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Exploration of the *in vitro* anti-HIV and cyclin-dependent kinase 2 (CDK2) inhibitory activities of new 6-aryl-pyrimidines and their nitroso analogues

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Abstract: A new series of 2-amino-4-benzyloxy-6-(2-fluorophenyl)pyrimidine derivatives (**14-22**) were synthesized *via* Suzuki-Miyaura cross-coupling reaction, with the aim of developing novel HIV non-nucleoside reverse transcriptase inhibitors. All the synthesized compounds were structurally confirmed by spectral analyses. The compounds were evaluated for their antiviral activity against the replication of HIV-1 and HIV-2 in MT-4 cells using an MTT assay. Compound **20** exhibited IC_{50} value of 1.83 μM with SI = 10.3 against HIV-1. In a docking study, **20** interacted with several amino acids in the reverse transcriptase (RT) binding site of HIV-1, and the results suggest that **20** can be considered as a new lead in the development of antiviral agents.The structure activity relationship (SAR) of these new analogues was studied as well. In addition, the CDK2 inhibitory activity of the 5-nitrosopyrimidine analogues **23-27** and **35-38** was evaluated.

Keywords: Anti-HIV activity, Arylpyrimidines, CDK2, Molecular docking study, QSAR, Suzuki cross-coupling reaction

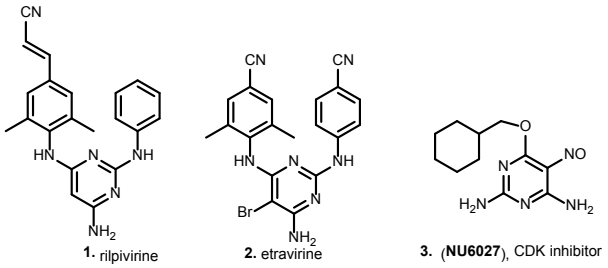
1. Introduction

Pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acids to their current use in the chemotherapy of AIDS. Pyrimidines possess several pharmaceutical applications, including antitumor [1-6], antimicrobial [7-10], antihypertensive [11] in addition to their cardiovascular [12,13] and

diuretic [14,15] properties. Some pyrimidine analogues exhibited potent antiviral activity against a wide spectrum of unrelated viruses, such as poliovirus [16], herpes virus [17] and anti-HIV agents [18-20], whereas two recent diarylpyrimidines (DAPY), rilpivirine (**1**) [21] and etravirine (**2**) [22, 23] have been classified as non-nucleoside reverse transcriptase inhibitors (NNRTI's), meanwhile Chen *et al.* [24] have reported a new class of diarylpyrimidines (CHX-DAPYs) as potent NNRTI's. Further, several pyrimidine derivatives exhibited significant antitumor activity *e.g.* imatinib mesylate (Gleevec) [25], is the tyrosine kinase inhibitor, which contains a 4-pyridylsubstituted 2-aminopyrimidine.

Some pyrimidine derivatives were reported to act as inhibitors of cyclin-dependent kinases (CDK's) and which thereby can provide useful therapeutic compounds for use in treatment of tumours or other cell proliferation disorders. Recently, Melguizo *et al.* [26] have reported that 4-alkoxy-5-nitrosopyrimidines are useful building block for the generation of biologically active compounds. Compound **3** (NU6027) was considered as a competitive inhibitor for both cyclin-dependent kinases, cdk1 and cdk2 [27], with IC_{50} values of 2.9±0.1 μM and 2.2±0.6 μM against cdk1/cyclin B1 and cdk2/cyclinA3, respectively. Furthermore, other pyrimidine derivatives were reported as potent acid pump antagonists (APAs) [28], meanwhile Jian *et al.* [29] have reviewed the biological and medicinal significance of pyrimidines extensively.

The discovery and development of DAPY derivatives as next-generation NNRTI drugs, and in continuation of our ongoing work on the synthesis of pyrimidines as new anti-HIV agents [30-35], we report here the synthesis of new series of pyrimidines having aryl residues, *via* Suzuki cross-coupling reaction and evaluation of their anti-HIV activity as well as CDKs inhibition activity of some synthesized analogues together with the SAR and molecular modeling study.



2. Experimental section

General

Melting points were recorded on melting point apparatus (VEEGO), or a Büchi melting point apparatus B-545 (BÜCHI Labortechnik AG, Switzerland) and are uncorrected. ^{1}H and ^{13}C NMR spectra was recorded on a Bruker (Avance III, Germany) spectrospin-400 and 600 MHz (^{1}H), 100 and 150.91 MHz (^{13}C) spectrometers using ($CDCl_3$) containing tetramethylsilane as internal standard (chemical shifts in δ, ppm). Heteronuclear assignments were verified by ^{1}H, ^{13}C HSQC NMR experiments. Microanalytical data were obtained with a Vario elemental apparatus (Shimadzu, Japan). Microanalytical data were obtained with a Vario elemental apparatus (Shimadzu, Japan). Column chromatography was carried out with silica gel powder (230-400 mesh size) by using appropriate solvents.

2.1. *General procedure the preparation of new 6-arylpyrimidine analogues* **(14-22)** *via Suzuki reaction*

To a stirred suspension of 2-amino-4-(benzyloxy)-6-chloropyrimidine (**4**) (100 mg, 0.42 mmol) in *n*-propanol (15 mL) was added arylboronic acid (0.42 mmol) and stirred at room temperature for 15 min until all solids were dissolved. To this solution was added $Pd(OAc)_2$ (360 mg, 0.11 mmol), Ph_3P (128 mg, 0.49 mmol) and 2M aq. solution of Na_2CO_3 (4 mL). The reaction mixture was heated under

reflux for 8-16 h, and monitored by TLC (eluent: ethyl acetate/ hexane). After cooling, the reaction mixture was filtered, and the filtrate was evaporated to dryness. The solid product was washed with cold ether and recrystallized from DMF-ether or EtOH to give the desired product.

2.1.1. *2-Amino-4-benzyloxy-6-(2-fluorophenyl) pyrimidine **(14)***

From 2-fluorophenylboronic acid (**5**) (59 mg). Yield: 107 mg (67 %); MP: 246-248 ^{o}C; R_f= 0.45; ^{1}H NMR (DMSO-d_6): δ7.62-7.55 (m, 2H, $H_{arom.}$), 7.44-7.33 (m, 7H, $H_{arom.}$), 7.11 (bs, 2H, NH_2), 6.15 (s, 1H, H-5), 5.31 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6): δ 170.2 ($C_{pyrimid.}$-4), 162.6 ($C_{pyrimid.}$-6), 159.9 ($C_{pyrimid.}$-2), 148.0 (d, J = 251 Hz, C-F), 136.1 (C_{benzyl}-1), 131.9,131.4, 128.7, 128.3, 128.0, 127.7 ($C_{arom.}$), 94.3 ($C_{pyrimid.}$-5), 67.2 (CH_2); MS (FAB), *m/z* = 295/297 $[M+H]^+$; Elemental Analysis for $C_{17}H_{14}FN_3O$ (295.31); Cal: C, 69.14; H, 4.78; N, 14.23%; Found: C, 68.97; H, 4.64; N, 14.03%.

2.1.2. *2-Amino-4-benzyloxy-6-(3-fluorophenyl) pyrimidine **(15)***

From 3-fluorophenylboronic acid (**6**) (59 mg). Yield: 110 mg (69 %); MP: 248-250 ^{o}C; R_f = 0.46; ^{1}H NMR (DMSO-d_6): δ 7.64-7.55 (m, 2H, $H_{arom.}$), 7.49-7.33 (m, 7H, $H_{arom.}$), 7.10 (bs, 2H, NH_2), 6.15 (s, 1H, H-5), 5.32 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6): δ 171.1 ($C_{pyrimid.}$-4.), 163.5 ($C_{pyrimid.}$-6), 160.8 ($C_{pyrimid.}$-2), 159.6 (d, J = 252 Hz, C-F), 138.3 (C_{benzyl}-1), 136.2 ($C_{arom.}$-1'), 132.6, 132.1, 129.4, 129.2, 128.9, 128.6, 128.4 ($C_{arom.}$), 94.8 ($C_{pyrimid.}$-5), 67.6 (CH_2); MS (FAB), *m/z* = 295/297 $[M+H]^+$; Elemental Analysis for $C_{17}H_{14}FN_3O$ (295.31); Cal: C, 69.14; H, 4.78; N, 14.23%; Found: C, 68.85; H, 4.70; N, 14.11%.

2.1.3. *2-Amino-4-benzyloxy-6-(4-fluorophenyl) pyrimidine**(16)***

From 4-fluorophenylboronic acid (**7**) (59 mg, 0.42 mmol). Yield: 99 mg (62 %); MP: 246-248 ^{o}C; R_f = 0.46; ^{1}H NMR (DMSO-d_6): δ 7.64-7.55 (m, 2H, $H_{arom.}$),7.47-7.33 (m, 7H, $H_{arom.}$), 7.10 (bs, 2H, NH_2), 6.15 (s, 1H, H-5), 5.32 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6): δ 170.2 ($C_{pyrimid.}$-4), 162.6 ($C_{pyrimid.}$-6), 159.9 ($C_{pyrimid.}$-2), 158.3 (d, J = 250 Hz, C-F), 136.6 (C_{benzyl}-1), 131.9 ($C_{arom.}$-1'), 131.9, 131.4, 128.7, 128.3, 128.1, 127.7 ($C_{arom.}$), 94.3 ($C_{pyrimid.}$-5), 67.2 (CH_2); MS (FAB), *m/z* = 295/297 $[M+H]^+$; Elemental Analysis for $C_{17}H_{14}FN_3O$ (295.03); Cal: C, 69.14; H, 4.78; N, 14.23%; Found: C, 69.31; H, 4.69; N, 13.98%.

2.1.4 2-*Amino-4-benzyloxy-6-(4-nitrophenyl) pyrimidine(**17**)*

From 4-nitrophenylboronic acid (**8**) (70 mg). Yield: 115 mg (67 %); MP: 167-170 ^{o}C; R_f = 0.42; ^{1}H NMR (DMSO-d_6): δ 7.64-3.38 (m, 9H, $H_{arom.}$), 7.10 (bs, 2H, NH_2), 6.15 (s, 1H, H-5), 5.32 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6): δ170.2 ($C_{pyrimid.}$-4), 162.6 ($C_{pyrimid.}$-6), 159.9 ($C_{pyrimid.}$-2), 145.5 (C-NO_2), 140.1 ($C_{arom.}$-1'), 136.1 (C_{benzyl}-1), 131.4, 131.3, 128.7, 128.3, 128.1, 127.9, 127.7 ($C_{arom.}$); 94.3 ($C_{pyrimid.}$-5), 67.2 (CH_2); MS (FAB), *m/z* = 295/297 $[M+H]^+$; Elemental Analysis for- $C_{17}H_{14}N_4O_3$ (322.32); Cal: C, 63.35; H, 4.38; N, 17.38%; Found: C, 63.02; H, 4.21; N, 16.98%.

2.1.5. *2-Amino-4-benzyloxy-6-(4-ethoxycarbonylphenyl)pyrimidine(**18**)*

From 4-ethoxycarbonylphenylboronic acid (**9**) (82 mg). Yield: 145 mg (79 %); MP: 168-170 ^{o}C (dec.); R_f = 0.56; ^{1}H NMR (DMSO-d_6): δ 7.96-7.87 (m, 1H, $H_{arom.}$), 7.84-7.38 (m, 8H, $H_{arom.}$), 7.15 (bs, 2H, NH_2), 6.14 (s, 1H, H-5), 5.31 (s, 2H, CH_2), 4.19 (q, 2H, J = 7.1 Hz, CH_2CH_3), 1.26 (t, 3H, J = 7.1 Hz,CH_2CH_3); ^{13}C NMR (DMSO-d_6): δ 190.3 (CO_2Et), 170.2 ($C_{pyrimid.}$-4), 164.9 ($C_{pyrimid.}$-6), 162.6 ($C_{pyrimid.}$-2), 147.1 ($C_{arom.}$-1'), 140.2 (C_{benzyl}-1), 132.0, 131.9, 131.4, 128.7, 128.3, 128.0, 127.8 ($C_{arom.}$), 94.3 ($C_{pyrimid.}$-5), 67.3 (CH_2), 62.6 (CH_2CH_3), 11.1 (CH_2CH_3); MS (FAB), *m/z* = 350 $[M+H]^+$; Elemental Analysis for $C_{20}H_{19}N_3O_3$ (349.38); Cal: C, 68.75; H, 5.48; N, 12.03%; Found: C, 68.55; H, 5.31; N, 11.84%.

2.1.6. *2-Amino-4-benzyloxy-6-(4-hydroxyphenyl)pyrimidine(**19**)*

From 4-hydroxyphenylboronic acid (**10**) (58 mg). Yield: 120 mg (76 %); MP: 160-162 ^{o}C; R_f = 0.38; ^{1}H NMR (DMSO-d_6): δ 7.64-7.30 (m, 9H, $H_{arom.}$), 7.10 (bs, 2H, NH_2), 6.14 (s, 1H, H-5), 5.31 (br s., 3H, OH+CH_2); ^{13}C NMR (DMSO-d_6): δ 170.2 ($C_{pyrimid.}$-4), 162.6 ($C_{pyrimid.}$-6), 158.6 (C-OH+$C_{pyrimid.}$-2), 136.1 (C_{benzyl}-1), 131.4 ($C_{arom.}$-1'), 128.7, 128.5, 128.3, 128.0, 127.7, 127.4 ($C_{arom.}$), 115.4 (C-3+ $C_{arom.}$-3'+$C_{arom.}$-5'), 94.3 ($C_{pyrimid.}$-5), 67.3 (CH_2); Elemental Analysis for $C_{17}H_{15}N_3O_2$ (293.32); Cal: C, 69.61; H, 5.15; N, 14.33%; Found: C, 69.40; H, 5.02; N, 14.02%.

2.1.7. 2-*Amino-4-oxybenzyl-6-(3-cyanophenyl) pyrimidine(**20**)*

From 3-cyanophenylboronic acid (**11**) (62 mg). Yield: 128 mg (79 %); MP: 154-157 ^{o}C (dec.); R_f = 0.58; ^{1}H NMR (DMSO-d_6): δ 7.88 (d, 1H, $J_{5',6'}$ = 7.1 Hz, $H_{arom.}$-6'), 7.63-7.56 (m, 2H, $H_{arom.}$), 7.44-7.36 (m, 6H, $H_{arom.}$), 7.10 (bs, 2H, NH_2), 6.14 (s, 1H, H-5), 5.31 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6): δ 170.2 ($C_{pyrimid.}$-4), 162.7 ($C_{pyrimid.}$-6), 159.9 ($C_{pyrimid.}$-2), 136.1 (C_{benzyl}-1), 132.0 ($C_{arom.}$-1'), 128.7, 128.6, 128.3, 128.0, 127.9 ($C_{arom.}$), 117.1 (CN), 112.9 (*C*-CN), 67.3 (CH_2); Elemental Analysis for $C_{18}H_{14}N_4O$ (302.33); Cal: C, 71.51; H, 4.67; N, 18.53%; Found: C, 71.23; H, 4.69; N, 18.36%.

2.1.8. *2-Amino-4-benzyloxy-6-(3,4-dimethoxyphenyl)pyrimidine(**21**)*

From 3,4-dimethoxyphenylboronic acid (**12**) (76 mg). Yield: 129 mg (73 %); MP: 178-180 ^{o}C; R_f = 0.50; ^{1}H NMR (DMSO-d_6): δ 7.64-7.36 (m, 7H, $H_{arom.}$), 7.10 (bs, 2H, NH_2), 6.14 (s, 1H, H-5), 5.31 (s, 2H, CH_2), 3.87 (s, 3H, 2×OMe); ^{13}C NMR (DMSO-d_6): δ 170.2 ($C_{pyrimid.}$-4), 162.6 ($C_{pyrimid.}$-6), 159.9 ($C_{pyrimid.}$-2), 150.6 (*C*-OMe), 136.1 (C_{benzyl}-1), 131.1 ($C_{arom.}$-1'), 131.9, 131.4, 128.7, 128.3, 128.0, 127.9 ($C_{arom.}$), 120.7 ($C_{arom.}$-2'), 110.9 ($C_{arom.}$-3'), 106.4 ($C_{arom.}$-6'), 94.3 ($C_{pyrimid.}$-5), 67.3 (CH_2), 55.9 (2×OMe); Elemental Analysis for $C_{19}H_{19}N_3O_3$ (337.37); Cal;: C, 67.64; H, 5.68; N, 12.46%; Found: C, 67.42; H, 5.55; N, 12.16%.

2.1.9. 2-*Amino-4-benzyloxy-6-(3,4-difluorophenyl)pyrimidine(**22**)*

From 3,4-difluorophenylboronic acid (**13**) (66 mg). Yield: 107 mg (64 %); MP: 258-260 ^{o}C; R_f = 0.48; ^{1}H NMR (DMSO-d_6): δ 7.90 (m, 1H, $H_{arom.}$-2'), 7.79 (m, 1H, $H_{arom.}$-6'), 7.36-7.19 (m, 6H, $H_{arom.}$), 7.11 (bs, 2H, NH_2), 6.70 (s, 1H, H-5), 5.29 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6): δ 170.2 ($C_{pyrimid.}$-4), 162.7 ($C_{pyrimid.}$-6), 159.9 ($C_{pyrimid.}$-2), 141.0 (d, J = 250 Hz, 2×C-F), 136.1 (C_{benzyl}-1), 133.0 ($C_{arom.}$-1'), 131.4, 128.7, 128.3, 128.1, 127.9 ($C_{arom.}$), 118.4 ($C_{arom.}$-6'), 110.3 ($C_{arom.}$-3'), 94.3 ($C_{pyrimid.}$-5), 67.3 (CH_2); Elemental Analysis for $C_{17}H_{13}N_3O$ (313.30); Cal: C, 65.17; H, 4.18; N, 13.41%; Found: C, 64.89; H, 4.02; N, 13.13%.

2.2. *General procedure the preparation of 2-amino-6-aryl-4-benzyloxy5-nitrosopyrimidine analogues (**23-27**)*

Compounds **14-16**, **20** and **21** (1.00 mmol) were dissolved in a mixture of water (1:1) and 30% AcOH (6 mL) with stirring. To this mixture, a soln. of $NaNO_2$ (68 mg, 1.00 mmol) in water (4 mL) was added dropwise at ambient temperature within 10 min. The mixture was then heated at 80 ^{o}C for 2 h. After cooling, the resulting precipitate was filtered, washed several times with water and dried in a vaccum desiccator over P_2O_5 to give the desired nitroso products.

2.2.1. *2-Amino-4-benzyloxy-6-(2-fluorophenyl)-5-nitrosopyrimidine (**23**)*

From **14** (295 mg). Yield: 207 mg (64 %); MP: 257-260 ^{o}C; R_f = 0.40; ^{1}H NMR (DMSO) δ 7.72-7.71 (m, 2H, $H_{arom.}$), 7.47-7.30 (m, 7H, $H_{arom.}$), 6.78 (bs, 2H, NH_2), 5.28 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6): δ 167.9 ($C_{pyrimid.}$-2), 160.1 ($C_{pyrimid.}$-6), 159.5 ($C_{pyrimid.}$-4), 150.1 (d, J= 250 Hz, C-F), 140.1 (C_5-NO), 136.0 (C_{benzyl}-1), 130.7, 130.0, 129.6, 128.5, 128.3, 128.0, 127.5,

124.3, 116.3 ($C_{arom.}$), 66.0 (CH_2); MS (FAB), *m/z* = 324/326 $[M+H]^+$; Elemental Analysis for $C_{17}H_{13}FN_4O_2$ (324.32); Cal: C, 62.96; H, 4.04; N, 17.28%; Found: C, 62.78; H, 3.97; N, 17.09%.

2.2.2. *2-Amino-4-benzyloxy-6-(3-fluorophenyl)-5-nitrosopyrimidine (**24**)*

From **15** (295 mg). Yield: 220 mg (68 %); MP: 260-263 °C; R_f = 0.39; ^{1}H NMR (DMSO) δ 7.62-7.51 (m, 2H, $H_{arom.}$), 7.48-7.23 (m, 7H, $H_{arom.}$), 6.75 (bs, 2H, NH_2), 5.29 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6): δ 167.4 ($C_{pyrimid.}$-2), 160.0 ($C_{pyrimid.}$-6), 159.1 ($C_{pyrimid.}$-4), 153.7 (d, *J* = 248 Hz, C-F), 140.0 (C_5-NO), 136.5 (C_{benzyl}-1), 133.9, 129.0, 128.7, 127.2, 123.6, 116.2, 115.3 ($C_{arom.}$), 65.8 (CH_2); MS (FAB), *m/z* = 324/326 $[M+H]^+$; Elemental Analysis for $C_{17}H_{13}FN_4O_2$ (324.32); Cal: C, 62.96; H, 4.04; N, 17.28%; Found: C, 62.80; H, 3.93; N, 16.95%.

2.2.3. *2-Amino-4-benzyloxy-6-(4-fluorophenyl)-5-nitrosopyrimidine (**25**)*

From **16** (295 mg). Yield: 243 mg (75 %); MP: 258-261 °C; R_f = 0.41; ^{1}H NMR (DMSO) δ 7.80-7.53 (m, 4H, $H_{arom.}$), 7.39-7.30 (m, 5H, $H_{arom.}$), 6.70 (bs, 2H, NH_2), 5.28 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6): δ 167.0 ($C_{pyrimid.}$-2), 161.8 (d, *J* = 250 Hz, C-F), 160.0 ($C_{pyrimid.}$-6), 159.5 ($C_{pyrimid.}$-4), 140.0 (C_5-NO), 136.3 (C_{benzyl}-1), 130.4, 129.5, 128.5, 127.8, 122.0, 117.0 ($C_{arom.}$), 65.5 (CH_2); MS (FAB), *m/z* = 324/326 $[M+H]^+$; Elemental Analysis for $C_{17}H_{13}FN_4O_2$ (324.32); Cal: C, 62.96; H, 4.04; N, 17.28%; Found: C, 62.82; H, 3.98; N, 17.13%.

2.2.4. *2-Amino-4-benzyloxy-6-(3-cyanophenyl)-5-nitrosopyrimidine (**26**)*

From **20** (302 mg). Yield: 243 mg (79 %); MP: 161-163 °C; R_f = 0.50; ^{1}H NMR (DMSO) δ 7.91-7.71 (m, 4H, $H_{arom.}$), 7.49-7.30 (m, 5H, $H_{arom.}$), 6.79 (bs, 2H, NH_2), 5.26 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6): δ 166.7 ($C_{pyrimid.}$-2), 160.3 ($C_{pyrimid.}$-6), 159.3 ($C_{pyrimid.}$-4), 139.6 (C_5-NO), 136.2 (C_{benzyl}-1), 133.6, 131.9, 130.2, 129.0, 128.0, 127.6 ($C_{arom.}$), 118.8 (CN), 114.0 (*C*-CN), 65.3 (CH_2); MS (FAB), *m/z* = 332 $[M+H]^+$; Elemental Analysis for $C_{18}H_{13}N_5O_2$ (331.34); Cal: C, 65.25; H, 3.95; N, 21.14%; Found: C, 65.02; H, 3.88; N, 20.92%.

2.2.5. *2-Amino-4-benzyloxy-6-(3,4-dimethoxyphenyl)-5-nitrosopyrimidine (**27**)*

From **21** (337 mg). Yield: 252 mg (75 %); MP: 188-190 °C; R_f = 045; ^{1}H NMR (DMSO) δ 7.60-7.40 (m, 4H, $H_{arom.}$), 7.35-7.11 (m, 3H, $H_{arom.}$), 6.89 (bs, 2H, NH_2), 5.28 (s, 2H, CH_2); 55.7 (2×OMe); ^{13}C NMR (DMSO-d_6): δ 166.3 ($C_{pyrimid.}$-2), 160.1 ($C_{pyrimid.}$-6), 159.1 ($C_{pyrimid.}$-4), 150.2, 149.9 (2x*C*-OMe), 139.3 (C_5-NO), 136.5 (C_{benzyl}-1), 130.0, 129.0, 127.1, 125.9, 111.2, 109.2 ($C_{arom.}$), 65.3 (CH_2); 55.8 (2×OMe); MS (FAB), *m/z* = 389 $[M+Na]^+$; Elemental Analysis for $C_{19}H_{18}N_4O_4$ (366.38); Cal: C, 62.29; H, 4.95; N, 15.29%; Found: C, 61.98; H, 4.88; N, 15.08%.

2.3. *General procedure the preparation of 4-aryl-6-methoxy-2-(dimethylamino)-5-nitrosopyrimidine analogues (**35-38**)*

These compounds were prepared according to the procedure for preparation of **23-27** from the analogues **31-34** (1.00 mmol) and a soln. of $NaNO_2$.

2.3.1. *4-(2-Fuorophenyl)-6-methoxy-2-(dimethylamino)-5-nitrosopyrimidine analogues (**35**)*

From **31** (247 mg). Yield: 218 mg (79 %); MP: 260-263 °C; R_f = 0.40; ^{1}H NMR (DMSO) δ 7.79-7.70 (m, 2H, $H_{arom.}$), 7.48 (m, 1H, $H_{arom.}$), 7.28 (m, 1H, $H_{arom.}$), 3.85 (s, 3H, OMe), 3.11 (s, 6H, NMe_2); ^{13}C NMR (DMSO-d_6): δ 170.1 ($C_{pyrimid.}$-2), 160.2 ($C_{pyrimid.}$-6), 159.2 ($C_{pyrimid.}$-4), 155.9 (d, *J* = 249 Hz, (C-F), 139.9 (C_5-NO), 130.5, 129.5, 124.5, 122.9, 115.2 ($C_{arom.}$), 53.4 (OMe), 36.7 (NMe_2); MS (FAB), *m/z* = 276/278 $[M+H]^+$; Elemental Analysis for $C_{13}H_{13}FN_4O_2$ (276.27); Cal: C, 56.52; H, 4.74; N, 20.28%; Found: C, 56.38; H, 4.66; N, 20.10%.

2.3.2. *4-(4-Nitrophenyl)-6-methoxy-2-(dimethylamino)-5-nitrosopyrimidine analogues (**36**)*

From **32** (274 mg). Yield: 221 mg (73 %); MP: 160-162 °C; R_f = 0.35; ^{1}H NMR (DMSO) δ 8.31 (d, 2H, $J_{3',5'}$ = 7.8 Hz, $H_{arom.}$-3'+$H_{arom.}$-5'), 8.01 (d, 2H, $J_{2',6'}$ = 7.8 Hz, $H_{arom.}$-2'+$H_{arom.}$-6'), 3.90 (s, 3H, OMe), 3.17 (s, 6H, NMe_2); ^{13}C NMR (DMSO-d_6): δ 170.8 ($C_{pyrimid.}$-2), 160.0 ($C_{pyrimid.}$-6), 159.7 ($C_{pyrimid.}$-4), 148.4 ($C_{arom.}$-NO_2), 139.8 (C_5-NO), 131.5 ($C_{arom.}$-1'), 128.1 ($C_{arom.}$-2'+$C_{arom.}$-6'), 124.3 ($C_{arom.}$-3'+$C_{arom.}$-5'), 53.5 (OMe), 36.9 (NMe_2); MS (FAB), *m/z* = 304 $[M+H]^+$; Elemental Analysis for $C_{13}H_{13}N_5O_4$ (303.28); Cal: C, 51.49; H, 4.32; N, 23.09%; Found: C, 51.29; H, 4.26; N, 22.89%.

2.3.3. *3-(2-(Dimethylamino)-6-methoxy-5-nitrosopyrimidin-4-yl)benzoic acid (**37**)*

From **33** (273 mg). Yield: 208 mg (69 %); MP: 279-281 °C; R_f = 0.52; ^{1}H NMR (DMSO) δ 10.55 (s, 1H, CO_2H, exchangable with D_2O), 8.28-8.05 (m, 4H, $H_{arom.}$), 3.88 (s, 3H, OMe), 3.19 (s, 6H, NMe_2); ^{13}C NMR (DMSO-d_6): δ 167.9 ($C_{pyrimid.}$-2), 160.2 ($C_{pyrimid.}$-6), 159.0 ($C_{pyrimid.}$-4), 139.8 (C_5-NO), 132.5, 130.9, 129.7, 129.0, 128.5 ($C_{arom.}$), 52.9 (OMe), 36.5 (NMe_2); MS (FAB), *m/z* = 303 $[M+H]^+$; Elemental Analysis for $C_{14}H_{14}N_4O_4$ (302.29); Cal: C, 55.63; H, 4.67; N, 18.53%; Found: C, 55.48; H, 4.55; N, 18.40%.

2.3.4. *5-(2-(Dimethylamino)-6-methoxy-5-nitrosopyrimidin-4-yl)furan-2-carboaldehyde (**38**)*

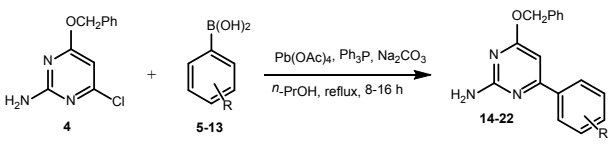
From **34** (247 mg). Yield: 199 mg (72 %); MP: 256-258 °C; R_f = 0.52; ^{1}H NMR (DMSO) δ 10.52 (s, 1H, CHO); 8.50 (d, 1H, *J* = 5.3 Hz, H_{furan}-4'), 7.95 (d, 1H, *J* = 5.3 Hz, H_{furan}-3'), 4.20 (s, 3H, OMe), 2.91 (s, 6H, NMe_2); ^{13}C NMR (DMSO-d_6): δ 178.0 (CHO), 169.0 (C-4), 168.1 (C-2), 162.3 (C-6), 161.4 (C_{furan}-1'), 152.2 (*C*-CHO), 139.7 (C-5), 124.5 (C_{furan}-3'), 111.6 (C_{furan}-2'), 53.9 (OMe), 38.2 (NMe_2); MS (FAB), *m/z* = 299 $[M+Na]^+$; Elemental Analysis for $C_{12}H_{12}N_4O_4$ (276.25); Cal: C, 52.17; H, 4.38; N, 20.28%; Found: C, 51.95; H, 4.26; N, 20.05%.

3. Results and discussion

3.1. Chemistry

2-Amino-4-(benzyloxy)-6-chloropyrimidine (**4**) was selected as a starting material for the synthesis of new pyrimidine derivatives, employing Suzuki-Miyaura cross-coupling reaction [36], aiming to examine their anti-HIV activity. Thus, treatment of **4** with various arylboronic acids (aryl: 2-fluoro- **5**, 3-fluoro- **6**, 4-fluoro- **7**, 4-nitro- **8**, 4-ethoxycarbonyl- **9**, 4-hydroxy- **10**, 3-cyano- **11**, 3,4-dimethoxy- **12**and 3,4-difluoro- **13**) in the presence of $Pd(OAc)_2$, Ph_3P and Na_2CO_3 in refluxing *n*-propanol afforded **14-22** in 79-62% yield (Scheme 1).

The structures of **14-22** were identified by the ^{1}H and ^{13}C NMR spectra, which showed rather similar patterns for the pyrimidine scaffold. The spectra were characterized by the presence of additional aromatic proton and carbon atoms, indicative for arylation of the analogue **4**. In the ^{1}H NMR spectra of **14-19**, the low field multiplets at the regions δ 7.64-7.55 ppm were assigned for nine aromatic protons, indicated by their integration. Compound **20** revealed a doublet at δ 7.88 ppm ($J_{5',6'}$ = 7.1 Hz) assigned for $H_{arom.}$-6', while the two multiplets at the regions δ 6.63-7.56 and

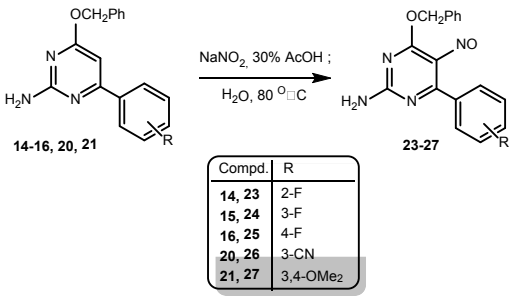


Compd.	R	R	Compd.
5, 14	2-F	4-OH	10, 19
6, 15	3-F	3-CN	11, 20
7, 16	4-F	3,4-OMe_2	12, 21
8, 17	4-NO_2	3,4-F_2	13, 22
9, 18	4-CO_2Et		

Scheme 1. Synthesis of 2-amino-6-aryl-4-benzyloxy-pyrimidine analogues

7.44-7.36 ppm were attributed to two and six aromatic protons, respectively. However, the aromatic protons of **21** and **22** were fully analysed (*c.f.* Experimental section). In the ^{13}C NMR spectra of **14-22**, the low field signals at the regions δ 170.2-171.1 and 164.9-162.6 ppm were assigned to carbon atoms 4 and 6 of the pyrimidine backbone, respectively, while C-2 and C-5 of the same ring appeared at the regions δ 162.6-158.6 and 94.8-94.3 ppm, respectively. The aromatic carbon atoms appeared at the regions δ 132.6-127.4 ppm, whereas C-1 and C-1' carbons of the benzyl and aromatic ring at C-6 resonated at the regions δ 140.2-136.1 and 147.1-131.1 ppm, respectively. Furthermore, C-F signals of **14-16** and **22** appeared as doublets at δ 148.0, 159.6, 158.3 and 141.0 (2xC-F) ppm ($J_{C,F}$ ~ 250 Hz), respectively. The other aromatic and substituents carbon atoms were fully analysed (*c.f.* Experimental section).

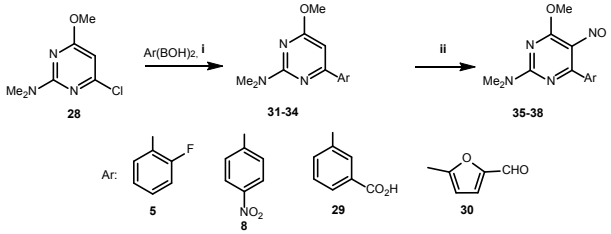
Recently, Hardcastle *et al.* [27] have synthesized a series of 4-alkoxy-2,6-diamino-5-nitrosopyrimidine derivatives whereas some of these analogues showed significant inhibition activity against the cyclin-dependent kinase (CDK). Such result prompted us to modify our new synthesized 6-arylpyrimidine derivatives to prepare their nitroso analogues, aiming for evaluation of their inhibition activity against cyclin-dependent kinases 1 and 2. Thus, treatment of compounds **14-16**, **20** and **21** with aq. solution of $NaNO_2$ in 30% AcOH at 80 °C gave after purification the nitroso analogues **22-26** in 70-79 % yield (Scheme 2).



Scheme 2. Synthesis of 2-amino-6-aryl-4-benzyloxy-5-nitrosopyrimidine analogues (**23-27**)

The structures of **22-26** were established by ^1H, ^{13}C NMR and mass spectra, as they showed similar pattern of pyrimidine and aromatic protons. In the ^{13}C NMR spectra of **23-27**, C-2 and C-4 of the pyrimidine scaffold resonated in the regions δ 167.9-166.3 and 159.5-159.1 ppm, while C-5 and C-6 appeared in the regions δ 140.1-139.3 and 160.3-160.0 ppm, respectively (*c.f.* Experimental Section). Moreover, all the synthesized compounds were further identified by ^1H, ^{13}C HSQC [37] spectroscopic study.

Recently, we have synthesized 2-amino-4-aryl-6-methoxy-*N,N*-dimethylpyrimidines (**31-34**) *via* Suzuki-Miyaura cross-coupling reaction, from 2-amino-4-chloro-6-methoxy-*N,N*-dimethyl-pyrimidine (**28**), with evaluation of their antimicrobial activity [38]. Nitrosation of **31-34** by treatment with $NaNO_2$ afforded the nitroso analogues **35-38** in 69-79 % yield (Scheme 2). However, in view of promising anti-HIV activity of **20** as well as the activity of nitrosopyrimidines against the CDKs [27], we encouraged for exploring our work for evaluation of our new synthesized analogues for their inhibitory activity against HIV and CDKs. The structures of **31-34** were determined from their ^1H, ^{13}C NMR and mass spectra. In the ^{13}C NMR spectra of **31-34**, C_5-NO resonated in lower fields (δ 139.9-139.7 ppm), in comparison for those of the starting materials (δ 102.3-91.4 ppm) [38].



Scheme 3. *Reagents and conditions:* (i) $Pb(OAc)_2$, Ph_3P, Na_2CO_3, ^{n}-PrOH, reflux, 8-14 h; (ii) $NaNO_2$, 30% AcOH; H_2O, 80 °C

3. Bioactivities

3.1. *In vitro anti-HIV activity*

Compounds **14-22** and **31-34** were tested for their inhibitory activity against HIV-1 (strain III_B) and HIV-2 (strain ROD) in human MT-4 cell cultures on a Microculture Tetrazolium (MTT) [39]. The results are summarized in Table 1, where the data for nevirapine [40] was included for comparison purposes. The cytotoxicity of the compounds was determined in parallel. Compound **20** was found to be the only compound from the series inhibiting HIV-1 (III_B strain) replication in cell cultures with IC_{50} of 1.83 μM and CC_{50} of 18.9 μM resulting with selectivity index (SI) of 10.3 at non-toxic concentration. The remaining compounds exhibited poor activity with SI <1. However, introduction of cyano group at the phenyl ring conjugated pyrimidine backbone considerably increased the anti-HIV activity, in comparison to the effectiveness of other functional groups. This inhibitory activity can be shown clearly by the new anti-HIV drugs, rilpivirine (**1**), and etravirine (**2**), since their QSAR and docking study with RT HIV supported this argument.

From the data of Table 1, we concluded that the substitution of the amino group at C-2 and benzyloxy residue at C-4 of the pyrimidine scaffold of **13-22** by the dimethylamino group (at C-2) and methoxy (at C-4) residues as in **31-34,** did not showed any enhancement of the activity against HIV.

Table 1. *In vitro* anti HIV-1 and HIV-2 activity and cytotoxicity of new pyrimidine derivatives

Compd.	HIV-1 (III_B) IC_{50} (μM)	HIV-2 (ROD) IC_{50} (μM)	CC_{50} (μM)	SI (III_B)	SI (ROD)
14	>61.95	>61.95	61.95	<1	<1
15	>53.48	>53.48	53.48	<1	<1
16	>65.95	>65.95	65.95	<1	<1
17	>65.95	>65.95	65.95	<1	<1
18	>56.70	>56.70	56.70	<1	<1
19	>76.68	>76.68	76.68	<1	<1
20	1.83	>18.9	18.9	10.3	<1
21	>65.70	>65.70	65.70	<1	<1
22	>76.68	>76.68	76.68	<1	<1
26	>55.23	>55.23	55.23	<1	<1
27	>85.45	>85.45	85.45	<1	<1
28	>41.22	>41.22	40.22	<1	<1
29	>31.29	>31.29	31.29	<1	<1
Nevirapine	0.027	>4.00	>4.00	>147	X1

Anti HIV-1 activity measured with strain III_B; anti HIV-2 activity measured with strain ROD; IC_{50} (μM) compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1 and HIV-2 induced cytopathic effect; CC_{50}(μM) compound concentration that reduced the viability of mock-infected MT-4 cells by 50%; SI: selectivity index (CC_{50}/IC_{50}).

3.2. *Cyclin-dependent kinase (CDK) inhibitory activity*

CDK2 inhibitory activity of 5-nitropyrimidine analogues **23-27** and **35-38** prepared above were shown in Table 2, together with those of Olomoucine as a reference compound, using the method described in ref. [41]. CDK2 is one of CDK family protein which has a proline directed serine/threonine kinase activity, phosphorylating serine or threonine residue ahead of proline.

In general, 4-benzyloxy compounds **23-27** showed better CDK2 inhibitory activities than the corresponding 4-methoxy analogues **35-38**. Compound **24** having a 3-fluorophenyl group at C-6 of the 5-nitropyrimidine backbone exhibited remarkable activity (IC_{50} = 8.1 μM), in comparison to Olomoucine as reference compound ((IC_{50} = 7.0 μM). Compounds **23**, and **25-27** showed mild activity against CDK2 activity ranging from 11.9-16.0 μM and the rest

of compounds had no significant activity.

Table 2. Inhibition of cdk2 by 5-nitrosopyrimidine derivatives

Compd.	IC_{50} (μM)
23	15.2
24	8.1
25	11.9
26	36.3
27	16.0
35	76
36	>100
37	>100
38	29.3
Olomoucine	7.0

3.3. Molecular docking analysis

In the docking study, the X-ray crystal structure of HIV-1 reverse transcriptase (RT); PDB ID: 3dlg) was obtained from the Protein Data Bank (http://www.rcsb.org) [42]. The Graphical User Interface program ‘’AutoDock Tools’’ as well as autodock-4.2.6 program were used to prepare, run, and analyse the docking simulations. Kollman united atom charges, solvation parameters and polar hydrogens were added into the receptor PDB file for the preparation of protein in docking simulation [43].

Compound **20** has been selected to show its binding to the enzyme pocket, where the molecular modeling analysis has suggested a role for conformational flexibility. Thus, conformational changes and torsion angles in compound **20** (Figs.1 and 2, Table 3) are achieved by variation of four torsion angles (τ1 - τ4). Stimulation of molecular dynamics supported the hypothesis that the molecular flexibility allows torsional changes (wiggling) of **20** into the non-nucleoside binding site (NNBS) of RT. Exploitation of favourable components of inhibitor conformational flexibility (such as torsional flexibility about strategically located chemical bonds) can be a powerful drug design concept, especially for designing drugs that will be effective against rapidly mutating targets [44, 45].

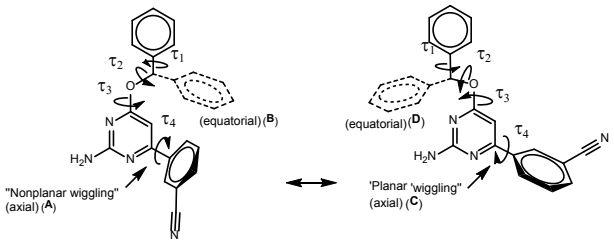


Fig. 1. Flexibility hypothesis with four torsion angles (τ1-τ4) define the conformation of **20**

Table 3. Calculated torsion angles (τ1, τ2, τ3, and τ4) bonds rotations of compound **20**

Conform	Torsion angle (°)			
	τ1	τ2	τ3	τ4
A	93	149	- 179	- 20
B	- 83	- 93	177	- 19
C	0.1	- 180	180	0
D	- 84	- 96	177	- 20

Table 3 shows that ’non-planar wiggling’ (**A**, axial) conform having a slightly lower energy than the other conforms: ’non-planar wiggling’ (**B**, equatorial), ’non-planar wiggling’ (**C**, equatorial), and ’non-planar wiggling’ (**D**, equatorial), supported by relative torsion angles energies shown in Figure 4 as well as thermodynamic energies (Table 3). Such difference in energies between (**A**) and the other conforms would lead to the conclusion that the ‘’non-planar wiggling’’ of axial axis mode of **20** is the most favourable conformation to the NNBS of RT [44, 46].

Based on the above data, Figure 1 shows that

20 with a flexible 'non-planar wigging' (axial) mode (**A**) is the favoured conformation, where its aromatic ring is fitted into an arene-rich subpocket surrounded by the aromatic side chains of Tyr318, and Tyr180. The pyrimidine backbone is located in the middle of the binding pocket, anchoring the NH_2 group at C-2 of pyrimidine ring in a favourable position for hydrogen bonding with the OH group of the Tyr318 aromatic residue of RT. On the other hand, a hydrophobic interaction only between the benzyl group of **20**, with a 'non-planar wiggling' (equatorial) conformation (**B**), and Trp229 aromatic residue of RT was observed (Fig. 3). Overall, the combination of hydrophobic interaction and π-π stacking appears to govern the binding of **20** (**A**) with HIV RT (binding energy -9.78 kcal mol^{-1}, Ki 68.36 nM, Intermolecular energy - 0.58 kcal mol^{-1}, torsional energy 1.49 kcal mol^{-1}, and unbounded extended energy - 0.30 kcal mol^{-1}). The thermodynamic study [47] also predicted that flexibility of the RT inhibitor is essential for its

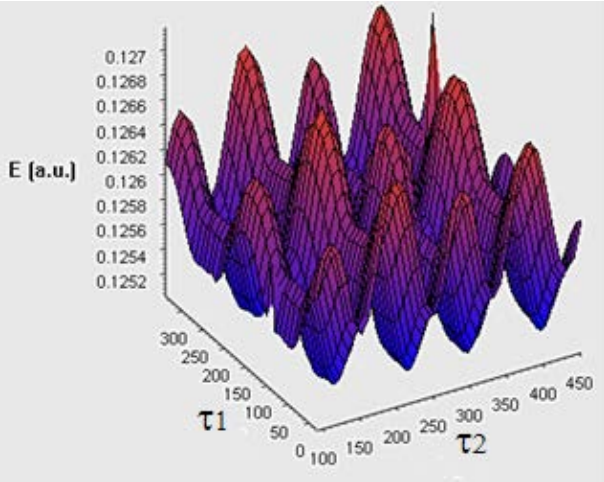


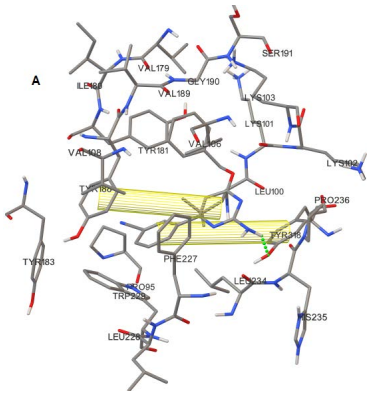
Fig. 2. 3D variation in conformational energy of compound **20** as a function of the torsion angles τ1 and τ2. The fairly shallow energetic barrier (especially for τ1 rotation) permits easy interconversion among inhibitor conformations. Angles rotations around 360°, calculated by the PM6 Hamiltonian.

favourable binding to mutant enzyme. A classic lock and key model for inhibitor binding requires the ideal inhibitor to fill its complementary binding pocket, maximizing favourable interactions with the target. For this reason, we have studied the thermodynamic relative conformational populations of the conforms (Table 4), calculated at B3LYP/6-3+G(d,p) level of theory, and then selected the most stable conform for binding with RT.

Table 4. The total electronic energies (E), zero point vibrational energies (E + ZPE), thermodynamics quantities (G, H), relative ΔG differences in vacuum and relative conformational population in vacuum for the conforms of **20**.

Conf-orm	E	E+ZPE	G	H	ΔG* kcal. mol^{-1}	Conform (%)
A	- 988.65	- 988.37	- 988.42	- 988.35	0	81.06
B	- 988.65	- 988.37	- 988.42	- 988.35	1.12	10.90
C	- 988.65	- 988.37	- 988.42	- 988.35	1.32	7.92
D	- 988.65	- 988.37	- 988.41	- 988.35	3.90	0.11

A: 'Non-planar wiggling' (axial); B: 'Non-planar-wiggling' (equatorial); C: 'Planar-wiggling' (axial); D: 'Planar-wiggling' (equatorial); E: total energy (a.u.), G: Free energy (a.u.), H: enthalpy (a.u.), * ΔG: free energies with respect to the more stable conform **A**.



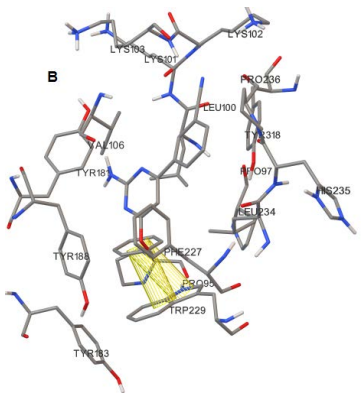


Fig. 3.(I) Compound **20** (**A**) shows a hydrogen bond: OH group of Tyr310 with NH_2 group of pyrimidine backbone. In addition, hydrophobic interactions between the phenyl moiety of **20** at C-6 and Tyr318, as well as pyrimidine ring with Tyr180 of RT enzyme residues were observed. (II) Compound **20** (**B**) shows a hydrophobic interaction between the benzyl group and Trp229 of RT residue.

3.4. Structure–activity relationship (SAR)

SAR has been used widely to predict the hazard of untested chemicals with already tested chemicals by developing statistical relationships between molecular structure linear descriptors and biological activity [48]. Consequently, hydrophobicity is the vital character affecting the biological activity of arylpyrimidine derivatives, such as log P (the hydrophobic parameter), polarizability (P), the steric parameters volume and surface area (grid), and dihedral angles. It is important to select appropriate molecular descriptors for SAR, such as electronic, hydrophobic, and the geometry [49] parameters. The selected quantum parameters of the pyrimidine inhibitors are listed in Table 5.

The SAR were constructed by performing a multidimensional linear regression analysis using the BILIN software developed by Kubinyi [50]. All geometries of **14-22** are minimized with the semi empirical RM1 Hamiltonian.

Table 5. The molecular descriptors used in the QSAR study, and the dihedral angles

	Log P	Surface area (grid)	Polari-zability	E_{HOMO}	E_{LUMO}	Dihedral angle (τ2)	$E_{Binding}$	E_{Total}
14	4.26	514.89	32.07	-8.902	-0.231	50.38	-3380	-86838
15	4.26	515.58	32.07	-8.994	-0.304	45.89	-3381	-86839
16	4.26	515.37	32.07	-8.974	-0.323	44.85	-3381	-86840
17	4.07	543.98	34.00	-9.248	-1.113	46.27	-3348	-94932
18	4.05	598.40	37.75	-9.005	-0.474	45.98	-3924	-93222
19	3.83	522.54	32.80	-8.786	-0.090	43.90	-3332	-83221
20	4.15	543.61	34.01	-9.106	-0.534	45.07	-3503	-83293
21	3.61	596.47	37.10	-8.860	-0.214	46.85	-3808	-97610
22	4.40	519.75	31.98	-9.097	-0.560	45.49	-3445	-97763

To obtain the SAR models, we have correlated the quantum parameters with each other and the biological activity (IC_{50}). Therefore, it is important in SAR study to establish the relationship between IC_{50} and numerous parameters by regression models [51]. By the multiple regression analysis, we found that the calculated IC_{50} of compounds **14-16** and **18-22** are almost in accordance with their observed IC_{50} values (Table 5).

Table 5. The observed and calculated IC_{50} of arylpyrimidine derivatives.

Compd	IC_{50} obs.	IC_{50} calcd.	Residual
14	61.95	60.73	1.22
15	53.48	55.39	-1.71
16	65.95	66.05	-0.10
18	56.70	54.00	2.70
19	76.68	75.16	1.52
20	1.83	2.04	-0.21
21	65.70	68.89	-3.19
22	57.08	57.12	-0.04

5. Conclusion

In summary, we have synthesized and evaluated a new series of 6-arylpyrimidines **14-22** and their 5-nitroso analogues **23-27** and **35-38** with evaluation of their anti-HIV activity. Compound **20** is the most active analogue from the series against HIV-1, which being a promising agent for further structural modification and pharmacological evaluation. The 5-nitrosopyrimidine analogues were evaluated for their inhibitory activity against CDK2, where compound **24** exhibited a remarkable activity in comparison to Olomoucine as a reference compound. Therefore, **24** serves as a new lead for inhibition of CDK2, which subjected for further pharmacological evaluations.

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