A
Summary of Ph.D thesis entitled

Design, Synthesis and Biological Studies of some Novel Diazepines and Pyrimidines

By
R. Ramajayam

Guided by
Dr. (Mrs.) Rajani Giridhar
M.Pharm., Ph. D.

Pharmacy Department
Faculty of Technology and Engineering
The M.S.University of Baroda
Vadodara-390 001

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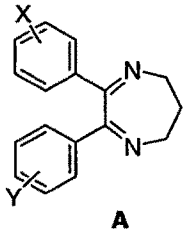
Summary

The thesis is presented in two parts: **Part A** concerning the syntheses, anticancer and antiplatelet activity of **1,4-diazepines** and **Part B** concerning the work on syntheses, biological studies and molecular modeling studies of **pyrimidines** for anti HIV activity.

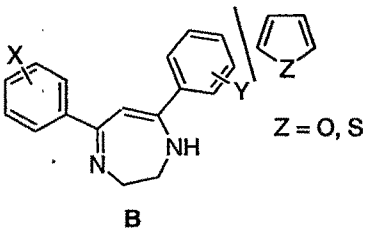
Part A

1,4-diazepines

Since the discovery of benzodiazepines about five decades ago, as CNS active drugs, several studies have been undertaken to understand the SAR profile of 1,4-diazepines. Ring systems having fused of carbo/heterocyclic rings to 1,4-diazepine at various positions and isolated 1,4-diazepines having different types of substituents at different positions have been synthesized and evaluated for various pharmacological activities. The multifarious activities of the diazepines are mainly governed by the position of the nitrogen atom atoms in the ring and the types of additional rings and substituents present in the seven membered ring. Hence, it would not be safe to make any generalization as far as the bioactivity of 1,4-diazepines are concerned. Comparatively, reports on monocyclic diazepines are scarce particularly with respect to biological activity. In this thesis two types of monocyclic diazepines were synthesized and evaluated for anticancer and antiplatelet activity.



A

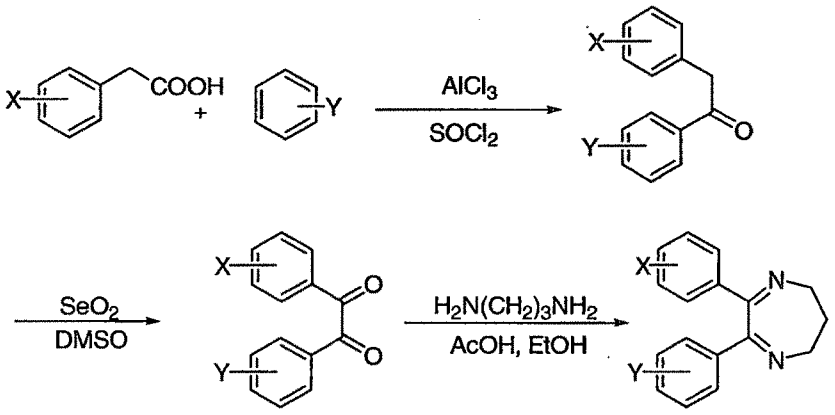


B

2,3-diaryl-6,7-dihydro-5*H*-1,4-diazepines (A): It was aimed to synthesize the unexplored and formidable syntheses of 6,7-dihydro monocyclic diazepines as observed in the chemical literature.

5,7-diaryl-2,3-dihydro-1*H*-1,4-diazepines (B): Unlike the earlier class of diazepines, 2,3-dihydro diazepines are chemically well explored but have received less attention with respect to pharmacological activity.

2,3-diaryl-6,7-dihydro-5*H*-1,4-diazepines (A): The 2,3-diaryl-6,7-dihydro-5*H*-1,4-diazepines are synthesized by the given **Scheme-1** below:



Scheme-1

Commercially available phenylacetic acid derivatives were used without purification. The expensive phenylacetic acid derivatives were synthesized by either Kindler modified Willgerodt reaction or acid hydrolysis of substituted benzyl cyanides. The substituted phenylacetyl chloride was reacted with substituted benzenes through Friedel-Crafts acylation to afford the ethanone. The ethanones were oxidized to the desired ethanedione using selenium dioxide as the oxidizing agent. The desired ethanedione derivatives thus obtained were cyclized with 1,3-propanediamine to afford the respective diazepines.

General Procedure for the synthesis of 1,2-substituted diaryl-1-ethanones (42a-r)

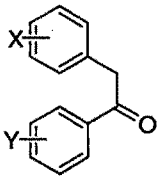
2-chloro/3-chloro/4-chloro/4-nitro/4-methyl phenylacetic acid (1 mol) was dissolved in excess quantity of thionyl chloride (2 mol) and allowed to reflux on steam bath for 3 hrs. The excess of thionyl chloride was recovered under

vacuum. The resulting acid chloride was cooled and added dropwise into the cooled mixture of $AlCl_3$ (1.5 mol) and substituted aromatic compounds. The reaction mixture was stirred for 45 min. at room temperature followed by refluxing for 1 hr. The reaction mixture is quenched with cold-HCl, extracted with chloroform (3 x 20 mL). The combined organic extracts were washed with sodium bicarbonate solution, water and dried over anhydrous sodium sulphate. Recrystallization from methanol after solvent removal gave the ethanone derivatives. The results are summarized in **Table I.**

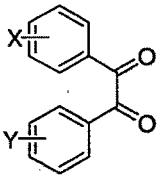
General procedure for synthesis of 1,2-diaryl ethanedione derivatives (43a-r)

Selenium dioxide (0.15 mol) was added into the solution of ethanone derivatives (0.1 mol) in DMSO (15 ml) and irradiated in the microwave oven for the specified time as given in Table (I). The hot mixture was filtered to remove the selenium metal and filtrate was poured over crushed ice. The resulting precipitate was filtered, dried and recrystallized from methanol to get the ethanedione derivatives. The results are summarized in **Table I.**

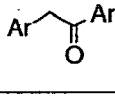
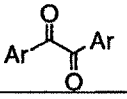
Table 1. General structure of 1,2-diarylethanone and ethanedione derivatives



42a-r



43a-r

Compound	X	Y	IR (cm^{-1})		Reaction Time (Sec)
					
42a, 43a	H	H			30
42b, 43b	H	4-CH_3	1680	1666	30
42c, 43c	H	4-Br	1686	1668	95
42d, 43d	H	4-F	1685	1664	80
42e, 43e	H	4-OCH_3	1674	1668	35
42f, 43f	H	4-SCH_3	1681	1666	35

42g, 43g	4-Cl	4-CH_3	1676	1666	50
42h, 43h	4-Cl	4-Cl	1690	1658	100
42i, 43i	4-Cl	4-F	1683	1663	80
42j, 43j	4-Cl	4-SCH_3	1672	1652	115
42k, 43k	4-Cl	H	1684	1667	55
42l, 43l	4-Cl	4-Br	1689	1664	155
42m, 43m	4-NO_2	H	1685	1662	20
42n, 43n	4-NO_2	4-CH_3	1680	1661	35
42o, 43o	2-Cl	4-Cl	1691	1674	40
42p, 43p	2-Cl	4-SCH_3	1670	1659	40
42q, 43q	4-CH_3	4-CH_3	1685	1660	40
42r, 43r	3-Cl	4-CH_3	1685	1677	55

General Procedure for synthesis of 2,3-diaryl-6,7-dihydro-5*H*-1,4-diazepines (44a-r)

The equimolar mixture of ethanedione derivatives, 1,3-propanediamine and glacial acetic acid (1:1:1) was dissolved in ethanol (30 ml). The mixture was allowed to reflux for 24–30 hrs. The reaction was monitored throughout by TLC and the solvent was evaporated under vacuum after completion. The resulting sticky compound was stripped with silica gel and chromatography with benzene gives a liquid product, with partial recovery of starting material, which on trituration with petroleum ether gives a solid product. This was recrystallized from a suitable solvent to afford the desired compounds **(44a-r).** The spectral analyses of title compounds are given in **Table 2.**

Table 2

Compd. No	**IR (cm^{-1})**	**NMR (δ)**	**Mass (m/z)**	**CHN(%) (Found)**
44a	1605 (C=N)	2.31-2.40 (m, 2H, N-CH_2-CH_2-), 3.47 (br, 4H, N-CH_2- CH_2-), 7.2-7.6 (m, 10H, ArH)	249 (M+1)	—

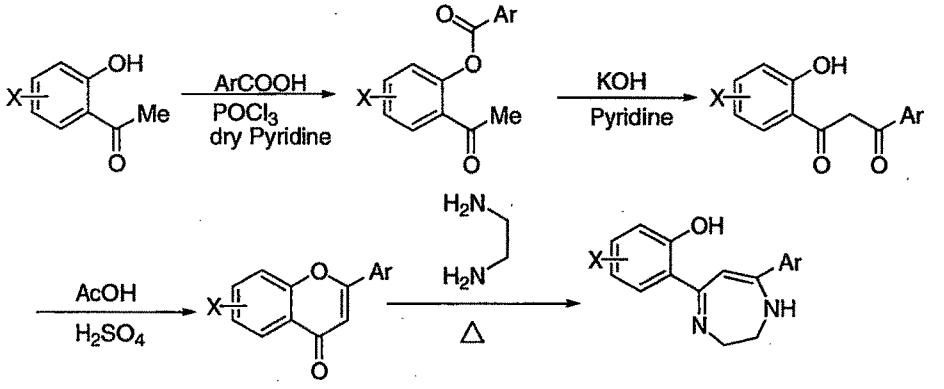
44b	1608 (C=N)	2.32 (s, 3H, CH_3), 2.34-2.41(m, 2H, N-CH_2-$C\underline{H}_2$-), 3.5 (br, 4H, N-$C\underline{H}_2$-CH_2-), 7.11-7.64 (m, 9H, ArH)	263 (M+1)	—
44β	1598 (C=N)	2.33-2.41(m, 2H, N-CH_2-$C\underline{H}_2$-), 3.5 (br, 4H, N-$C\underline{H}_2$-CH_2-), 7.1-7.6 (m, 9H, ArH)	329 (M+2)	—
44d	1598 (C=N)	2.31-2.4 (m, 2H, N-CH_2-$C\underline{H}_2$-), 3.5 (br, 4H,N-$C\underline{H}_2$-CH_2-), 6.9-7.6 (m, 9H, ArH)	—	—
44e	1600 (C=N)	3.77 (s, 3H, OCH_3), 2.31-2.38 (m, 2H, N-CH_2-$C\underline{H}_2$-), 3.2 (br, 4H, N-$C\underline{H}_2$-CH_2-), 6.8-7.3 (m, 9H, ArH)	279 (M+1)	—
44f	1593 (C=N)	2.4 (s, 3H, SCH_3), 2.34-2.37 (m, 2H, N-CH_2-$C\underline{H}_2$-), 3.5 (br, 4H, N-$C\underline{H}_2$-CH_2-), 7.1-7.5 (m, 9H, ArH)	295 (M+1)	—
44g	1610 (C=N)	2.3 (s, 3H, CH_3), 2.32-2.39 (m, 2H, N-CH_2-$C\underline{H}_2$-), 3.4 (br, 4H, N-$C\underline{H}_2$-CH_2-), 7.1-7.5 (m, 8H, ArH)	—	C, 72.84 (72.83) H, 5.77 (5.62) N, 9.44 (9.49)
44h	1614 (C=N)	2.31-2.40 (m, 2H, N-CH_2-$C\underline{H}_2$-), 3.5 (br, 4H, N-$C\underline{H}_2$-CH_2-), 7.2-7.5 (m, 8H, ArH)	—	C, 64.37 (64.24) H, 4.45 (3.97) N, 8.83 (8.98)
44i	1614 (C=N)	2.32-2.39 (m, 2H, N-CH_2-$C\underline{H}_2$-), 3.5 (br, 4H, N-$C\underline{H}_2$-CH_2-), 6.98-7.31 (m, 8H, ArH)	—	C, 67.89 (68.12) H, 4.69 (4.16) N, 9.31 (9.54)
44j	1610 (C=N)	2.45 (s, 3H, SCH_3), 2.28-2.43 (m, 2H, N-CH_2-$C\underline{H}_2$-), 3.51 (br,	—	C, 65.74 (65.91) H, 5.21 (4.72) N, 8.52 (8.82)

		4H, N-C<u>H</u>$_2$-CH$_2$-), 7.1-7.7 (m, 8H, ArH)		
44k	1611 (C=N)	2.32-2.41(m, 2H, N-CH$_2$-C<u>H</u>$_2$-), 3.5 (br, 4H, N-C<u>H</u>$_2$-CH$_2$-), 7.2-7.6 (m, 9H, ArH)	283 (M+1)	—
44l	1612 (C=N)	2.3-2.4 (m, 2H, N-CH$_2$-C<u>H</u>$_2$-), 3.5 (br, 4H, N-C<u>H</u>$_2$-CH$_2$-), 7.2-7.5 (m, 8H, ArH)	—	C, 56.46 (57.05) H, 3.9 (3.33) N, 7.75 (8.19)
44m	1608 (C=N)	2.3-2.4 (m, 2H, N-CH$_2$-C<u>H</u>$_2$-), 3.5 (br, 4H, N-C<u>H</u>$_2$-CH$_2$-), 7.2-8.1 (m, 9H, ArH)	293 (M$^+$)	—
44n	1595 (C=N)	2.33 (s, 3H, CH$_3$), 2.34-2.4 (m, 2H, N-CH$_2$-C<u>H</u>$_2$-), 3.58 (br, 4H, N-C<u>H</u>$_2$-CH$_2$-), 7.1-8.1 (m, 8H, ArH)	—	—
44o	1608 (C=N)	2.43-2.52 (m, 2H, N-CH$_2$-C<u>H</u>$_2$-), 3.58-3.62 (t, 2H, *J* =12 Hz, N-C<u>H</u>$_2$-CH$_2$-), 3.70-3.74 (t, 2H, *J* = 12 Hz, N-C<u>H</u>$_2$-CH$_2$-), 7.22-7.68 (m, 8H, ArH)	—	C, 64.37 (64.75) H, 4.45 (3.95) N, 8.83 (9.12)
44p	1610 (C=N)	δ 2.43 (s, 3H, SCH$_3$), 2.46-2.49 (m, 2H, N-CH$_2$-C<u>H</u>$_2$-), 3.60-3.63 (t, 2H, *J* = 12 Hz, N-C<u>H</u>$_2$-CH$_2$-), 3.70-3.73 (t, 2H, *J* =12 Hz, N-C<u>H</u>$_2$-CH$_2$-), 7.1-7.3 (m, 8H, ArH)	329 (M+1)	—
44q	1608 (C=N)	2.32 (s, 6H, (CH$_3$)$_2$), 2.34-2.37 (m, 2H, N-CH$_2$-C<u>H</u>$_2$-), 3.5 (br, 4H, N-C<u>H</u>$_2$-CH$_2$-), 7.11-7.52 (m, 8H, ArH)	277 (M+1)	—

44r	1608 (C=N)	2.33 (s, 3H, CH_3), 2.35-2.40 (m, 2H, N-CH_2-$C\underline{H}_2$-), 3.53 (br, 4H, N-$C\underline{H}_2$-CH_2-), 7.1-7.7 (m, 8H, ArH)	297 (M+1)	—

5,7-Diaryl-2,3-dihydro-1*H*-1,4-diazepines (B)

It was envisaged to synthesize 5,7-diaryl-2,3-dihydro-1*H*-1,4-diazepines **(B)** **(Scheme 2)** as discussed previously. For the preparation of such a system 1,3-diarylpropane-1,3-dione was required which could be cyclised to 2,3-dihydro-1,4-diazepine.



Scheme 2

General Procedure for 2-acetyl-1-(benzoyloxy) benzene derivatives (46a-s)

To a cold solution of 2-hydroxyacetophenone (0.05 mol) and substituted benzoic acid (0.05 mol) in pyridine (30ml), $POCl_3$ (0.07 mol) was added slowly. The reaction mixture was stirred at room temperature for 3 hr and then poured over ice and dil. HCl. The solid obtained was filtered and used directly in the next step without purification.

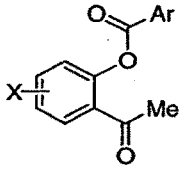
General procedure for 1-(2-hydroxyphenyl)-3-subtitutedphenyl-1,3-propanediones (47a-s)

To a solution of substituted 2-acetyl-1-(benzoyloxy)benzene (2 g) in dry pyridine (20 ml) powdered KOH (0.8 g) was added and the mixture was stirred at room temperature till a thick yellow paste was obtained. The paste was diluted with water; the yellow solid obtained was filtered, dried and used in the next step without purification.

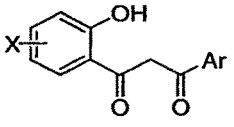
General procedure for 2-(substituted phenyl)chromen-4-one derivatives (48a-s)

To a solution of 1-(2-hydroxyphenyl)-3-subtitutedphenyl-1,3-propanedione (2.0 g) in glacial acetic acid (30 ml) was added few drops of con. H_2SO_4 and the reaction mixture were refluxed for 2hrs. After cooling to room temperature the reaction mixture was poured over crushed ice. The solid obtained was filtered, washed with water, dried and recrystallized from methanol.

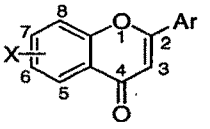
Table 3: The carbonyl stretching of compounds (46a-s), (47a-s) and (48a-s)



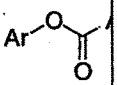
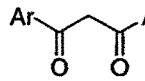
46a-s



47a-s



48a-s

Compound	X	Ar	IR (cm^{-1})		
					chromene
46a, 47a, 48a	H	C_6H_5	1735	1614	1645
46b, 47b, 48b	H	4-$CH_3C_6H_4$	1735	1616	1637
46c, 47c, 48c	H	3-$CH_3C_6H_4$	1732	1612	1637
46d, 47d, 48d	H	4-$CH_3OC_6H_4$	1724	1616	1649
46e, 47e, 48e	H	3-$CH_3OC_6H_4$	1741	1621	1652

46f, 47f, 48f	H	3-CH_3O,4-$CH_3OC_6H_3$	1732	1604	1652
46g, 47g,48g	H	4-ClC_6H_4	1739	1625	1662
46h,47h, 48h	H	3-ClC_6H_4	1739	1618	1641
46i,47i, 48i	H	2-ClC_6H_4	1743	1608	1652
46j,47j, 48j	H	2-Cl,4-ClC_6H_3	1751	1620	1650
46k,47k,48k	H	4-BrC_6H_4	1739	1614	1666
46l,47l,48l	H	4-FC_6H_4	1741	1625	1639
46m,47m, 48m	H	2-Furyl	1732	1616	1662
46n,47n, 48n	H	2- Thienyl	1732	1620	1639
46o,47o, 48o	6-CH_3O	C_6H_5	1738	1627	1641
46p,47p, 48p	6-CH_3O	4-$CH_3C_6H_4$	1724	1614	1647
46q,47q, 48q	6-CH_3O	4-ClC_6H_4	1726	1608	1635
46r,46r, 48r	6-CH_3O	3-ClC_6H_4	1737	1617	1642
46s,47s, 48s	6-CH_3O	4-$CH_3OC_6H_4$	1735	1612	1647

General procedure for Synthesis of 5,7-substituted diaryl-2,3-dihydro-1*H*-1,4-diazepines (49a-s)

A mixture of 2-phenylchromen-4-one derivatives **(48a-s)** (1 g) and 70% aqueous ethylenediamine (20 ml) was refluxed for 1hr on an oil bath. After 2 hrs the reaction mixture was cooled and 25 ml of cold water was added to the cooled reaction mixture. The solid obtained was filtered, dried and recrystallized from methanol to afford **(49a-s)**. The spectral analyses of title compounds were given in **Table 4.**

Table 4

Compd. No	**IR (cm^{-1})**	**NMR (δ)**	**Mass (m/z)**	**CHN(%) (Found)**
49a	3231, 3000, 1605	3.65 (br, 2H, CH_2), 3.9 (br, 2H, CH_2), 5.71(s, 1H, CH), 8.35 (br, 1H, NH), 6.45-7.74 (m, 9H, ArH)	264 (M^+)	—

49b	3176, 2916, 1595	2.39 (s, 3H, CH_3), 3.72 (br, 2H, CH_2), 3.82 (br, 2H, CH_2), 5.8 (s, 1H, CH), 9.8 (br, 1H, NH), 6.53-7.62 (m, 8H, ArH)	—	—
49c	3232, 3000, 1608	2.45 (s, 3H, CH_3), 3.62 (br, 2H, CH_2), 3.9 (br, 2H, CH_2), 5.7 (s, 1H, CH), 8.27 (br, 1H, NH), 6.48-7.65 (m, 9H, ArH)	279 (M+1)	—
49d	3200, 3000, 1604, 1255, 1031	3.63 (br, 2H, CH_2), 3.88 (bs, 5H, CH_2 &OCH_3), 5.7 (s, 1H, CH), 8.25 (br, 1H, NH), 6.45-7.65 (m, 8H, ArH)	—	C, 73.45 (73.49) H, 6.16 (6.13) N, 9.52 (9.64)
49e	3200, 2985, 1600, 1230, 1048	3.63 (br, 2H, CH_2), 3.9 (bs, 5H, CH_2 & OCH_3), 5.72 (s, 1H, CH), 8.25 (br, 1H, NH), 6.45-7.66 (m, 8H, ArH)	295 (M+1)	—
49f	3217, 2929, 1602, 1251, 1024,	3.63 (br, 2H, CH_2), 3.9 (bs, 2H, CH_2), 3.88 (s, 3H, OCH_3), 5.73 (s, 1H, CH), 8.25 (br, 1H, NH), 6.5-7.6 (m, 7H, ArH)	—	—
49g	3203, 3000, 1598	3.75 (br, 2H, CH_2), 3.85 (br, 2H, CH_2), 5.74 (s, 1H, CH), 7.74 (br, 1H, NH), 6.61-7.59 (m, 8H, ArH)	—	—
49h	3200, 2916, 1610	3.62 (br, 2H, CH_2), 3.92 (br, 2H, CH_2), 5.7 (s, 1H, CH), 8.28 (br, 1H, NH), 6.5-7.7 (m, 8H, ArH)	298 (M^+)	—

49i	3232, 2916, 1598	3.63 (br, 2H, CH_2), 4.0 (br, 2H, CH_2), 5.37 (s, 1H, CH), 8.3 (br, 1H, NH), 6.49-7.64 (m, 8H, ArH)	298 (M^+)	—
49j	3087, 3000, 1598	3.71 (br, 2H, CH_2), 3.97 (br, 2H, CH_2), 5.41 (s, 1H, CH), 7.54 (br, 1H, NH), 6.46-7.45 (m, 7H, ArH)	333 (M^+)	—
49k	3064, 3000, 1635	3.69 (br, 2H, CH_2), 3.89 (br, 2H, CH_2), 5.65 (s, 1H, CH), 8.11(br, 1H, NH), 6.46-7.54 (m, 8H, ArH)	345 (M+2)	—
49l	3203, 3000, 1604	δ 3.65 (br, 2H, CH_2), 3.9 (br, 2H, CH_2), 5.68 (s, 1H, CH), 8.38 (br, 1H, NH), 6.5-7.8 (m, 8H, ArH)	282 (M^+)	—
49m	3286, 3000, 1608	3.65 (br, 2H, CH_2), 3.9 (br, 2H, CH_2), 6.1 (s, 1H, CH), 8.28 (br, 1H, NH), 6.53-7.96 (m, 7H, ArH)	254 (M^+)	—
49n	3203, 3000, 1595	3.6 (br, 2H, CH_2), 3.9 (br, 2H, CH_2), 5.92 (s, 1H, CH), 8.3 (br, 1H, NH), 6.5-7.8 (m, 7H, ArH)	—	—
49o	3423, 3100, 1633	3.65 (br, 2H, CH_2), 3.8 (br, 2H, CH_2), 3.71 (s, 3H, OCH_3), 5.68 (s, 1H, CH), 7.57 (br, 1H, NH), 6.7-7.55 (m, 8H, ArH)	295 (M+1)	—

49p	3288, 3000, 1608, 1218, 1040	2.38 (s, 3H, CH_3), 3.69 (br, 2H, CH_2), 3.78 (br, 2H, CH_2), 3.71(s, 3H, OCH_3), 5.68 (s, 1H, CH), 7.49 (br, 1H, NH), 6.75-7.47 (m, 7H, ArH)	309 (M+1)	—
49q	3299, 3000, 1595, 1271, 1095	3.69 (br, 2H, CH_2), 3.92 (br, 2H, CH_2), 3.73 (s, 3H, OCH_3), 5.65 (s, 1H, CH), 7.87 (br, 1H, NH), 6.77-7.58 (m, 7H, ArH)	—	—
49r	3257, 3000, 1612, 1218, 1040	—	—	—
49s	3222, 3000, 1604, 1257, 1033	3.66 (br, 2H, CH_2), 3.90 (br, 2H, CH_2), 3.72 (s, 6H, OCH_3), 5.69 (s, 1H, CH), 7.88 (br, 1H, NH), 6.75-7.47 (m, 7H, ArH)	325 (M+1)	—

Biological Studies

The biological work carried out has been discussed under following sub heads:

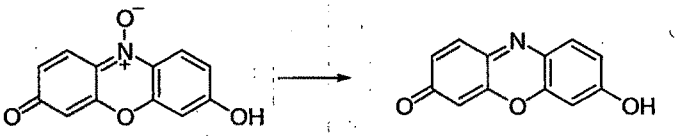
A. Antiproliferative activity

B. Antiplatelet activity

A. Antiproliferative activity

Initial evaluation of 2,3-diaryl-6,7-dihydro-5*H*-1,4-diazepines and 5,7-diaryl-2,3-dihydro-1*H*-1,4-diazepines were carried out at Memorial Sloan Kettering Cancer Center (MSKCC), New York (USA). The cytotoxic effects of the above said compounds were tested using Alamar blue assay. Alamar blue (Resazurin) is nontoxic, commonly employed as an indicator of cell number and viability,

since it is reduced to a (Resorufin) pink fluorescent dye in the medium by cell activity (possibly by oxygen consumption through metabolism). Alamar blue reduction was measured on CCD-based optical imaging reader.



Resazurin (blue) Resorufin (pink)

The various human leukemic cancer cell lines Jurkat, HL60, Molt3, NCEB-1 and K562 were incubated with various concentrations of the 2,3 and 5,7-diaryldiazepines for 72 h (48h plus 24h with dye). The results of IC_{50} values of the compound are summarized in **Table 5 and 6.**

Table 5: Antiproliferative activity IC_{50} (μM) values of 2,3-diaryl-6,7-dihydro-5*H*- 1,4-diazepines (44a-r)

Compound	Jurkat	HL60	Molt-3	NCEB-1	K562
44a	>100	>100	>100	>100	>100
44b	48.6	45	31.25	70	64
44c	40.59	41	24.37	54	49
44d	71.87	59	82	75	68
44e	NT	>100	NT	>100	>100
44f	NT	>100	NT	>100	>100
44g	30.1	39	22.6	>100	27.22
44h	51.78	47	27.6	54	39.19
44i	33.72	46	22.47	57	43.83
44j	26.67	30	21.4	40	36.59
44k	29	40	22.64	56	44
44l	48.26	67	28.75	>100	51.54
44m	39.73	32	25.13	65	49.18
44n	>100	>100	>100	>100	>100
44o	44	38	21	45	37.79
44p	NT	>100	NT	82.88	>100
44q	NT	>100	NT	>100	>100
44r	NT	>100	NT	>100	>100

Table 6: Antiproliferative activity IC_{50} (μM) values of 5,7-diaryl-2,3-dihydro-1*H*-1,4-diazepines (49a-s)

Compound	Jurkat	HL60	Molt-3	NCEB-1	K562
49a	10.59	60	11.4	>100	9.12
49b	50.79	>100	40.81	86	24.3
49c	8.47	22	11.6	100	4.55
49d	>100	>100	31	>100	20.85
49e	5.39	23	14.33	26	8.28
49f	N. T	>100	N. T	>100	>100
49g	81.58	>100	64	>100	65
49h	>100	>100	>100	>100	>100
49i	7.66	26	12.09	23	10.12
49j	N. T	>100	N. T	>100	>100
49k	N. T	>100	N. T	>100	>100
49l	22.75	80	38.48	70	29.46
49m	>100	>100	>100	>100	88.78
49n	N. T	>100	N. T	>100	>100
49o	N. T	88.24	N. T	>100	85.26
49p	N. T	>100	N. T	>100	>100
49q	N. T	>100	N. T	>100	>100
49r	N. T	N. T	N. T	N. T	N. T
49s	N. T	>100	N.T	>100	>100

N.T = Not tested

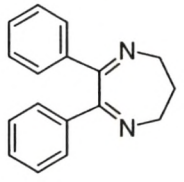
Looking to the results of antileukemic activity of 2,3-diaryl-6,7-dihydro-5*H*- 1,4-diazepines **(44a-r)** and 5,7-diaryl-2,3-dihydro-1*H*- 1,4-diazepines **(49a-s)** it is reflected that the former category of compounds do not show any significant activity. In comparison, among the 5,7-diaryl-2,3-dihydro-1*H*- 1,4-diazepines **(49a-s)** compound **49a** showed significant cytotoxic activity with IC_{50} values of 9.12 and 10.59 μM against K562 and Jurkat cell lines respectively. Introduction of methyl group at meta position of the diaryldiazepine nucleus as in compound **49c**, demonstrated high cytotoxic activity against the K562 and Jurkat cell lines with an IC_{50} values of 4.55 and 8.47 μM respectively. Compound **49e**, in which the methyl group was replaced with a greater electron donating group, a methoxy group, was also found to be cytotoxic but significantly less so

than the compound **49c**. Results indicate that among the halo substituted diaryldiazepines, compound **49i**, bearing a chloro substituent at ortho position showed significant activity with IC_{50} value of 7.66 μM against Jurkat cell line. Substitution of methoxy at 5th position of the phenyl ring attached to the 4th position of the dazepine does not show any significant activity **(49o-s)**. Also, there was no interesting activity when one of the aryl rings was replaced by furan **(49m)** and thiophene ring **(49n)**.

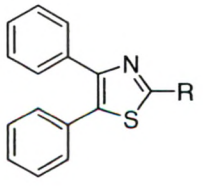
B. Antiplatelet activity

The evaluation of antiplatelet activity for 2,3-diaryl-6,7-dihydro-5*H*-1,4-diazepine **(44a-r)** in this study was due to the following reason:

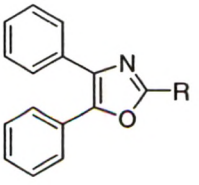
Looking to the chemical features of 2,3-diaryl-1,4-diazepines, the antiplatelet activity can be speculated, as the compounds possess both the features for such activity reported in case of certain diazepines (described in the introduction chapter) and some diaryl heterocycles **(A-C)**.



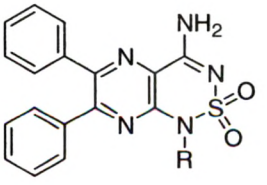
44a-r



A



B



C

The 2,3-diaryl-6,7-dihydro-5H-1,4-diazepines **(44a-r)** were evaluated to inhibit platelet aggregation of female Sprague-Dawley rat platelet-rich plasma induced by arachidonic acid. 500 μM of test compounds **(44a-r)** were used in the

preliminary screening. Aspirin was used as standard drug and showed maximum inhibition (97%) at 10mM. In this screening **44c, 44d, 44h, 44i, 44k, 44m** and **44o** were found to be moderately active **(Table 7)**.

Table 7 Antiplatelet activity (500 µM)

Compound	% inhibition
44a	18.5
44b	35.7
44c	42.5
44d	60.7
44e	35.7
44f	34
44g	34
44h	44.3
44i	77.8
44j	36.4
44k	44.6
44l	43
44m	52.6
44n	22
44o	58.2
44p	N.T
44q	22.1
44r	0

N.T = Not tested

Hence, these compounds were subjected to further screening at low concentrations (100 µM). The results of the screening are displayed in **Table 8.**

Table 8 Antiplatelet activity (100 µM)

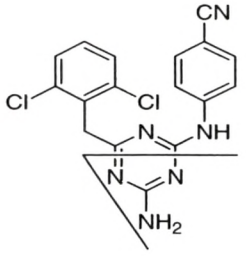
Compound	% inhibition
44c	18.5
44d	18.8
44h	24
44i	36.4
44k	24
44m	24.2
44o	28.3
Aspirin	14

From these studies, **44i** was the most active one with 36.4% inhibition. Compound **44o** showed 28.3% inhibition with two fold greater activity as compared to aspirin (14%). The compound **44h, 44k** and **44m** showed moderately good activity.

Part B

4,6-diaryl-2-aminopyrimidines

Incorporation of a structural fragment or pharmacophore into a molecule is the one of the approaches adopted in searching for lead compounds. Common fragments present in various compounds contribute to similarity in biological activity. However, they usually exhibit different potencies. The common fragments, guanidine and the diaryl wings, present in the structures of the diaryl pyrimidines, an NNRTI class of compounds, were recognized by us.



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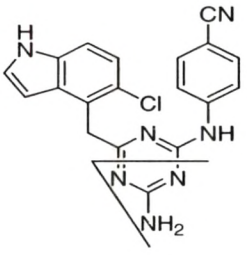

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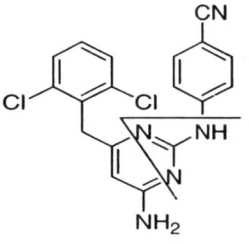

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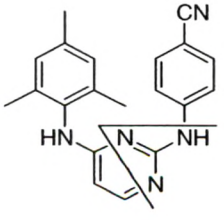

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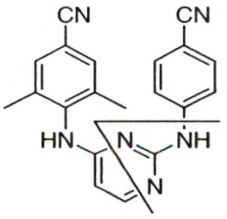

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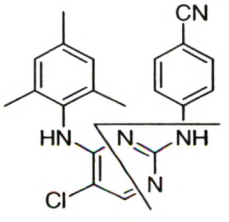

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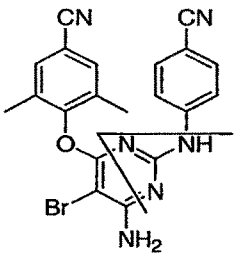

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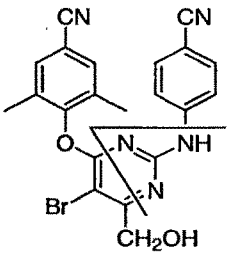
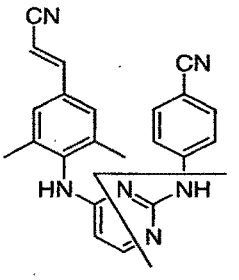
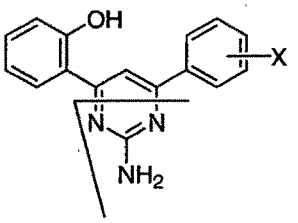


VIII



IX



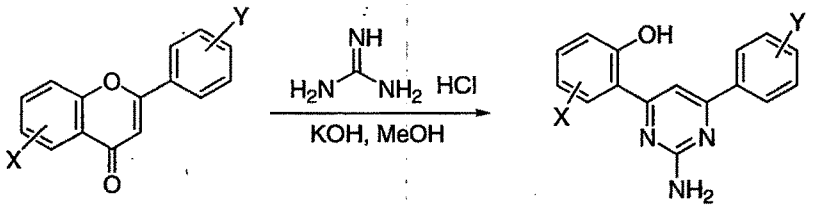
Proposed compounds (50a-q)

Compounds containing such common fragments can be synthesized by reacting the starting materials containing such fragments or by incorporating the fragments during the synthesis. It is also known that diaryl pyrimidines could be synthesized from a common intermediate chromen-4-one derivatives **(47a-q)**, from which we reported the synthesis of different 5,7-diaryl diazepines presented in Part I of this thesis. The reaction of flavones with guanidine hydrochloride leads to the formation of the diaryl pyrimidines which contain both the common fragments.

Hence, it was proposed to synthesize diaryl pyrimidines and evaluate their anti-HIV activity.

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

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)**. The spectral analyses of the title compounds are given in **Table 9.**



Scheme 3

Table 9

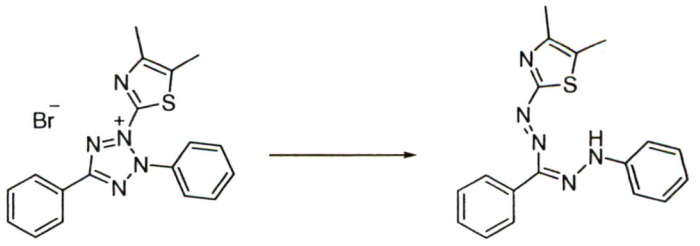
Compd. No	IR (cm^{-1})	NMR (δ)	Mass (m/z)	CHN(%) (Found)
50a	3508, 3354, 3205, 1625	5.37 (s, 2H, NH_2), 6.92-8.06 (m, 10H, ArH+ C-5 pyrimidine), 14.3 (br, 1H, OH)	264 (M+1)	—
50b	3500, 3330, 3197, 1610	2.43 (s, 3H, CH_3), 5.74 (bs, 2H, NH_2), 6.93-8.0 (m, 9H, ArH+C-5 pyrimidine), 13.5 (br, 1H, OH)	278 (M+1)	—
50c	3490, 3394, 3200, 1629	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)	—	—
50d	3492, 3327, 3150, 1615, 1249, 1026	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)	294 (M+1)	—
50e	3400, 3313, 3176, 1647, 1236, 1029	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)	—	—

50f	3431, 3313, 3190, 1643, 1259, 1026	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)	324(M+1)	—
50g	3502, 3340, 3217, 1641	6.28 (bs, 2H, NH_2), 6.92-8.07 (m, 9H, ArH+ C-5 pyrimidine), 13.5 (br, 1H, OH)	298 (M+1)	—
50h	3502, 3346, 3203, 1616	5.24 (s, 2H, NH_2), 6.92-8.05 (m, 9H, ArH+ C-5 pyrimidine), 13.5 (br, 1H, OH)	—	—
50i	3489, 3371, 3180, 1631	δ 6.44 (bs, 2H, NH_2), 6.91-7.80 (m, 9H, ArH+ C-5 pyrimidine), 13.8 (br, 1H, OH)	—	—
50j	3502, 3338, 3217, 1641	6.66 (bs, 2H, NH_2), 6.89-7.64 (m, 8H, ArH+ C-5 pyrimidine)	332 (M^+)	—
50k	3519, 3367, 3200, 1639	δ 6.1 (s, 2H, NH_2), 6.95-7.93 (m, 9H, ArH+ C-5 pyrimidine)	—	—
50l	3490, 3321, 3201, 1647	5.5 (bs, 2H, NH_2), 6.93-8.11 (m, 9H, ArH+ C-5 pyrimidine)	282 (M+1)	—
50m	3417, 3286, 3163, 1635	5.3 (s, 2H, NH_2), 6.59-7.87 (m, 8H, ArH/furyl+ C-5 pyrimidine), 14.3 (br, 1H, OH)	254 (M+1)	—
50n		5.27 (s, 2H, NH_2), 6.92-7.84 (m, 8H,		—

	3502, 3438, 3232, 1647	ArH/thienyl+ C-5 pyrimidine), 13.5 (br, 1H, OH)	270 (M+1)	
50o	3492, 3311, 3190, 1633, 1218, 1093	—	—	—
50p	3475, 3305, 3160, 1631, 1218, 1220, 1074	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)	—	—
50q	3444, 3332, 3220, 1643, 1431, 1218, 1091	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)	—	—

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.



The most active ones are compounds **50a** and **50c** with IC_{50} of 3.22 and 3.45μM respectively (**Table 9**). However their selectivity indices are rather weak by 10 and 4 respectively.

Table 9: 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.

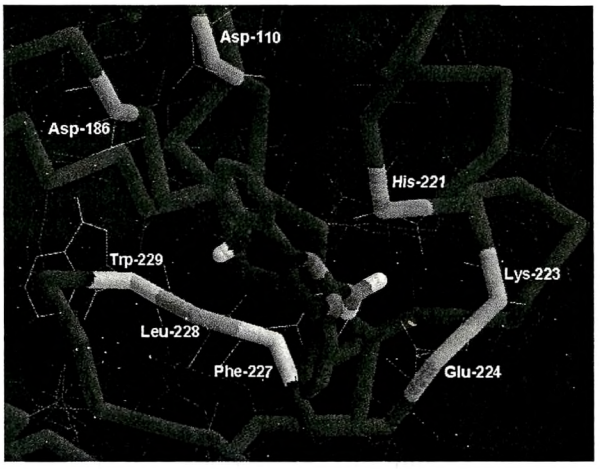
[b]Concentration required to reduce MT-4 cell viability by 50%.

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

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. 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.

Conclusion

The 2,3-diaryl-6,7dihydro-5*H*-1,4-diazepines screened for both antileukemic and antiplatelet activities. From the results of the above studies, some compounds this class of diazepines showed very good antiplatelet activity when arachidonic acid induced platelet aggregation. Further studies are required to prove their mechanism of action. This group of compounds lack antileukemic activity.

The antileukemic results of 5,7-diaryl-2,3-dihydro-1*H*-1,4-diazepines indicate that a few compounds show promising activity. They can be further optimized by introducing different substitutions in both aryl rings and diazepines ring.

In case of the 4,6-diaryl-2-aminopyrimidine, we hypothesize 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.