8

EXPERIMENTAL WORK

The work carried out has been discussed under the following three main heads:

8.1 Synthesis of 4,6-diaryl-2-amino pyrimidine derivatives

8.2 Anti-HIV activity and

8.3 Docking studies

8.1 Synthesis of 4,6-diaryl-2-amino pyrimidine derivatives (50a-q)

4-(2-Hydroxyphenyl)-6-phenyl-2-aminopyrimidine (50a)

A mixture of 2-phenylchromen-4-one **(48a)** (0.5 g, 0.0022 mol), guanidine hydrochloride (0.7 g, 0.0073 mol) and sodium hydroxide (1 g) was refluxed in methanol (30 ml) for 10 hours. After removal of excess methanol the reaction mixture was poured on crushed ice containing acetic acid. The solid so obtained was filtered, washed with water and recrystallized from methanol to yield the title product **(50a)**, (0.25 g, 42.2%), m.p. 174-76 °C.

Anal:

TLC	: 0.34 ($CHCl_3$)
IR (KBr)	: 3508, 3354, 3205, 1625, 1230, 890 and 760 cm^{-1}
PMR	: δ 5.37 (s, 2H, NH_2), 6.92-8.06 (m, 10H, ArH+ C-5 pyrimidine), 14.3 (br, 1H, OH)
MS	: m/z 264 (M+1)

4-(2-Hydroxyphenyl)-6-(4-methylphenyl)-2-amino pyrimidine (50b)

2-(4-Methylphenyl)chromen-4-one **(48b)** (0.4 g, 0.0016 mol) was dissolved in a mixture of guanidine hydrochloride (1 g, 0.01 mol) and potassium hydroxide (1 g) in methanol (30 ml). The mixture was refluxed for 10 hours. The workup

procedure was similar to that for compound **(50a)**. Recrystallization from methanol yields **(50b)**, (0.13 g, 27.68%), m.p. 191-92 °C.

Anal:

TLC	: 0.33 ($CHCl_3$)
IR (KBr)	: 3500, 3330, 3197, 1610, 1363, 1228, 890 and 750 cm^{-1}
PMR	: δ 2.43 (s, 3H, CH_3), 5.74 (bs, 2H, NH_2), 6.93-8.0 (m, 9H, ArH+ C-5 pyrimidine), 13.5 (br, 1H, OH)
MS	: m/z 278 (M+1)

4-(2-Hydroxyphenyl)-6-(3-methylphenyl)-2-aminopyrimidine (50c)

Guanidine hydrochloride (1 g, 0.01 mol) was added to the refluxing mixture of 2-(3-methylphenyl)chromen-4-one **(48c)** (0.5 g, 0.0021 mol), and potassium hydroxide (1 g) in methanol (30 ml) for 10 hours. After the usual experimental procedure followed by recrystallization from methanol, the desired compound **(50c)**, (0.25 g, 42.6%), m.p. 124-26 °C, was obtained.

Anal:

TLC	: 0.41 ($CHCl_3$)
IR (KBr)	: 3490, 3394, 3200, 1629, 1357, 1240 and 750 cm^{-1}
PMR	: δ 2.46 (s, 3H, CH_3), 5.35 (s, 2H, NH_2), 6.92-7.88 (m, 9H, ArH+ C-5 pyrimidine), 14.2 (br, 1H, OH)

4-(2-Hydroxyphenyl)-6-(4-methoxyphenyl)-2-aminopyrimidine (50d)

A mixture of 2-(4-methoxyphenyl)chromen-4-one **(48d)** (0.5 g, 0.0019 mol), guanidine hydrochloride (1 g, 0.01 mol) and potassium hydroxide (1 g) was refluxed in methanol (30 ml) for 10 hours. The reaction mixture was processed by the procedure as described for the compound **(50a)** and followed by recrystallization from methanol to yield **(50d)**, (0.26 g, 44.0%), m.p. 161-63 °C.

Anal:

TLC	: 0.31 ($CHCl_3$)
IR (KBr)	: 3492, 3327, 3150, 1615, 1249, 1026, 830 and 767 cm^{-1}

PMR : δ 3.88 (s, 3H, OCH_3), 5.4 (s, 2H, NH_2), 6.76-8.24 (m, 9H, ArH+ C-5 pyrimidine), 14.5 (br, 1H, OH)

MS : m/z 294 (M+1)

4-(2-Hydroxyphenyl)-6-(3-methoxyphenyl)-2-aminopyrimidine (50e)

2-(3-Methoxyphenyl)chromen-4-one **(48e)** (0.5 g, 0.0019 mol) was dissolved in a mixture of guanidine hydrochloride (1 g, 0.01 mol) and potassium hydroxide (1 g) in methanol (30 ml). The mixture was refluxed for 10 hours. The workup procedure was similar to compound **(50a)**. Recrystallization from methanol yields **(50e)**, (0.35 g, 60.34%), m.p. 203-06 °C.

Anal:

TLC : 0.32 ($CHCl_3$)

IR (KBr) : 3400, 3313, 3176, 1647, 1236, 1029, 792 and 763 cm^{-1}

PMR : δ 3.92 (s, 3H, OCH_3), 6.0 (bs, 2H, NH_2), 6.92-7.89 (m, 9H, ArH+ C-5 pyrimidine), 14.2 (br, 1H, OH)

4-(2-Hydroxyphenyl)-6-(3,4-dimethoxyphenyl)-2-aminopyrimidine (50f)

Guanidine hydrochloride (1 g, 0.01 mol) was added to the refluxing mixture of 2-(3,4-dimethoxyphenyl)chromen-4-one **(48f)** (0.5 g, 0.0017 mol) and potassium hydroxide (1 g) in methanol (30 ml) for 10 hours. After the usual experimental procedure followed by recrystallization from methanol the desired compound **(50f)**, (0.3 g, 53%), m.p. 221-23 °C, was obtained.

Anal:

TLC : 0.32 ($CHCl_3$)

IR (KBr) : 3431, 3313, 3190, 1643, 1259, 1026, 812 and 758 cm^{-1}

PMR : δ 3.89 (s, 6H, $(OCH_3)_2$), 6.9 (s, 2H, NH_2), 7.07-8.25 (m, 8H, ArH+ C-5 pyrimidine), 14.12 (s, 1H, OH)

MS : m/z 324 (M+1)

4-(2-Hydroxyphenyl)-6-(4-chlorophenyl)-2-aminopyrimidine (50g)

A mixture of 2-(4-chlorophenyl)chromen-4-one **(48g)** (0.5 g, 0.0019 mol), guanidine hydrochloride (1 g, 0.01 mol) and potassium hydroxide (1 g) was refluxed in methanol (30 ml) for 10 hours. The reaction mixture was processed by the procedure as described for the compound **(50a)** and followed by recrystallization from methanol to yield **(50g)**, (0.22 g, 37.93%), m.p. 239-41 °C.

Anal:

TLC	: 0.31 ($CHCl_3$)
IR (KBr)	: 3502, 3340, 3217, 1641, 1309, 1089, 837 and 750cm^{-1}
PMR	: δ 6.28 (bs, 2H, NH_2), 6.92-8.07 (m, 9H, ArH+ C-5 pyrimidine), 13.5 (br, 1H, OH)
MS	: m/z 298 (M+1)

4-(2-Hydroxyphenyl)-6-(3-chlorophenyl)-2-aminopyrimidine (50h)

2-(3-Chlorophenyl)chromen-4-one **(48h)** (0.5 g, 0.0019 mol) was dissolved into a mixture of guanidine hydrochloride (1 g, 0.01 mol) and potassium hydroxide (1 g) in methanol (30 ml). The mixture was refluxed for 10 hours. The workup procedure was similar to compound **(50a)**. Recrystallization from methanol yielded **(50h)**, (0.2 g, 34.9%), m.p. 183-85 °C.

Anal:

TLC	: 0.36 ($CHCl_3$)
IR (KBr)	: 3502, 3346, 3203, 1616, 1421, 1230, 856 and 757 cm^{-1}
PMR	: δ 5.24 (s, 2H, NH_2), 6.92-8.05 (m, 9H, ArH+ C-5 pyrimidine), 13.5 (br, 1H, OH)

4-(2-Hydroxyphenyl)-6-(2-chlorophenyl)-2-aminopyrimidine (50i)

Guanidine hydrochloride (1 g, 0.01 mol) was added to the refluxing mixture of 2-(2-chlorophenyl)chromen-4-one **(48i)** (0.5 g, 0.0019 mol) and potassium hydroxide (1 g) in methanol (30 ml) for 10 hours. After the usual experimental

procedure followed by recrystallization from methanol the desired compound **(50i)**, (0.25 g, 44%), m.p. 177-79 °C, was obtained.

Anal:

TLC	: 0.48 (5% MeOH in $CHCl_3$)
IR (KBr)	: 3489, 3371, 3180, 1631, 1325, 1230, 856 and 757 cm^{-1}
PMR	: δ 6.44 (bs, 2H, NH_2), 6.91-7.80 (m, 9H, ArH+ C-5 pyrimidine), 13.8 (br, 1H, OH)

4-(2-Hydroxyphenyl)-6-(2,4-dichlorophenyl)-2-aminopyrimidine (50j)

A mixture of 2-(2,4-dichlorophenyl)chromen-4-one **(48j)** (0.5 g, 0.0017 mol), guanidine hydrochloride (1 g, 0.01 mol) and potassium hydroxide (1 g) was refluxed in methanol (30 ml) for 10 hours. The reaction mixture was processed by the procedure as described for the compound **(50a)** and on recrystallization from methanol yielded **(50j)**, (0.19 g, 33.0%), m.p. 230-31 °C.

Anal:

TLC	: 0.49 (5% MeOH in $CHCl_3$)
IR (KBr)	: 3502, 3338, 3217, 1641, 1224, 1141, 817 and 750 cm^{-1}
PMR	: δ 6.66 (bs, 2H, NH_2), 6.89-7.64 (m, 8H, ArH+ C-5 pyrimidine)
MS	: m/z 332 (M^+)

4-(2-Hydroxyphenyl)-6-(4-bromophenyl)-2-aminopyrimidine (50k)

2-(4-Bromophenyl)chromen-4-one **(48k)** (1 g, 0.0033 mol) was dissolved in a mixture of guanidine hydrochloride (1 g, 0.01 mol) and potassium hydroxide (1 g) in methanol (30 ml). The mixture was refluxed for 10 hours. The workup procedure was similar to that for compound **(50a)**. Recrystallization of the crude product from methanol yielded **(50k)**, (0.38 g, 34.5%), m.p. 212-14 °C.

Anal:

TLC	: 0.52 (5% MeOH in $CHCl_3$)
IR (KBr)	: 3519, 3367, 3200, 1639, 1421, 1012, 850 and 748 cm^{-1}

PMR : δ 6.1 (s, 2H, NH_2), 6.95-7.93 (m, 9H, ArH+ C-5 pyrimidine)

4-(2-Hydroxyphenyl)-6-(4-fluorophenyl)-2-aminopyrimidine (50l)

Guanidine hydrochloride (1 g, 0.01 mol) was added to the refluxing mixture of 2-(4-fluorophenyl)chromen-4-one **(48l)** (0.5 g, 0.002 mol) and potassium hydroxide (1 g) in methanol (30 ml) for 10 hours. After the usual experimental procedure followed by recrystallization from methanol the desired compound **(50l)**, (0.18 g, 30.76%), m.p. 221-23 °C, was obtained.

Anal:

TLC : 0.42 ($CHCl_3$)

IR (KBr) : 3490, 3321, 3201, 1647, 1220, 831 and 750 cm^{-1}

PMR : δ 5.5 (bs, 2H, NH_2), 6.93-8.11 (m, 9H, ArH+ C-5 pyrimidine)

MS : m/z 282 (M+1)

4-(2-Hydroxyphenyl)-6-(2-furyl)-2-aminopyrimidine (50m)

A mixture of 2-(2-furyl)chromen-4-one **(48m)** (0.5 g, 0.0023 mol), guanidine hydrochloride (1 g, 0.01 mol) and potassium hydroxide (1 g) was refluxed in methanol (30 ml) for 10 hours. The reaction mixture was processed by the procedure as described for compound **(50a)** which was the followed by recrystallization from methanol to yield **(50m)**, (0.3 g, 50.27%), m.p. 211-13 °C.

Anal:

TLC : 0.5 ($CHCl_3$)

IR (KBr) : 3417, 3286, 3163, 1635, 1305, 1220, 1014, 852 and 750 cm^{-1}

PMR : δ 5.3 (s, 2H, NH_2), 6.59-7.87 (m, 8H, ArH/furyl+ C-5 pyrimidine), 14.3 (br, 1H, OH)

MS : m/z 254 (M+1)

4-(2-Hydroxyphenyl)-6-(2-thienyl)-2-aminopyrimidine (50n)

2-(2-thienyl)chromen-4-one **(48n)** (1 g, 0.0043 mol) was dissolved into a mixture of guanidine hydrochloride (1.5 g, 0.015 mol) and potassium hydroxide (1 g) in methanol (30 ml). The mixture was refluxed for 10 hours. The workup procedure was similar to compound **(50a)**. Recrystallization from methanol yielded **(50n)**, (0.29 g, 24.78%), m.p. 182-84 °C.

Anal:

TLC	: 0.28 ($CHCl_3$)
IR (KBr)	: 3502, 3438, 3232, 1647, 1431, 1363, 1226, 825 and 752 cm^{-1}
PMR	: δ 5.27 (s, 2H, NH_2), 6.92-7.84 (m, 8H, ArH/thienyl+ C-5 pyrimidine), 13.5 (br, 1H, OH)
MS	: m/z 270 (M+1)

4-(2-Hydroxy-5-methoxyphenyl)-6-phenyl-2-aminopyrimidine (50o)

Guanidine hydrochloride (1 g, 0.01 mol) was added to a refluxing mixture of 2-phenyl-6-methoxychromen-4-one **(48o)** (0.5 g, 0.0019 mol) and potassium hydroxide (1 g) in methanol (30 ml) for 10 hours. After the usual experimental procedure followed by recrystallization from methanol the desired compound **(50o)**, (0.5 g, 45%), m.p. 195-96 °C, was obtained.

Anal:

TLC	: 0.68 (5% MeOH in $CHCl_3$)
IR (KBr)	: 3492, 3311, 3190, 1633, 1218, 1093, 823 and 769 cm^{-1}

4-(2-Hydroxy-5-methoxyphenyl)-6-(4-methylphenyl)-2-aminopyrimidine (50p)

A mixture of 2-(4-methylphenyl)-6-methoxychromen-4-one **(48p)** (0.5 g, 0.0018 mol), guanidine hydrochloride (1.2 g, 0.012 mol) and potassium hydroxide (1.2 g) was refluxed in methanol (30 ml) for 10 hours. The reaction mixture was processed by the procedure as described for compound **(50a)** which was

followed by recrystallization from methanol to yield **(50p)**, (0.3 g, 52.6%), m.p. 210-12 °C.

Anal:

TLC	: 0.48 (5% MeOH in $CHCl_3$)
IR (KBr)	: 3475, 3305, 3160, 1631, 1218, 1220, 1074, 833 and 763 cm^{-1}
PMR	: δ 2.43 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 5.32 (s, 2H, NH_2), 6.94- 7.96 (m, 8H, ArH+ C-5 pyrimidine)

4-(2-Hydroxy-5-methoxyphenyl)-6-(4-chlorophenyl)-2-aminopyrimidine (50q)

2-(4-chlorophenyl)-6-methoxychromen-4-one **(48q)** (0.5 g, 0.0017 mol) was dissolved in a mixture of guanidine hydrochloride (1 g, 0.01 mol) and potassium hydroxide (1 g) in methanol (30 ml). The mixture was refluxed for 10 hours. The workup procedure was similar as for compound **(50a)**. Recrystallization from methanol yields **(50q)**, (0.08 g, 14%), m.p. 234-35 °C.

Anal:

TLC	: 0.47 (5% MeOH in $CHCl_3$)
IR (KBr)	: 3444, 3332, 3220, 1643, 1431, 1218, 1091, 815 and 765 cm^{-1}
PMR	: δ 3.87 (s, 3H, OCH_3) 7.32 (s, 2H, NH_2), 6.91-8.38 (m, 8H, ArH+ C-5 pyrimidine), 13.5 (s, 1H, OH)

8.2 Anti-HIV-1 activity

The antiviral activity of the test compounds was tested against HIV-IIIB by using MTT colorimetric assay[235]. Briefly, various concentrations of the test compounds were added to wells of a flat-bottom microtiter plate. Subsequently, virus and MT-4 cells were added to a final concentration of 200 CCID50/well and 30 000 cells/well, respectively. To determine the toxicity of the test compound, mock-infected cell cultures, containing an identical compound concentration range were incubated in parallel with the virus infected cell

cultures. After 5 days of incubation (37 °C, 5% CO_2), the viability of the cells was determined using MTT. The results of drug susceptibility assays were expressed as an EC_{50} defined as the concentration of drug at which there was 50% infection compared with the drug-free control. Toxicity results are expressed as CC_{50}, defined as the concentration of drug at which the cell viability was reduced by 50% compared to the drug-free control. The results are summarized in **Table 8.**

8.3 Docking Studies

Docking simulations was performed on a red hat linux 9.0 work station. RT crystal structures were obtained from the Protein Data Bank[237]. Autodock 3.0[236] was used in this study to perform the docking simulations. All water molecules and magnesium ion were removed from the original Protein Data Bank files.

All single bonds of a substrate were allowed to rotate freely. The Lammarckian Genetic Algorithm (LGA) was used to explore the energy landscape. The hybrid search technique consists of a global optimizer modified from a genetic algorithm with 2-point crossover, random mutation, and a local optimizer with a Solis and Wets algorithm. A docking box of 40x40x50 points with a grid spacing of 0.9444 Å was used in the calculations. Random conditions were used in the settings of seed, initial quaternion, coordinates and torsions. A 0.2 Å step was used for translation and a 25-degree was used for quaternion and torsion. The maximum number of energy evaluation was set to 250,000. The rate of gene mutation was 0.02 and the rate of crossover was 0.8. The number of docking runs was 100. So a total of 100 docking configurations were determined in each docking calculation. The "preferable" docking configuration was chosen based on the lowest empirical binding free energy.