7

Results and Discussion

The work carried out in this section is discussed under the following three main heads:

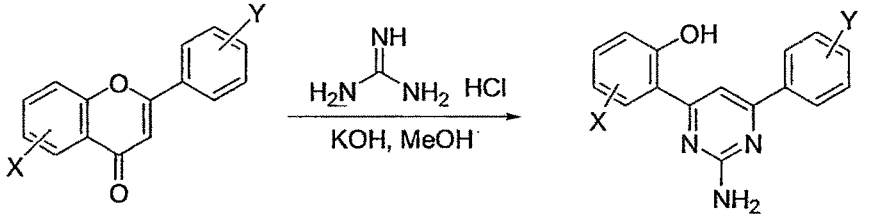
7.1 Synthesis of 4,6-diaryl-2-amino pyrimidine derivatives

7.2 Anti-HIV activity and

7.3 Docking studies

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

The 2-(substituted phenyl)chromen-4-one derivatives on refluxing with guanidine hydrochloride in methanol in presence of potassium hydroxide furnished 2-amino diaryl pyrimidines **(Scheme 3)**.

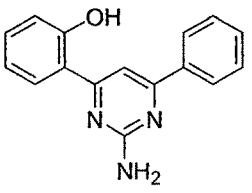


Scheme 3

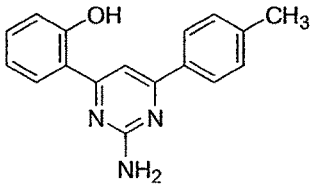
All the compounds so synthesized were characterized on the basis of their IR, NMR and Mass spectra. The purity was confirmed by TLC.

4-(2-Hydroxyphenyl)-6-phenyl-2-aminopyrimidine **(50a)** showed a characteristic peak for OH stretching at 3205 cm^{-1} in the IR spectrum. The asymmetric and symmetric stretching NH bands were observed at 3508 and 3354 cm^{-1} respectively. The characteristic C=N stretching of the pyrimidine ring system found at 1625 cm^{-1}. The PMR spectrum displayed a sharp singlet and a broad peak at δ 5.37 and 14.3 for amino and hydroxyl group, respectively. The

C-5 pyrimidine ring proton and nine aromatic protons appeared as a multiplet at 6.92-8.06. The M+1 peak was found at 264 (m/z) in the mass spectrum.



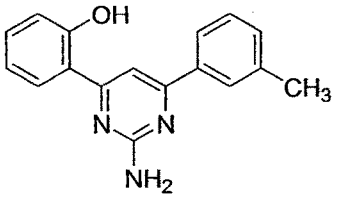
50a



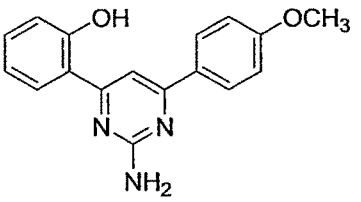
50b

The IR spectrum of the compound **(50b)** showed asymmetric and symmetric NH stretching bands at 3500 and 3330 cm^{-1} respectively. The OH stretching and C=N stretching of the pyrimidine was found at 3197 and 1610 cm^{-1} respectively. The PMR spectrum displayed a sharp singlet for methyl protons at δ 2.43. The nine aromatic protons including C-5 pyrimidine ring proton appeared as a multiplet at δ 6.93-8.0. A broad singlet and a broad hump were found at δ 5.74 and 13.5 for amine and hydroxyl group respectively. The mass spectrum shows the M+1 peak at 278 (m/z).

4-(2-Hydroxyphenyl)-6-(3-methylphenyl)-2-aminopyrimidine **(50c)** showed a characteristic peak at 3200 (OH str), and 3490 and 3394 cm^{-1} (NH str) in the IR spectrum. Also, the C=N stretching of the pyrimidine ring was found at 1629 cm^{-1}. The PMR spectrum displayed two sharp singlets at δ 2.46 and 5.35 for methyl and amine protons respectively. A multiplet for aromatic protons and pyrimidine C-5 proton were found at δ 6.92-7.88. A broad hump was observed at δ 14.2 for the hydroxyl proton.



50c

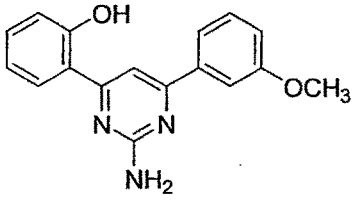


50d

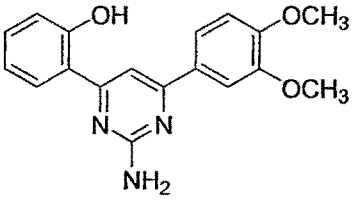
The IR spectrum of the compound **(50d)** showed a distinctive peak at 3150 cm^{-1} for OH stretching and asymmetric and symmetric NH stretching bands at 3492

and 3327 cm^{-1} respectively. A strong band at 1615 cm^{-1} for C=N stretching of the pyrimidine ring and asymmetric and symmetric vibrational bands for OCH_3 were displayed at 1249 and 1026 cm^{-1} respectively. The PMR spectrum displayed a sharp singlet for methoxy protons at δ 3.88. A singlet and a broad hump were found at δ 5.4 and 14.5 for amine and hydroxyl group, respectively. The nine aromatic protons including the C-5 pyrimidine ring proton appeared as a multiplet at δ 6.76-8.24. The mass spectrum shows M+1 peak at 294 (m/z).

4-(2-Hydroxyphenyl)-6-(3-methoxyphenyl)-2-aminopyrimidine **(50e)** showed a characteristic peak at 3176 for OH stretching and 3400 and, 3313 cm^{-1} (NH str) in the IR spectrum. Also, the C=N stretching of the pyrimidine ring was found at 1647 cm^{-1}. The asymmetric and symmetric vibrational bands for OCH_3 were displayed at 1236 and 1029 cm^{-1} respectively. The PMR spectrum displayed a sharp singlet and a broad singlet at δ 3.92 and 6.0 for methoxy and amine protons respectively. A multiplet for aromatic protons and pyrimidine C-5 proton was found at δ 6.92-7.89. A broad hump was observed at 14.2 for hydroxyl proton.



50e

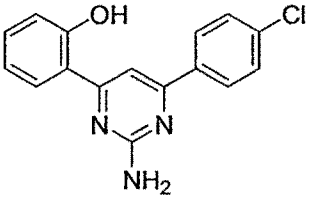


50f

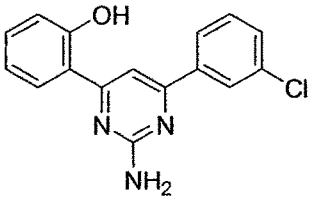
Compound **(50f)** showed a characteristic peak at 3190 cm^{-1} (OH str), asymmetric and symmetric peaks at 3431 and 3313 cm^{-1} for NH stretching respectively. A sharp asymmetric and symmetric peak was observed at 1259 and 1026 cm^{-1} for OCH_3 stretching respectively. Also, the C=N stretching of the pyrimidine ring was found at 1643 cm^{-1}. The PMR spectrum displayed a sharp singlet for six protons at δ 3.89 and amine protons at 6.9. The pyrimidinyl proton and seven aryl protons appeared as a multiplet at δ 7.07-8.25. A sharp singlet was

observed at δ 14.12 for the hydroxyl proton. The mass spectrum shows M+1 peak at 324 (m/z).

4-(2-Hydroxyphenyl)-6-(4-chlorophenyl)-2-aminopyrimidine **(50g)** showed a characteristic peak for OH stretching at 3217 cm^{-1} in the IR spectrum. The asymmetric and symmetric stretching NH bands were observed at 3502 and 3340 cm^{-1} respectively. A characteristic C=N stretching of the pyrimidine ring system was found at 1641 cm^{-1}. The PMR spectrum displayed a broad singlet and a broad hump at δ 6.28 and 13.5 for amino and hydroxyl group respectively. The C-5 of the pyrimidine ring proton and the eight aromatic protons appeared as a multiplet at δ 6.92-8.07. The M+1 peak was found at 298 (m/z) in the mass spectrum.



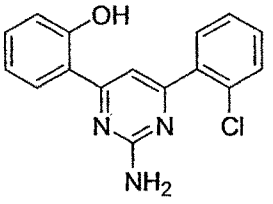
50g



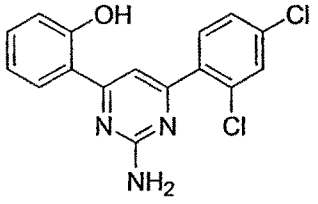
50h

The IR spectrum of the compound **(50h)** showed a distinctive peak at 3203 cm^{-1} for OH stretching, asymmetric and symmetric NH stretching bands at 3502 and 3346 cm^{-1} respectively. A strong band at 1616 cm^{-1} for C=N stretching of the pyrimidine ring was observed. A singlet and a broad hump were found at δ 5.24 and 13.5 for amine and hydroxyl group respectively. The nine aromatic protons including C-5 pyrimidine ring proton appeared as a multiplet at δ 6.92-8.05.

4-(2-Hydroxyphenyl)-6-(2-chlorophenyl)-2-aminopyrimidine **(50i)** showed a characteristic peak at 3180 for OH stretching and 3489and, 3371 cm^{-1} (NH str) in the IR spectrum. The C=N stretching of the pyrimidine ring was found at 1631 cm^{-1}. The PMR spectrum displayed a broad singlet at δ 6.44 for amine protons. A multiplet for aromatic protons and pyrimidine C-5 proton were found at δ 6.91-7.80. A broad hump was observed at δ 13.8 for the hydroxyl proton.



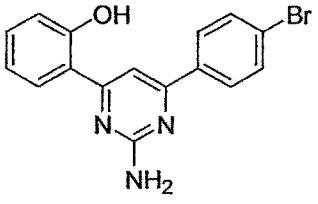
50i



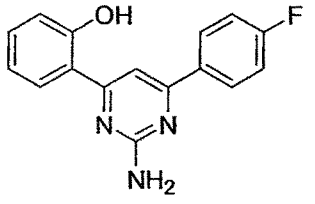
50j

Compound **(50j)** showed a characteristic peak at 3217 cm^{-1} (OH str), asymmetric and symmetric peaks at 3502 and 3338 cm^{-1} for N-H stretching respectively. The C=N stretching of the pyrimidine ring was found at 1641 cm^{-1}. The PMR spectrum displayed a broad singlet for amine protons at δ 6.66. A pyrimidinyl ring C-5 proton and seven aryl protons appeared as a multiplet at δ 6.89-7.64. The mass spectrum shows M^+ peak at 332 (m/z).

4-(2-Hydroxyphenyl)-6-(4-bromophenyl)-2-aminopyrimidine **(50k)** showed a characteristic peak for OH stretching at 3200 cm^{-1} in the IR spectrum. The asymmetric and symmetric stretching NH bands were observed at 3519 and 3367 cm^{-1} respectively. A characteristic C=N stretching of the pyrimidine ring system found at 1639 cm^{-1}. The PMR spectrum displayed a broad hump at δ 6.1. The C-5 pyrimidine ring proton and eight aromatic protons appeared as a multiplet at δ 6.95-7.93.



50k

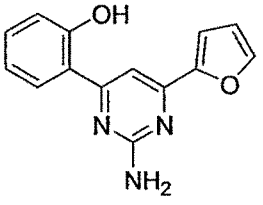


50l

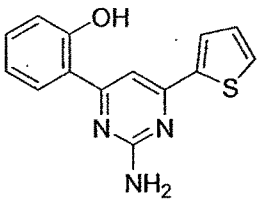
The IR spectrum of the compound **(50l)** showed a distinctive peak at 3201 cm^{-1} for OH stretching, asymmetric and symmetric NH stretching bands were seen at 3490 and 3321 cm^{-1} respectively. A strong band at 1647 cm^{-1} for C=N stretching of the pyrimidine ring. The PMR spectrum displayed a broad singlet for two amine protons at δ 5.50 and the nine aromatic protons including C-5

pyrimidine ring proton appeared as multiplet at δ 6.93-8.11. The mass spectrum shows M+1 peak at 282 (m/z).

4-(2-Hydroxyphenyl)-6-(2-furyl)-2-aminopyrimidine **(50m)** showed a characteristic peak at 3163 for OH stretching and 3417 and 3286 cm^{-1} (NH str) in the IR spectrum. The C=N stretching of the pyrimidine ring was found at 1635 cm^{-1}. The PMR spectrum displayed a broad singlet at δ 5.30 for amine protons. A multiplet for aryl/furyl and pyrimidine C-5 protons was found at δ 6.59-7.87. A broad hump was observed at δ 14.3 for hydroxyl proton. The mass spectrum shows M+1 peak at 254 (m/z).



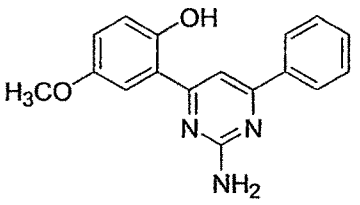
50m



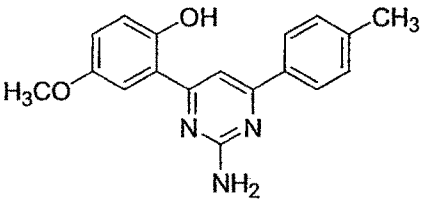
50n

Compound **(50n)** showed a characteristic peak at 3232 cm^{-1} (OH str), asymmetric and symmetric peaks were seen at 3502 and 3438 cm^{-1} for NH stretching respectively. The C=N stretching of the pyrimidine ring was found at 1647 cm^{-1}. The PMR spectrum displayed a broad singlet for amine protons at δ 5.27. The aryl/thienyl and pyrimidinyl ring C-5 protons appeared as a multiplet at δ 6.92-7.84. A broad hump was observed at δ 13.5 for the hydroxyl proton. The mass spectrum shows M+1 peak at 270 (m/z).

4-(2-Hydroxy-5-methoxyphenyl)-6-phenyl-2-aminopyrimidine **(50o)** showed a characteristic peak at 3190 for OH stretching and 3492 and 3311 cm^{-1} (NH str) in the IR spectrum. The C=N stretching of the pyrimidine ring was found at 1633 cm^{-1}. The asymmetric and symmetric vibrational bands for OCH_3 were displayed at 1218 and 1093 cm^{-1} respectively.



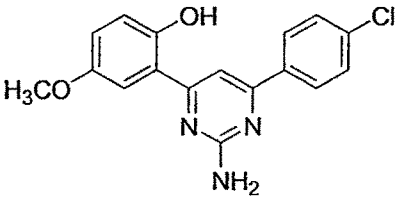
50o



50p

Compound **(50p)** showed a characteristic peak at 3160 cm^{-1} (OH str), asymmetric and symmetric peaks at 3475 and 3305 cm^{-1} for NH stretching respectively. A sharp asymmetric and symmetric peak was observed at 1218 and 1074 cm^{-1} for OCH_3 stretching respectively. The C=N stretching of the pyrimidine ring was found at 1631 cm^{-1}. The PMR spectrum displayed two sharp singlets for methyl and methoxy protons at δ 2.43 and 3.84 respectively. The amine protons were observed as a broad singlet at δ 5.32. The pyrimidinyl proton and seven aryl protons appeared as a multiplet at δ 6.94-7.96.

4-(2-Hydroxy-5-methoxyphenyl)-6-(4-chlorophenyl)-2-aminopyrimidine **(50q)** showed a characteristic peak for OH stretching at 3220 cm^{-1} in the IR spectrum. The asymmetric and symmetric stretching NH bands were observed at 3444 and 3332 cm^{-1} respectively. A characteristic C=N stretching for the pyrimidine ring system was found at 1643 cm^{-1}.

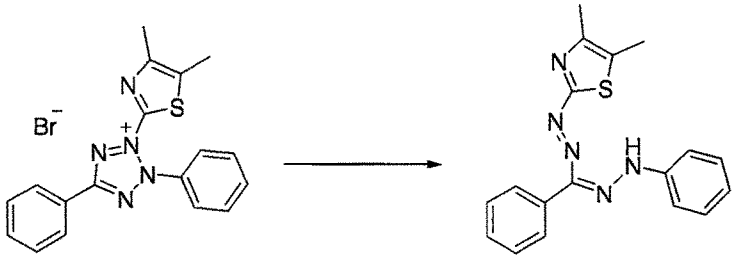


50q

A sharp asymmetric and symmetric peak was observed at 1218 and 1091 cm^{-1} for OCH_3 stretching respectively. The PMR spectrum displayed a sharp singlet and a broad hump at δ 3.87 and 7.32 for methoxy and amine protons respectively. A sharp singlet was observed at δ 13.5 for the hydroxyl proton. The C-5 pyrimidine ring proton and seven aromatic protons appeared as a multiplet at δ 6.91-8.38.

7.2 Anti-HIV activity

The anti-HIV studies of 4,6-diaryl-2-aminopyrimidines were carried out at Rega Institute for Medical Research, Katholieke Universiteit Leuven, Belgium. Inhibition of the HIV-induced cytopathic effect was used as the end point. The viability of both HIV- and mock-infected cells was assessed spectrophotometrically via the *in situ* reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into formazan[234, 235].



MTT (yellow) formazan (purple)

The most active ones are compounds **50a** and **50c** with IC_{50} of 3.22 and 3.45μM respectively (**Table 8**). However their selectivity indices are rather weak by 10 and 4 respectively.

Table 8: The anti-HIV activity, cytotoxicity and selectivity index of 4,6-diaryl-2-aminopyrimidines.

Compound	EC_{50}(μM)[a] HIV-1- III_B	CC_{50}(μM)[b]	SI[c]
50a	3.22	31.28	10
50b	>125	>125	-
50c	3.45	12.98	4
50d	>92.22	92.22	-
50e	>78.3	>78.3	-
50f	>7.36	7.36	-
50g	>74.7	>74.7	-
50h	>125	>125	-
50i	>4	>4	-
50j	>4	>4	-
50k	>20	>20	-
50l	>67.48	67.48	-
50m	>125	>125	-

50n	5.59	5.3	-
50o	>4	>4	-
50p	>20	>20	-
50q	>20	>20	-

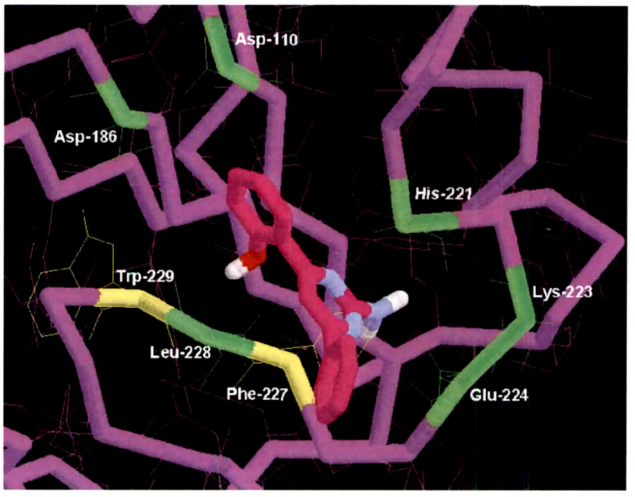
[a]Concentration required to reduce HIV-1 induced cytopathic effect by 50% in MT-4 cells.

[b]Concentration required to reduce MT-4 cell viability by 50%.

[c]Selectivity index : ratio CC_{50}/EC_{50}

7.3 Docking Studies

As discussed in the Aims and Objectives Section Part B, the synthesized 4,6-diaryl-2-aminopyrimidines are chemically similar to the DAPY analogs of NNRTIs. So we sought to validate our hypothesis by performing automated docking studies of the active compounds using Autodock 3.0[236]. After 100 runs, the lowest docked energy conformation was found to be -7.83 kcal/mol.



Compound 50a

Inspection of conformation of the compound **50a** at the non nucleoside binding site (NNBS) led to the following conclusions: The phenyl ring attached to the 4th position of the pyrimidine ring interacts with Asp 110 (distance 4.43 Å) and Asp

186 (distance 3.61 Å) which are at proximity to the polymerase active site. The hydrogen of the hydroxyl group of the phenyl ring forms a hydrogen bond with Leu 228 (distance 2.0 Å). The amino group hydrogens hydrogen bond with Glu 224 (distance 3.32 Å) and His 221 (distance 3.28 Å). The phenyl groups show lack of hydrophobic binding with the surrounding residues of NNBS viz., Tyr 181, Tyr 188, Trp 229 (distance 10.74 Å), Phe 227 (4.64 Å). The weak interactions are highlighted in yellow color.

It is therefore hypothesized that a linker group between one of the aromatic residues and the pyrimidine ring in the ligand would provide better binding interactions which may therefore contribute to better antiHIV-1 activity.