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SYNTHESIS OF NOVEL PYRROLOPYRIMIDINE DERIVATIVES AS CDK2 INHIBITORS

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ABSTRACT

The reaction of pyrrolopyrimidin-4-thiones (1a-g) with ethyl chloroacetate afforded the corresponding esters 2a-g which were further treated with hydrazine hydrate to obtain the acetohydrazides 3a-g in order to synthesize several pyrrolopyrimidine derivatives 4a-g – 8a-j. All the newly synthesized compounds were confirmed by the elemental analyses and further supported by the spectral data. Thirty-five compounds of the newly synthesized pyrrolopyrimidine derivatives were selected by National Cancer Institute (NCI) for single dose testing against 60 cell lines. Compounds 2a, 8a, 8b, 8g and 8i showed the highest anti-cancer activity. The action of these compounds by molecular docking studies against CDK2 was interpreted in the current study.

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Introduction

Cancer is considered as one of the major health problemsin the world, especially, in low- and middle-income countries (LMIC) where approximately 70% of deaths from cancer occur. [1-3] Worldwide, cancer is the second leading cause of death and about one in six deaths is due to cancer. [4] Cancer in the Middle East is on the rise and it is the fourth cause of death on the region. [5, 6] As a result of poor healthcare access, unhealthy lifestyles and limited access for treatment, cancer incidence increases much faster than any region in the world. In the region, the vast majority of cancers are diagnosed at an advanced stage when curing them was improbable, leading to higher mortality rates and increased health-care costs.

Current cancer therapies encounter poor therapeutic outcomes and serious side effects, in addition to acquired resistance to drugs used for treatment. Furthermore, there is an urgent need for the discovery of new cancer-targeted drugs. Generally, Protein kinases have crucial roles in regulating cellular growth and survival, [7-9] from them; cyclin-dependent kinases (CDKs) are a family of protein kinases whose activity depends on association with a non-catalytic regulatory subunit called a cyclin. There are 20 different CDKs in the human genome which have been recently renamed as Cdk1 through to Cdk20. [10] CDKs promote chromosomal DNA replication and their mutation lead to abnormal CDK activity and consequently uncontrolled cellular proliferation that is what happens in many tumors. [10, 11]

Therefore, great efforts have been expended in developing CDK inhibitors as anticancer agents. [12-23] **Figure 1a** shows some examples of nonselective CDK inhibitors, [12-19] while **figure 1b** illustrates some of selective CDK2 inhibitors. [20-23].

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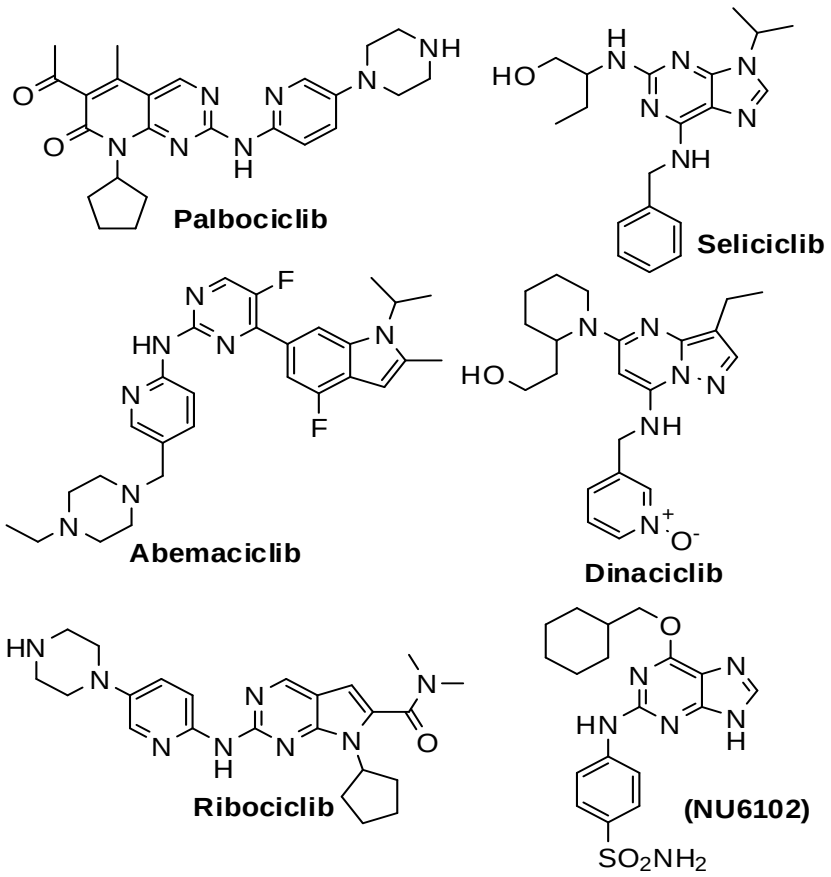


Figure 1a. Non-Selective CDK inhibitors.

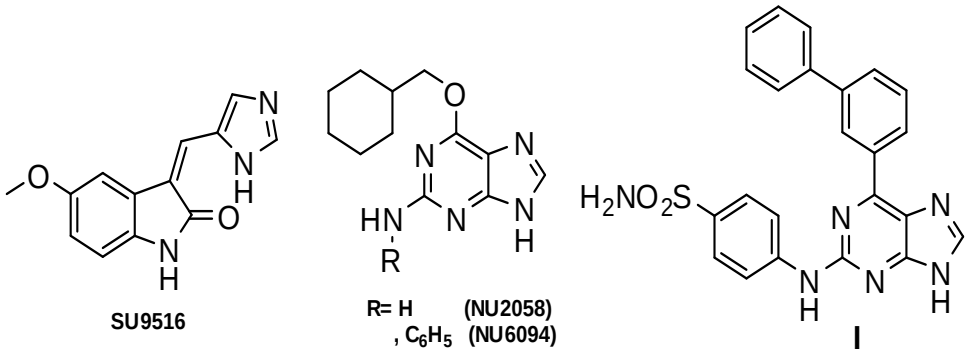


Figure 1b. Selective CDK2 inhibitors.

It's observed that the highest common chemical structure factor of the approved drugs for anti-cancer treatment and clinical trials involves purine and their analogs. Moreover, literature survey indicated that pyrroles and pyrrolo[2,3-*d*] pyrimidines are of considerable interest in drug discovery. Particularly, Pyrrolo[2,3-*d*] pyrimidines as 7-deazapurines possess remarkable biological activity due to their resemblance to cellular purines, especially, [24-29] as anticancer (**figure 1c**) and antiviral agents. [30-37]

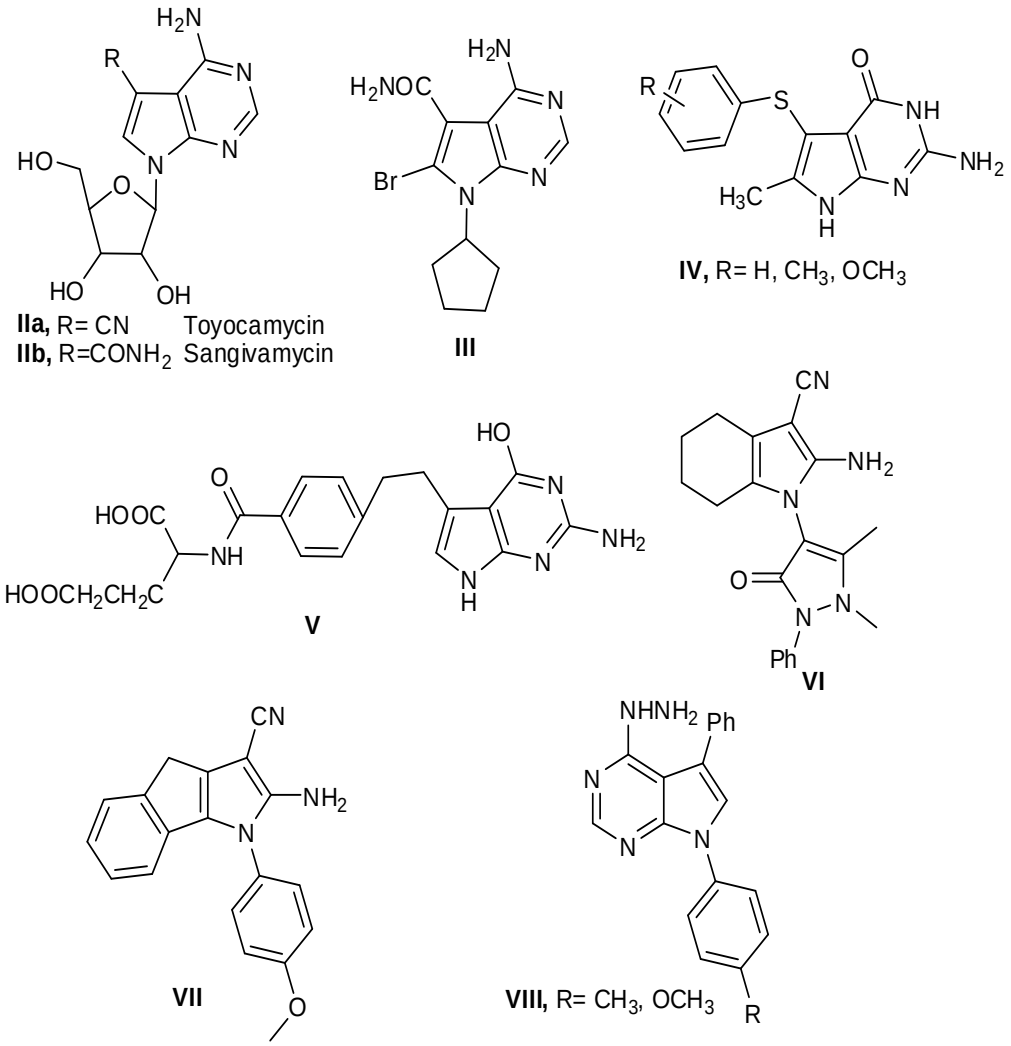


Figure 1c. Pyrrolopyrimidines and their analoges as anticancer agents.

The above findings motivated us to continue our previous work, [29] and introduce this study that involves synthesis of new pyrrolopyrimidines as purine analogs to investigate their anticancer activity against 60 different human tumor cell lines, representing cancers of the lung, colon, CNS, ovary, breast, prostate, and kidney, in addition to leukemia and melanoma. Finally, the binding mode of the synthesized compounds with CDK2 will be predicted using molecular docking studies.

Materials and Methods

Synthesis of lead compounds

All commercial chemicals used as starting materials and reagents in this study were purchased from Merck (Darmstadt, Germany) and were of reagent grade. All melting points were uncorrected and measured using Electro-thermal IA 9100 apparatus (Shimadzu, Japan); IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (USA), Faculty of Science, Cairo University, Cairo, Egypt. ^{1}H-NMR spectra were determined on a Varian Mercury (300 MHz) spectrometer (Varian UK) and chemical shifts were expressed as ppm against TMS as internal reference (The Main Chemical Warfare Laboratories, Almaza, Cairo, Egypt). Mass spectra were recorded on 70 eV (EI Ms-QP 1000 EX, Shimadzu, Japan), Faculty of Science, Cairo University, Cairo, Egypt. Microanalyses were operated using Vario, Elmentar apparatus (Shimadzu, Japan), Organic Microanalysis Unit, Faculty of Science, Cairo University, Cairo, Egypt. Column Chromatography was performed on (Merck) Silica gel 60 (particle size 0.06-0.20 mm). All compounds prepared in this paper are new and confirmed with spectral data except **1a-g** were previously prepared by the authors. [37-39]

General procedure for the synthesis of compounds 2a-g: Ethyl chloroacetate (0.01mol) was added drop wise to a hot solution of pyrrolopyrimidin-4-thiones (**1a-g**) and sodium hydroxide in ethanol as a solvent. The mixture was refluxed for two hours, then it filtered and the filtrate poured onto ice and left for one hour. The formed solid was collected and recrysallized from ethanol.

Ethyl-2-(5,6-diphenyl-7-(4-methylphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetate (2a): Yield: 82%; m.p.: 223-225 °C; IR (KBr) υ (cm^{-1}): 1726 (C=O), 1576 (C=N), 1315 (C-O); MS (EI) m/z:479 (M+, 35%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 1.2 (t, 3H, CH_2-CH_3*), 2.23 (s, 3H, Ph-CH_3), 4.4 (q, 2H, OCH_2*-CH_3), 4.5 (s, 2H, S-CH_2), 6.9-7.7 (m, 14H, Ar-H), 8.7 (s, 1H, C_2-H); Anal. Calcd for $C_{29}H_{25}N_3O_2S$ (479.59): C, 72.65; H, 5.30; N, 8.77; S, 6.68%. Found: C, 72.80; H, 5.11; N, 8.65; S, 6.44%.

Ethyl-2-(5,6-diphenyl-7-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetate (2b): Yield: 87%; m.p.: 212-214 °C; IR (KBr) υ (cm^{-1}):1733 (C=O), 1605 (C=N), 1321 (C-O); MS (EI) m/z:495 (M+, 51%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 1.26 (t, 3H, CH_2-CH_3*), 3.47 (s, 3H, Ph-OCH_3), 4.28 (q, 2H, OCH_2*-CH_3), 4.29 (s, 2H, S-CH_2), 6.9-7.5 (m, 14H, Ar-H), 8.87 (s, 1H, C_2-H); Anal. Calcd for $C_{29}H_{25}N_3O_3S$ (495.59): C, 70.30; H, 5.05; N, 8.48; S, 6.46%. Found: C, 70.22; H, 5.34; N, 8.62; S, 6.77%.

Ethyl-2-(5-phenyl-7-(4-methylphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetate (2c): Yield: 60%; m.p.: 198-200 °C; IR (KBr) υ (cm^{-1}):1729 (C=O), 1599 (C=N), 1291 (C-O); MS (EI) m/z:403 (M+, 12.5%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 1.31 (t, 3H, CH_2-CH_3*), 2.32 (s, 3H, Ph-CH_3), 4.32 (q, 2H, OCH_2*-CH_3), 4.4 (s, 2H, S-CH_2), 7.0-7.8 (m, 10H, Ar-H), 9.1 (s, 1H, C_2-H); Anal. Calcd for $C_{23}H_{21}N_3O_2S$ (403.50): C, 68.49; H, 5.21; N, 10.42; S, 7.94%. Found: C, 68.70; H, 5.03; N, 10.15; S, 8.21%.

Ethyl-2-(5-phenyl-7-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetate (2d): Yield: 87%; m.p.: 188-190 °C; IR (KBr) υ (cm^{-1}): 1733 (C=O), 1595 (C=N), 1317 (C-O); MS (EI) m/z: 419 (M+, 32.7%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 1.3 (t, 3H, CH_2-CH_3*), 3.4 (s, 3H, Ph-OCH_3), 4.2 (q, 2H, OCH_2*-CH_3), 4.44 (s, 2H, S-CH_2), 7.0-7.9 (m, 10H, Ar-H), 9.08 (s, 1H, C_2-H); Anal. Calcd for $C_{23}H_{21}N_3O_3S$ (419.50): C, 65.87; H, 5.01; N, 10.02; S, 7.64%. Found: C, 66.03; H, 5.11; N, 9.85; S, 7.91%.

Ethyl-2-(7-cyclohexyl-5-phenyl-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetate (2e): Yield: 53%; m.p.: 219- 221 °C; IR (KBr) υ (cm^{-1}): 1708 (C=O), 1587 (C=N), 1311 (C-O); MS (EI) m/z: 395 (M+, 36.2 %); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 1.2 (t, 3H, CH_2-CH_3*), 1.4 - 1.92 (m, 10H, cyclohexyl), 3.9 (m, 1H, CH-N cyclohexyl), 4.3 (q, 2H, OCH_2*-CH_3), 4.42 (s, 2H, S-CH_2), 7.0-7.8 (m, 6H, Ar-H), 9.05 (s, 1H, C_2-H); Anal. Calcd for $C_{22}H_{25}N_3O_2S$ (395.52): C, 66.84; H, 6.33, N, 10.63, S, 8.10 %. Found: C, 67.09; H, 5.98; N, 10.51, S, 8.14%.

Ethyl-2-(7-(4-chlorophenyl)-5-phenyl-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetate (2f): Yield: 51%; m.p.: 237- 239 °C; IR (KBr) υ (cm^{-1}):1734 (C=O), 1576 (C=N), 1300 (C-O); MS (EI) m/z: 423 (M+, 37.8 %), 425 (M+2, 12%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 1.27 (t, 3H, CH_2-CH_3*), 4.2 (q, 2H, OCH_2*-CH_3), 4.37 (s, 2H, S-CH_2), 6.8-7.7 (m, 10H, Ar-H), 8.89 (s, 1H, C_2-H); Anal. Calcd for $C_{22}H_{18}ClN_3O_2S$ (423.92): C, 62.41; H, 4.26, Cl, 8.27, N, 9.93, S, 7.57 %. Found: C, 62.09; H, 3.88; Cl, 8.56, N, 9.67, S, 7.66 %.

Ethyl-2-(7-(3-chlorophenyl)-5,6-diphenyl-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetate (2g): Yield: 52%; m.p.: 228-230 °C; IR (KBr) υ (cm^{-1}):1717 (C=O), 1596 (C=N), 1303 (C-O); MS (EI) m/z: 499 (M+, 16 %), 501 (M+2, 5%); H^1-NMR (DMSO-d6, 300 MHz) δ (ppm): 1.11 (t, 3H, CH_2-CH_3*), 4.3 (q, 2H, OCH_2*-CH_3), 4.9 (s, 2H, S-CH_2), 6.9-7.9 (m, 14H, Ar-H), 8.8 (s, 1H, C_2-H); Anal. Calcd for $C_{28}H_{22}ClN_3O_2S$ (500.01): C, 67.20; H, 4.40, Cl, 7.0, N, 8.40, S, 6.40 %. Found: C, 66.98; H, 4.58; Cl, 7.14, N, 8.61, S, 6.59 %.

General procedure for the synthesis of compounds 3a-g: A mixture of compound **2a-g** (0.01 mol) and hydrazine hydrate (5 mL,0.015 mol, 98%) was heated under reflux in dry ethanol (30 mL) for four hours, cooled, poured onto ice-water to give precipitate which was filtered off, dried, and recrystallized from methanol to give compounds **3a-g**.

2-(5,6-diphenyl-7-(4-methylphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetohydrazide (3a): Yield: 89%; m.p.: 234-236 °C; IR (KBr) υ (cm^{-1}): 3373, 3352 (NH_2), 3212 (N-H), 1654 (C=O), 1591 (C=N); MS (EI) m/z: 465 (M+, 28.3%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 2.23 (s, 3H, Ph-CH_3), 3.8 (s, 2H, S-CH_2), 4.53 (s, 2H, NH_2, D_2O exchangeable), 7.0-8.2 (m, 14H, Ar-H), 9.3 (s, 1H, C_2-H), 10.22 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{27}H_{23}N_5OS$ (465.57): C, 69.68; H, 4.95; N, 15.05; S, 6.88%. Found: C, 69.83; H, 5.23; N, 15.36; S, 6.45%.

2-(5,6-diphenyl-7-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetohydrazide (3b): Yield: 96%; m.p.: 227-229 °C; IR (KBr) υ (cm^{-1}): 3740, 3720 (NH_2), 3454 (N-H), 1780 (C=O), 1603 (C=N), 1242 (C-O); MS (EI) m/z: 481 (M+, 9.3%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.5 (s, 3H, Ph-OCH_3), 4.3 (s, 2H, S-CH_2), 4.57 (s, 2H, NH_2, D_2O exchangeable), 6.9-7.9 (m, 14H, Ar-H), 8.98 (s, 1H, C_2-H), 9.1 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{27}H_{23}N_5O_2S$ (481.57): C, 67.36; H, 4.78; N, 14.55; S, 6.65%. Found: C, 67.71; H, 4.44; N, 14.69; S, 6.98%.

2-(5-phenyl-7-(4-methylphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetohydrazide (3c): Yield: 92%; m.p.: 210-212 °C; IR (KBr) υ (cm^{-1}):3416, 3383 (NH_2), 3195 (N-H), 1640 (C=O), 1576 (C=N); MS (EI) m/z: 389 (M+, 48%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 2.31 (s, 3H, Ph-CH_3), 4.3 (s, 2H, S-CH_2), 4.6 (s, 2H, NH_2, D_2O exchangeable), 6.8-7.9 (m, 10H, Ar-H), 9.08 (s, 1H, C_2-H), 9.2 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{21}H_{19}N_5OS$ (389.47): C, 64.78; H, 4.88; N, 17.99; S, 8.23%. Found: C, 64.71; H, 5.11; N, 17.62; S, 8.46%.

2-(5-phenyl-7-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetohydrazide (3d): Yield: 97%; m.p.: 203-205 °C; IR (KBr) υ (cm^{-1}): 3429, 3377 (NH_2), 3186 (N-H), 1646 (C=O), 1593 (C=N), 1328 (C-O); MS (EI) m/z: 405 (M+, 30.4%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.5 (s, 3H, Ph-OCH_3), 4.31 (s, 2H, S-CH_2), 4.5 (s, 2H, NH_2, D_2O exchangeable), 6.9-7.9 (m, 10H, Ar-H), 9.11 (s, 1H, C_2-H), 9.2 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{21}H_{19}N_5O_2S$ (405.47): C, 62.22; H, 4.69; N, 17.28; S, 7.90%. Found: C, 62.41; H, 4.43; N, 17.69; S, 8.15%.

2-(7-cyclohexyl-5-phenyl-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetohydrazide (3e): Yield: 81%; m.p.: 231- 233 °C; IR (KBr) υ (cm^{-1}):3379, 3320 (NH_2), 3194 (N-H), 1637 (C=O), 1601 (C=N); MS (EI) m/z: 381 (M+, 71.8 %); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 1.43 - 2.0 (m, 10H, cyclohexyl), 3.6 (m, 1H, CH-N cyclohexyl), 3.9 (s, 2H, S-CH_2),4.4 (s, 2H, NH_2, D_2O exchangeable), 7.0-7.9 (m, 6H, Ar-H), 9.03 (s, 1H, C_2-H), 9.12 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{20}H_{23}N_5OS$ (381.49): C, 62.99; H, 6.04; N, 18.37, S, 8.40%. Found: C, 63.18; H, 6.35, N, 18.61, S, 8.22 %.

2-(7-(4-chlorophenyl)-5-phenyl-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetohydrazide (3f): Yield: 82%; m.p.: 216- 218 °C; IR (KBr) υ (cm^{-1}): 3394, 3371 (NH_2), 3244 (N-H), 1658 (C=O), 1604 (C=N); MS (EI) m/z: 409 (M+, 41.5 %), 411 (M+2, 14.8%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm):3.5 (s, 2H, S-CH_2), 4.7 (s, 2H, NH_2, D_2O exchangeable), 7.0-7.9 (m, 10H, Ar-H), 9.1 (s, 1H, C_2-H), 9.24 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{20}H_{16}ClN_5OS$ (409.89): C, 58.68; H, 3.91, Cl, 8.56, N, 17.11, S, 7.82 %. Found: C, 58.39; H, 3.85; Cl, 8.36, N, 17.33, S, 7.61 %.

2-(7-(3-chlorophenyl)-5,6-diphenyl-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetohydrazide (3g): Yield: 78%; m.p.: 237-239 °C; IR (KBr) υ (cm^{-1}): 3412, 3320 (NH_2), 3237 (N-H), 1663 (C=O), 1596 (C=N); MS (EI) m/z: 485 (M+, 25 %), 487 (M+2, 9.2%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 4.05 (s, 2H, S-CH_2), 5.0 (s, 2H, NH_2, D_2O exchangeable), 6.7-7.8 (m, 14H, Ar-H), 9.1 (s, 1H, C_2-H), 11.8 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{26}H_{20}ClN_5OS$ (485.99): C, 64.33; H, 4.12, Cl, 7.22, N, 14.43, S, 6.60 %. Found: C, 64.58; H, 4.37; Cl, 7.12, N, 14.67, S, 6.50 %.

General procedure for the synthesis of compounds 4a-g: A mixture of compound **3a-g** (0.002mol) and ethyl cyanoacetate (0.002mol) was heated under reflux in dry ethanol (20 mL) for eight hours, cooled, poured onto ice-water to give precipitate which was filtered off, dried, and recrystallized from methanol to give compounds **4a-g**.

1-(3-Amino-5-hydroxy-1*H*-pyrazol-1-yl)-2-(5,6-diphenyl-7-(4-methylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ylthio)ethanone (4a): Yield: 71%; m.p.: 217-219 °C; IR (KBr) υ (cm^{-1}): 3492 (OH), 3382, 3364 (NH_2), 1716 (C=O), 1594 (C=N), 1283 (C-O); MS (EI) m/z: 532 (M+, 19.3%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 2.27 (s, 3H, Ph-CH_3), 4.05 (s, 2H, S-CH_2), 5.5 (s, 2H, NH_2, D_2O exchangeable), 6.8-7.8 (m, 15H, Ph-H+ H-pyrazole), 9.1 (s, 1H, C_2-H), 10.9 (s, 1H, OH, D_2O exchangeable); Anal. Calcd for $C_{30}H_{24}N_6O_2S$ (532.62): C, 67.67; H, 4.51; N, 15.79; S, 6.02%. Found: C, 67.86; H, 4.29; N, 16.03; S, 5.94%.

1-(3-Amino-5-hydroxy-1*H*-pyrazol-1-yl)-2-(5,6-diphenyl-7-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ylthio)ethanone (4b): Yield: 75%; m.p.: 241-243 °C;IR (KBr) υ (cm^{-1}): 3461 (OH), 3389, 3371 (NH_2), 1736 (C=O), 1604 (C=N), 1291 (C-O); MS (EI) m/z: 548 (M+, 53%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.4 (s, 3H, Ph-OCH_3), 4.11 (s, 2H, S-CH_2), 5.7 (s, 2H, NH_2, D_2O exchangeable), 6.9-8.0 (m, 15H, Ph-H+ H-pyrazole), 9.12 (s, 1H, C_2-H), 11.2 (s, 1H, OH, D_2O exchangeable); Anal. Calcd for $C_{30}H_{24}N_6O_3S$ (548.61): C, 65.69; H, 4.38; N, 15.33; S, 5.84%. Found: C, 65.29; H, 4.54; N, 15.62; S, 6.09%.

1-(3-Amino-5-hydroxy-1*H*-pyrazol-1-yl)-2-(5-phenyl-7-(4-methylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ylthio)ethanone (4c): Yield: 63%; m.p.: 233-235 °C; IR (KBr) υ (cm^{-1}): 3477 (OH), 3173, 3155 (NH_2), 1742(C=O), 1604 (C=N), 1369 (C-O); MS (EI) m/z: 456 (M+, 12.8%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 2.65 (s, 3H, Ph-CH_3), 3.98 (s, 2H, S-CH_2), 4.15 (s, 2H, NH_2, D_2O exchangeable), 6.9-7.8 (m, 11H, Ph-H+ H-pyrazole), 9.08 (s, 1H, C_2-H), 10.8 (s, 1H, OH, D_2O exchangeable); Anal. Calcd for $C_{24}H_{20}N_6O_2S$ (456.52): C, 63.16; H, 4.39; N, 18.42; S, 7.02%. Found: C, 63.41; H, 4.11; N, 18.62; S, 6.86%.

1-(3-Amino-5-hydroxy-1*H*-pyrazol-1-yl)-2-(5-phenyl-7-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) ethanone (4d): Yield: 82%; m.p.: 225-227 °C; IR (KBr) υ (cm^{-1}): 3444 (OH), 3383, 3365 (NH_2), 1704 (C=O), 1621 (C=N), 1311 (C-O); MS (EI) m/z: 472 (M+, 67%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.42 (s, 3H, Ph-OCH_3), 4.15 (s, 2H, S-CH_2), 5.8 (s, 2H, NH_2, D_2O exchangeable), 6.9-8.0 (m, 11H, Ar-H+ H-pyrazole), 9.08 (s, 1H, C_2-H), 11.43 (s, 1H, OH, D_2O exchangeable); Anal. Calcd for $C_{24}H_{20}N_6O_3S$ (472.52): C, 61.02; H, 4.24; N, 17.80; S, 6.78%. Found: C, 61.34; H, 4.04; N, 17.64; S, 6.33%.

1-(3-Amino-5-hydroxy-1*H*-pyrazol-1-yl)-2-(7-cyclohexyl-5-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ylthio)ethanone (4e): Yield: 54%; m.p.: 245- 247 °C; IR (KBr) υ (cm^{-1}):3467 (OH), 3391, 3378 (NH_2), 1722 (C=O), 1606 (C=N), 1293 (C-O); MS (EI) m/z: 448 (M+, 71.8 %); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 1.4 - 1.8 (m, 10H, cyclohexyl), 3.4 (m, 1H, CH-N cyclohexyl), 4.09 (s, 2H, S-CH_2), 5.2 (s, 2H, NH_2, D_2O exchangeable), 7.0-7.9 (m, 7H, Ar-H+ H-pyrazole), 9.02 (s, 1H, C_2-H), 10.9 (s, 1H, OH, D_2O exchangeable); Anal. Calcd for $C_{23}H_{24}N_6O_2S$ (448.54): C, 61.61; H, 5.36; N, 18.75, S, 7.14%. Found: C, 61.34; H, 5.31, N, 18.81, S, 7.22 %.

1-(3-Amino-5-hydroxy-1*H*-pyrazol-1-yl)-2-(7-(4-chlorophenyl)-5-phenyl-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) ethanone (4f): Yield: 61%; m.p.: 228- 230 °C; IR (KBr) υ (cm^{-1}): 3444 (OH), 3409, 3387 (NH_2), 1729 (C=O), 1626 (C=N), 1318 (C-O); MS (EI) m/z: 476 (M+, 31 %), 478 (M+2, 11.8%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.98 (s, 2H, S-CH_2), 4.9 (s, 2H, NH_2, D_2O exchangeable), 6.9-8.2 (m, 11H, Ar-H+ H-pyrazole), 9.1 (s, 1H, C_2-H), 11.2 (s, 1H, OH, D_2O exchangeable); Anal. Calcd for $C_{23}H_{17}ClN_6O_2S$ (476.94): C, 57.98; H, 3.57, Cl, 7.35, N, 17.65, S, 6.72 %. Found: C, 58.23; H, 3.85; Cl, 7.37, N, 17.36, S, 6.60 %.

1-(3-Amino-5-hydroxy-1*H*-pyrazol-1-yl)-2-(7-(3-chlorophenyl)-5,6-diphenyl-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) ethanone (4g): Yield: 66%; m.p.: 255-257 °C; IR (KBr) υ (cm^{-1}): 3408-3061 (OH, NH_2), 1740 (C=O), 1591 (C=N), 1236 (C-O); MS (EI) m/z: 552 (M+, 21 %), 554 (M+2, 8%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 4.26 (s, 2H, S-CH_2), 5.2 (s, 2H, NH_2, D_2O exchangeable), 6.9-8.1 (m, 15H, Ar-H+ H-pyrazole), 8.97 (s, 1H, C_2-H), 11.1 (s, 1H, OH, D_2O exchangeable);

Anal. Calcd for $C_{29}H_{21}ClN_6O_2S$ (553.03): C, 63.04; H, 3.80, Cl, 6.33, N, 15.19, S, 5.79 %. Found: C, 62.88; H, 4.21; Cl, 6.30, N, 15.37, S, 5.50 %.

General procedure for the synthesis of compounds 5a-g: A mixture of compound **3a-g** (0.002 mol) and acetyl acetone (0.002 mol) was heated under reflux in dry ethanol (20 mL) for eight hours, cooled, poured onto ice-water to give precipitate which was filtered off, dried, and recrystallized from methanol to give compounds **5a-g**.

1-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-2-(5,6-diphenyl-7-(4-methylphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) ethanone (5a): Yield: 64%; m.p.: 218-220 °C; IR (KBr) υ (cm^{-1}): 1713 (C=O), 1596 (C=N); MS (EI) m/z: 529 (M+, 18.6%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 2.19 (s, 3H, Ph-CH_3), 2.39 (s, 3H, pyrazole-CH_3), 2.7 (s, 3H, pyrazole-CH_3), 4.09 (s, 2H, S-CH_2), 6.7-7.5 (m, 15H, Ar-H+ H-pyrazole), 9.8 (s, 1H, C_2-H); Anal. Calcd for $C_{32}H_{27}N_5OS$ (529.65): C, 72.59; H, 5.10; N, 13.23; S, 6.05%. Found: C, 72.81; H, 5.29; N, 13.36; S, 6.35%.

1-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-2-(5,6-diphenyl-7-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) ethanone (5b): Yield: 71%; m.p.: 209-211 °C; IR (KBr) υ (cm^{-1}): 1714 (C=O), 1587 (C=N), 1281 (C-O); MS (EI) m/z: 545 (M+, 84%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm):2.3 (s, 3H, pyrazole-CH_3), 2.41 (s, 3H, pyrazole-CH_3), 3.3 (s, 3H, Ph-OCH_3), 4.2 (s, 2H, S-CH_2), 6.8-7.8 (m, 15H, Ar-H+ H-pyrazole), 9.06 (s, 1H, C_2-H); Anal. Calcd for $C_{32}H_{27}N_5O_2S$ (545.65): C, 70.46; H, 4.95; N, 12.84; S, 5.87%. Found: C, 70.75; H, 4.98; N, 12.66; S, 5.78%.

1-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-2-(5-phenyl-7-(4-methylphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) ethanone (5c): Yield: 65%; m.p.: 217-219 °C; IR (KBr) υ (cm^{-1}): 1750 (C=O), 1599 (C=N); MS (EI) m/z: 453 (M+, 71.6%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm):2.37 (s, 3H, Ph-CH_3), 2.39 (s, 3H, pyrazole-CH_3), 2.48 (s, 3H, pyrazole-CH_3), 4.5 (s, 2H, S-CH_2), 6.9-8.1 (m, 11H, Ar-H+ H-pyrazole), 9.3 (s, 1H, C_2-H); Anal. Calcd for $C_{26}H_{23}N_5OS$ (453.56): C, 68.87; H, 5.08; N, 15.45; S, 7.06%. Found: C, 68.75; H, 5.21; N, 15.80; S, 6.96%.

1-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-2-(5-phenyl-7-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) ethanone (5d): Yield: 81%; m.p.: 244-246 °C; IR (KBr) υ (cm^{-1}): 1726 (C=O), 1593 (C=N), 1332 (C-O); MS (EI) m/z: 469 (M+, 32%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm):2.27 (s, 3H, pyrazole-CH_3), 2.39 (s, 3H, pyrazole-CH_3), 3.5 (s, 3H, Ph-OCH_3), 4.3 (s, 2H, S-CH_2), 6.8-8.0 (m, 11H, Ar-H+ H-pyrazole), 9.2 (s, 1H, C_2-H); Anal. Calcd for $C_{26}H_{23}N_5O_2S$ (469.56): C, 66.52; H, 4.90; N, 14.93; S, 6.82%. Found: C, 66.49; H, 4.83; N, 14.99; S, 6.55%.

1-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-2-(7-cyclohexyl-5-phenyl-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) ethanone (5e): Yield: 48%; m.p.: 249- 250 °C; IR (KBr) υ (cm^{-1}): 1737 (C=O), 1621 (C=N); MS (EI) m/z: 445 (M+, 50 %); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 1.5 - 2.0 (m, 10H, cyclohexyl), 2.2 (s, 3H, pyrazole-CH_3), 2.35 (s, 3H, pyrazole-CH_3), 3.5 (m, 1H, CH-N cyclohexyl), 3.9 (s, 2H, S-CH_2), 7.0-8.1 (m, 7H, Ar-H+ H-pyrazole), 9.1 (s, 1H, C_2-H); Anal. Calcd for $C_{25}H_{27}N_5OS$ (445.58): C, 67.42; H, 6.07; N, 15.73, S, 7.19%. Found: C, 67.18; H, 6.32, N, 15.61, S, 7.22 %.

1-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-2-(7-(4-chlorophenyl)-5-phenyl-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) ethanone (5f): Yield: 66%; m.p.: 230- 232 °C; IR (KBr) υ (cm^{-1}): 1712 (C=O), 1614 (C=N); MS (EI) m/z: 473 (M+, 25 %), 475 (M+2, 8.6%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm):2.2 (s, 3H, pyrazole-CH_3), 2.4 (s, 3H, pyrazole-CH_3), 4.15 (s, 2H, S-CH_2), 7.0-7.9 (m, 11H, Ar-H+ H-pyrazole), 9.1 (s, 1H, C_2-H); Anal. Calcd for $C_{25}H_{20}ClN_5OS$ (473.98): C, 63.42; H, 4.23, Cl, 7.40, N, 14.80, S, 6.77 %. Found: C, 63.76; H, 4.58; Cl, 7.31, N, 14.99, S, 6.49 %.

1-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-2-(7-(3-chlorophenyl)-5,6-diphenyl-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) ethanone (5g): Yield: 78%; m.p.: 211-213 °C; IR (KBr) υ (cm^{-1}): 1711 (C=O), 1584 (C=N); MS (EI)m/z: 549 (M+, 44 %), 551 (M+2, 15.6%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm):2.2 (s, 3H, pyrazole-CH_3), 2.46 (s, 3H, pyrazole-CH_3), 4.22 (s, 2H, S-CH_2), 7.0-8.2 (m, 15H, Ar-H+ H-pyrazole), 9.37 (s, 1H, C_2-H); Anal. Calcd for $C_{31}H_{24}ClN_5OS$ (550.07): C, 67.63; H, 4.37, Cl, 6.38, N, 12.75, S, 5.83 %. Found: C, 67.57; H, 4.30; Cl, 6.12, N, 12.61, S, 6.05 %.

General procedure for the synthesis of 1-(5-hydroxy-3-methyl-1*H*-pyrazol-1-yl)-2-(5,6-diphenyl-7-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) ethanone (6): A mixture of compound **3b** (0.002 mol) and ethyl acetoacetate (0.002 mol) was heated under reflux in dry ethanol (20 mL) for eight hours, cooled, poured onto ice-water to give precipitate which was filtered off, dried, and recrystallized from methanol to give compounds **6**. Yield: 81%; m.p.: 210-212 °C; IR (KBr) υ (cm^{-1}): 3319 (O-H), 1716 (C=O), 1590 (C=N), 1275 (C-O); MS (EI) m/z: 547 (M+, 22%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 2.4 (s, 3H, pyrazole-CH_3), 4.23 (s, 3H, Ph-OCH_3), 4.29 (s, 2H, S-CH_2), 6.9-8.2 (m, 15H, Ar-H+ H-pyrazole), 9.13 (s, 1H, C_2-H); Anal. Calcd for $C_{31}H_{25}N_5O_3S$ (547.63): C, 68.01; H, 4.57; N, 12.80; S, 5.85%. Found: C, 67.82; H, 4.29; N, 12.62; S, 5.65%.

General procedure for the synthesis of compounds 7a-g

A mixture of compound **3a-g** (0.002mol) and KOH (0.002mol) in dry ethanol (30 mL) was cooled and five mL of CS_2 was added with stirring then the mixture was heated under reflux for eight hours, then the solvent was evaporated and the residue was dissolved in cold water then acidified with hydrochloric acid. The formed precipitate was filtered, dried then recrystallized from ethanol to give compounds **7a-g**.

5-[(5,6-diphenyl-7-(4-methylphenyl)-pyrrolo[2,3-*d*] pyrimidin-4-yl) sulfanylmethyl]-1,3,4-oxadiazole-2-thiol (7a): Yield: 83%; m.p.: 245-247 °C; IR (KBr) υ (cm^{-1}): 3199 (N-H), 1604 (C=N), 1113 (C-O), 1451, 1244, 1017, 759 (C=S); MS (EI) m/z: 507 (M+, 29%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 2.3 (s, 3H, CH_3), 4.19 (s, 2H, S-CH_2), 6.9-8.1 (m, 14H, Ar-H), 9.06 (s, 1H, C_2-H), 11.2 (s, 1H, SH); Anal. Calcd for $C_{28}H_{21}N_5OS_2$(507.63): C, 66.27; H, 4.14; N, 13.81; S, 12.62%. Found: C, 65.98; H, 4.49; N, 13.72; S, 12.85%.

5-[(5,6-diphenyl-7-(4-methoxyphenyl)-pyrrolo[2,3-*d*] pyrimidin-4-yl) sulfanylmethyl]-1,3,4-oxadiazole-2-thiol (7b): Yield: 92%; m.p.: 234-236 °C; IR (KBr) υ (cm^{-1}): 3164 (N-H), 1609 (C=N), 1196 (C-O), 1422, 1207, 1021, 791 (C=S); MS (EI) m/z: 523 (M+, 72%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.23 (s, 3H, OCH_3), 4.27 (s, 2H, S-CH_2), 7.0-8.1 (m, 14H, Ar-H), 9.02 (s, 1H, C_2-H), 11.1 (s, 1H, SH); Anal. Calcd for $C_{28}H_{21}N_5O_2S_2$ (523.63): C, 64.24; H, 4.02; N, 13.38; S, 12.24%. Found: C, 64.38; H, 4.39; N, 13.55; S, 12.08%.

5-[(5-phenyl-7-(4-methylphenyl)-pyrrolo[2,3-*d*] pyrimidin-4-yl) sulfanylmethyl]-1,3,4-oxadiazole-2-thiol (7c): Yield: 66%; m.p.: 240-242 °C; IR (KBr) υ (cm^{-1}): 3203 (N-H), 1604 (C=N), 1113 (C-O), 1451, 1244, 1017, 816 (C=S); MS (EI) m/z: 431 (M+, 16.5%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 2.36 (s, 3H, CH_3), 4.14 (s, 2H, S-CH_2), 7.0-8.1 (m, 10H, Ar-H), 9.03 (s, 1H, C_2-H), 11.27 (s, 1H, SH); Anal. Calcd for $C_{22}H_{17}N_5OS_2$ (431.53): C, 61.25; H, 3.94; N, 16.24; S, 14.85%. Found: C, 61.45; H, 3.91; N, 16.55; S, 14.76%.

5-[(5-phenyl-7-(4-methoxyphenyl)-pyrrolo[2,3-*d*] pyrimidin-4-yl) sulfanylmethyl]-1,3,4-oxadiazole-2-thiol (7d): Yield: 82%; m.p.: 219-221 °C; IR (KBr) υ (cm^{-1}): 3127 (N-H), 1598 (C=N), 1140 (C-O), 1434, 1217, 1033, 808 (C=S); MS (EI) m/z: 447 (M+, 47.8%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.5 (s, 3H, OCH_3), 4.1 (s, 2H, S-CH_2), 7.0-8.0 (m, 10H, Ar-H), 9.0 (s, 1H, C_2-H), 11.32 (s, 1H, SH); Anal. Calcd for $C_{22}H_{17}N_5O_2S_2$(447.53): C, 59.06; H, 3.80; N, 15.66; S, 14.32%. Found: C, 58.80; H, 4.13; N, 15.54; S, 14.08%.

5-[(7-cyclohexyl-5-phenyl-pyrrolo[2,3-*d*] pyrimidin-4-yl) sulfanylmethyl]-1,3,4-oxadiazole-2-thiol (7e): Yield: 42%; m.p.: 254- 256 °C; IR (KBr) υ (cm^{-1}): 3186 (N-H), 1590 (C=N), 1119 (C-O), 1424, 1210, 1033, 805 (C=S); MS (EI) m/z: 423 (M+, 16%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 1.5 - 2.0 (m, 10H, cyclohexyl), 3.5 (m, 1H, CH-N cyclohexyl), 4.19 (s, 2H, S-CH_2), 7.1-8.1 (m, 6H, Ar-H), 9.1 (s, 1H, C_2-H), 11.2 (s, 1H, SH); Anal. Calcd for $C_{21}H_{21}N_5OS_2$ (423.55): C, 59.57; H, 4.96; N, 16.55, S, 15.13%. Found: C, 59.38; H, 5.03, N, 16.70, S, 15.20 %.

5-[(7-(4-chlorophenyl)-5-phenyl-pyrrolo[2,3-*d*] pyrimidin-4-yl) sulfanylmethyl]-1,3,4-oxadiazole-2-thiol (7f): Yield: 72%; m.p.: 229- 231 °C; IR (KBr) υ (cm^{-1}): 3199 (N-H), 1595 (C=N), 1395 (C-O), 1484, 1236, 1047, 760 (C=S); MS (EI) m/z: 451 (M+, 18.2 %), 453 (M+2, 6.6%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 4.1 (s, 2H, S-CH_2), 7.0-7.9 (m, 10H, Ar-H), 9.1 (s, 1H, C_2-H), 11.0 (s, 1H, SH); Anal. Calcd for $C_{21}H_{14}ClN_5OS_2$ (451.95): C, 55.88; H, 3.10, Cl, 7.76, N, 15.52, S, 14.19 %. Found: C, 55.96; H, 2.88; Cl, 7.51, N, 15.67, S, 14.49 %.

5-[(7-(3-chlorophenyl)-5,6-diphenyl-pyrrolo[2,3-*d*] pyrimidin-4-yl) sulfanylmethyl]-1,3,4-oxadiazole-2-thiol (7g): Yield: 79%; m.p.: 258-260 °C; IR (KBr) υ (cm^{-1}):3234 (N-H), 1604 (C=N), 1165 (C-O), 1451, 1242, 1027, 762 (C=S); MS (EI)m/z: 527 (M+, 50.9 %), 529 (M+2, 17.6%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.43 (s, 2H, S-CH_2), 6.8-7.8 (m, 14H, Ar-H), 9.2 (s, 1H, C_2-H), 11.7 (s, 1H, SH); Anal. Calcd for $C_{27}H_{18}ClN_5OS_2$ (528.05): C, 61.36; H, 3.41, Cl, 6.63, N, 13.26, S, 12.12 %. Found: C, 61.50; H, 3.39; Cl, 6.42, N, 13.64, S, 12.05 %.

General procedure for the synthesis of compounds (8a-j): A mixture of compound **3a-g** (0.002 mol) and appropriate aldhyde (0.002 mol) in dry ethanol (30 mL) was heated under reflux for eight hours, cooled, poured onto ice-water to give precipitate which was filtered off, dried, and recrystallized from ethanol to give compounds **8a-j**.

N'-(2-Nitrobenzylidene)-2-(5-phenyl-7-(4-methylphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetohydrazide (8a): Yield: 70%; m.p.: 246-248 °C; IR (KBr) υ (cm^{-1}): 3329 (N-H), 1687 (C=O), 1599 (C=N), 1560, 1376 (NO_2); MS (EI) m/z: 522 (M+, 29.3%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 2.23 (s, 3H, Ph-CH_3), 4.02 (s, 2H, S-CH_2), 6.9-8.2 (m, 14H, Ar-H), 8.59(s, 1H, CH=N), 9.1 (s, 1H, C_2-H), 11.03 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{28}H_{22}N_6O_3S$ (522.58): C, 64.37; H, 4.21; N, 16.09; S, 6.13%. Found: C, 64.62; H, 4.12; N, 16.02; S, 6.16%.

N'-(2-Nitrobenzylidene)-2-(5,6-diphenyl-7-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetohydrazide (8b): Yield: 75 %; m.p.: 194-196 °C; IR (KBr) υ (cm^{-1}): 3428 (N-H), 1682 (C=O), 1615 (C=N), 1563, 1347 (NO_2), 1317 (C-O); MS (EI) m/z: 614 (M+, 71%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.52 (s, 3H, Ph-OCH_3), 4.2 (s, 2H, S-CH_2), 6.9-8.0(m, 18H, Ar-H), 8.5 (s, 1H, CH=N), 9.25 (s, 1H, C_2-H), 11.2 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{34}H_{26}N_6O_4S$ (614.67): C, 66.45; H, 4.23; N, 13.68; S, 5.21%. Found: C, 66.69; H, 4.01; N, 13.42; S, 5.11%.

N'-(4-Nitrobenzylidene)-2-(5,6-diphenyl-7-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetohydrazide (8c): Yield: 87 %; m.p.: 189-190 °C; IR (KBr) υ (cm^{-1}): 3430 (N-H), 1677 (C=O), 1610 (C=N), 1559, 1341 (NO_2), 1320 (C-O); MS (EI) m/z: 614 (M+, 16.4%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.5 (s, 3H, Ph-OCH_3), 4.32 (s, 2H, S-CH_2), 6.9-8.0 (m, 18H, Ar-H), 8.71 (s, 1H, CH=N), 9.2 (s, 1H, C_2-H), 10.9 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{34}H_{26}N_6O_4S$ (614.67): C, 66.45; H, 4.23; N, 13.68; S, 5.21%. Found: C, 66.75; H, 4.46; N, 13.35; S, 5.19%.

N'-(4-Nitrobenzylidene)-2-(5-phenyl-7-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetohydrazide (8d): Yield: 75 %; m.p.: 217-219 °C; IR (KBr) υ (cm^{-1}): 3338 (N-H), 1690 (C=O), 1603 (C=N), 1572, 1349 (NO_2), 1282 (C-O); MS (EI) m/z: 538 (M+, 59%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.39 (s, 3H, Ph-OCH_3), 4.26 (s, 2H, S-CH_2), 6.8-7.8 (m, 148H, Ar-H), 8.67 (s, 1H, CH=N), 9.17 (s, 1H, C_2-H), 11.06 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{28}H_{22}N_6O_4S$ (538.58): C, 62.45; H, 4.09; N, 15.61; S, 5.95%. Found: C, 62.79; H, 4.22; N, 15.40; S, 5.65%.

N'-(4-Nitrobenzylidene)-2-(5,6-diphenyl-7-(4-methylphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetohydrazide (8e): Yield: 86 %; m.p.: 249-251 °C; IR (KBr) υ (cm^{-1}): 3331 (N-H), 1685 (C=O), 1579 (C=N), 1556, 1344 (NO_2); MS (EI) m/z: 598 (M+, 28%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 2.4 (s, 3H, Ph-CH_3), 4.38 (s, 2H, S-CH_2), 6.8-8.1 (m, 18H, Ar-H), 8.6 (s, 1H, CH=N), 9.17 (s, 1H, C_2-H), 11.2 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{34}H_{26}N_6O_3S$ (598.67): C, 68.23; H, 4.35; N, 14.05; S, 5.35%. Found: C, 68.56; H, 4.19; N, 14.09; S, 5.12%.

N'-(4-Chlorobenzylidene)-2-(5,6-diphenyl-7-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetohydrazide (8f): Yield: 82 %; m.p.: 185-187 °C; IR (KBr) υ (cm^{-1}): 3406 (N-H), 1693 (C=O), 1601 (C=N), 1307 (C-O); MS (EI) m/z: 603 (M+, 55.8%), 605 (M+2, 18%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.41 (s, 3H, Ph-OCH_3), 4.3 (s, 2H, S-CH_2), 7.0-8.0 (m, 18H, Ar-H), 8.62 (s, 1H, CH=N), 9.2 (s, 1H, C_2-H), 10.82 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{34}H_{26}ClN_5O_2S$ (604.12): C, 67.66; H, 4.31; Cl, 5.80; N, 11.61; S, 5.31%. Found: C, 67.49; H, 4.66; Cl, 5.95; N, 11.46; S, 5.10%.

N'-(4-Dimethylaminobenzylidene)-2-(5,6-diphenyl-7-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetohydrazide (8g): Yield: 97 %; m.p.: 200-202 °C; IR (KBr) υ (cm^{-1}): 3747 (N-H), 1733 (C=O), 1596 (C=N), 1363 (C-O); MS (EI) m/z: 612 (M+, 7%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.4 (s, 6H, N(CH_3)$_2$), 3.67 (s, 3H, Ph-OCH_3), 4.3 (s, 2H, S-CH_2), 7.0-8.1 (m, 18H, Ar-H), 8.59 (s, 1H, CH=N), 9.04 (s, 1H, C_2-H), 10.9 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{36}H_{32}N_6O_2S$ (612.74): C, 70.59; H, 5.23; N, 13.73; S, 5.23%. Found: C, 70.70; H, 5.16; N, 13.54; S, 5.40%.

N'-(4-Dimethylaminobenzylidene)-2-(5-phenyl-7-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetohydrazide (8h): Yield: 93 %; m.p.: 182-184 °C; IR (KBr) υ (cm^{-1}): 3349 (N-H), 1701 (C=O), 1590 (C=N), 1321 (C-O); MS (EI) m/z: 536 (M+, 9.8%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.38 (s, 6H, N(CH_3)$_2$), 3.7 (s, 3H, Ph-OCH_3), 4.33 (s, 2H, S-CH_2), 6.9-8.1 (m, 14H, Ar-H), 8.5 (s, 1H, CH=N), 9.2 (s, 1H, C_2-H), 11.07 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{30}H_{28}N_6O_2S$ (536.65): C, 67.16; H, 5.22; N, 15.67; S, 5.97%. Found: C, 67.37; H, 5.06; N, 15.94; S, 6.20%.

N'-(4-Dimethylaminobenzylidene)-2-(5-phenyl-7-(4-methylphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetohydrazide (8i): Yield: 89 %; m.p.: 235-237 °C; IR (KBr) υ (cm^{-1}): 3376 (N-H), 1692 (C=O), 1597 (C=N), 1267 (C-O); MS (EI) m/z: 520 (M+, 39%); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 2.6 (s, 3H, Ph-CH_3), 3.5 (s, 6H, N(CH_3)$_2$), 4.4 (s, 2H, S-CH_2), 6.8-8.0 (m, 14H, Ar-H), 8.52 (s, 1H, CH=N), 9.26 (s, 1H, C_2-H), 11.1 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{30}H_{28}N_6OS$ (520.65): C, 69.23; H, 5.38; N, 16.15; S, 6.15%. Found: C, 69.37; H, 5.56; N, 16.46; S, 6.09%.

N'-(4-Dimethylaminobenzylidene)-2-(5-phenyl-7-cyclohexyl-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-ylthio) acetohydrazide (8j): Yield: 81 %; m.p.: 256-258 °C; IR (KBr) υ (cm^{-1}): 3426 (N-H), 1664 (C=O), 1593 (C=N); MS (EI) m/z: 512 (M+, 44.3 %); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm):1.5 - 2.0 (m, 10H, cyclohexyl), 3.3 (s, 6H, N(CH_3)$_2$), 3.5 (m, 1H, CH-N cyclohexyl), 4.26 (s, 2H, S-CH_2), 6.8-7.8 (m, 10H, Ar-H), 8.5 (s, 1H, CH=N), 9.2 (s, 1H, C_2-H), 11.0 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{29}H_{32}N_6OS$ (512.67): C, 67.97; H, 6.25; N, 16.41; S, 6.25%. Found: C, 67.67; H, 6.0; N, 16.72; S, 6.54%.

Assessment of anticancer activity

The NCI-60 Human Tumor Cell Lines Screen

The operation of this screen utilizes 60 different human tumor cell lines, representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney cancers.

Selection Guidelines in NCI

Structures are generally selected for screening based on their ability to add diversity to the NCI small molecule compound collection (according to the protocol of the drug evaluation branch of the NCI, Bethesda, USA. [40, 41]

From our 46 newly synthesized pyrrolopyrimidines35 compounds were selected for single-dose testing. **NCI60** single-dose testing is performed in all 60 cell linesaccording to the reported assay, [42, 43] compounds were dissolved in DMSO: glycerol (9:1) and are stored in a -70°C freezer. The prepared compounds were added at single concentration of 10^{-5} M and the culture was incubated for 48 hrs. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers.

Interpretation of One-Dose Data

The One-dose data is reported as a mean of graph of the percent growth of treated cells. The number reported for the One-dose assay is growth relative to the no-drug control, and relative to the time zero number of cells. This allows detection of both growth inhibition (values between 0 and 100) and lethality (values less than 0). For example, a value of 100 means no growth inhibition. A value of 40 would mean 60% growth inhibition. A value of 0 means no net growth over the course of the experiment. A value of -40 would mean 40% lethality. A value of -100 means all cells are dead. [42]

Molecular docking

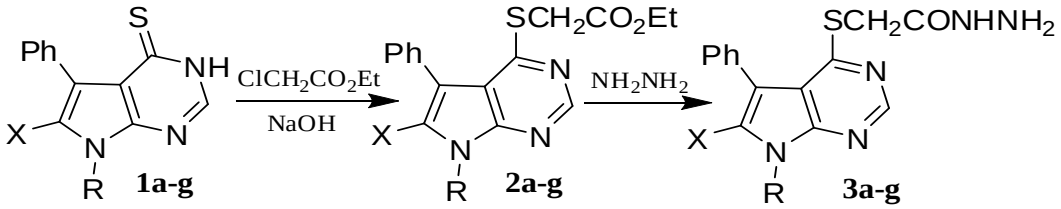
All compounds were built using MOE 2014.09and saved in molecular data base file. [44] The crystal structure of CDK2 was obtained from protein data bank (PDBID: 5ANJ). [45] Protein was energy minimized and 3-D protonated by using the structure preparation module of MOE. The co-crystallized bound compound and water molecules were removed from the crystal structure. The site of docking was identified and the database containing all the tested compounds was docked using rigid receptor as a docking protocol and alpha triangle as a placement method. Two rescoring functions were selected, London dG and GBVI/WSA dG. Force field was used as a refinement. The free binding energy (kcal/mol) was computed and only the best scored pose for each compound was selected.

Results and Discussion

Chemistry

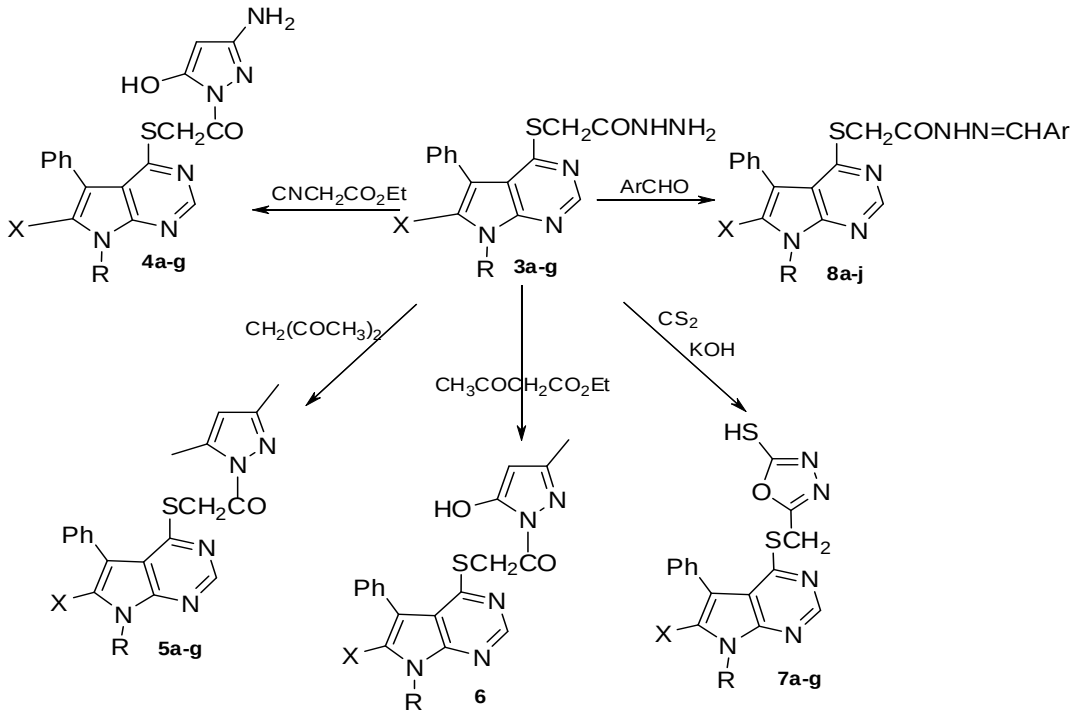
Pyrrolopyrimidin-4-thiones **(1a-g)** were prepared as reported before. [37-39] These compounds were utilized for the preparation of the corresponding esters **2a-g** by reaction with ethyl chloroacetate in presence of sodium hydroxide as revealed in **Scheme 1**. The structures of the produced esters were confirmed by IR and ^{1}HNMR where the presence of C=O absorption frequency band (ν, cm^{-1}) in IR spectrum at 1708 – 1734 cm^{-1} and the appearance of 2 characteristics signals in ^{1}H-NMR (DMSO-d_6) (δ, ppm) representing ester protons (OCH_2CH_3), one triplet at δ1.2- 1.3 ppm and the other is quartet at δ4.2-4.3ppm in addition to S-CH_2CO protons at δ4.2- 4.4 ppm.

The produced esters **2a-g** were reacted with hydrazine hydrate to afford the hydrazide derivatives **3a-g** (as revealed in **Scheme 1)** which were characterized by appearance of 2 IR absorption bands (ν, cm^{-1}) corresponding to NH_2 and NH at ranges 3320-3429 cm^{-1} and 3186-3244 cm^{-1}, in addition to the shift of C=O band to 1637-1663 cm^{-1} indicating amide carbonyl. These hydrazides were also characterized using ^{1}H-NMR by the disappearance of ester signals and the appearance of D_2O exchangeable signals of NH_2 (δ 4.2-4.7 ppm) and NH (δ 9.1-9.24 ppm).



Scheme 1. Synthesis of compounds **2a-g – 3a-g.**

The hydrazide derivatives **3a-g** were used as starting materials for preparation of several new heterocycles **4a-g – 7a-g** (**Scheme 2**) via reaction with ethyl cyanoacetate, acetyl acetone, ethyl acetoacetate and CS_2, respectively, using the reported methods. [46-50] Finally Schiff bases **8a-j** were prepared by reaction of hydrazide derivatives **3a-g** with the appropriate aldhyde, each of the produced compounds was characterized by mass, IR and ^{1}H-NMR analysis.



Scheme 2. Synthesis of compounds **4a-g – 8a-j.**

Anticancer activity

In an effort to discover new anti-cancer agent as CDK2 inhibitor, Herein, a series of novel pyrrolo[2,3-*d*] pyrimidine derivatives were synthesized and 35 compounds of the synthesized compounds were selected to be tested for their anticancer activity via **NCI60** single-dose testing (showed in **tables 1-4**). The results revealed that pre-virtual screening results are false-positive results as all the tested compounds showed no anti-cancer activity except five compounds showed moderate activity against certain cell lines (< 50% growth or > 50% inhibition) which are **2a, 8a, 8b, 8g** and **8i** as revealed in **table 5** and **figures 2a, b** (for the highest two active compounds **2a** and **8b).**

Table 1. Anticancer testing results for compounds **2a-g – 3a-g** (growth percent against 60 cell lines).

Panel/Cell Line	Growth Percent									
	2a	**2c**	**2e**	**2f**	**2g**	**3a**	**3b**	**3c**	**3f**	**3g**
Leukemia:										
CCRF-CEM	67.01	96.63	92.52	102.45	94.36	93.52	106.91	95.35	106.08	89.30
HL-60(TB)	55.91	100.32	90.20	96.70	98.20	83.41	83.55	91.68	96.27	100.35
K-562	37.99*	79.23	80.74	88.71	88.61	75.75	70.39	83.26	94.21	83.54
MOLT-4	67.38	96.74	87.41	87.46	79.43	66.06	90.90	87.10	96.44	78.78
RPMI-8226	37.83*	86.93	91.73	93.13	89.74	81.48	64.41	91.45	100.29	90.98
SR	80.44	99.27	91.62	91.07	83.88	90.13	90.34	92.54	91.35	82.54
Non-Small Cell Lung Cancer:										
A549/ATCC	72.45	89.39	84.39	84.78	95.12	80.65	82.57	88.77	86.53	92.02
EKVX	43.87*	93.21	101.93	100.20	96.55	84.38	79.84	100.06	90.99	101.40
HOP-62	64.82	76.16	82.70	84.83	80.04	74.98	78.99	79.67	81.35	78.64
HOP-92	49.44*	73.46	86.41	70.48	65.76	64.21	61.27	76.13	78.36	63.03
NCI-H226	77.84	88.42	86.19	87.76	89.82	81.96	77.84	89.68	79.43	91.21
NCI-H23	74.96	88.37	101.60	102.12	90.44	102.20	92.06	98.53	102.47	87.96
NCI-H322M	94.41	97.29	86.66	89.40	87.13	84.33	84.85	93.81	94.51	77.32
NCI-H460	76.63	106.40	98.93	111.27	98.98	102.63	94.08	113.68	110.67	98.89
NCI-H522	47.33*	84.02	78.88	83.80	73.62	73.53	70.33	72.36	70.01	68.20
Colon Cancer:										
COLO 205	63.66	99.06	91.95	92.60	87.86	87.46	86.65	103.44	99.37	88.84
HCC-2998	75.79	106.09	104.49	132.05	93.16	104.08	102.54	87.88	118.60	82.74
HCT-116	53.54	85.30	90.43	94.57	96.00	81.97	82.61	89.46	89.39	94.25
HCT-15	106.29	103.89	98.15	110.18	101.84	101.99	90.50	117.71	95.87	105.06
HT29	63.92	94.04	97.13	105.12	88.92	94.07	90.02	92.43	101.11	89.69
KM12	50.19	100.58	101.09	102.70	94.41	94.51	96.88	97.33	112.10	98.04
SW-620	81.24	99.65	85.63	97.25	91.03	97.61	92.87	98.19	105.25	89.80
CNS Cancer:										
SF-268	86.16	103.89	105.16	109.42	108.66	96.49	105.73	101.25	104.46	98.43
SF-295	76.20	96.44	100.68	95.70	96.84	90.08	92.53	89.29	97.03	98.76
SF-539	86.25	101.30	93.20	91.64	90.07	91.42	93.47	99.24	102.53	90.59
SNB-19	63.37	89.23	93.38	91.70	87.33	98.81	90.60	84.60	102.73	86.61
SNB-75	87.81	102.44	81.50	93.13	79.29	77.70	78.13	97.34	92.84	78.28
U251	49.08*	90.24	91.78	95.54	95.51	87.41	87.71	83.08	95.47	92.48
Melanoma:										
LOX IMVI	78.44	93.39	97.87	89.50	94.82	85.26	89.49	100.14	87.58	92.16
MALME-3M	62.15	98.33	92.53	101.08	86.48	107.99	91.57	100.04	96.17	104.14
M14	75.65	95.26	91.63	101.21	95.04	96.03	93.13	89.66	99.04	87.51
MDA-MB-435	74.22	109.39	96.41	106.86	95.16	96.71	94.14	100.81	109.41	93.96
SK-MEL-2	82.35	101.28	95.38	105.74	89.13	88.68	95.66	89.20	109.76	91.38
SK-MEL-28	87.57	99.74	99.34	103.96	90.72	106.03	94.94	101.72	106.26	88.23
SK-MEL-5	44.93*	98.80	91.09	97.22	93.49	85.02	70.55	105.11	105.19	93.14
UACC-257	51.87	85.70	82.46	88.06	88.11	79.55	84.68	83.85	94.75	84.51
UACC-62	57.05	82.90	79.91	68.00	65.37	76.88	74.75	85.61	81.66	70.82
Ovarian Cancer:										
IGROV1	79.86	97.83	102.31	86.04	81.58	80.85	77.61	92.94	94.54	85.02
OVCAR-3	66.38	100.53	101.61	110.68	93.20	100.51	91.57	109.95	113.71	96.25
OVCAR-4	80.45	98.02	106.72	102.36	94.91	93.40	93.30	100.84	116.01	101.96
OVCAR-5	93.79	104.24	85.06	95.19	96.52	85.88	89.43	97.77	95.89	93.88
OVCAR-8	54.53	91.98	93.11	95.80	93.86	85.74	80.16	92.35	95.39	93.50
NCI/ADR-RES	98.43	108.92	106.33	112.72	93.04	104.22	107.38	105.11	104.39	92.52
SK-OV-3	56.03	64.86	78.40	74.86	75.66	77.39	71.32	72.47	77.58	69.60
Renal Cancer:										

786-0	80.93	98.44	99.48	95.47	104.25	92.72	92.76	94.68	93.29	93.53
A498	75.21	105.25	112.78	117.89	108.20	101.98	102.41	112.94	105.62	114.75
ACHN	90.75	96.97	93.02	85.17	83.67	93.76	84.07	95.63	94.45	90.12
CAKI-1	75.55	87.13	79.44	76.35	83.33	78.30	81.60	87.41	87.23	77.89
RXF 393	104.97	111.86	97.70	110.69	106.07	108.50	88.59	114.67	93.66	100.25
SN12C	64.34	95.41	101.33	103.10	79.40	98.38	98.47	95.12	110.66	83.66
TK-10	78.55	112.70	104.59	118.94	116.21	104.00	103.28	112.84	109.71	117.11
UO-31	73.43	75.22	61.73	60.72	66.63	60.68	66.36	69.87	70.10	58.09
Prostate Cancer:										
PC-3	51.92	84.98	82.55	85.46	87.63	80.36	78.13	87.46	94.35	83.20
DU-145	74.37	101.54	106.75	115.78	103.79	99.49	93.11	102.95	99.03	96.59
Breast Cancer:										
MCF7	52.47	96.54	97.34	93.24	95.84	91.92	78.38	105.70	101.56	88.86
MDA-MB-231/ATCC	85.73	89.31	95.00	87.01	99.60	85.86	89.60	92.94	99.12	88.31
HS 578T	81.68	101.17	96.42	111.27	87.03	102.65	95.64	97.11	110.73	91.27
BT-549	61.76	82.72	94.21	107.14	98.85	79.65	90.93	82.71	99.73	92.01
T-47D	9.51*	79.25	86.76	82.62	75.81	60.69	69.08	74.84	75.82	75.01
MDA-MB-468	51.93	97.92	102.61	81.38	107.68	122.36	68.71	99.68	91.57	80.78
Mean	**69.14**	**94.59**	**93.14**	**95.95**	**90.89**	**89.24**	**86.66**	**94.16**	**97.00**	**89.13**
Delta	**59.63**	**29.73**	**31.41**	**35.23**	**25.52**	**28.56**	**25.39**	**24.29**	**26.99**	**31.04**
Range	**96.78**	**47.84**	**51.05**	**71.33**	**50.84**	**61.68**	**46.11**	**47.84**	**48.59**	**59.02**

* Growth < 50%

Table 2. Anticancer testing results for compounds **4a-g – 5a-g** (growth percent against 60 cell lines).

Panel/Cell Line	**Growth Percent**									
	4a	**4b**	**4c**	**4f**	**4g**	**5a**	**5b**	**5c**	**5f**	**5g**
Leukemia:										
CCRF-CEM		94.21	102.01	111.63	99.77	95.95	85.71	102.69	104.38	95.67
HL-60(TB)	101.61	88.23	97.82	105.07	122.63	104.23	91.94	104.13	85.24	131.71
K-562	77.14	83.14	91.60	89.52	107.04	61.75	59.19	89.31	110.17	99.96
MOLT-4	87.26	110.21	95.18	98.87	85.19	94.13	96.18	105.49	106.69	99.94
RPMI-8226	81.90	65.01	103.48	112.53	102.71	74.80	57.38	101.89	105.49	96.71
SR	102.88	117.29	95.05	98.76	111.34	105.79	97.19	112.50	104.41	114.53
Non-Small Cell Lung Cancer:										
A549/ATCC	92.51	96.42	93.53	97.01	89.94	95.35	90.64	93.77	88.58	93.30
EKVX	87.23	79.31	100.92	76.76	86.34	71.15	64.82	107.03	84.17	77.86
HOP-62	87.60	84.50	86.39	81.31	82.12	70.06	79.36	90.23	82.95	78.55
HOP-92	74.71	74.28	81.11	77.04	67.78	69.76	57.41	82.75	72.15	74.57
NCI-H226	88.82	63.42	89.60	83.00	60.88	75.39	80.79	79.63	66.57	78.02
NCI-H23	90.83	88.62	96.08	83.89	82.36	70.81	70.45	75.89	86.01	81.23
NCI-H322M	100.67	97.14	97.84	94.24	95.74	93.56	95.32	103.56	91.13	96.84
NCI-H460	103.13	97.91	114.97	102.99	104.93	96.07	90.10	106.41	107.34	102.64
NCI-H522	79.64	77.69	85.52	78.36	90.56	77.66	70.77	88.38	82.92	81.48
Colon Cancer:										
COLO 205	94.38	92.86	93.84	110.16	103.10	98.74	82.65	126.76	115.35	109.96
HCC-2998	112.79	103.84	107.25	98.67	98.23	93.94	89.41	95.04	99.11	96.04
HCT-116	78.15	79.89	91.77	96.26	94.82	78.41	77.09	99.16	92.13	92.75
HCT-15	100.93	99.83	114.41	94.52	94.30	88.05	84.25	102.70	90.11	103.39
HT29	93.99	96.60	100.51	113.27	112.41	93.59	91.32	104.76	102.22	98.17
KM12	98.12	89.63	105.21	98.79	93.69	86.34	81.64	96.70	96.95	95.13
SW-620	90.30	101.28	98.54	98.60	103.65	90.93	88.01	101.61	96.71	98.25
CNS Cancer:										
SF-268	106.57	98.22	107.43	92.99	100.66	91.83	93.76	94.26	93.11	98.35
SF-295	89.31	87.30	92.24	92.01	93.90	83.38	78.98	93.14	82.77	92.11
SF-539	96.02	97.05	99.77	94.66	98.66	91.27	96.77	99.43	97.25	88.76
SNB-19	91.85	91.81	91.78	90.58	100.53	91.21	86.35	102.34	93.54	101.40
SNB-75	97.11	94.24	92.54	98.98	100.24	89.17	77.01	92.63	97.18	97.45
U251	86.97		89.19							
Melanoma:										
LOX IMVI	97.68	97.74	101.45	82.52	90.98	83.57	81.93	85.17	87.00	93.67
MALME-3M	97.62	103.58	103.33	105.62	101.74	105.74	94.62	108.44	103.01	103.76

M14	94.32	94.44	97.62	112.37	97.04	103.43	90.28	106.53	100.66	102.43
MDA-MB-435	105.97	93.20	118.03	107.60	104.20	99.69	95.05	106.09	104.44	97.70
SK-MEL-2	107.65	92.09	98.45	104.10	113.42	106.52	108.74	115.43	99.72	94.46
SK-MEL-28	101.21	106.42	103.52	117.51	112.16	106.59	102.42	111.68	106.85	111.29
SK-MEL-5	87.34	74.20	102.08	98.83	84.20	85.23	78.24	99.54	84.13	90.19
UACC-257	94.06	96.98	86.21	119.35	97.81	108.60	96.88	105.36	111.22	98.02
UACC-62	77.37	78.33	83.11	79.35	81.77	69.14	79.42	85.28	78.98	73.69
Ovarian Cancer:										
IGROV1	79.76	87.19	96.62	89.50	93.43	76.61	83.73	88.82	85.41	91.93
OVCAR-3	91.93	86.75	113.71	100.01	96.10	87.23	78.10	97.17	99.42	97.68
OVCAR-4	96.03	93.07	110.09	93.00	100.42	80.21	78.89	96.32	104.85	90.56
OVCAR-5	101.35	92.17	101.55	99.87	95.35	96.39	94.67	103.48	94.33	95.45
OVCAR-8	83.18	89.65	95.65	101.94	93.21	84.60	79.83	99.54	91.17	90.59
NCI/ADR-RES	105.03	108.91	92.46	88.53	96.89	87.41	84.39	86.58	89.49	93.47
SK-OV-3	79.52	91.92	74.08	89.91	97.67	68.42	80.76	84.10	101.33	89.59
Renal Cancer:										
786-0	96.59	93.61	101.23	97.18	100.05	92.32	89.32	98.95	88.82	94.74
A498	113.74	85.79	125.71	96.16	106.54	71.31	67.05	111.61	105.07	115.37
ACHN	91.22	98.18	93.24	94.41	92.42	91.23	95.36	98.19	87.59	84.53
CAKI-1	88.38	81.63	98.78	69.35	74.06	64.15	82.17	75.87	69.99	68.81
RXF 393	96.61	75.00	107.47	112.41	98.09	106.39	91.07	117.70	82.35	104.53
SN12C	99.23	79.73	91.70	90.36	95.78	82.57	82.57	105.59	95.72	92.62
TK-10	103.58	115.13	107.29	126.05	135.36	124.87	108.04	122.21	122.81	120.14
UO-31	71.92	76.65	75.62	68.32	69.22	68.80	73.77	69.00	69.75	66.48
Prostate Cancer:										
PC-3	79.40	66.03	89.19	87.73	84.08	70.01	64.62	89.47	80.77	84.04
DU-145	108.33	104.83	114.56	104.42	107.14	100.75	93.59	107.49	106.92	104.92
Breast Cancer:										
MCF7	86.74	71.65	93.61	78.58	86.61	65.08	63.56	93.37	82.25	93.84
MDA-MB-231/ATCC	83.29	83.73	95.59	79.50	75.48	73.84	83.93	80.71	73.36	76.32
HS 578T	105.97	101.65	114.53	91.37	96.85	86.50	87.45	94.57	96.56	94.17
BT-549	98.97	85.12	98.32	99.12	97.30	85.64	73.27	104.05	90.20	91.97
T-47D	76.57	71.38	88.23	74.75	90.08	59.97	60.41	98.68	77.11	80.62
MDA-MB-468	91.76	51.72	107.40	90.92	81.77	73.37	57.70	93.60	81.02	88.54
Mean	**92.96**	**89.46**	**98.27**	**95.27**	**95.44**	**86.43**	**82.99**	**98.18**	**93.00**	**94.08**
Delta	**21.04**	**37.74**	**24.19**	**26.95**	**34.56**	**26.46**	**25.61**	**29.18**	**26.43**	**27.60**
Range	**41.82**	**65.57**	**51.63**	**57.73**	**74.48**	**64.90**	**51.36**	**57.76**	**56.24**	**65.23**

Table 3. Anticancer testing results for compounds **6 – 7a-g** (growth percent against 60 cell lines).

Panel/Cell Line	**Growth Percent**				
	6	**7b**	**7c**	**7f**	**7g**
Leukemia:					
CCRF-CEM	97.72	95.59	98.21	90.23	99.96
HL-60(TB)	96.87	93.05	102.40	101.30	104.83
K-562	69.38	66.04	93.14	92.29	93.71
MOLT-4	93.01	95.64	98.79	98.14	91.70
RPMI-8226	77.25	70.05	92.49	98.02	99.60
SR	97.86	95.47	104.06	104.91	97.00
Non-Small Cell Lung Cancer:					
A549/ATCC	89.66	96.85	102.24	98.49	97.27
EKVX	73.75	79.68	80.04	92.03	83.03
HOP-62	75.64	99.26	78.31	91.05	69.07
HOP-92	50.80	88.16	86.63	92.95	98.62
NCI-H226	73.30	90.75	91.23	89.48	85.35
NCI-H23	78.98	84.87	88.95	92.68	89.34
NCI-H322M	100.71	100.42	98.10	97.92	94.73
NCI-H460	96.35	97.57	103.11	99.39	99.91
NCI-H522	80.67	81.36	93.38	89.51	93.91
Colon Cancer:					
COLO 205	96.93	92.28	97.47	99.15	90.52
HCC-2998	94.33	98.44	100.77	103.81	106.47

HCT-116	92.18	86.04	93.55	93.83	99.03
HCT-15	89.84	114.57	120.30	116.81	118.79
HT29	97.47	91.35	103.82	107.29	98.37
KM12	94.86	92.62	100.28	98.75	96.34
SW-620	93.53	93.10	96.94	95.23	100.20
CNS Cancer:					
SF-268	91.90	94.14	93.38	97.53	100.28
SF-295	81.39	94.43	103.01	102.10	100.79
SF-539	96.28	92.37	89.92	94.71	94.48
SNB-19	90.05	90.74	94.54	94.64	96.90
U251	88.92	84.85	97.68	93.76	99.79
Melanoma:					
LOX IMVI	90.03	89.03	92.59	90.15	92.58
MALME-3M	97.05	92.97	95.77	98.52	94.67
M14	94.47	96.25	101.26	100.10	100.23
MDA-MB-435	99.34	93.94	99.10	103.69	102.82
SK-MEL-2	96.71	96.25	104.36	105.21	87.80
SK-MEL-28	108.46	111.07	118.02	115.21	113.68
SK-MEL-5	74.57	87.43	103.07	104.08	102.46
UACC-257	102.34	109.94	112.71	104.65	109.79
UACC-62	77.88	86.76	88.56	89.14	83.76
Ovarian Cancer:					
IGROV1	88.01	92.85	92.38	92.27	87.66
OVCAR-3	83.36	86.74	102.67	101.31	97.37
OVCAR-4	93.80	87.17	93.32	95.35	94.15
OVCAR-5	95.26	96.12	103.93	104.05	99.63
OVCAR-8	86.08	83.74	100.15	97.49	99.67
NCI/ADR-RES	86.93	94.52	96.79	99.31	101.69
SK-OV-3	70.25	79.27	86.04	78.19	77.01
Renal Cancer:					
786-0	94.95	97.15	98.28	102.24	102.67
A498	72.61	78.56	94.84	94.73	112.64
ACHN	96.86	94.87	95.44	92.71	88.42
CAKI-1	84.85	85.16	76.55	76.18	70.77
RXF 393	85.34	109.12	113.91	125.39	115.75
SN12C	85.95	93.49	95.97	92.82	95.45
TK-10	113.17	107.57	121.53	113.59	122.59
UO-31	74.03	79.89	72.57	73.00	68.09
Prostate Cancer:					
PC-3	70.00	75.45	89.91	88.15	85.81
DU-145	99.09	96.69	106.13	105.79	103.75
Breast Cancer:					
MCF7	73.91	79.48	94.48	93.69	105.30
MDA-MB-231/ATCC	80.88	91.90	82.17	94.32	79.69
HS 578T	90.62	95.61	95.09	94.50	91.19
BT-549	87.63	95.99	102.10	106.55	94.50
T-47D	70.72	88.57	84.30	90.59	84.33
MDA-MB-468	64.40	70.44	105.86	108.28	112.31
Mean	**87.27**	**91.25**	**96.99**	**97.58**	**96.24**
Delta	**36.47**	**25.21**	**24.42**	**24.58**	**28.15**
Range	**62.37**	**48.53**	**48.96**	**52.39**	**54.50**

Table 4. Anticancer testing results for compounds **8a-j** (growth percent against 60 cell lines).

Panel/Cell Line	**Growth Percent**									
	8a	**8b**	**8c**	**8d**	**8e**	**8f**	**8g**	**8h**	**8i**	**8j**
Leukemia:										
CCRF-CEM	110.72	90.08	98.55	103.89	98.39	93.38	74.60	92.13	96.12	97.85
HL-60(TB)	103.16	80.50	100.04	103.20	106.21	102.75	107.11	102.09	94.96	105.02
K-562	91.64	77.00	66.80	109.66	74.38	80.68	95.42	100.37	117.28	103.92
MOLT-4	98.09	102.06	104.89	108.10	98.46	96.97	99.56	108.62	107.96	100.85
RPMI-8226	116.51	70.42	84.11	108.01	87.31	87.36	65.40	96.55	93.74	96.36

SR	105.13	105.27	107.08	108.44	105.03	104.82	107.38	107.66	112.41	99.52
Non-Small Cell Lung Cancer:										
A549/ATCC	98.10	83.70	88.80	105.12	97.53	93.08	99.03	99.49	97.02	102.87
EKVX	81.52	86.15	86.99	96.99	75.16	86.99	76.27	85.54	79.28	84.33
HOP-62	95.24	74.03	100.11	99.98	85.19	85.53	94.61	90.92	88.76	84.30
HOP-92	87.61	38.83*****	88.15	98.31	72.08	66.85	56.78	66.60	44.96*****	76.68
NCI-H226	76.09	48.34*****	81.44	84.19	82.40	76.63	63.94	88.47	61.21	73.26
NCI-H23	87.59	77.10	75.95	88.89	75.97	68.25	80.55	85.09	81.06	77.09
NCI-H322M	103.72	94.97	101.61	105.94	98.30	97.25	90.75	97.81	96.22	97.26
NCI-H460	104.56	93.57	99.95	112.71	99.64	102.36	87.97	107.68	106.77	108.62
NCI-H522	88.33	77.88	81.35	91.46	87.18	83.49	76.20	90.81	81.63	91.61
Colon Cancer:										
COLO 205	113.07	89.29	110.47	109.38	101.78	104.33	93.54	104.11	95.66	106.53
HCC-2998	99.12	94.58	93.28	97.67	96.97	93.21	101.98	92.90	95.34	94.33
HCT-116	95.69	70.03	83.49	105.17	85.46	87.78	71.12	88.80	88.38	98.20
HCT-15	97.45	85.41	100.68	107.64	89.67	105.14	91.02	98.19	88.87	106.02
HT29	113.85	86.46	96.97	113.79	101.87	94.41	89.64	94.49	96.71	107.56
KM12	99.45	87.52	89.82	100.91	89.15	89.13	88.40	97.35	94.43	94.47
SW-620	101.49	91.51	101.51	103.40	95.09	98.99	94.60	94.65	99.60	99.92
CNS Cancer:										
SF-268	95.05	92.95	90.95	98.47	91.43	88.50	96.62	95.77	98.39	94.98
SF-295	97.22	80.36	88.70	104.74	85.05	92.90	87.62	92.25	88.54	99.45
SF-539	97.49	78.77	94.69	97.92	99.72	93.83	97.97	96.71	97.34	101.42
SNB-19	95.66	85.57	98.34	99.67	93.07	99.95	90.71	96.25	93.59	95.65
U251	100.43	79.62	85.65	103.55	94.85	91.57	93.61	102.30	92.18	101.05
Melanoma:										
LOX IMVI	88.91	88.38	88.90	94.20	85.12	88.42	99.25	95.93	89.95	83.02
MALME-3M	110.28	95.60	112.40	110.34	108.68	106.59	102.78	98.02	101.76	100.21
M14	107.68	91.88	106.42	107.96	102.47	99.46	88.53	93.89	95.37	100.64
MDA-MB-435	107.20	94.67	96.60	108.75	103.55	98.56	89.31	100.10	107.20	108.52
SK-MEL-2	109.40	105.97	112.91	98.05	103.84	106.83	93.17	99.61	103.23	96.59
SK-MEL-28	112.60	97.73	107.52	110.10	111.83	103.84	98.89	106.15	105.96	101.47
SK-MEL-5	97.77	71.26	90.04	100.23	89.42	86.89	72.78	92.10	87.37	92.78
UACC-257	119.27	100.82	99.23	107.26	116.58	95.06	103.56	108.74	101.44	103.40
UACC-62	87.82	75.58	85.54	97.50	77.00	86.69	75.72	89.58	91.54	85.15
Ovarian Cancer:										
IGROV1	98.77	83.87	88.58	107.53	77.31	87.86	89.28	96.43	86.13	93.73
OVCAR-3	102.88	80.87	84.87	104.70	87.62	85.94	79.30	103.47	95.15	96.18
OVCAR-4	97.53	92.86	91.29	101.53	86.96	91.97	85.44	99.76	99.67	95.50
OVCAR-5	104.74	96.30	101.20	99.18	99.32	98.32	92.79	99.06	98.75	93.12
OVCAR-8	101.62	76.68	82.58	102.99	87.12	85.29	88.73	94.63	91.89	100.18
NCI/ADR-RES	91.34	86.01	90.94	91.29	88.61	87.06	103.94	96.43	92.19	82.06
SK-OV-3	92.77	76.13	81.25	108.92	96.11	72.98	88.02	101.36	83.59	91.78
Renal Cancer:										
786-0	98.15	85.11	93.40	95.33	91.57	92.48	91.32	94.35	97.14	98.44
A498	74.98	49.08*****	74.39	107.56	81.60	75.64	85.20	94.22	88.07	116.12
ACHN	102.69	92.13	98.58	104.61	89.77	100.59	83.65	98.64	101.70	94.27
CAKI-1	81.34	83.07	78.87	89.69	71.61	87.91	86.13	91.23	88.83	82.59
RXF 393	109.80	65.26	101.23	113.07	105.12	97.25	65.16	85.00	78.10	96.05
SN12C	91.27	83.83	93.25	101.95	89.47	98.99	84.55	101.28	98.96	101.23
TK-10	130.89	112.89	114.49	107.74	123.80	107.91	107.76	101.94	128.02	117.09
UO-31	74.37	81.95	69.12	79.41	65.39	74.49	71.47	71.80	69.68	67.50
Prostate Cancer:										
PC-3	94.02	65.86	77.10	96.35	76.15	83.35	64.82	87.01	83.20	86.48
DU-145	104.82	96.08	96.77	113.55	103.90	95.41	98.84	107.07	105.98	110.82
Breast Cancer:										
MCF7	41.42*****	40.30*****	73.98	92.49	77.40	84.99	66.44	86.86	70.84	86.66
MDA-MB-231/ATCC	86.30	80.25	83.84	102.45	79.06	82.10	80.39	89.05	86.76	87.73
HS 578T	93.99	87.12	97.23	105.24	89.51	93.01	98.20	97.56	95.87	102.93
BT-549	93.88	79.47	93.74	97.82	81.38	85.18	75.91	90.38	94.37	94.08
T-47D	77.15	63.98	78.01	98.69	76.87	79.86	74.19	96.30	86.70	88.50

MDA-MB-468	78.60	35.35*****	71.50	98.00	91.57	77.62	43.07*****	84.57	72.00	92.27
Mean	**96.92**	**82.01**	**91.80**	**102.06**	**91.24**	**90.93**	**86.46**	**95.36**	**92.64**	**95.70**
Delta	**55.50**	**46.66**	**25.00**	**22.65**	**25.85**	**24.08**	**43.39**	**28.76**	**47.68**	**28.20**
Range	**89.47**	**77.54**	**47.69**	**34.38**	**58.41**	**41.06**	**64.69**	**42.14**	**83.06**	**49.59**

* Growth < 50%

Table 5. Compounds showing moderate activity < 50% growth (> 50% inhibition).

Cpd	Panel/Cell Line	Growth %	Cpd	Panel/Cell Line	Growth %
2a	**Leukemia:**		8a	**Breast Cancer:** MCF7	41.42
	K-562	37.99	8b	**Breast Cancer:**	
	RPMI-8226	37.83		MCF7	40.30
	Non-Small Cell Lung Cancer:			MDA-MB-468	35.35
	EKVX	43.87		**Renal Cancer:** A498	49.08
	HOP-92	49.44		**Non-Small Cell Lung Cancer:**	
	NCI-H522	47.33		HOP-92	38.83
	CNS Cancer: U251	49.08		NCI-H226	48.34
	Melanoma: SK-MEL-5	44.93	8g	**Breast Cancer:** MDA-MB-468	43.07
	Breast Cancer: T-47D	9.5	8i	**Non-Small Cell Lung Cancer:** HOP-92	44.96

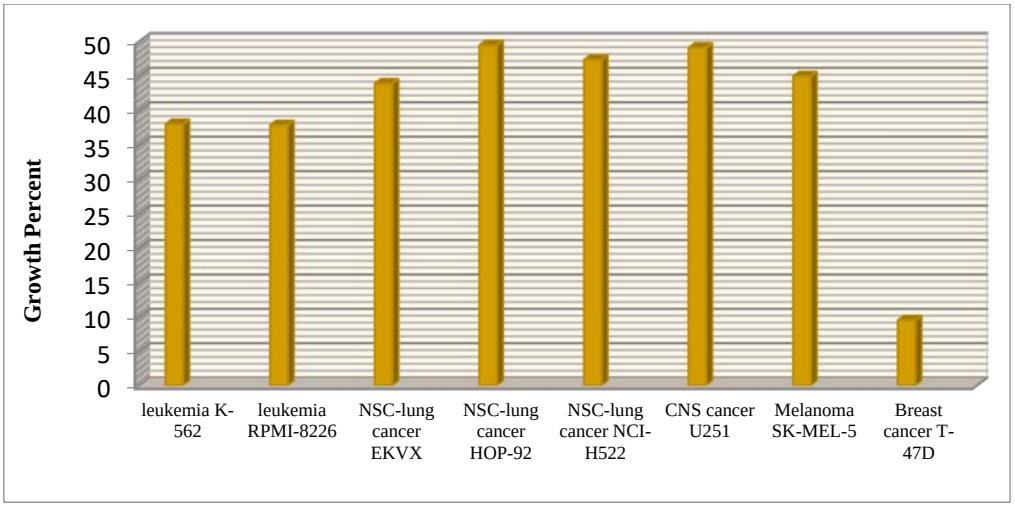


Figure 2a. Compound **2a** showing moderate activity against seven different cell lines and high activity against Breast Cancer T-47D.

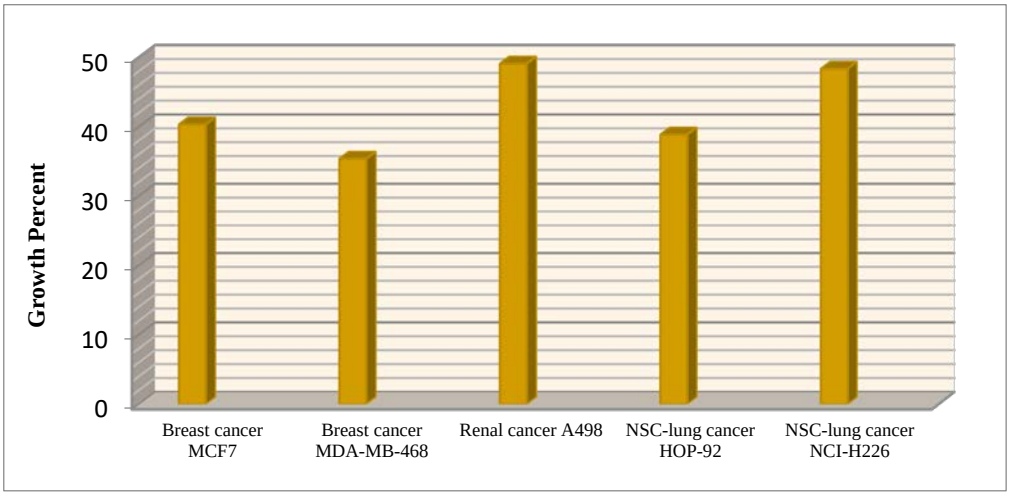


Figure 2b. Compound **8b** showing moderate activity against five different cell lines.

Comparing the mean of % growth against the tested cancer types, it was observed that pyrrolopyrimidines **2a, 3a, b, g, 4b, 5a, b, 6, 8b** exhibited improved mean values ranging from 69.14 to 89.46% **(Figure 3a).** The most active one is pyrrolopyrimidine **2a** with mean growth 69.14%, it has diphenyl groups at five and six positions, ethyl thioacetate moiety at position 4 and 4-methylphenyl at position seven which is clear to be the minimum requirements for adequate activity. Any modification at

positions four, six and seven lead to decrease the activity (**Figure 3b**). On the other hand, by looking to the activity against cell lines, compounds **2a, 8a, b, g, i** were found to achieve % growth < 50 against certain cell lines (**Table 5**). These results were consistent with docking results (next section).

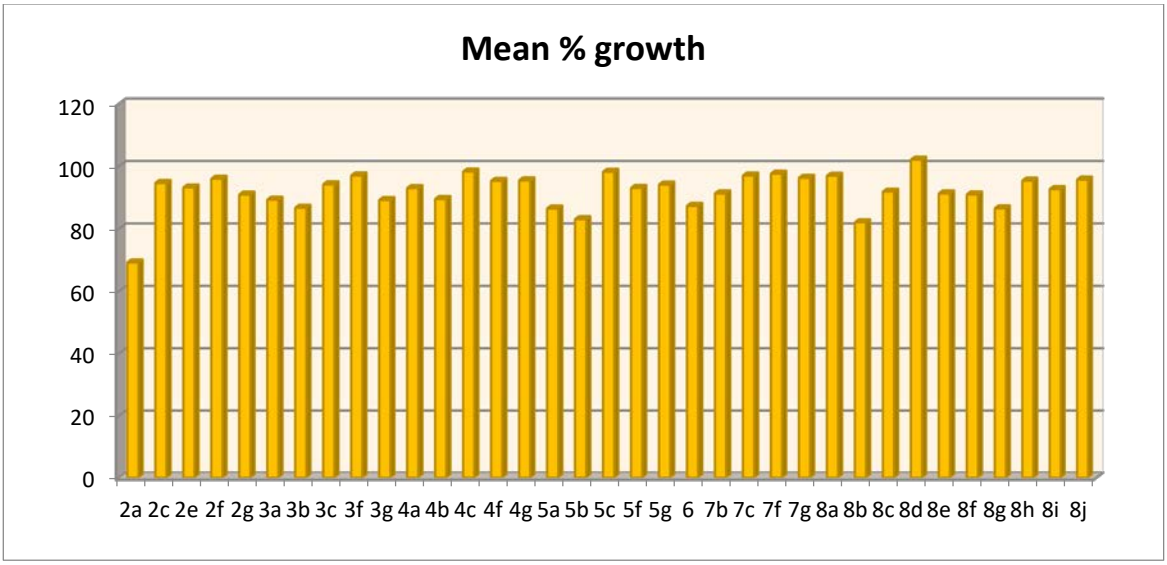


Figure 3a. Mean % growth of all the tested compounds.

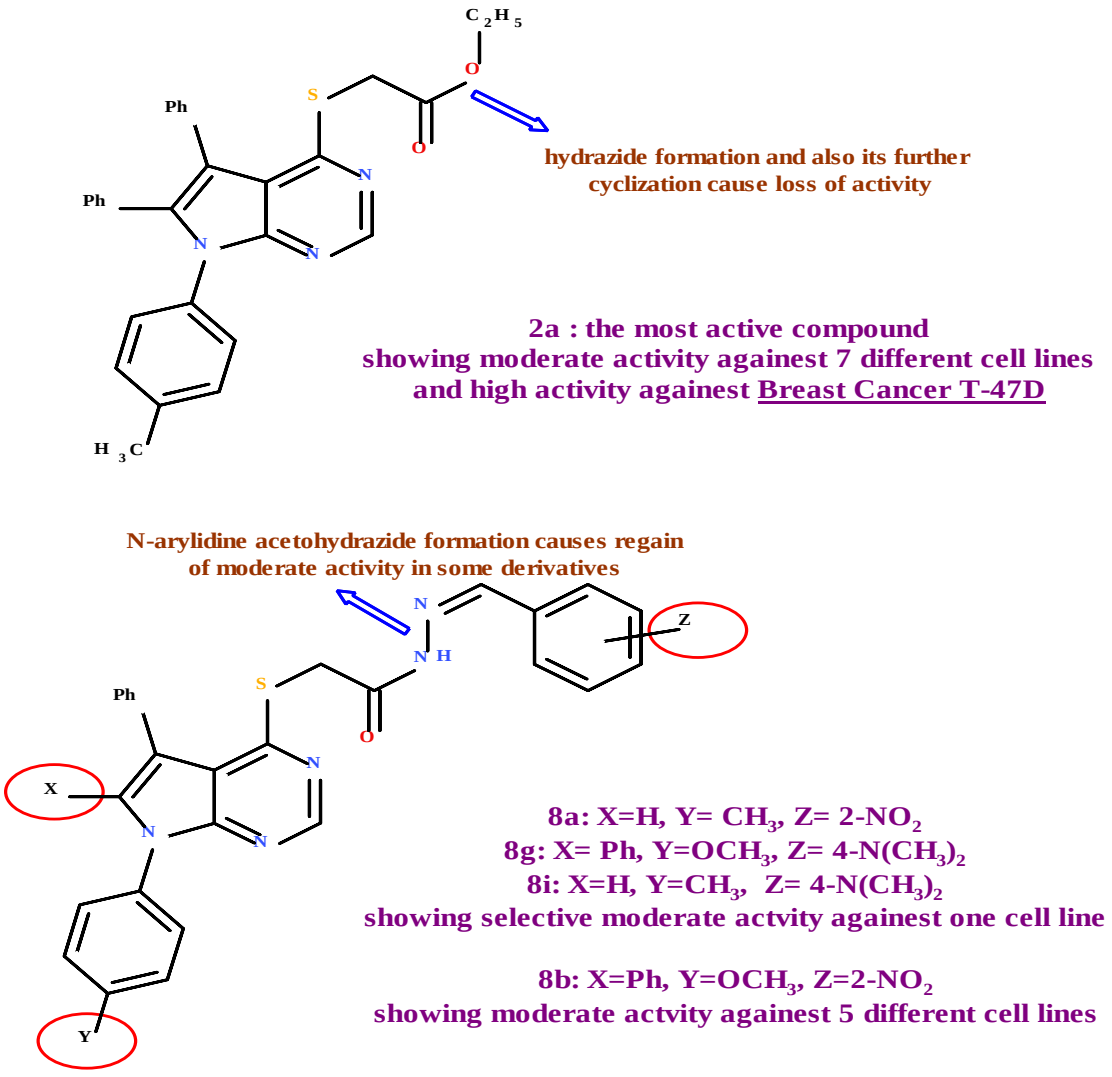


Figure 3b.SAR of the most active compounds **2a, 8a, 8b, 8g** and **8i.**

Molecular docking results

The binding modes of the highly active compounds into CDK2 ATP binding site were computed. The docking results were compared to the reported ligand, N-(9*H*-purin-6-yl) thiophene-2-carboxamide.

Briefly, CDK2 has 298 amino acid residues including four binding sites; the adenosine triphosphate (ATP) binding pocket (Site I), two non-competitive binding sites (Site II and III) and allosteric binding site (Site IV) (**as shown in figure 4**). [51, 52]

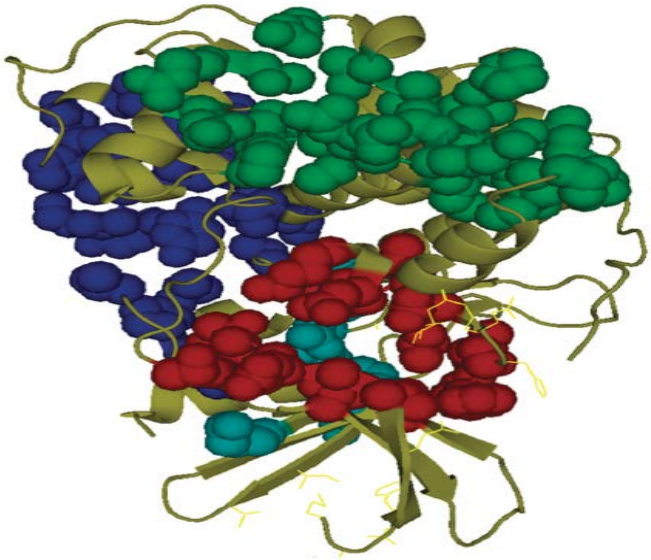


Figure 4. Four binding sites of CDK2. The ATP binding site (Site I) is shown in red, (Sites II and III) are shown in green and blue respectively and allosteric binding site (Site IV) is denoted in cyan. [52a]

The former site is the best binding site where inhibitors bind by competing with ATP. It is located at a deep cleft between the amino- and carboxy-terminal lobes as well as it is highly characterized by a hydrophobic feature. The Important residues involved in the CDK2 ATP-binding pocket are 10–15, 18, 31, 33, 64, 80–83, 86, 129–133, 145 and 160. [53] The prominent effects to stabilize the binding within this site are H-bond interactions formed with the hinge region including Glu 81 and Leu 83 in addition to the hydrophobic interactions. [52a]

According to the docking results, pyrrolo[2,3-*d*] pyrimidines **2a**, **8a**, **8b**, **8g** and **8i** showed good docking score (-6.84 to -4.86 Kcal/mol) and binding energy (London dG -11.87 to -10.57 Kcal/mol) which is better than the reported inhibitor (docking score -4.84 Kcal/mol and London dG -9.79 Kcal/mol), as revealed in **Table 6.**

Table 6. Results for molecular docking studies of compounds **2a, 8a, 8b, 8g** and **8i** CDK2 (PDB ID: 5ANJ).

Compound	Docking Score (S) Kcal/mol	RMSD	E score1 (London dG) Kcal/mol	E score 2 Kcal/mol	Binding interaction (Receptor-Ligand)
2a	-6.84	0.93	-11.83	-6.84	Leu83-(C=O) (H-b, 2.8 A°) Leu83-(S) (H-b, 3.91 A°) Phe82-(CH_2-S) (Pi-H,4.73A°) Gly13-(4-CH_3-Ph)(H-Pi,4.48 A°)
8a	-6.29	1.58	-11.87	-6.29	Leu83-(S) (H-b, 4.13 A°) Gly13-(4-CH_3-Ph) (H-Pi, 3.94 A°) Glu8-(2-NO_2-Ph) (H-b, 3.21 A°)
8b	-5.41	2.13	-11.77	-5.41	Lys33-(5-Ph) (Cation-Pi, 4.62 A°) Gly 11-(C=O) (H-b, 3.27A°)
8g	-6.31	1.98	-10.59	-6.31	Leu83-(S) (H-b, 3.54 A°) Ile10-(5-Ph)(H-Pi, 4.31A°)
8i	-4.59	2.00	-10.57	-4.59	Ile10-(4-CH3-Ph)(H-Pi, 4.57Ao)
ZXC	-4.84	1.96	-9.79	-4.84	Leu83-(N3pyrimidine) (H-b, 3.42 A°) Ile10-(pyrimidine) (H-Pi, 4.2 A°) Lys33-(N7pyrimidine, O) (H-b, 3.01, 2.88 A°) Glu81(N9, imidazole)(H-b, 2.97 A°)

From the obtained results compounds **2a** and **8b**, have the higher anti-cancer activity, also have the best docking results, this can be explained by interpretation of docking results as follow.

All these five compounds share the ligand (ZXC) by having bond with one residue except **8g** has bonds with two residues where it forms a hydrogen bond with Leu83 and arene-H binding with Ile10 whose bond lengths are 3.54 and 4.31A°,

respectively, longer than that of ZXC (3.42 and 4.2 Aº), this explains its moderate activity against only one cell line (**Figure 5d**).

Compound **8b** displays cation-arene binding with Lys33 that measures 4.72 Aºcompared to 3.01, 2.88 Aº for ZXC and hydrogen bond with Gly11 (3.27Aº), also its docking score (S) and RMSD values interpret moderate activity against five cell lines.

Compounds **2a** and **8a** revealed hydrogen bonds with Leu 83 which is considered a critical type of binding for protein stabilization. Compound **2a** which possess the higher activity (**Figure 5a, 6**) has two distinctive hydrogen bonds that are formed between C=O and sulfur with NH and C=O of Leu 83 whose bond length are 2.80 and 3.91 Aº, respectively, relative to one hydrogen bond of ligand (ZXC) of bond length 3.42 Aº. While compound **8a** showed one hydrogen bond with the respected residue measured with inactive bond length (4.13 Aº)**.** Moreover, compound **2a** forms arene-H binding with Phe 82 and Gly 13. It also has the best docking score (S) and RMSD values. This may explain the higher activity of **2a** as it has moderate activity against 7 cell lines and high activity against one cell line.

Compound **8i** showed only arene-H binding with Ile10 with bond length 4.57Aº (relative to 4.2 Aº of ZXC), this explaines its moderate activity against only one cell line **(Figure 5e)**.

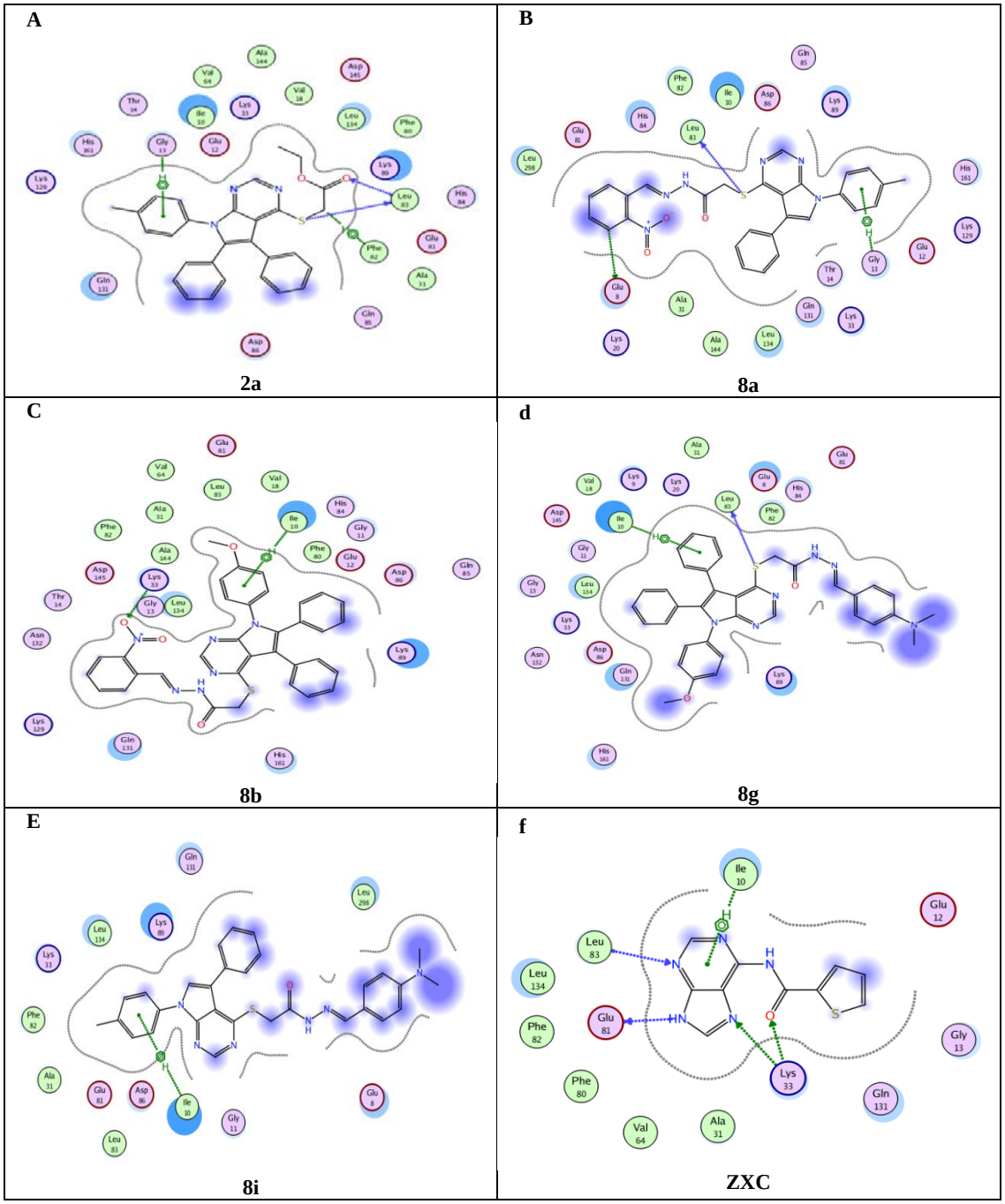


Figure 5. 2D binding mode of compounds **2a, 8a, 8b, 8g, 8i**and **ZXC** withCDK2 ATP binding site.

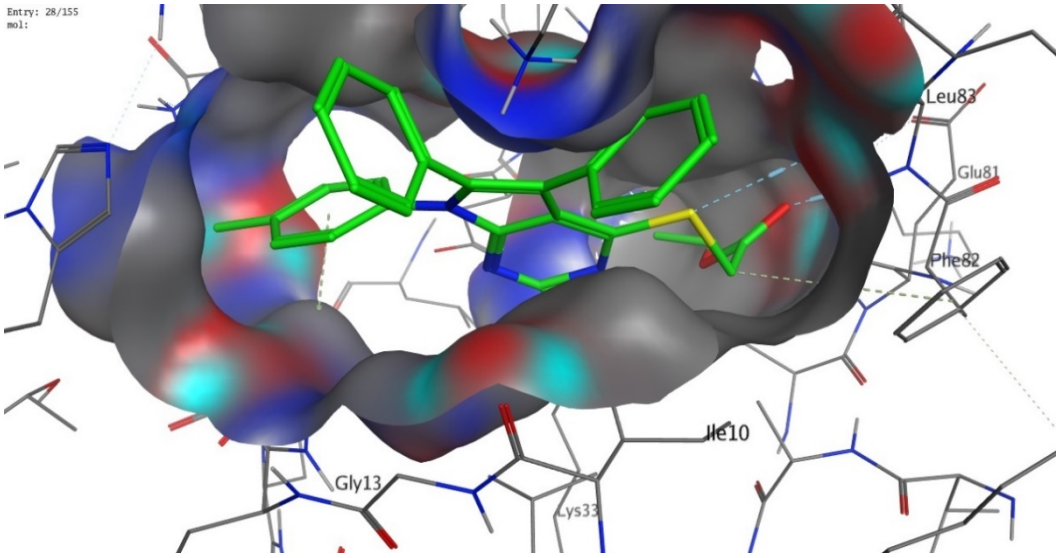


Figure 6. Molecular model for binding of **2a** forming 2 distinctive hydrogen bonds with Leu 83 and arene-H binding with Phe 82 and Gly 13.

Conclusion

In the present research, the early reported pyrrolopyrimidin-4-thiones were used to prepare 46 new pyrrolo[2,3-*d*] pyrimidinederivatives and 35 compounds were selected for single dose anti-cancer testing. The biological result defined that five compounds showed only moderate activity. Docking was carried out for these compounds with CDK2 and the results were consistent with the biological results. Compound **2a** exhibited the highest potency, especially against breast cancer T-47D cell line achieving minimum growth percent (9.51%) and RMSD (0.93). This made compound **2a** a potential scaffold for the drug discovery of anti-cancer agents.

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