325.37): m/z(% rel. Int.), 325.12, 220.11 (100%); Anal. Calcd for $C_{17}H_{19}N_5O_2$: C, 62.75; H, 5.89; N, 21.52; Found: C, 62.85; H, 5.75; N, 21.46.

*2.1.2.38. Ethyl 2-(benzylamino)-4-(4-chlorophenyl)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate **(9f)***. The titled compound was separated as yellow powder (0.26 g, 46%); m.p. 133–135 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, J = 8.1 Hz, 2H, Ar H), 8.39 (s, 1H, pyrazole), 7.54 (d, J = 8.1 Hz, 2H, Ar H), 7.48–7.28 (m, 5H, Ar H), 5.96 (s, 1H, NH D_2O exchangeable), 4.82 (s, 2H, N-CH_2), 4.41 (q, J = 7.0 Hz, 2H, CH_2CH_3), 1.46 (t, J = 7.0 Hz, 3H, CH_2CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.22, 157.78, 153.68, 147.37, 145.68, 138.39, 136.75, 133.40, 129.57, 128.85, 128.63, 126.95, 126,61, 98.93, 59.32, 42.60, 14.58; FT-IR (ύ max, cm^{-1}): 3346 (NH), 3063 (CH aromatic), 2928 (CH aliphatic), 1745 (C=O ester); MS: (Mwt.: 407.85): m/z(% rel. Int.), 409.36 [M^+ + 2, (23.6%)], 407.74 [M^+, (69.2%)], 44.10 (100%); Anal. Calcd for $C_{21}H_{18}ClN_5O_2$: C, 61.84; H, 4.45; N, 17.17; Found: C, 61.76; H, 4.61; N, 17.25.

*2.1.2.39. Ethyl 4-(4-chlorophenyl)-2-(phenethylamino)pyrazolo[1,5 a][1,3,5]triazine −8-carboxylate **(9g)***. The titled compound was separated as faint yellow powder (0.32 g, 55%); m.p. 158–160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, J = 8.5 Hz, 2H, Ar H), 8.37 (s, 1H, pyrazole H), 7.54 (d, J = 8.5 Hz, 2H, Ar H), 7.35–7.25 (m, 5H, Ar H), 5.69 (s, 1H, NH D_2O exchangeable), 4.42 (q, J = 7.4 Hz, 2H, –CH_2CH_3), 3.92 (t, J = 6.2 Hz, 2H, CH_2), 3.04 (t, J = 6.4 Hz, 2H, CH_2), 1.44 (t, J = 7.4 Hz, 3H, CH_2CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.78, 157.85, 152.21, 148.89, 146.85, 139.58, 137.22, 132.27, 129.62, 128.95, 127.97, 126.99, 126.31, 98.75, 60.21, 42.66, 35.33, 14.40; FT-IR (ύ max, cm^{-1}): 3350 (NH), 3078 (CH aromatic), 2920 (CH aliphatic), 1750 (C=O ester); MS: (Mwt.: 421.88): m/z(% rel. Int.), 423.74 [M^+ + 2, (12.8%)], 421.49 [M^+, (36.4%)], 194.87 (100%); Anal. Calcd for $C_{22}H_{20}ClN_5O_2$: C, 62.63; H, 4.78; N, 16.60; Found: C, 62.71; H, 4.82; N, 16.51.

*2.1.2.40. Ethyl 4-(4-chlorophenyl)-2-(piperidin-1-yl)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate **(9h)***. The titled compound was separated as faint yellow powder (0.31 g, 58%); m.p. 162–164 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, J = 8.7 Hz, 2H, Ar H), 8.33 (s, 1H, pyrazole H), 7.54 (d, J = 8.7 Hz, 2H, Ar H), 4.38 (q, J = 7.1 Hz, 2H, CH_2CH_3), 4.02 (br. s, 4H, piperidine H), 1.73 (br. s, 6H, piperidine H), 1.42 (t, J = 7.1 Hz, 3H, CH_2CH_3).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.29, 157.51, 152.30, 149.41, 143.38, 133.35, 130.65, 129.49, 128.29, 99.23, 60.42, 47.20, 29.33, 24.40, 14.40; FT-IR (ύ max, cm^{-1}): 3125 (CH aromatic), 2930 (CH aliphatic), 1745 (C=O ester); MS: (Mwt.: 385.85): m/z(% rel. Int.), 387.73 [M^+ + 2, (15.7%)], 385.46 [M^+, (44.8%)], 308.42 (100%); Anal. Calcd for $C_{19}H_{20}ClN_5O_2$: C, 59.14; H, 5.22; N, 18.15; Found: C, 59.22; H, 5.18; N, 18.19.

*2.1.2.41. Ethyl 4-(4-chlorophenyl)-2-(pyrrolidin-1-yl)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate **(9i)***. The titled compound was separated as faint yellow powder (0.29 g, 56%); m.p. 193–195 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, J = 8.7 Hz, 2H, ArH), 8.35 (s, 1H, pyrazole H), 7.68 (d, J = 8.7 Hz, 2H, Ar H), 4.37 (q, J = 7.1 Hz, 2H, CH_2CH_3), 3.85 (br s, 4H, $N(CH_2)_2$) 2.13 (br s, 4H, pyrrolidine $(CH_2)_2$), 1.44 (t, J = 7.1 Hz, 3H, CH_2CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.96, 155.73, 153.76, 148.81, 144.66, 132.80, 131.64, 129.41, 127.80, 98.10, 60.22, 47.53, 25.43, 14.71; FT-IR (ύ max, cm^{-1}): 3105 (CH aromatic), 2915 (CH aliphatic), 1750 (C=O ester); MS: (Mwt.: 371.82): m/z(% rel. Int.), 373.53 [M^+ + 2, (12.9%)], 371.68 [M^+, (35.4%)], 193.93 (100%); Anal. Calcd for $C_{18}H_{18}ClN_5O_2$: C, 58.14; H, 4.88; N, 18.84; Found: C, 58.21; H, 4.92; N, 18.75.

*2.1.2.42. Ethyl 4-(4-chlorophenyl)-2-(isopropylamino)pyrazolo[1,5-a][1,3,5]triazine −8-carboxylate **(9j)***. The titled compound was separated as faint yellow powder (0.26 g, 52%); m.p. 156–158 °C; ^{1}H NMR (400 MHz, $CDCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, J = 8.0 Hz, 2H, Ar H), 8.37 (s, 1H, pyrazole H), 7.56 (d, J = 8.0 Hz, 2H, Ar H), 5.52 (s, 1H, NH D_2O exchangeable), 4.46 (q, J = 7.0 Hz, 2H, CH_2CH_3), 4.2 (m,1H, $CH(CH_3)_2$), 1.43 (t, J = 7.0 Hz, 3H, CH_2CH_3), 1.28 (d, J = 6.3 Hz, 6H, $(CH_3)_2$);^{13}C NMR (101 MHz, $CDCl_3$) δ 163.33, 157.22, 153.03, 148.82, 144.64, 131.62, 130.89, 128.56, 127.78, 98.89, 59.75, 43.07, 22.73, 14.30; FT-IR (ύ max, cm^{-1}): 3317 (NH), 3103 (CH aromatic), 2924 (CH aliphatic), 1750 (C=O ester); MS: (Mwt.: 359.81): m/z(% rel. Int.), 361.74 [M^+ + 2, (17.4%)], 359.66 [M^+, (52.6%)], 194.12 (100%); Anal. Calcd for $C_{17}H_{18}ClN_5O_2$: C, 56.75; H, 5.04; N, 19.46; Found: C, 56.83; H, 5.15; N, 19.72.

*2.1.2.43. Ethyl 2-(benzylamino)-4-(4-bromophenyl)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate **(9k)***. The titled compound was separated as greenish yellow powder (0.29 g, 52%); m.p. 154–156 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, J = 8.3 Hz, 2H, Ar H), 8.38 (s, 1H, pyrazole H), 7.70 (d, J = 8.3 Hz, 2H, Ar H), 7.47–7.29 (m, 5H, Ar H), 6.01 (s, 1H, NH D_2O exchangeable), 4.82 (s, 2H, N-CH_2), 4.41 (q, J = 7.1 Hz, 2H, CH_2CH_3), 1.43 (t, J = 7.1 Hz, 3H, CH_2CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ163.32, 157.75, 153.72, 147.29, 145.62, 138.71, 136.79, 133.41, 129.72, 128.89, 128.66, 127.23, 126.52, 98.97, 59.89, 42.63, 14.52; FT-IR (ύ max, cm^{-1}): 3481 (NH), 3093 (CH aromatic), 2977 (CH aliphatic), 1748 (C=O ester); MS: (Mwt.: 452.30): m/z(% rel. Int.), 454.34 [M^+ + 2, (33.2%)], 452.16 [M^+, (35.4%)], 378.26 (100%); Anal. Calcd for $C_{21}H_{18}BrN_5O_2$: C, 55.76; H, 4.01; N, 15.48; Found: C, 55.82; H, 4.15; N, 15.52.

*2.1.2.44. Ethyl 4-(4-bromophenyl)-2-(phenethylamino)pyrazolo[1,5-a][1,3,5] triazine-8-carboxylate **(9l)***. The titled compound was separated as greenish yellow powder (0.38 g, 66%); m.p. 147–150 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, J = 8.4 Hz, 2H, Ar H), 8.37 (s, 1H, pyrazole H), 7.54 (d, J = 8.4 Hz, 2H, Ar H), 7.38–7.25 (m, 5H, Ar H), 5.66 (s, 1H, NH D_2O exchangeable), 4.40 (q, J = 7.2 Hz, 2H, CH_2CH_3), 3.92 (t, J = 6.6 Hz, 2H, CH_2), 3.05 (t, J = 6.5 Hz, 2H, CH_2), 1.41 (t, J = 7.2 Hz, 3H, CH_2CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.73, 157.82, 152.29, 148.82, 146.65, 137.53, 137.35, 132.28, 129.58, 128.87, 127.92, 126.64, 126.25, 98.76, 60.45, 42.65, 35.35, 14.43; FT-IR (ύ max, cm^{-1}): 3317 (NH), 3065 (CH aromatic), 2918 (CH aliphatic), 1745 (C=O ester); MS: (Mwt.: 466.33): m/z(% rel. Int.), 468.36 [$M^+ + 2$, (43.8%)], 466.25 [M^+, (45.2%)], 309.23 (100%); Anal. Calcd for $C_{22}H_{20}BrN_5O_2$: C, 56.66; H, 4.32; N, 15.02; Found: C, 56.58; H, 4.43; N, 15.18.

2.1.2.45. Ethyl 4-(4-bromophenyl)-2-(piperidin-1-yl)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate ***(9m)***. The titled compound was separated as greenish yellow powder (0.29 g, 55%); m.p. 168–170 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.49 (d, J = 8.6 Hz, 2H, Ar H), 8.33 (s, 1H, pyrazole H), 7.71 (d, J = 8.6 Hz, 2H, Ar H), 4.38 (q, J = 7.1 Hz, 2H, CH_2CH_3), 4.02 (br s, 4H, piperidine H), 1.73 (br s, 6H, piperidine H), 1.42 (t, J = 7.1 Hz, 3H, CH_2CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.32, 157.52, 152.32, 149.35, 143.52, 133.37, 130.85, 129.53, 128.32, 99.21, 60.45, 47.12, 29.45, 24.38, 14.62; FT-IR (ύ max, cm^{-1}): 3120 (CH aromatic), 2915 (CH aliphatic), 1750 (C=O ester); MS: (Mwt.: 430.30): m/z(% rel. Int.), 432.24 [$M^+ + 2$, (47.3%)], 430.19 [M^+, (43.9%)], 309.23 (100%); Anal. Calcd for $C_{19}H_{20}BrN_5O_2$: C, 53.03; H, 4.68; N, 16.28; Found: C, 53.12; H, 4.57; N, 16.35.

2.1.2.46. Ethyl 4-(4-bromophenyl)-2-(pyrrolidin-1-yl)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate ***(9n)***. The titled compound was separated as greenish yellow powder (0.23 g, 45%); m.p. 190–192 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, J = 8.7 Hz, 2H, Ar H), 8.35 (s, 1H, pyrazole H), 7.72 (d, J = 8.7 Hz, 2H, Ar H), 4.39 (q, J = 7.1 Hz, 2H, CH_2CH_3), 3.82 (br s, 4H, $N(CH_2)_2$) 2.07 (br s, 4H, pyrrolidine $(CH_2)_2$), 1.43 (t, J = 7.1 Hz, 3H, CH_2CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.98, 155.75, 153.79, 148.83, 144.68, 132.79, 131.67, 129.40, 127.84, 98.01, 60.21, 47.56, 25.41, 14.72; FT-IR (ύ max, cm^{-1}): 3108 (CH aromatic), 2919 (CH aliphatic), 1750 (C=O ester); MS: (Mwt.: 416.27): m/z(% rel. Int.), 418.28 [$M^+ + 2$, (23.7%)], 416.14 [M^+, (27.2%)], 248.22 (100%); Anal. Calcd for $C_{18}H_{18}BrN_5O_2$: C, 51.94; H, 4.36; N, 16.82; Found: C, 51.87; H, 3.22; N, 16.77

2.1.2.47. Ethyl 4-(4-bromophenyl)-2-(isopropylamino)pyrazolo[1,5-a][1,3,5]triazine −8-carboxylate ***(9o)***. The titled compound was separated as greenish yellow powder (0.27 g, 54%); m.p. 180–182 °C; ^1H NMR (400 MHz, $CDCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, J = 8.0 Hz, 2H, Ar H), 8.36 (s, 1H, pyrazole H), 7.72 (d, J = 8.0 Hz, 2H, Ar H), 5.46 (s,1H, NH D_2O exchangeable), 4.43 (q, J = 7.0 Hz, 2H, CH_2CH_3), 4.15(m, 1H, $CH(CH_3)_2$), 1.42 (t, J = 7.0 Hz, 3H, CH_2CH_3), 1.36 (d, J = 6.3 Hz, 6H, $(CH_3)_2$) ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.35, 157.20, 153.06, 148.80, 144.65, 131.63, 130.90, 128.55, 127.75, 98.90, 59.79, 43.05, 22.75, 14.32; FT-IR (ύ max, cm^{-1}): 3305 (NH), 3125 (CH aromatic), 2947 (CH aliphatic), 1750 (C=O ester); MS: (Mwt.: 404.26): m/z(% rel. Int.), 406.17 [$M^+ + 2$, (62.5%)], 404.21 [M^+, (65.1%)], 163.33 (100%); Anal. Calcd for $C_{17}H_{18}BrN_5O_2$: C, 50.51; H, 4.49; N, 17.32; Found: C, 50.75; H, 4.61; N, 17.35.

2.1.2.48. General procedure for the preparation of ***10a-c***. To the compounds ethyl 2-(ethylthio)-4-substituted phenylpyrazolo[1,5-a][1,3,5]triazine-8-carboxylate **(7a, 7c)** (1 equiv., 1.5 mmol), an appropriate amine (benzylamine or 2-phenylethylamine) (5 equivalent, 7.5 mmol) was added. The mixture was fused in oil bath at 150 °C for 5–7 h. The reaction mixture was then triturated with diethyl ether and the precipitate was collected by filtration and purified by flash column chromatography on silica gel, using a mixture of hexane/ethyl acetate (4:6) as eluent to give the titled compounds **(10a-c).**

2.1.2.49. N-phenethyl-2-(phenethylamino)-4-phenylpyrazolo[1,5-a][1,3,5]triazine-8-carboxamide ***(10a)***. The titled compound was separated as yellow powder (0.43 g, 61%); m.p. 190–192 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.49 (d, J = 7.5 Hz, 2H, Ar H), 8.47 (s, 1H, pyrazole H), 7.82 (s, 1H, NH D_2O exchangeable), 7.66–7.20 (mm, 13H, Ar H), 5.85 (s, 1H, NH D_2O exchangeable), 3.84 (t, J = 6.3 Hz, 2H, CH2), 3.46 (t, J = 6.2 Hz, 2H, CH2), 2.96 (t, J = 6.6 Hz, 2H, CH2), 2.87 (t, J = 6.7 Hz, 2H, CH2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.61, 156.89, 150.03, 147.70, 144.69, 138.13, 133.54, 130.91, 129.02, 128.57, 127.07, 126.69, 126.28, 101.58, 42.21, 39.96, 34.99; FT-IR (ύ max, cm^{-1}): 3398 (NH), 3175 (NH), 3060 (CH aromatic), 2948 (CH aliphatic), 1650 (C=O amide); MS: (Mwt.: 462.55): m/z(% rel. Int.), 462.43, 163.07 (100%); Anal. Calcd for $C_{28}H_{26}N_6O$: C, 72.71; H, 5.67; N, 18.17; Found: C, 72.85; H, 5.83; N, 18.21.

2.1.2.50. N-benzyl-2-(benzylamino)-4-phenylpyrazolo[1,5-a][1,3,5]triazine-8- carboxamide ***(10b)***. The titled compound was separated as yellow powder (0.38 g, 58%); m.p. 217–219 ^{0}C; ^1H NMR (400 MHz, $CDCl_3$)) δ 8.55 (d, J = 7.7 Hz, 2H, Ar H), 8.52 (s, 1H, pyrazole H), 8.05 (s, 1H, NH D_2O exchangeable), 7.69–7.57 (m, 3H, Ar H), 7.41–7.22 (m, 10*H*, Ar H), 6.05 (s, 1H, NH D_2O exchangeable), 4.68 (d, J = 5.3 Hz, 2H, CH_2), 4.52 (d, J = 5.8 Hz, 2H, CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.60, 156.82, 150.01, 147.72, 144.63, 138.14, 133.50, 130.85, 129.05, 128.53, 127.02, 126.62, 126.22, 101.55, 42.20, 39.27; FT-IR (ύ max, cm^{-1}): 3389 (NH), 3185 (NH), 3065 (CH aromatic), 2945 (CH aliphatic), 1652 (C=O amide); MS: (Mwt.: 434.49): m/z(% rel. Int.), 434.33, 135.07 (100%); Anal. Calcd for $C_{26}H_{22}N_6O$: C, 71.87; H, 5.10; N, 19.34; Found: C, 71.75; H, 5.21; N, 19.39.

2.1.2.51. 4-(4-Chlorophenyl)-N-phenethyl-2-(phenethylamino)pyrazolo[1,5-a] [1,3,5] triazine-8-carboxamide ***(10c)***. The titled compound was separated as yellow powder (0.38 g, 55%); m.p. 205–207 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, J = 8.3 Hz, 2H, Ar H), 8.39 (s, 1H, pyrazole H), 7.79 (s, 1H, NH D_2O exchangeable), 7.52 (d, J = 8.3 Hz, 2H, Ar H), 7.36–7.20 (m, 10*H*, Ar H), 5.71 (s, 1H, NH D_2O exchangeable), 3.84 (t, J = 5.5 Hz, 2H, CH_2), 3.48 (t, J = 6.0 Hz, 2H, CH_2), 2.91 (t, J = 6.1 Hz, 2H, CH_2), 2.85(t, J = 5.6 Hz, 2H, CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.59, 156.85, 150.05, 147.75, 144.65, 138.12, 133.52, 130.95, 129.07, 128.55, 127.05, 126.65, 126.25, 101.53, 42.25, 39.95, 34.90; FT-IR (ύ max, cm^{-1}): 3420 (NH), 3190 (NH), 3054 (CH aromatic), 2924 (CH aliphatic), 1651 (C=O amide); MS: (Mwt.: 496.99): m/z (% rel. Int.), 498.79 [$M^+ + 2$, (14.7%)], 496.58 [M^+, (44.1%)], 91.09 (100%); Anal. Calcd for $C_{28}H_{25}ClN_6O$: C, 67.67; H, 5.07; N, 16.91; Found: C, 67.65; H, 5.15; N, 16.85.

2.2. Biological evaluation

2.2.1. Antiproliferative activity in-vitro against NCI 60-cell lines

The NCI *in-vitro* anticancer screening is a two-stage process, beginning with the evaluation of all compounds against the full NCI 60 cell line panel representing leukemia, NSCLC, melanoma, colon cancer, CNS cancer, breast cancer, ovarian cancer, renal cancer and prostate cancer at a single dose of 10 μM following the standard NCI protocol [24]. The output from the single dose screen is reported as a mean graph.

2.2.2. In vitro cyclin dependent kinase2 inhibitory activity

The in vitro enzyme inhibition determination for the synthesized compounds was carried out in BPS Bioscience Corporation, San Diego, CA, USA (www.bpsbioscience.com). The CDK2/CyclinA2 activity at single dose concentration of 10 μM was performed, where Histone H1 (BPS#52043) served as the enzyme source & Kinase-Glo Plus Luminescence kinase assay kit (Promega#V3772) was used. The assay was performed using Kinase-Glo Plus Luminescence kinase assay kit (Promega). The compounds were diluted in 10% DMSO and 5 μl of the dilution was added to a 50 μl reaction so that the final concentration of DMSO is 1% in all of the reaction. All of the enzymatic reactions were conducted at 30 °C for 40 min. The 50 µl reaction mixture contains 40 mM Tris, pH 7.4, 10 mM $MgCl_2$, 0.1 mg/ml BSA, 1 mM DTT, 10 mM ATP, Kinase substrate and the enzyme (CDK2/CyclinA2). After the enzymatic reaction, 50 µl of Kinase-Glo Plus Luminescence kinase assay solution (Promega) was added to each reaction and incubate the plate for 5 min at room temperature. Luminescence signal was measured using a Bio Tek *Synergy* 2 microplate reader.

2.3. *In silico methods*

2.3.1. *Molecular docking*

Molecular docking study was conducted using C-Docker software in the interface of Accelry's discovery studio 2.5 (Accelrys Inc., San Diego, C.A, USA) at Faculty of pharmacy, Ain Shams university, drug design laboratory.

In this investigation, docking study and analysis of the binding modes of the target compounds were conducted to interpret the biological results and to gain further vision into binding orientations and interactions. The selected docking pose among the ten retrieved possible ones was chosen based on the similarity of its binding mode to that of the lead compound.

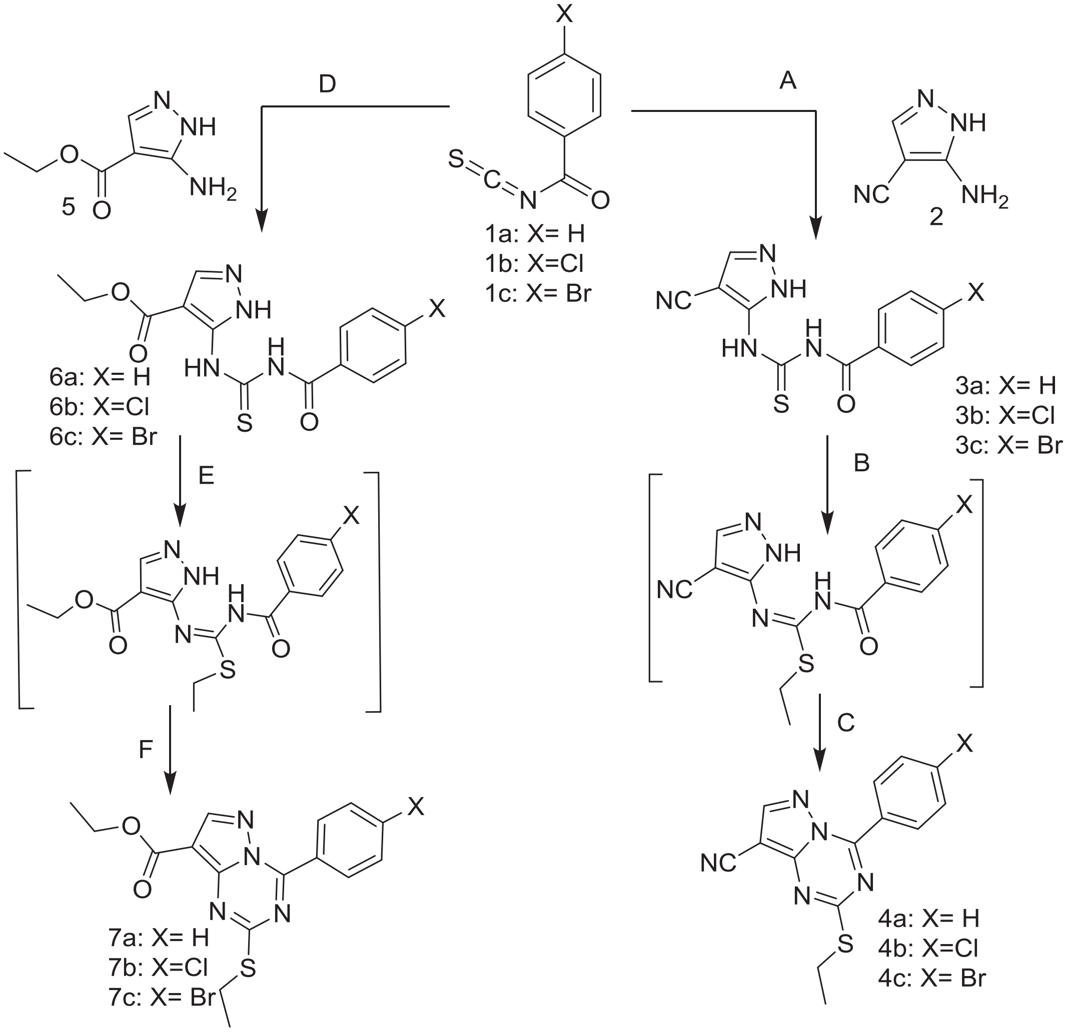
The X-ray crystal structure of CDK2 co-crystallized with Roscovitine **(**PDB code: 3ddq**)** was obtained. It is notable that Roscovitine forms the crucial hydrogen bonds with CDK2 through Leu 83 [25].

2.3.2. *Validation of docking protocol*

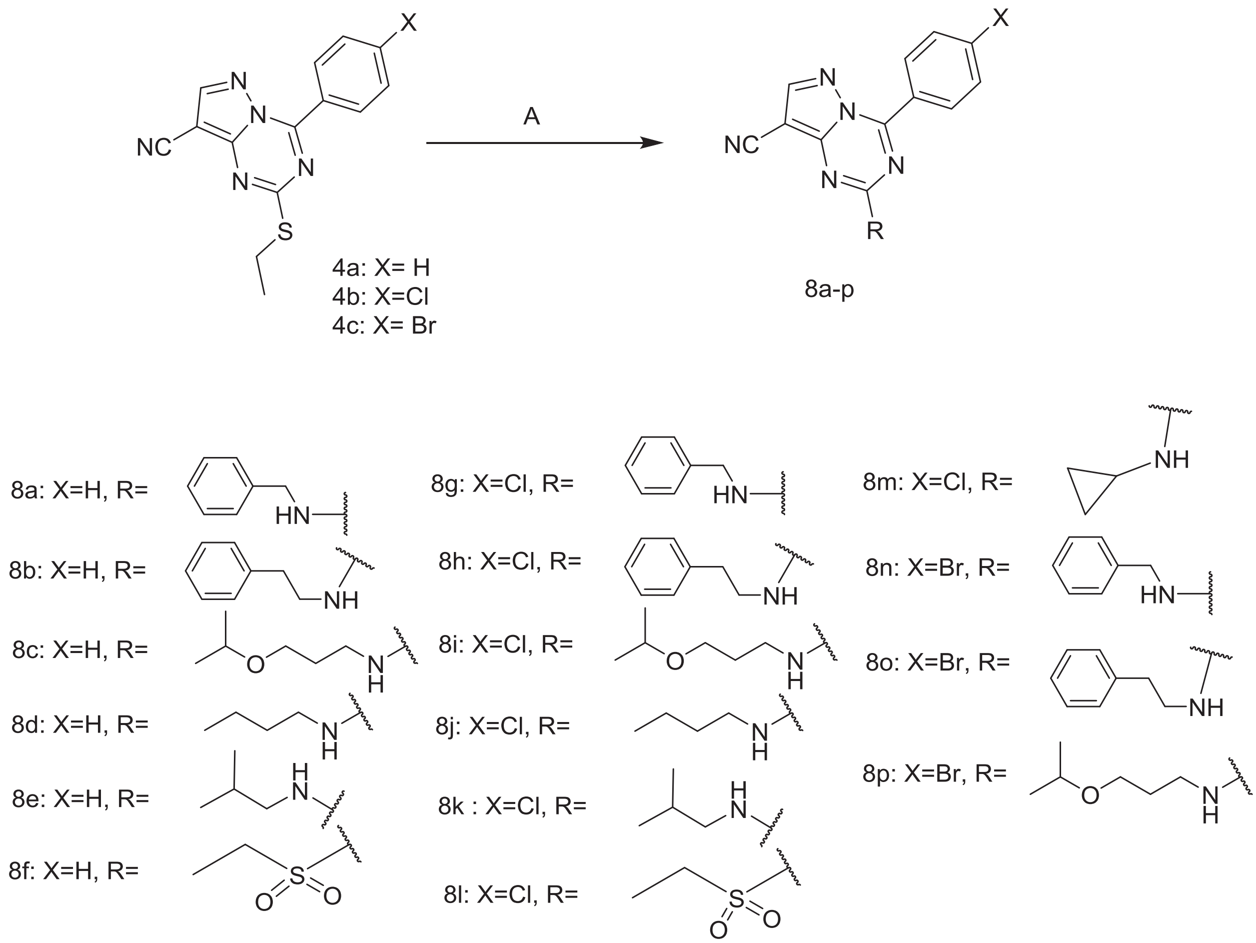
Validation of C-Docker protocol used in this study was performed by re-docking of the lead compound roscovitine in the CDK2 active site. This was followed by alignment of the X-ray bioactive conformer of the lead compound roscovitine with the best-fitted pose achieved from the docking run [26]. The alignment showed good coincidence between them with RMSD = 1.624, indicating the ability of the used docking protocol to give valid docking poses

2.3.3. *Absorption, distribution, metabolism and excretion (ADME) study*

Computer aided ADMET study was performed by using the Accelrys Discovery studio 2.5 software. The ultimate goal of *in silico* ADMET is to predict disposition behavior of compounds in the whole body by assembling all kinetic processes in one global model and hence it is expected to reduce the risk of late-stage attrition of drug development and to optimize screening and testing by looking only at the promising compounds. The study is based on the chemical structure of the molecule and involves the calculation of certain descriptors including; aqueous solubility level (Aq Sol), human intestinal absorption level (HIA), blood brain barrier penetration level (BBB), cytochrome P450 2D6 inhibition (CYP2D6), plasma protein binding level (PPB) and hepatotoxicity (Hepa Tox).



Scheme 1. Synthesis of compounds 4a-c and 7a-c. Reagents and conditions: (A) acetone, reflux, 2–3 h, (B) NaH, EtBr, DMF, rt, 30–45 min, (C) reflux, 45–60 min., (D) MeCN, reflux, 1.5–2 h, (E) NaH, EtBr, DMF, rt, 30 min, (F) DMF, reflux, 45–60 min.



Scheme 2. Synthesis of compounds 8a-p. Reagents and conditions: (A) Amines, fusion, 1–1.5 h, 150 °C.

3. Results and discussion

3.1. Chemistry

The designed compounds were synthesized according to the chemical pathways outlined in schemes 1, 2 and 3.

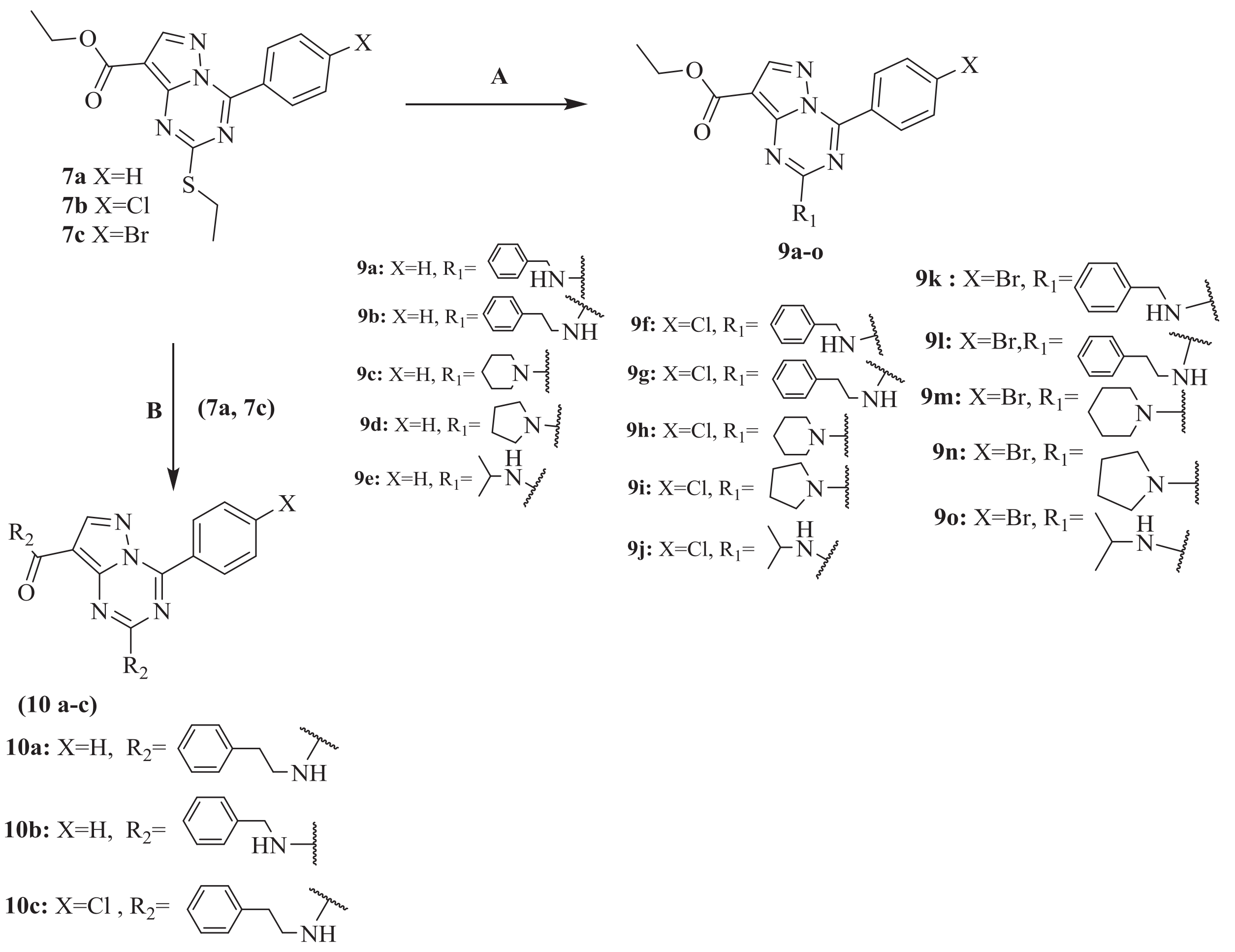
The targeted compounds (**4a-c**) were synthesized by suitable several steps starting from chlorination of benzoic acids and its derivatives with thionyl chloride to form the corresponding acid chloride. These acid chlorides were treated with ammonium thiocyanate to produce 4- un/substituted benzoyl isothiocyanates **(1)**. On the other hand, ethoxymethylenemalononitrile and hydrazine hydrate 99% were heated under reflux in absolute ethanol to give 5-amino-1H-pyrazole-4-carbonitrile **(2).** Then both benzoyl isothiocyanates derivatives and compound **2** were stirred in dry acetone at room temperature then heated under reflux to give the respective thiourea derivatives **(3a-c)** which were verified by their ^{1}HNMR spectra, where an extra peak of exchangeable NH of thiourea group appeared at about δ of 14.03 ppm.

The thiourea derivatives **(3a-c)** were treated with ethyl bromide in the presence of sodium hydride in DMF and stirred at room temperature to generate the ethyl sulfanyl intermediates, which subsequently undergoes cyclization by heating under reflux to produce the desired pyrazolotriazine compounds **(4a-c).** The structures of compounds **(4a-c)** were confirmed with ^{1}HNMR spectra, where the characteristic peak of NH of the thiourea group disappeared and an extra two peaks of ethyl sulfanyl group appeared in the aliphatic region as quartet and triplet around δ of 3.33 and 1.51 ppm respectively.

The targeted compounds **7a-c** were synthesized by the same procedures starting from ethyl 5-amino-1H-pyrazole-4-carboxylate (**5**). Compound **5** was prepared as reported by Schmidt et al. [22] using ethyl 2-cyano-3-ethoxyacrylate and hydrazine in ethanol. The product (**5**) was separated in high purity and was confirmed by its reported melting point and its spectral and analytical data [22]. Compound (**5)** was subjected to the reaction with 4-un/substituted benzoyl isothiocyanates (**1a-c**) in acetonitrile to give thiourea derivatives (**6a-c**) [23]. The synthesized compounds (**6a-c**) were structurally elucidated by different analytical and spectral data. ^{1}HNMR signals were consistent with protons of the targeted compounds. The spectra showed two equally integrated signals at around δ 14.03 and 9.2 ppm representing the D_2O exchangeable protons of (NH) thiourea protons, in addition to the signals of the protons of the new aromatic ring system. FT-IR spectra revealed stretching signal of C=O amide around 1680 cm^{-1} Then thiourea derivatives (**6a-c**) were cyclized in the presence of ethyl bromide and NaH in DMF stirred at rt. for 30 min, to give the carbamimidothioate intermediates which were heated under reflux for 45–60 min to produce the desired compounds **(7a-c).**

The structures of compounds **(7a-c)** were confirmed by different analytical and spectral data. ^{1}HNMR signals were consistent with protons of the targeted compounds. The spectra showed additional peak at aliphatic region at around δ 3.30 and 1.49 ppm corresponding to the newly ethyl group, also disappearance of peaks corresponding to NH of thiourea. FT-IR revealed stretching band of C=O ester around 1750 cm^{-1} and absence of C=O of amide. The molecular weights of the titled compounds match their calculated ones.

Literature revealed the reaction of pyrazolo[1,5-*a*][1,3,5]triazine with appropriate amines by fusion and in this study, the 4-(4-un/substitutedphenyl)-2-(ethylthio)pyrazolo[1,5-*a*][1,3,5]triazine-8-carbonitril **(4a-c)** and primary alphatic amines, were fused at 150 °C for 1–1.5 h



Scheme 3. Synthesis of compounds 9a-o and 10a-c. Reagents and conditions: (A) Amine, fusion, 1.5–2 h, 150 °C, (B) Amine, fusion, 5–7 h, 150 °C.

to give corresponding pyrazolo[1,5-a] [1,3,5]triazine-8-carbonitrile **(8a-p)**. ^{1}HNMR of the entitled compounds were characterized by the preservation of the characteristic peaks of aromatic pyrazole protons and peak of exchangeable NH at δ 5.5–6.5 ppm. In addition to the appearance of the new peaks of the aryl protons for compounds **(8a**, **8b**, **8g**, **8h**, **8n** and **8o)** at δ 7.01–7.92, and the appearance of peaks in the aliphatic region at δ of 3.8 and 1.0 ppm for compounds **(8c**, **8d**, **8e**, **8i**, **8j**, **8k**, **8m** and **8p)**.

During the reaction of 3-aminoacetanilide **(A)** with ethyl sulfanyl pyrazolo[1,5-*a*][1,3,5]triazine **(4a, b)**, the expected amine derivative **(B)** was not obtained upon fusion for more than 1.5 h. Though, the sulfoxide derivative of the ethyl sulfanyl group **(8f, 8l)** were obtained instead. This could be explained by weak nucleophilicity of aromatic amine compared with aliphatic amine, as shown in Fig. 1.

The formation of this sulfoxide derivative **(8f, 8l)** was confirmed through ^{1}HNMR spectra which revealed the appearance of the two peaks in the aliphatic protons as quartet and triplet at δ 4.00 and 1.7 ppm respectively, which are more deshielded compared to the starting ethyl sulfanyl derivatives **(4a, 4b)** that appeared in δ 3.3 and 1.5 ppm due to the presence of the more electron withdrawing SO_2 group. Moreover, the absence of the additional aromatic protons, two NH, and acetyl moiety peaks confirmed the failure of the formation of the acetamidophenylamino derivatives of pyrazolo[1,5-*a*][1,3,5]triazine. FT-IR spectrum of compounds **(8f and l)** showed the appearance of peaks at 1257 and 1301 cm^{-1} respectively which represent SO_2 group, which is not found in the starting ethylsulfanyl compound **(4a)**. Additionally, the mass spectrum of the product **(8f and l)** revealed a molecular ion peaks of $m/z = 313.21$ and 347.48 respectively, corresponding to their molecular weights, while the starting sulfanyl derivative **(4a and 4b)** have molecular weights of 281.34 and 315.78 respectively.

After that an optimization of compounds **7a-c** was performed by nucleophilic substitution of ethylsulfanyl group of pyrazolo[1,5-*a*] [1,3,5]triazine with different amines to give compounds **9a-o.** this reaction was performed by fusion at 150 °C in oil bath. The control of the concentration of amines and time of reaction are required to prevent the reaction of ester group too.

The structures of compounds **9a-o** were confirmed by different analytical and spectral data. ^{1}HNMR spectra revealed the characteristic signals of the targeted compounds. First, disappearance of the two ethyl signals of ethylthio group and appearance of signals corresponding to different amines. Regarding compounds **9b, 9g and 9l** additional aliphatic protons showed at around δ 3.05 and 3.92 in addition to the signals of protons of the newly introduced aromatic rings and singlet peak at δ around 5.7 ppm representing the D_2O exchangeable protons of NH linker. Compounds **9a, 9f and 9k** showed one additional singlet peak at around δ 4.8 ppm corresponding to benzylic protons in addition to protons of the newly introduced aromatic rings and singlet peak at around δ 6.0 ppm representing the D_2O exchangeable protons of NH linker. Compounds **9c, 9h and 9m** showed two singlet peaks at around δ 4.02 and 1.7 ppm representing the protons of the piperidine rings. Compounds **9d, 9i, and 9n** showed two broad singlet peaks equally integrated at around δ 3.8 and 2.08 ppm representing pyrrolidine ring. Finally, compounds **9e, 9j and 9o** showed the appearance of signal of the methyl protons which usually overlaps with signal of methyl protons of ester group and the peak of proton near to the amine group appears as multiplet at around δ 4.15 ppm. Mass spectra of the titled compounds revealed that the molecular ion peaks are matched with

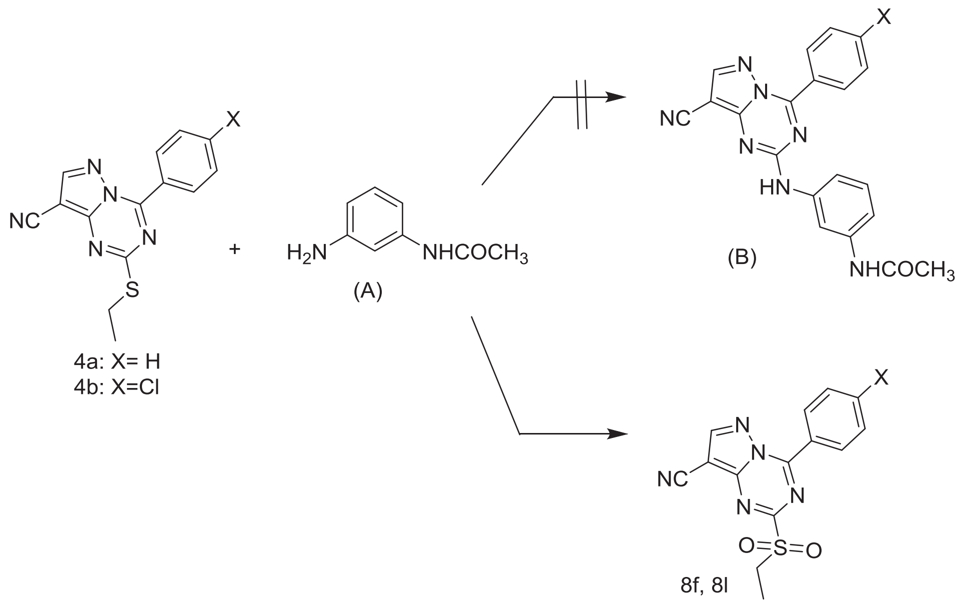


Fig. 1. Formation of compounds **(8f, 8l).**

their molecular weights, also compounds **(9f-j)** showed the presence of M^+ and $M^+ + 2$ peaks with relative intensity ratio of 3:1 corresponding to chlorine isotopes In addition, compounds **(9k-o)** showed the presence of M^+ and $M^+ + 2$ peaks with relative intensity ratio of 1:1 corresponding to bromine isotopes.

Further optimization of ester group occurred by direct fusion of benzylamine or 2-phenylethylamine with compounds **(7a, 7c)** at 150 °C in oil bath to give compounds **(10a-c)**. However, herein the concentration of amines is 5 equivalents and the time of reaction is longer so both ethylthio and ester groups reacted, so amines are added on both sides of compounds.

The structures of compounds **(10a-c)** were confirmed by different analytical and spectral data. ^{1}HNMR showed the disappearance of signals corresponding to ethylthio and ester groups. In addition to the appearance of signals of protons of the newly introduced aromatic rings, compounds **(10a, c)** showed two singlet peaks at 7.8 and 5.8 ppm corresponding to D_2O exchangeable protons of NH linkers, also four equally integrated peaks at aliphatic region representing two ethyl groups. Compound **(10b)** showed two singlet peaks at around 6.05 and 8.05 ppm corresponding to D_2O exchangeable protons of NH linkers and peaks of newly aromatic rings also appeared, in addition to two singlet peaks at δ around 4.62 and 4.52 ppm.

3.2. Biological evaluation

3.2.1. Antiproliferative activity in vitro against NCI 60-cell lines

Twenty of the final compounds were selected by the National Cancer Institute (NCI), NIH Bethesda, Maryland, USA (www.dtp.nci.nih.gov) under the Developmental Therapeutic Program (DTP), namely (**7a, 7b, 8c, 8f-h, 8j-m, 9a, 9f-k, 9m, 10a, 10c**). The obtained results were presented in Tables 1–3 in the supplementary part.

From the results obtained we can conclude that the 4-chloro phenyl derivative bearing benzyl amine group **(9f)** showed the highest cell growth inhibition with mean growth inhibition percentage of 41.77%. It exhibited broad spectrum and good anti-proliferative activity against several NCI cell panel as illustrated in Fig. 2.

Moreover, compound **(10c)**, bearing 2-phenylethan-1-amine in one side and phenylethyl amide in the other side together with 4-chloro substituted phenyl group, also exhibited considerable cell growth inhibition with mean growth inhibition percent of 33.32%. It exhibited broad spectrum and good inhibitory activity against certain cancer cells as illustrated in Fig. 3.

On the other hand, when comparing the results of compounds with their structures we observed that; compounds **(9a, 9f, 9k)**, which bear the unsubstituted analogue, chloro substituted, and the bromo

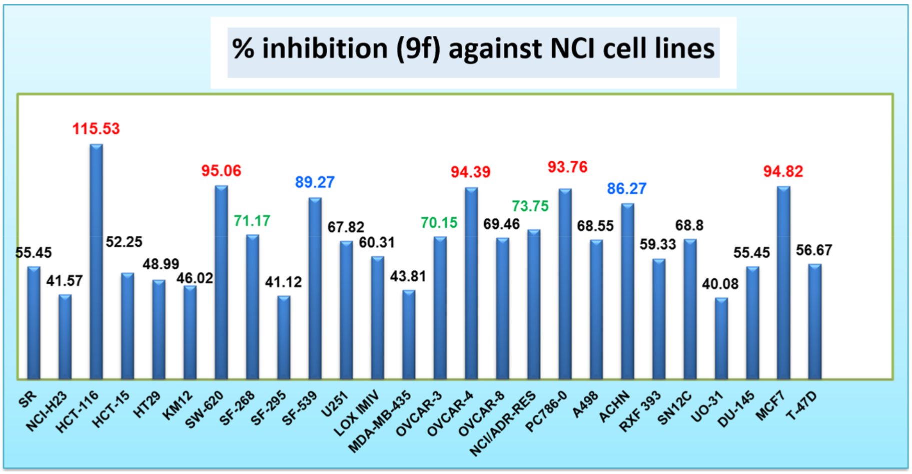


Fig. 2. %Inhibition of compound **(9f)** against 26 cell line from 56 NCI cell line that exhibit % inhibition range from 40% to 115%.

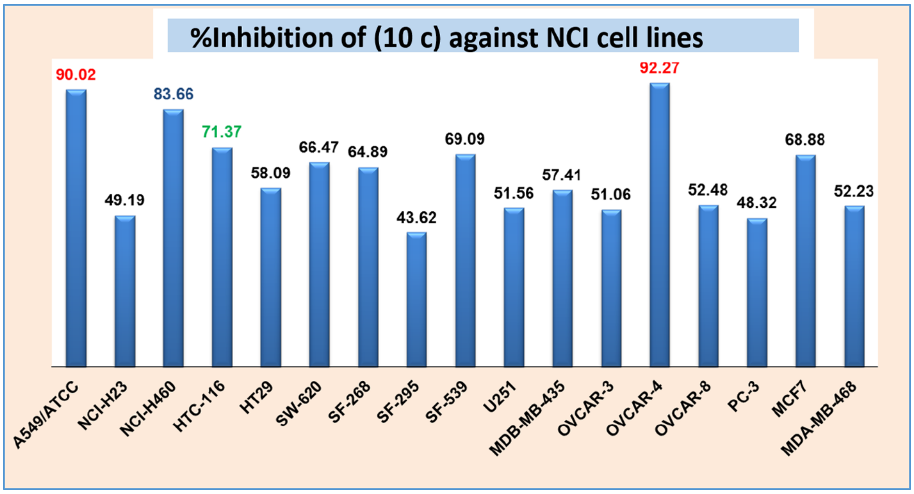


Fig. 3. % inhibition of compound **10c** against 17 cell line from 56 NCI cell line that exhibit % inhibition range from 43% to 92%.

substituted one respectively if ordered in their antiproliferative activity descendingly, their order will be **9f, 9k** then **9a**. So the chloro substituted analogue is the most active, then the bromo and the least is the unsubstituted analogue. Likewise, compounds **(7b, 8f),** bearing chloride group, exhibited more activity than unsubstituted ones **(7a, 8l).** Accordingly, we can say that the presence of chloride enhanced the activity of compounds **(7b, 9g and 10c)** on the selected NCI cell lines.

3.2.2. *In-vitro cyclin dependent kinase2 inhibitory activity*

The percentage inhibition of the enzymatic activity caused by tested compounds against CDK2/CyclinA kinases at a single concentration of 10 μM was investigated. Compounds **(8a, 8g, 8h, 8n, 9f, 9g, 9h, 9k, 9m, 10a and 10c)** were selected as representatives that exhibited significant CDK2-cyclin A inhibition percent at 10 μM concentration for further investigation for dose related CDK2-cyclin A enzymatic inhibition at 5 different concentrations (1 nM-10 nM-100 nM- 1 μM- 10 μM) to subsequently calculate their IC_{50} value. The results were shown in Table 4.

An overview of the results of CDK2 enzymatic activity at 10 μM concentration, compound **(9f)** bearing 4-chloro substituted phenyl group and benzyl amine and ester side chain has demonstrated good CDK2 inhibition (82.38%). Also compound **(10c)** bearing 4-chloro substituted and 2-phenylethan-1-amine in both sides exhibited good CDK2 inhibition (81.96%). On the other hand, several other investigated compounds namely **(8g, 8h, 9a, 9b, 9c, 9h, 9g, 9k, 9l, 9 m, 10a, and 10b)** exhibited significant CDK2 inhibition (above 70%).

From the above studies, structure activity relationship among the newly synthesized pyrazolo[1,5-*a*][1,3,5]triazine derivatives can be concluded as follows

- In general, the 8-carboxylate compounds have more inhibitory activity than the 8-carbonitrile congeners (**7a** compared to **4a**, 66.75% and 53.32% respectively, **9a** compared to **8a**, 70.01% and 68.34% respectively).
- Also, the 2-amino derivatives are more active than the 2-ethyl sulphide analogues (8a compared to **4a**, 68.34% and 53.32% respectively, **9a** compared to **7a,** 70.01% and 66.75% respectively).
- 4 -Chloro substitution at phenyl ring enhanced the CDK2 inhibitory activity as shown by compounds **(8g, 8h)** (72.25, 74.01%) **(9f-j)** (61.96–82.38%) respectively when compared to bromo substitution **(8n, 8o)** (6153, 52.84%) **(9k-o)** (58.65–78.07%) respectively or unsubstituted compounds **(9a-e)** (51.93–72.42%) respectively.
- Replacement of 2-ethylthio group of pyrazolo [1,5-*a*][1,3,5]triazine derivatives with phenyl alkyl amine enhanced the activity compared to ethyl thio and aliphatic or cyclic amines.
- Optimization of the 8-carboxylate ester group into amide group was carried out to increase the hydrophilicity of the side chain oriented in the solvent accessible region, which resulted in increase in CDK2 inhibitory activity, (**9a** compared to **10a**, 70.01% and 75.56%, respectively, **9b** compared to **10b**, 72.42% and 73.89%, respectively and **9g** compared to **10c**, 74.73% and 81.96%, respectively).

3.3. *In silico results*

3.3.1. *Results of docking of the target compounds into CDK2 active sites*

The docking of the synthesized compounds into CDK2 active site revealed that the designed compounds kept the key interactions done by the lead compound roscovitine co-crystallized with CDK2/cyclin A (PDB: code **3ddq)** which was reported to be essential for activity and being sandwiched between the side chains of Leu134 and Ile10 [25] (Fig. 4).

Docking of the target compounds showed that synthesized compounds have similar binding mode with comparable docking scores to the lead compound.

The performed docking study showed interesting results. Most of the targeted compounds **(4b, 4c, 7a-c, 8h, 8j, 8k, 8m, 8o, 9c-f, 9h-k, 9m-o)** form a conserved hydrogen bond pattern where the N atom of pyrazolo ring accepts proton from the backbone Leu 83 residue in the hinge region, which is crucial for CDK2 inhibitory activity. The two compounds **(9f and 9k)** have significant percentage inhibition of CDK2 (82.38 and 78.07% respectively) may be due to presence of aromatic amine and halide group which appear in all significant active compounds. In addition, compound (**9f**) which has the highest percentage inhibitory activity has the highest docking score among the series (Figs. 5 and 6).

Furthermore, the five compounds **(4a, 8f, 8i, 8l and 8p)** which showed lower inhibitory activity against CDK2/cyclinA2 (53.32, 27.73, 16, 42.12 and 36.63% respectively), were not bound to Leu83 and that may explain their very poor biological activity against CDK2.

It has been reported that several roscovitine bioisosteres compounds were potent though they had a reversed binding with CDK2 to that of roscovitine. For target compounds **(8a, 8g, 8n, 9a, 9b, 6f, 9g, and 10a-c)**, possessed the reversed binding mode of roscovitine where the amino NH– was bound to Leu 83 through hydrogen bond in the hinge region, their CDK2 inhibitory activity were (68, 72, 61, 72.42, 70.01, 73.49, 74.73, 75.56, 73.89 and 81.96%, respectively). In addition, the activity of these compounds may be due to the presence of another hydrogen bond with backbone Leu83 residue through the ester group in them. On the other hand, compound **(10c)** has the highest docking score and the most active between them, that may be due to the presence of another

Table 4
Percentage inhibition of CDK2 enzymatic activity of targeted compounds and calculated IC_{50} of compounds **8a, g, h, n, 9f, g, h, k, m, 10a, c.**

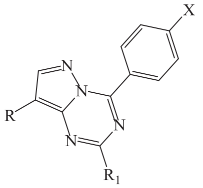
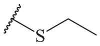
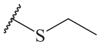
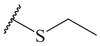
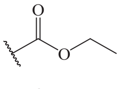
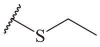
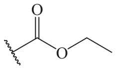
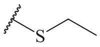
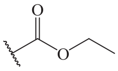
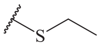
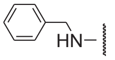
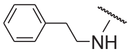
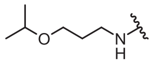
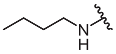
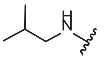
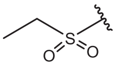
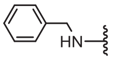
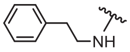
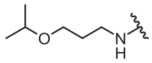
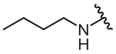
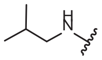
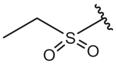
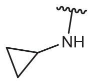
Compound number				CDK_2% inhibition at 10 μM	CDK2/Cyclin A IC_{50} (μM)
	X	R	R_1		
4a	H	CN		53.32	ND
4b	Cl	CN		27.21	ND
4c	Br	CN		36.65	ND
7a	H			66.75	ND
7b	Cl			69.74	ND
7c	Br			67.62	ND
8a	H	CN		68.34	2.84
8b	H	CN		57.25	ND
8c	H	CN		28.36	ND
8d	H	CN		17.45	ND
8e	H	CN		54.67	ND
8f	H	CN		27.73	ND
8g	Cl	CN		72.25	3.58
8h	Cl	CN		74.01	1.89
8i	Cl	CN		16.00	ND
8j	Cl	CN		23.85	ND
8k	Cl	CN		2.00	ND
8l	Cl	CN		42.12	ND
8m	Cl	CN		10.35	ND

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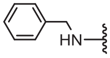
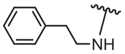
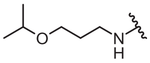
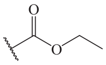
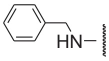
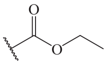
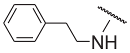
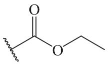
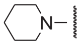
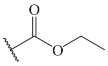
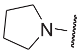
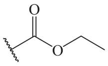
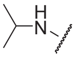
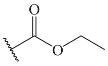
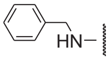
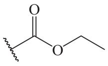
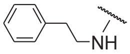
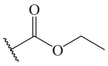
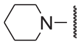
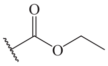
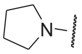
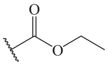
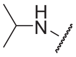
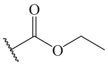
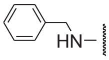
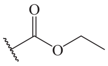
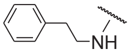
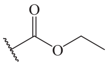
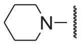
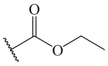
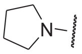
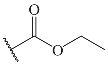
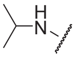
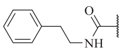
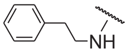
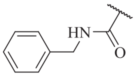
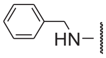
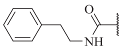
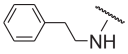
Table 4 (*continued*)

8n	Br	CN		61.53	3.11
8o	Br	CN		52.84	ND
8p	Br	CN		36.63	ND
9a	H			70.01	ND
9b	H			72.42	ND
9c	H			71.21	ND
9d	H			51.93	ND
9e	H			68.14	ND
9f	Cl			82.38	1.85
9g	Cl			74.73	2.11
9h	Cl			74.28	3.51
9i	Cl			67.35	ND
9j	Cl			61.96	ND
9k	Br			78.07	2.3
9l	Br			73.49	ND
9m	Br			74.55	6.8
9n	Br			58.65	ND
9o	Br			61.40	ND
10a	H			75.56	8.5
10b	H			73.89	ND
10c	Cl			81.96	2.09
Roscovitine	-	-	-	89.6	ND

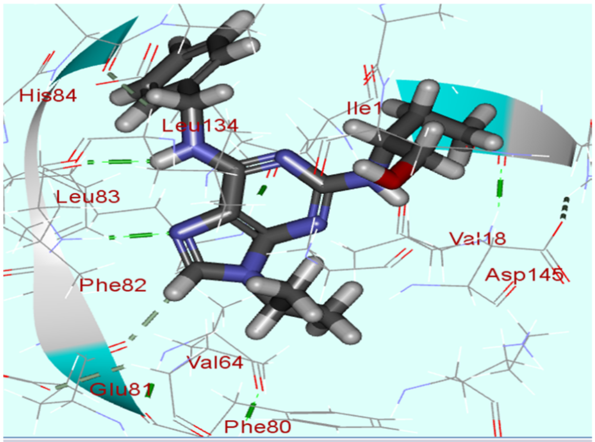


Fig. 4. 3D interaction diagram of the top docking pose of the roscovitine in the active site

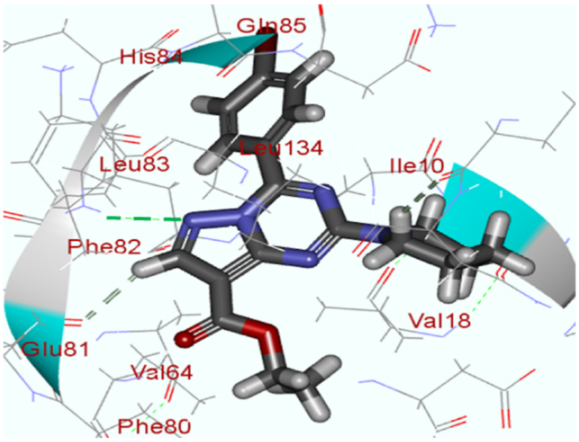


Fig. 5. 3D interaction diagram of the top docking pose of the compound **9f**

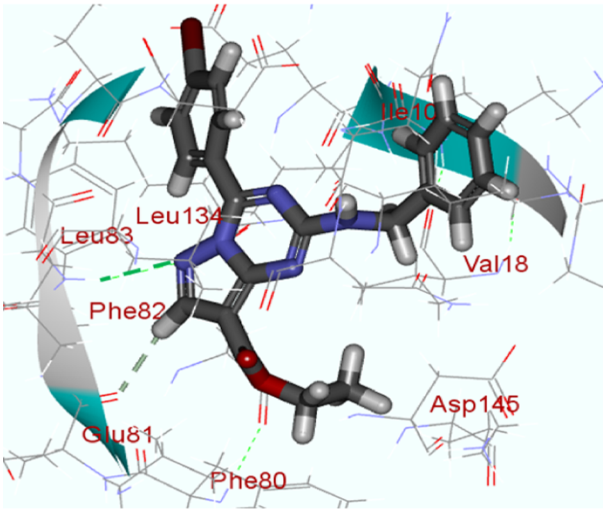


Fig. 6. 3D interaction diagram of the top docking pose of the compound **9k.**

hydrogen bonding with Leu83 through O-carbonyl group and the presence of chloride group in it which significantly increases the activity. In addition, the presence of hydrophobic groups on both sides of the scaffold is favorable to occupy two deep hydrophobic pockets made up of Phe82, Val64 and His84, Gln85 (Fig. 7).

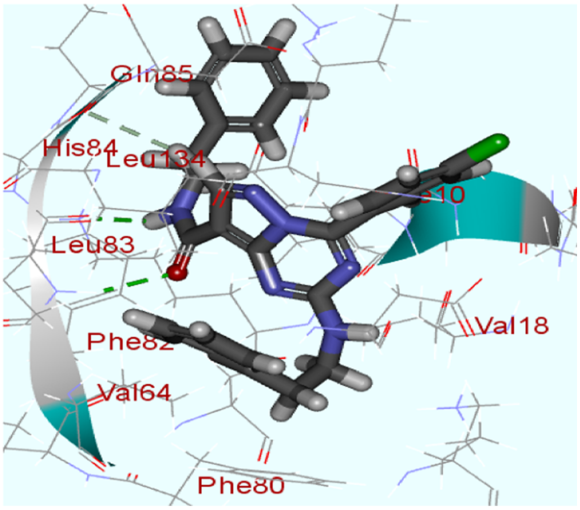


Fig. 7. 3D interaction diagram of the top docking pose of the compound **10c.**

3.3.2. In silico ADMET study

Most of the aqueous solubility of compounds is low except compound **(8c)** which had good aqueous solubility level and compounds **9l, 9k, 9g and 10c** which are very low but soluble. All compounds showed HIA level 0 which means that these compounds are expected to be well absorbed, except of **10a and 10c** which bear the same amine group. Blood brain barrier penetration level of compounds was found to be 1 or 2 which indicated high to medium BBB penetration; except compound **(8c)** showed low level of BBB penetration and compounds **8f, 8l, 9l, 10a and 10c** which showed undefined penetration. The CYP2D6 score predicts the inhibitory and non-inhibitory character of the given chemical structure on Cytochrome P450 2D6 enzyme. Seventeen of the compounds with 0 level were predicted as non-inhibitors; hence no drug-drug interactions would be expected upon administration of these compounds.

The PBB level of half of the compounds was > 95%, thus these drugs would exhibit longer $t_{1/2}$ and hence less frequent drug administration. Other compounds were varied between level 0 and 1. The hepatotoxicity level of half of compounds was 0 which indicate non-toxic compound, but Further experimental studies are required to determine the hepatotoxic dose levels for compounds with hepatotoxicity level. The calculated parameters from the ADMET study are illustrated in **Table 5** in the supplementary part.

4. Conclusion

Thirty-four novel pyrazolo[1,5-*a*][1,3,5]triazine derivatives **8a-p, 9a-o, and 10a-c** were synthesized by fusion via nucleophilic substitution of ethylsulfanyl group of pyrazolo[1,5-*a*][1,3,5]triazine **(4a-c, and 7a-c)** with different amines. Compounds **7a, 7b, 8c, 8f-h, 8j-m, 9a, 9f-k, 9m, 10a, 10c** were selected by NCI, USA and screened against 60 cancer cell lines. It was found that, Ethyl 2-(benzylamino)-4-(4-chlorophenyl)pyrazolo[1,5-a][1,3,5]triazine-8-carboxylate (**9f**) showed the highest cell growth inhibition with mean growth inhibition percentage of 41.77%, and compound 4-(4-Chlorophenyl)-*N*-phenethyl-2-(phenethylamino)pyrazolo[1,5-a][1,3,5] triazine-8-carboxamide **(10c)**, also exhibited considerable cell growth inhibition with mean growth inhibition percent of 33.32%. Furthermore, all compounds **(4a-c, 7a-c, 8a-p, 9a-o, and 10a-c)** were screened for their *In-vitro* Cyclin dependent kinase2 inhibitory activity. **9f** was the most active compound followed by **10c** which matches the NCI results. In general, the chloro substituted analogues are the most active, then the bromo and the least are the unsubstituted analogues. Also, the 8-carboxylate compounds have more inhibitory activity than the 8-carbonitrile congeners. Molecular docking study indicated the significant interactions between compounds **9f and 10c** and CDK2 enzyme. Thereby, it could be demanded that pyrazolo triazine derivatives represented a talented starting point for further study as anticancer drug.

Acknowledgements

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103239.

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