Scaffold Repurposing Reveals New Nanomolar Phosphodiesterase Type 5 (PDE5) Inhibitors Based on Pyridopyrazinone Scaffold: Investigation of In Vitro and In Silico Properties

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Supplementary Materials

Table of content

S1. Training set

150 reported PDE5 inhibitors (**t1–150**) were used as training set in this study

Table S1. Key structure of training set (**t1–150**)

Training set		PDE5 IC_{50} (nM)		Ref.
Compound code	**Compound code in Ref.**	**Experimental**	**Predicted**	
t1 (1)	Sildenafil	2.2	30.0	[1]
t2 (2)	Vardenafil	1.0	30.7	[1]
t3 (3)	Tadalafil	1.2	22.9	[1]
t4 (4)	18	13.4	21.9	[2]
t5	Benzamidenafil	1.1	24.8	[3]
t6	Avanafil	5.2	16.5	[5]
t7	Homosildenafil	3.8	31.3	[5]
t8	Hydroxyhomosildenafil	3.4	29.3	[5]
t9	Acetildenafil	7.6	15.7	[5]
t10	Hydroxyacetildenafil	58.4	100.7	[5]
t11	Thiosildenafil	0.6	13.5	[5]
t12	Carbodenafil	5.2	29.5	[6]
t13	Udenafil	8.3	24.7	[6]
t14	Norneosildenafil	5.8	16.6	[3]
t15	Thioaildenafil	0.6	45.5	[3]
t16	Nitrodenafil	67.4	61.8	[3]
t17	Dimethylacetildenafil	51.2	54.6	[3]
t18	Zaprinast	300.0	421.4	[7]
t19	9a	3.0	23.2	[4]
t20	9b	11.0	48.8	[4]
t21	10	3.0	21.2	[4]
t22	6c	150.0	253.0	[8]
t23	6e	60.0	88.7	[8]
t24	6f	70.0	93.8	[8]
t25	6h	100.0	272.9	[8]
t26	6i	140.0	101.0	[8]
t27	6l	50.0	95.5	[8]
t28	6m	370.0	126.5	[8]
t29	6n	320.0	384.4	[8]
t30	6o	70.0	88.8	[8]
t31	6q	530.0	145.6	[8]
t32	6r	200.0	163.2	[8]
t33	6s	510.0	358.6	[8]
t34	6t	500.0	141.6	[8]
t35	6u	38.0	38.3	[8]
t36	6v	310.0	408.4	[8]
t37	27	0.3	18.2	[2]
t38	28	0.3	16.3	[2]
t39	30	0.3	12.3	[2]
t40	6	0.3	13.3	[9]
t41	29	0.4	19.4	[2]
t42	26	0.9	15.7	[2]

Training set		PDE5 IC_{50} (nM)		Ref.
Compound code	**Compound code in Ref.**	**Experimental**	**Predicted**	
t43	25	1.0	20.7	[2]
t44	9	1.3	20.9	[2]
t45	17	1.6	28.9	[2]
t46	10	2.0	31.0	[2]
t47	35	2.3	20.0	[2]
t48	36	2.4	28.0	[2]
t49	11	2.5	30.1	[2]
t50	13	2.7	19.1	[2]
t51	31	2.9	47.1	[2]
t52	15	3.0	31.2	[2]
t53	19	3.5	27.3	[2]
t54	16	4.6	20.5	[2]
t55	34	14.5	60.9	[2]
t56	12	20.3	32.0	[2]
t57	22	22.4	20.1	[2]
t58	37	26.7	20.1	[2]
t59	14	28.5	40.2	[2]
t60	23	33.3	103.2	[2]
t61	33	33.9	36.2	[2]
t62	32	36.5	39.3	[2]
t63	20	52.5	90.6	[2]
t64	21	149.0	214.0	[2]
t65	24	224.0	375.2	[2]
t66	38	534.0	162.7	[2]
t67	2	0.9	18.7	[9]
t68	3	1.1	17.8	[9]
t69	4	10.6	23.8	[9]
t70	5	0.1	13.0	[9]
t71	1	51.0	57.5	[2]
t72	2	25.0	24.1	[2]
t73	3	43.0	94.4	[2]
t74	9	4.0	21.4	[10]
t75	10	0.8	15.6	[10]
t76	11	16.3	68.9	[10]
t77	12	0.3	14.3	[10]
t78	13	1.1	15.8	[10]
t79	14	1.2	21.9	[10]
t80	15	0.9	14.7	[10]
t81	16	0.2	30.2	[10]
t82	17	0.1	20.2	[10]
t83	18	0.1	15.1	[10]
t84	19	0.1	15.2	[10]

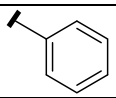
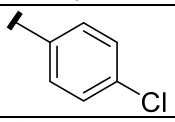
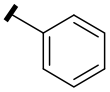
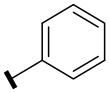
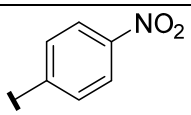
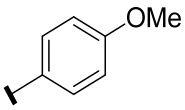
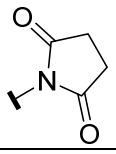
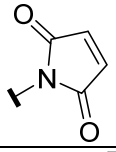
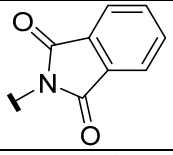
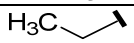
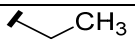
Training set		PDE5 IC_{50} (nM)		Ref.
Compound code	**Compound code in Ref.**	**Experimental**	**Predicted**	
t85	20	3.0	22.3	[10]
t86	21	1.1	30.8	[10]
t87	22	2.6	16.1	[10]
t88	23	0.7	12.6	[10]
t89	24	0.4	13.4	[10]
t90	25	0.1	17.1	[10]
t91	26	0.3	14.4	[10]
t92	27	0.1	19.2	[10]
t93	7a	0.3	23.4	[1]
t94	7b	0.4	15.5	[1]
t95	7c	4.3	18.4	[1]
t96	7d	15.0	30.9	[1]
t97	7e	4.1	61.4	[1]
t98	7f	0.6	12.6	[1]
t99	16f	410.0	257.5	[7]
t100	14h	160.0	158.1	[7]
t101	16h	65.0	102.8	[7]
t102	21d	350.0	146.5	[7]
t103	5a	9.3	23.8	[4]
t104	5b	4.9	21.5	[4]
t105	6	86.0	68.9	[4]
t106	7a	0.6	13.6	[4]
t107	7b	0.5	16.5	[4]
t108	11	17.0	53.0	[4]
t109	12	240.0	340.3	[4]
t110	13	190.0	354.2	[4]
t111	34a	177.0	123.1	[4]
t112	35c	235.0	247.3	[4]
t113	37a	52.5	100.6	[4]
t114	37b	39.7	61.4	[4]
t115	41	227.0	447.2	[4]
t116	Aminotadalafil	4.2	20.4	[5]
t117	4a	290.0	170.3	[11]
t118	4d	38.0	46.4	[11]
t119	7a	100.0	259.0	[11]
t120	7d	60.0	89.7	[11]
t121	8a	320.0	433.4	[11]
t122	8d	50.0	97.5	[11]
t123	9a	260.0	124.3	[11]
t124	9d	50.0	67.5	[11]
t125	10d	40.0	65.4	[11]
t126	8a	160.0	277.1	[4]

Training set		PDE5 IC_{50} (nM)		Ref.
Compound code	**Compound code in Ref.**	**Experimental**	**Predicted**	
t127	8b	170.0	169.1	[4]
t128	19b	36.9	25.3	[4]
t129	19c	12.7	24.9	[4]
t130	19d	7.2	23.6	[4]
t131	19e	8.7	22.7	[4]
t132	19f	3.9	20.3	[4]
t133	19g	80.2	205.9	[4]
t134	19h	50.2	58.5	[4]
t135	20h	97.5	76.9	[4]
t136	21c	56.4	57.6	[4]
t137	21f	19.9	42.0	[4]
t138	22f	61.0	71.7	[4]
t139	26a	10.5	49.8	[4]
t140	26b	2.9	19.2	[4]
t141	26c	5.2	22.6	[4]
t142	26d	21.4	102.0	[4]
t143	27b	512.0	456.6	[4]
t144	27c	383.0	351.5	[4]
t145	28b	27.6	71.1	[4]
t146	28c	155.0	242.1	[4]
t147	29b	8.4	20.7	[4]
t148	29c	7.0	24.6	[4]
t149	26e	30.3	21.2	[4]
t150	29e	36.4	45.3	[4]

S2. Test set

34 compounds of our research compounds with different substitutions (series **A–H**) were selected in this study

Table S2. Key structure of test set (series **A–H**)

Series	Compound	X	R_1	R_2
A	**5a**	O		-
	5b	O		-
	5c	S		-
B	**6a**	-		-
	6b	-		-
	6c	-		-
C	**7a**	-		-
	7b	-		-
	7c	-		-
D	**8a**	-		
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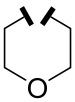
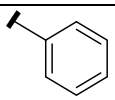
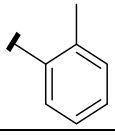
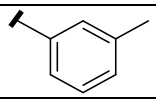
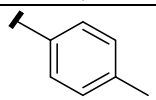
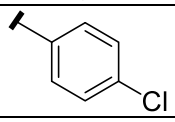
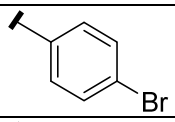
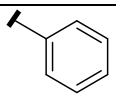
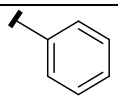
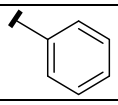
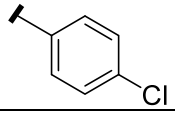
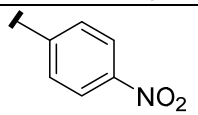
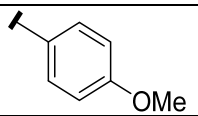
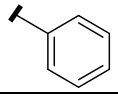
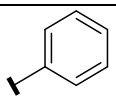
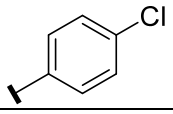
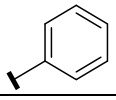
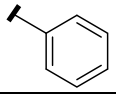
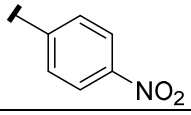
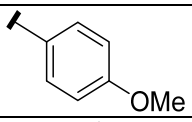
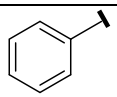
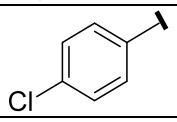
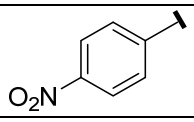
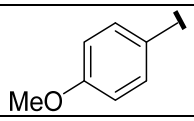
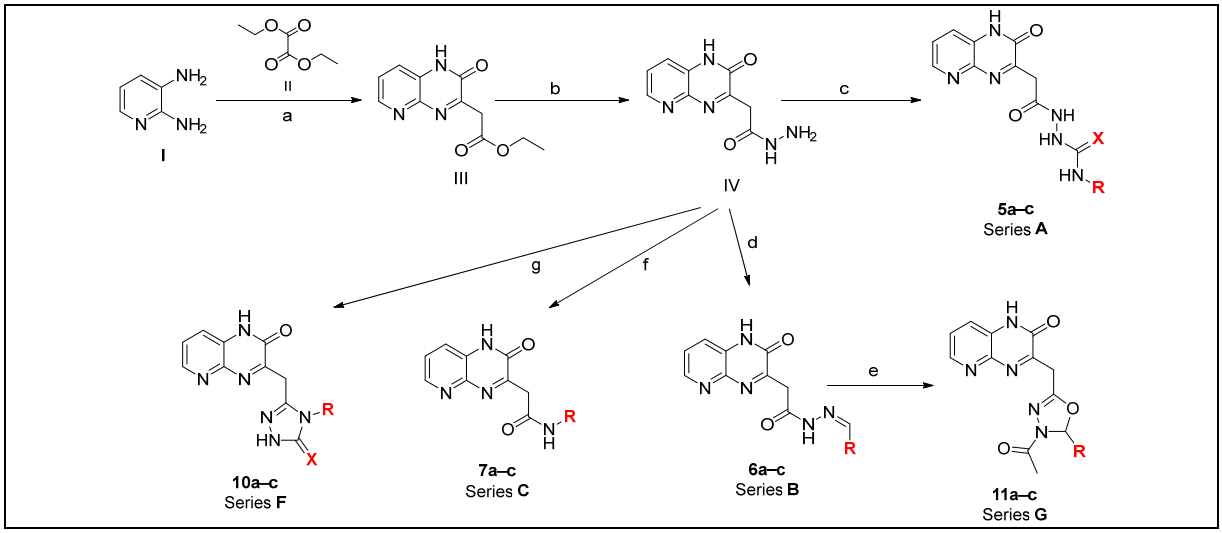
	8c	-		
	8d	-	H	
	8e	-	H	

Series	Compound	X	R_1	R_2
	8f	-	H	
	8g	-	H	
	28h	-	H	
	8i	-	H	
	8j	-		
E	**9a**	-	H	
	9b	-	H	
	9c	-	H	
	9d	-	H	
	9e	-		
F	**10a**	O		-
	10b	O		-
	10c	S		-
G	**11a**	-		-
	11b	-		-

Series	Compound	X	R_1	R_2
	11c	-		-
H	**12a**	-		-
	12b	-		-
	12c	-		-
	12e	-		-

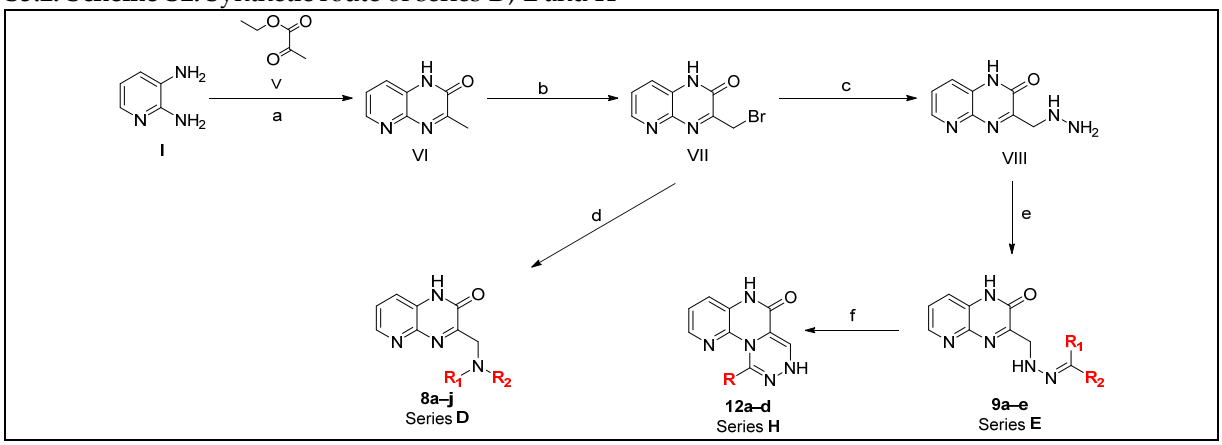
S3. Synthetic schemes

S3.1. Scheme S1: Synthetic route of series **A**, **B**, **C**, **F** and **G**.



Scheme S1. Reagents and conditions: **a**, Na, EtOH, AcOH, reflux, 30 min.; **b**, hydrazine hydrate, EtOH, reflux, 9 h; **c**, appropriate isocyanate or isothiocyanate, EtOH, reflux, 6 h; **d**, appropriate aromatic aldehyde, AcOH, reflux, 8 h; **e**, acetic anhydride, reflux, 2 h; **f**, appropriate cyclic acid anhydride, AcOH, reflux, 6 h; **g**, appropriate isocyanate or isothiocyanate, EtOH, reflux, 24 h.

S3.2. Scheme S2: Synthetic route of series **D**, **E** and **H**



Scheme S2. Synthetic route of series **D**, **E** and **H**. Reagents and conditions: **a**, EtOH, reflux, 10 h; **b**, Br_2, AcOH, NaOAc, reflux, 30 min; **c**, hydrazine hydrate, EtOH, reflux, 9 h; **d**, appropriate amine, EtOH, NaI, reflux, 10 h; **e**, appropriate aldehyde or ketone, AcOH, reflux, 8 h; **f**, Br_2, AcOH, NaOAc, rt, 3 h.

S4. Chemical experimental

Melting points were determined by open capillary tube method using Gallen Kamp melting point apparatus MFB-595-010M (Gallen Kamp, London, England) and were uncorrected. IR Spectra were recorded as potassium bromide discs on Schimadzu FT-IR 8400S spectrophotometer (Shimadzu, Kyoto, Japan) and expressed in wavenumber (ν) cm^{-1}. The ^1H NMR spectra were recorded on *Varian Mercury VX-300 NMR spectrometer at 300 MHz and **Bruker NMR spectrometer at 400 MHz. Chemical Shifts were quoted in δ as parts per million (ppm) downfield from tetramethylsilane (TMS) as internal standard. Mass spectra were recorded using Shimadzu Gas Chromatograph Mass spectrometer QP 1000 Ex (Shimadzu). TLC was carried out using Art. DC-Plastikfolien, Kieselgel 60F_{254} sheets (Merck, Darmstadt, Germany). Chloroform/MeOH was used as the developing solvent and the spots were visualized at 366 and 254 nm by UV Vilber Lourmat 77202 (Vilber, Marne La Vallee, France).

S4.1. Synthesis of 3-(ethoxycarbonyl methyl) pyrido[2,3-*b*]pyrazin-2(1*H*)one (III):
Absolute ethanol (25 ml) was added to sodium metal (2.3 g, 0.1 mol). Stand till reaction ceased and then a mixture of ethyl acetate (8.8 g, 9.8 ml, 0.1 ml) and diethyl oxalate (14.6 g, 16.2 ml, 0.1 mol) was slowly added. The mixture was stirred for 30 min. and then added to a solution of 2,3-diaminopyridine (**I**,10.9 g, 0.1 mol) in a mixture of hot ethanol (15 ml) and acetic acid (7 ml). The reaction mixture was heated on a steam bath for 10 min. After cooling, the formed precipitate was filtered and dried. The crude product was crystalized from ethanol to give the titled product **III**. Yield 43%. Mp 234-6 °C. IR υ_{max}, cm^{-1}: 3213 (NH), 3100 (CH Ar), 2922, 2850 (CH Aliph), 1697 (2C=O), 1647, 1602, 1562, 1540, 1521 (NH, C=N, C=C). **^1H NMR (400 MHz, DMSO-d_6): δ= 1.23 (t, 3H, $OCH_2C\mathbf{H_3}$), 2.41 (s, 2H, CH_2), 3.89 (q, 2H, $OC\mathbf{H_2}CH_3$), 7.11 (d, *J*= 7.3 Hz, 1H, H-8 Ar), 7.52 (t, 1H, H-7 Ar), 8.11 (d, *J*= 7.0 Hz, 1H, H-6 Ar), 11.49 (s, 1H, NH exchanged with D_2O). Anal. calcd. for $C_{11}H_{11}N_3O_3$ (233.22): C, 56.65; H, 4.75; N, 18.02. Found: C, 56.78; H, 4.88; N, 18.17.

S4.2. Synthesis of 3-hydrazino carbonyl methyl pyrido[2,3-*b*]pyrazin-2(1*H*)one (IV):
Hydrazine hydrate (7.00 g, 6.86 ml, 0.14 mol) was added to a solution of compound **2** (2.33 g, 0.01 mol) in ethanol (20 ml) and refluxed for 9 h. The reaction mixture was cooled in ice bath for 10 min. and filtered, washed with ethanol (5 ml) and dried. The crude product was crystalized from ethanol to give the titled product **IV**. Yield 71%. Mp 214-5 °C. IR υ_{max}, cm^{-1}: 3340, 3309 (NH_2, 2NH), 3032 (CH Ar), 2939, 2866 (CH Aliph), 1666 (2C=O), 1620, 1593, 1546, 1512 (NH, C=N, C=C). ^1H NMR (300 MHz, DMSO-d_6): δ= 2.33 (s, 2H, CH_2), 4.61 (s, 2H, NH_2 exchanged with D_2O), 7.21 (d, *J*= 7.1 Hz, 1H, H-8 Ar), 7.51 (t, 1H, H-7 Ar), 8.20 (d, *J*= 6.3 Hz, 1H, H-6 Ar), 10.89 (s, 1H, NH exchanged with D_2O), 11.50 (s, 1H, NH exchanged with D_2O). Anal. calcd. for $C_9H_9N_5O_2$ (219.20): C, 49.31; H, 4.14; N, 31.95. Found: C, 49.11; H, 4.23; N, 31.70.

S4.3. General procedure for synthesis of 3-[4′-((substituted)phenyl (thio)semicarbazido) carbonyl-methyl] pyrido[2,3-*b*]pyrazin-2(1*H*)ones (series A, 5a-c):
Appropriate isocyanate or isothiocyanate (0.3 mol) and compound **IV** (6.57 g, 0.03 mol) were refluxed with ethanol (60 ml) for 6 h. The precipitate was cooled, filtered and dried. The crude product was crystalized from acetic acid.

S4.3.1. 3-[4′-(Phenyl semicarbazido) carbonyl-methyl] pyrido[2,3-*b*]pyrazin-2(1*H*)one (5a):
Yield 55%. Mp 218-20 °C. IR υ_{max}, cm^{-1}: 3421, 3375, 3233 (4NH), 3018 (CH Ar), 2951, 2843 (CH Aliph), 1670 (3C=O), 1631, 1593, 1550, 1504 (NH, C=N, C=C). ^1H NMR (300 MHz, DMSO-d_6): δ= 2.21 (s, 2H, CH_2), 5.66 (s, 1H, NH exchanged with D_2O), 6.00 (s, 1H, NH exchanged with D_2O), 7.11 (d, *J*= 7.2 Hz, 1H, H-8 Ar), 7.20-7.45 (m, 6H, H-7,2′,3′,4′,5′,6′ Ar), 8.13 (d, *J*= 8.0 Hz, 1H, H-6 Ar), 10.87 (s, 1H, NH exchanged with D_2O), 11.52 (s, 1H, NH exchanged with D_2O). Anal. calcd. for $C_{16}H_{14}N_6O_3$ (338.32): C, 56.80; H, 4.17; N, 24.84. Found: C, 56.45; H, 4.00; N, 25.01.

S4.3.2. 3-[4′-(4-Chloro phenyl semicarbazido) carbonyl-methyl] pyrido[2,3-*b*]pyrazin-2(1*H*)one (5b)
Yield 54%. Mp 230-2 °C. IR υ_{max}, cm^{-1}: 3417, 3371, 3243 (4NH), 3024 (CH Ar), 2947, 2843 (CH Aliph), 1693 (3C=O), 1631, 1593, 1550, 1500 (NH, C=N, C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ= 2.26 (s, 2H, CH_2), 5.46 (s, 1H, NH exchanged with D_2O), 6.01 (s, 1H, NH exchanged with D_2O), 7.11 (d, *J*= 7.5 Hz, 1H, H-8 Ar), 7.19-7.51 (m, 5H, H-7,2′,3′,5′,6′ Ar), 8.20 (d, *J*= 6.4 Hz, 1H, H-6 Ar), 10.77 (s, 1H, NH exchanged with D_2O), 11.53 (s, 1H, NH exchanged with D_2O). Anal. calcd. for $C_{16}H_{13}ClN_6O_3$ (372.77): C, 51.55; H, 3.52; N, 22.55. *Found: C, 51.83; H, 3.78; N, 22.62.

S4.3.3. 3-[4′-(Phenyl thiosemicarbazido) carbonyl-methyl] pyrido[2,3-*b*]pyrazin-2(1*H*)one (5c):
Yield 54%. Mp 234-6 °C. IR υ_{max}, cm^{-1}: 3433, 3375, 3275 (4NH), 3028 (CH Ar), 2954, 2843 (CH Aliph), 1693 (2C=O), 1639, 1608, 1593, 1546, 1500 (NH, C=N, C=C), 1265 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6): δ= 2.22 (s, 2H, CH_2), 4.53 (s, 1H, NH exchanged with D_2O), 5.98 (s, 1H, NH exchanged with D_2O), 6.59-6.78 (m, 5H, H-2′,3′,4′,5′,6′ Ar), 7.12 (d, *J*= 7.2 Hz, 1H, H-8 Ar), 7.44 (t, 1H, H-7 Ar), 8.12 (d, *J*= 7.3 Hz, 1H, H-6 Ar), 10.55 (s, 1H, NH exchanged with D_2O), 11.43 (s, 1H, NH exchanged with D_2O). Anal. calcd. for C16H14N6O2S (354.39): C, 54.23; H, 3.98; N, 23.71. *Found: C, 54.52; H, 4.11; N, 23.90.

S4.4. General procedure for synthesis of 3-(N^2-(substituted)benzylidenehydrazino carbonyl methyl)pyrido[2,3-*b*]pyrazin-2(1*H*)ones (series B, 6a-c):
Appropriate aromatic aldehyde (0.01 mol) was added to a solution of compound **IV** (2.19 g, 0.01 mol) in glacial acetic acid (10 ml) and refluxed for 8 h. The mixture was allowed to cool and poured onto crushed ice (30 g). The formed precipitate was filtered, washed with water (5 ml) and filtered. The crude product was crystalized from ethanol.

S4.4.1. 3-(N^2-Benzylidenehydrazino carbonyl methyl)pyrido[2,3-*b*]pyrazin2(1*H*)one (6a):
Yield 65%. Mp 228-30 °C. IR υ_{max}, cm^{-1}: 3437, 3371 (2NH), 3018 (CH Ar), 2910, 2824 (CH Aliph), 1678 (2C=O), 1635, 1616, 1604, 1585, 1523 (NH, C=N, C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ= 2.31 (s, 2H, CH_2), 7.10 (d, *J*= 6.9 Hz, 1H, H-8 Ar), 7.45 (t, 1H, H-7 Ar), 7.77-7.91 (m, 5H, H-2′,3′,4′,5′,6′ Ar), 8.02 (s, 1H, N=CH), 8.30 (d, *J*= 6.1 Hz, 1H, H-6 Ar), 10.96 (s, 1H, NH exchanged with D_2O), 11.61 (s, 1H, NH exchanged with D_2O). Anal. calcd. for $C_{16}H_{13}N_5O_2$ (307.31): C,62.53; H, 4.26; N, 22.79. Found: C, 62.12; H, 4.70; N, 22.41.

S4.4.2. 3-(N^2-4-Nitro benzylidenehydrazino carbonyl methyl)pyrido[2,3-*b*]pyrazin2(1*H*)one (6b):
Yield 58%. Mp 232-4 °C. IR υ_{max}, cm^{-1}: 3390, 3285 (2NH), 3016 (CH Ar), 2923, 2819 (CH Aliph), 1659 (2C=O), 1628, 1600, 1585, 1573 (NH, C=N, C=C), 1512, 1323(NO_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ= 2.38 (s, 2H, CH_2), 7.03 (d, *J*= 7.2 Hz, 1H, H-8 Ar), 7.40 (t, 1H, H-7 Ar), 7.79 (d, *J*= 7.4 Hz, 2H, H-2′,6′ Ar), 8.11 (s, 1H, N=CH), 8.26-8.40 (m, 3H, H-6,3′,5′ Ar), 10.89 (s, 1H, NH exchanged with D_2O), 11.39 (s, 1H, NH exchanged with D_2O). Anal. calcd. for $C_{16}H_{12}N_6O_4$ (352.30):

S4.4.3. 3-(N^2-4-Methoxy benzylidenehydrazino carbonyl methyl)pyrido[2,3-*b*]pyrazin2(1*H*)one (6c):
Yield 61%. Mp 222-4 °C. IR υ_{max}, cm^{-1}: 3433, 3213 (2NH), 3032 (CH Ar), 2943, 2843 (CH Aliph), 1697 (2C=O), 1633, 1620, 1612, 1554, 1504 (NH, C=N, C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ= 2.40 (s, 2H, CH_2), 3.37 (s, 3H, OCH_3), 7.12 (d, *J*= 6.8 Hz, 1H, H-8 Ar), 7.34 (t, 1H, H-7 Ar), 7.50 (m, 4H, H-2′,3′,5′,6′ Ar), 8.09 (s, 1H, N=CH), 8.22 (d, *J*= 5.9 Hz, 1H, H-6 Ar), 10.99 (s, 1H, NH exchanged with D_2O), 11.34 (s, 1H, NH exchanged with D_2O). Anal. calcd. for $C_{17}H_{15}N_5O_3$ (337.33): C, 60.53; H, 4.48; N, 20.76. Found: C, 60.33; H, 4.73; N, 20.98.

S4.5. General procedure for synthesis of 3-[*N*-(substituted)carbamoyl methyl]pyrido[2,3-*b*]pyrazin-2(1*H*)ones (series C, 7a-c):

Appropriate cyclic acid anhydrides (0.005 mol) were added to solution of compound **IV** (1.1 g, 0.005 mol) in glacial acetic acid (10 ml) and refluxed for 6 h. After cooling, the mixture was poured onto crushed ice (30 g). The formed precipitate was filtered, washed with water (5 ml) and dried. The crude product was crystalized from ethanol.

S4.5.1. 3-[*N*-(Succinimido)carbamoyl methyl]pyrido[2,3-*b*]pyrazin-2(1*H*)one (7a):
Yield 82%. Mp 210-12 °C. IR υ_{max}, cm^{-1}: 3426, 3410 (2NH), 3063 (CH Ar), 2939, 2866 (CH Aliph), 1681 (4C=O), 1635, 1629, 1554, 1516 (NH, C=N, C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ= 2.12 (s, 2H, CH_2), 2.78 (t, 4H, C**H**$_2$C**H**$_2$), 7.12 (d, *J*= 7.3 Hz, 1H, H-8 Ar), 7.41 (t, 1H, H-7 Ar), 8.12 (d, *J*= 7.3 Hz, 1H, H-6 Ar), 10.49 (s, 1H, NH exchanged with D_2O), 11.50 (s, 1H, NH exchanged with D_2O). Anal. calcd. for $C_{13}H_{11}N_5O_4$ (301.26): C, 51.83; H, 3.68; N, 23.25. Found: C, 52.21; H, 3.80; N, 23.13.

S4.5.2. 3-[*N*-(Maleic imido)carbamoyl methyl]pyrido[2,3-*b*]pyrazin-2(1*H*)one (7b):
Yield 85%. Mp 213-5 °C. IR υ_{max}, cm^{-1}: 3341, 3290 (2NH), 3036 (CH Ar), 2947, 2866 (CH Aliph), 1659 (4C=O), 1647, 1620, 1600, 1585 (NH, C=N, C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ= 2.22 (s, 2H, CH_2), 5.54 (d, *J*= 7.8 Hz, 2H, C**H**=C**H**), 7.21 (d, *J*= 7.2 Hz, 1H, H-8 Ar), 7.44 (t, 1H, H-7 Ar), 8.10 (d, *J*= 7.0 Hz, 1H, H-6 Ar), 10.22 (s, 1H, NH exchanged with D_2O), 11.33 (s, 1H, NH exchanged with D_2O). Anal. calcd. For $C_{13}H_9N_5O_4$ (299.24): C, 52; H, 3.03; N, 23.40. Found: C, 51.92; H, 3.33; N, 23.76.

S4.5.3. 3-[*N*-(Phthalimido)carbamoyl methyl]pyrido[2,3-*b*]pyrazin-2(1*H*)one (7c):
Yield 78%. Mp 211-13 °C. IR υ_{max}, cm^{-1}: 3429, 3356 (2NH), 3020 (CH Ar), 2939, 2858 (CH Aliph), 1670 (4C=O), 1620, 1600, 1590, 1500 (NH, C=N, C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ= 2.20 (s, 2H, CH_2), 7.11 (d, *J*= 7.0 Hz, 1H, H-8 Ar), 7.34 (t, 1H, H-7 Ar), 7.66-798 (m, 4H, H-3′,4′,5′,6′ Ar), 8.21 (d, *J*= 7.2 Hz, 1H, H-6 Ar), 10.41 (s, 1H, NH exchanged with D_2O), 11.55 (s, 1H, NH exchanged with D_2O). Anal. calcd. for $C_{17}H_{11}N_5O_4$ (349.30): C, 58.45; H, 3.17; N, 20.05. Found: C, 58.12; H, 3.19; N, 19.80.

S4.6. General procedure for synthesis of 3-[4′-((substituted)phenyl)-5′ (thio)oxo-4′*H*-1′,2′,4′-triazol-3′-yl)methyl]pyrido[2,3-*b*]pyrazin-2(1*H*)ones (series F, 10a-c):
Appropriate isocyanate or isothiocyanate (0.12 mol) and compound **IV** (0.22 g, 0.001 mol) were refluxed with ethanol (50 ml) for 24 h. The precipitate was cooled, filtered and dried. The crude product was crystalized from acetic acid.

S4.6.1. 3-[4′-(Phenyl)-5′ oxo-4′*H*-1′,2′,4′-triazol-3′-yl)methyl]pyrido[2,3-*b*]pyrazin-2(1*H*)one (10a):
Yield 77%. Mp 220-2 °C. IR υ_{max}, cm^{-1}: 3422, 3375 (2NH), 3082 (CH Ar), 2951, 2843 (CH Aliph), 1689 (2C=O), 1647, 1593, 1546, 1500 (NH, C=N, C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ= 2.34 (s, 2H, CH_2), 4.31 (s, 1H, NH exchanged with D_2O), 7.11 (d, *J*= 8.0 Hz, 1H, H-8 Ar), 7.43 (t, 1H, H-7 Ar), 7.60-8.10 (m, 5H, H-2′,3′,4′,5′,6′ Ar), 8.21 (d, *J*= 7.1 Hz, 1H, H-6 Ar), 11.39 (s, 1H, NH exchanged with D_2O). Anal. calcd. for $C_{16}H_{12}N_6O_2$ (320.31): C, 60.00; H, 3.78; N, 26.24. Found: C, 60.23; H, 4.01; N, 26.54.

S4.6.2. 3-[4′-(4-Chloro phenyl)-5′ oxo-4′*H*-1′,2′,4′-triazol-3′-yl)methyl]pyrido[2,3-*b*]pyrazin-2(1*H*)one (10b):
Yield 68%. Mp 228-30 °C. IR υ_{max}, cm^{-1}: 3444, 3275 (2NH), 3032 (CH Ar), 2954, 2845 (CH Aliph), 1689 (2C=O), 1647, 1589, 1546, 1500 (NH, C=N, C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ= 2.31 (s, 2H, CH_2), 4.22 (s, 1H, NH exchanged with D_2O), 7.11 (d, *J*= 7.4 Hz, 1H, H-8 Ar), 7.34 (t, 1H, H-7 Ar), 7.55-7.91 (m, 4H, H-2′,3′,5′,6′ Ar), 8.26 (d, *J*= 7.3 Hz, 1H, H-6 Ar), 11.45 (s, 1H, NH exchanged with D_2O). Anal. calcd. for $C_{16}H_{11}ClN_6O_2$ (354.75): C, 54.17; H, 3.13; N, 23.69. *Found: C, 54.32; H, 3.22; N, 24.00.

S4.6.3. 3-[4′-(Phenyl)-5′ thioxo-4′*H*-1′,2′,4′-triazol-3′-yl)methyl]pyrido[2,3-*b*]pyrazin-2(1*H*)one (10c):
Yield 71%. Mp 219-22 °C. IR υ_{max}, cm^{-1}: 3444, 3275 (2NH), 3032 (CH Ar), 2954, 2846 (CH Aliph), 1689 (C=O), 1647, 1593, 1546, 1500 (NH, C=N, C=C), 1270 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6): δ= 2.28 (s, 2H, CH_2), 4.00 (s, 1H, NH exchanged with D_2O), 6.60-7.11 (m, 5H, H-2′,3′,4′,5′,6′ Ar), 7.21 (d, *J*= 7.2 Hz, 1H, H-8 Ar), 7.44 (t, 1H, H-7 Ar), 8.22 (d, *J*= 6.0 Hz, 1H, H-6 Ar), 11.51 (s, 1H, NH exchanged with D_2O). Anal. calcd. for $C_{16}H_{12}N_6OS$ (336.37): C, 57.13; H, 3.60; N, 24.98. *Found: C, 57.22; H, 3.61; N, 24.67.

S4.7. General procedure for synthesis of 3-[(3′-acetyl-2′- (substituted)phenyl-2′-3′-dihydro-1′,3′,4′-oxadiazolo-5′-yl) methyl[2,3-*b*]pyrazin-2(1*H*)ones (series G, 11a-c):
A mixture of compounds **6a-c** (0.01 mol) and acetic anhydride (6 ml) were refluxed for 2 h. the excess acetic anhydride was distilled off under reduced pressure. The obtained residue was triturated with petroleum ether (25 ml). The precipitate was filtered and dried. The crude product was crystalized from benzene.

S4.7.1. 3-[(3′-Acetyl-2′- phenyl-2′-3′-dihydro-1′,3′,4′-oxadiazolo-5′-yl) methyl[2,3-*b*]pyrazin-2(1*H*)one (11a):
Yield 53%. Mp 234-6 °C. IR υ_{max}, cm^{-1}: 3444 (NH), 3035 (CH Ar), 2947, 2846 (CH Aliph), 1693 (2C=O), 1639, 1627, 1589, 1523 (NH, C=N, C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ= 2.13 (s, 2H, CH_2), 2.34 (s, 3H, $COCH_3$), 6.23 (s, 1H, CH oxadiazole), 7.68-7.09 (m, 5H, H-2′,3′,4′,5′,6′ Ar), 7.13 (d, *J*= 7.1 Hz, 1H, H-8 Ar), 7.33 (t, 1H, H-7 Ar), 8.21 (d, *J*= 6.2 Hz, 1H, H-6 Ar), 11.51 (s, 1H, NH exchanged with D_2O). Anal. calcd. for $C_{18}H_{15}N_5O_3$ (349.34): C, 61.89; H, 4.33; N, 20.05. Found: C, 62.03; H, 4.11; N, 19.76.

S4.7.2. 3-[(3′-Acetyl-2′-(4-nitro phenyl)-2′-3′-dihydro-1′,3′,4′-oxadiazolo-5′-yl) methyl[2,3-*b*]pyrazin-2(1*H*)one (11b):
Yield 55%. Mp 240-2 °C. IR υ_{max}, cm^{-1}: 3337 (NH), 3043 (CH Ar), 2951, 2854 (CH Aliph), 1651 (2C=O), 1635, 1600, 1554, 1532 (NH, C=N, C=C), 1500, 1335 (NO_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ= 2.02 (s, 2H, CH_2), 2.33 (s, 3H, $COCH_3$), 6.50 (s, 1H, CH oxadiazole), 7.12 (d, *J*= 6.8 Hz, 1H, H-8 Ar), 7.34 (t, 1H, H-7 Ar), 7.41-7.80 (m, 4H, H-2′,3′,5′,6′ Ar), 8.13 (d, *J*= 5.8 Hz, 1H, H-6 Ar), 11.48 (s, 1H, NH exchanged with D_2O). Anal. calcd. for $C_{18}H_{14}N_6O_5$ (394.34): C, 54.82; H, 3.58, N, 21.31. *Found: C, 54.45; H, 3.42; N, 21.09.

S4.7.3. 3-[(3′-Acetyl-2′-(4-methoxy phenyl)-2′-3′-dihydro-1′,3′,4′-oxadiazolo-5′-yl) methyl[2,3-*b*]pyrazin-2(1*H*)one (11c):
Yield 47%. Mp 235-7 °C. IR υ_{max}, cm^{-1}: 3441 (NH), 3067 (CH Ar), 2931, 2843 (CH Aliph), 1693 (2C=O), 1627, 1600, 1551, 1508 (NH, C=N, C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ= 2.13 (s, 2H, CH_2), 2.34 (s, 3H, $COCH_3$), 3.65 (s, 3H, OCH_3), 6.71 (s, 1H, CH oxadiazole), 6.82-7.01 (m, 4H, H-2′,3′,5′,6′ Ar), 7.21 (d, *J*= 7.6 Hz, 1H, H-8 Ar), 7.42 (t, 1H, H-7 Ar), 8.23 (d, *J*= 6.3 Hz, 1H, H-6 Ar), 11.39 (s, 1H, NH exchanged with D_2O). MS: *m*/z% 379, M^+, (1.92%). Anal. calcd. for $C_{19}H_{17}N_5O_4$ (379.37): C, 60.15; H, 4.52; N, 18.46. *Found: C, 60.51; H, 4.69; N, 18.80.

S4.8. 3-Methylpyrido[2,3-b]pyrazin-2(1H)one (VI):
Ethyl pyruvate (**V**, 2.90 g, 2.77 ml, 0.025 mol) was added to a solution of 2,3-diaminopyridine (**I**, 2.75 g, 0.025 mol) in ethanol (30 ml) and refluxed for 10 h. Reaction mixture was cooled, filtered, washed with ethanol (5 ml) and dried. The crude product was crystalized from ethanol. Yield 59%, mp 278-280 °C, IR (KBr) [υ /cm^{-1}]: 3186 (NH), 3018 (CH Ar), 2943, 2839 (CH Aliph), 1685 (C=O), 1641, 1631, 1597, 1559, 1546, 1527 (C=N, NH, C=C). ^{1}HNMR *(300 MHz, DMSO-d_6): δ= 2.41 (s, 3H, CH_3), 7.48 (d, *J*= 8.4 Hz, 1H, H-8Ar), 7.67 (t, 1H, H-7Ar), 8.11 (d, *J*= 7.6 Hz, 1H, H-6Ar)), 12.44 (s, 1H, NH, exchanged with D_2O). Anal. Calcd. for $C_8H_7N_3O$ (161.16): C, 59.62; H, 4.38; N, 26.07. Found: C, 59.90; H, 4.14; N, 26.73.

S4.9. 3-Bromomethylpyrido[2,3-b]pyrazin-2(1H)one (VII):
Compound **VI** (3.22g, 0.02 mol) was dissolved in glacial acetic acid (10 ml) and anhydrous sodium acetate (1.64 g, 0.02 mol) was added. Bromine (3.04 g, 0.99 ml, 0.019 mol) was added dropwise and the mixture was heated on steam bath for 30 min. then cooled. The formed precipitate was filtered, washed with glacial acetic acid (5 ml) and dried. The crude product was crystalized from ethanol. Yield 43%, mp 237-239°C, IR (KBr) [υ /cm^{-1}]: 3414 (NH), 3055 (CH Ar), 2980, 2883 (CH Aliph), 1670 (C=O), 1647, 1616, 1558, 1544, 1508 (C=N, NH, C=C). ^{1}HNMR *(300 MHz, DMSO-d_6): δ= 3.10 (s, 2H, CH_2), 7.40 (d, *J*= 7.4 Hz, 1H, H-8Ar), 7.61 (t, 1H, H-7Ar), 8.35 (d, *J*= 6.1 Hz, 1H, H-6Ar), 11.50 (s, 1H, NH, exchanged with D_2O). MS (*m*/*z*) %; 240 (M^+) 0.53%, 242 (M^++2) 0.67%. Anal. Calcd. for $C_8H_6BrN_3O$ (240.06): C, 40.03; H, 2.52; N, 17.50. Found: C, 40.13; H, 2.47; N, 17.68.

S4.10. General procedure for synthesis of 3-substituted methylpyrido[2,3-b]pyrazin-2(1H)ones (series D, 8a-j):
Appropriate amine (0.02 mol) was added to a solution of compound **VII** (2.4 g, 0.01 mol) in ethanol (30 ml) containing sodium iodide (0.15 g, 0.001 mol) and refluxed for 10 h. The reaction mixture was cooled, formed precipitate filtered, washed with ethanol (5 ml) and dried. The crude product was crystalized from ethanol.

S4.10.1. 3-(N,N-Diethylamino)methylpyrido[2,3-b]pyrazin-2(1H)one (8a):
Yield 81%, mp 262-263°C, IR (KBr) [υ/cm^{-1}]: 3238 (NH), 3051 (CH Ar), 2924, 2852 (CH Aliph), 1672 (C=O), 1637, 1624, 1612, 1593, 1508 (C=N, NH, C=C). ^{1}HNMR *(300 MHz, DMSO-d_6): δ= 1.41 (t, 6H, 2x $CH_2C\mathbf{H}_3$), 2.29 (q, 4H, 2x $C\mathbf{H}_2CH_3$), 2.43 (s, 2H, CH_2), 7.11 (d, *J*= 7.4 Hz, 1H, H-8Ar), 7.30 (t, 1H, H-7Ar), 8.21 (d, *J*= 6.4 Hz, 1H, H-6Ar), 10.80 (s, 1H, NH, exchanged with D_2O). Anal. Calcd. for $C_{12}H_{16}N_4O$ (232.28): C, 62.05; H, 6.94; N, 24.12. Found: C, 62.18; H, 6.97; N, 24.13.

S4.10.2. 3-(Piperidin-1-yl))methylpyrido[2,3-b]pyrazin-2(1H)one (8b):
Yield 80%, mp 277-279 °C, IR (KBr) [υ/cm^{-1}]: 3387 (NH), 3066 (CH Ar), 2964, 2866 (CH Aliph), 1700 (C=O), 1649, 1612, 1591, 1508, 1500 (C=N, NH, C=C). ^{1}HNMR (400 MHz, DMSO-d_6): δ= 2.06-2.32 (m, 10H, piperidine H), 2.51 (s, 2H, CH_2), 7.31 (d, *J*=8.0 Hz, 1H, H-8Ar), 7.53 (t, 1H, H-7 Ar), 8.22 (d, *J*= 6.6 Hz, 1H, H-6 Ar), 11.13 (s, 1H, NH, exchanged with D_2O). Anal. Calcd. for $C_{13}H_{16}N_4O$ (244.29): C, 63.91; H, 6.60; N, 22.93. Found: C, 63.98; H, 6.64; N, 23.10.

S4.10.3. 3-(Morpholin-4-yl)methylpyrido[2,3-b]pyrazin-2(1H)one (8c):
Yield 74%, mp 234-235 °C, IR (KBr) [υ/cm^{-1}]: 3394 (NH), 3059 (CH Ar), 2920, 2840 (CH Aliph), 1674 (C=O), 1640, 1618, 1593, 1580, 1540 (C=N, NH, C=C). ^{1}HNMR *(300 MHz, DMSO-d_6): δ= 2.04 (t, 4H, morpholine H), 2.41 (s, 2H, CH_2), 3.30 (t, 4H, morpholine H), 7.33 (d, *J*= 8.0 Hz, 1H, H-8 Ar), 7.75 (t, 1H, H-7 Ar), 8.12 (d, *J*= 7.4 Hz, 1H, H-6 Ar), 10.89 (s, NH, exchanged with D_2O). Anal. Calcd. for $C_{12}H_{14}N_4O_2$ (246.27): C, 58.53; H, 5.73; N, 22.75. Found: C, 58.61; H, 5.71; N, 22.92.

S4.10.4. 3-Phenylaminomethylpyrido[2,3-b]pyrazin-2(1H)one (8d):
Yield 60%, mp 248-250 °C, IR (KBr) [υ/cm^{-1}]: 3404, 3385 (2NH), 3049 (CH Ar), 2926, 2852 (CH Aliph), 1672 (C=O), 1593, 1560, 1521, 1508 (C=N, NH, C=C). ^{1}HNMR (400 MHz, DMSO-d_6): δ= 3.10 (s, 2H, CH_2), 6.52-7.10 (m, 5H, H-2′, 3′, 4′, 5′, 6′ Ar), 7.21 (d, *J*= 9.6 Hz, 1H, H-8Ar), 7.50 (t, 1H, H-7Ar), 8.17 (d, *J*= 8.2 Hz, 1H, H-6Ar), 10.62 (s, 1H, NH, exchanged with D_2O), 11.51 (s, 1H, NH, exchanged with D_2O). Anal. Calcd. for $C_{14}H_{12}N_4O$ (252.27): C, 66.65; H, 4.79; N, 22.21. Found: C, 66.83; H, 4.74; N, 22.37.

S4.10.5. 3-(2-Methylphenyl)aminomethylpyrido[2,3-b]pyrazin-2(1H)one (8e):

Yield 64%, mp 296-298°C, IR (KBr) [υ/cm^{-1}]: 3414, 3385 (2NH), 3049 (CH Ar), 2924, 2854 (CH Aliph), 1672 (C=O), 1649, 1622, 1593, 1570, 1521 (C=N, NH, C=C). ^{1}HNMR (400 MHz, DMSO-*d*$_6$): δ= 2.13 (s, 3H, CH_3), 3.11 (s, 2H, CH_2), 6.30-7.00 (m, 4H, H-3′, 4′, 5′, 6′ Ar), 7.21 (d, *J*= 7.2 Hz, 1H, H-8Ar), 7.50 (t, 1H, H-7Ar), 8.16 (d, *J*= 8.4 Hz, 1H, H-6Ar), 10.80 (s, 1H, NH, exchanged with D_2O), 11.44 (s, 1H, NH, exchanged with D_2O). Anal. Calcd. for $C_{15}H_{14}N_4O$ (266.30): C, 67.65; H, 5.30; N, 21.04. Found: C, 67.82; H, 5.32; N, 21.08.

S4.10.6. 3-(3-Methylphenyl)aminomethylpyrido[2,3-b]pyrazin-2(1H)one (8f):
Yield 57%, mp 258-260 °C, IR (KBr) [υ/cm^{-1}]: 3414, 3388 (2NH), 3057 (CH Ar), 2922, 2852 (CH Aliph), 1674 (C=O), 1622, 1593, 1558, 1518 (C=N, NH, C=C). ^{1}HNMR (400 MHz, DMSO-*d*$_6$): δ= 2.15 (s, 3H, CH_3), 3.32 (s, 2H, CH_2), 6.12-6.99 (m, 4H, H-2′, 4′, 5′, 6′ Ar), 7.20 (d, *J*= 6.3 Hz, 1H, H-8 Ar), 7.50 (t, 1H, H-7 Ar), 8.19 (d, *J*= 8.3 Hz, 1H, H-6 Ar), 10.81 (s, 1H, NH, exchanged with D_2O), 11.50 (s, 1H, H NH, exchanged with D_2O). Anal. Calcd. for $C_{15}H_{14}N_4O$ (266.30): C, 67.65; H, 5.30; N, 21.04. Found: C, 67.84; H, 5.34;N, 21.06.

S4.10.7. 3-(4-Methylphenyl)aminomethylpyrido[2,3-b]pyrazin-2(1H)one (8g):
Yield 73%, mp 284-286 °C, IR (KBr) [υ/cm^{-1}]: 3369, 3350 (2NH), 3051 (CH Ar), 2926, 2856 (CH Aliph), 1672 (C=O), 1612, 1591, 1544, 1514 (C=N, NH, C=C). ^{1}HNMR *(300 MHz, DMSO-*d*$_6$): δ= 2.10 (s, 3H, CH_3), 2.41 (s, 2H, CH_2), 6.30-6.83 (m, 4H, H-2′, 3′, 5′, 6′ Ar), 7.22 (d, *J*= 6.7 Hz, 1H, H-8 Ar), 7.54 (t, 1H, H-7 Ar), 8.17 (d, *J*= 7.0 Hz, 1H, H-6 Ar), 10.09 (s, 1H, NH, exchanged with D_2O), 11.51 (s, 1H, NH, exchanged with D_2O). Anal. Calcd. for $C_{15}H_{14}N_4O$ (266.30): C, 67.65; H, 5.30; N, 21.04. Found: C, 67.80; H, 5.30;N, 21.18.

S4.10.8. 3-(4-Chlorophenyl)aminomethylpyrido[2,3-b]pyrazin-2(1H)one (8h):
Yield 66%, mp>300 °C, IR (KBr) [υ/cm^{-1}]: 3421, 3385 (2NH), 3093 (CH Ar), 2924, 2852 (CH Aliph), 1674 (C=O), 1647, 1624, 1593, 1570 (C=N, NH, C=C). ^{1}HNMR (400 MHz, DMSO-*d*$_6$): δ= 3.31 (s, 2H, CH_2), 6.42-7.12 (m, 4H, H-2′, 3′, 5′, 6′ Ar), 7.21 (d, *J*= 6.3 Hz, 1H, H-8 Ar), 7.59 (t, 1H, H-7 Ar), 8.11 (d, *J*= 6.2 Hz, 1H, H-6 Ar), 11.00 (s, 1H, NH, exchanged with D_2O), 11.59 (s, 1H, NH, exchanged with D_2O). Anal. Calcd. for $C_{14}H_{11}ClN_4O$ (286.72): C, 58.65; H, 3.87; N, 19.54. Found: C, 58.78; H, 3.92; N, 19.73.

S4.10.9. 3-(4-Bromophenyl)aminomethylpyrido[2,3-b]pyrazin-2(1H)one (8i):
Yield 79%, mp>300 °C, IR (KBr) [υ/cm^{-1}]: 3400, 3388 (2NH), 3101 (CH Ar), 2924, 2897 (CH Aliph), 1664 (C=O), 1649, 1618, 1593 (C=N, NH, C=C). ^{1}HNMR (400 MHz, DMSO-*d*$_6$): δ= 3.11 (s, 2H, CH_2), 6.36-7.20 (m, 4H, H-2′, 3′, 5′, 6′ Ar), 7.41 (d, *J*= 8.1 Hz, 1H, H-8 Ar), 7.69 (t, 1H, H-7 Ar), 8.22 (d, *J*= 6.0 Hz, 1H, H-6 Ar), 11.02 (s, 1H, NH, exchanged with D_2O), 11.61 (s, 1H, NH, exchanged with D_2O). Anal. Calcd. for $C_{14}H_{11}BrN_4O$ (331.17): C, 50.77; H, 3.35; N, 16.92. Found: C, 50.91; H, 3.37; N, 16.98.

S4.10.10. 3-(N,N-Diphenylamino)methylpyrido[2,3-b]pyrazin-2(1H)one (8j):
Yield 46%, mp 292-293°C, IR (KBr) [υ/cm^{-1}]: 3410 (NH), 3057 (CH Ar), 2924, 2852 (CH Aliph), 1662 (C=O), 1649, 1610, 1591, 1570, 1508 (C=N, NH, C=C). ^{1}HNMR (400 MHz, DMSO-*d*$_6$): δ= 3.32 (s, 2H, CH_2), 6.50-7.11 (m, 10H, Ar H), 7.21 (d, *J*= 7.2 Hz, 1H, H-8 Ar), 7.55 (t, 1H, H-7 Ar), 8.15 (d, *J*= 7.0 Hz, 1H, H-6 Ar), 11.44 (s, 1H, NH, exchanged with D_2O). Anal. Calcd. for $C_{20}H_{16}N_4O$ (328.37): C, 73.15; H, 4.91; N, 17.06. Found: C, 73.28; H, 4.97; N, 17.22.

S4.11. 3-Hydrazinomethylpyrido[2,3-b]pyrazin-2(1H)one (VIII):
Hydrazine hydrate (7.00 g, 6.86 ml, 0.14 mol) was added to a solution of compound **VII** (2.40 g, 0.01 mol) in ethanol (20 ml) and refluxed for 9 h. The mixture was cooled in ice bath for 10 min. and formed precipitate was filtered. The precipitate was washed with ethanol (5 ml) and dried. The crude product was crystallized from ethanol. Yield 73%, mp 243-245 °C, IR (KBr) [υ/cm^{-1}]: 3369, 3329 (NH_2, 2NH), 3066 (CH Ar), 2924, 2852 (CH Aliph), 1670 (C=O), 1612, 1591, 1570, 1508 (C=N, NH, C=C). ^{1}HNMR (400 MHz, DMSO-

d$_6$): δ= 2.51 (s, 2H, CH_2), 7.21 (d, *J*= 6.2 Hz, 1H, H-8 Ar), 7.50 (t, 1H, H-7 Ar), 8.22 (d, *J*= 8.2 Hz, 1H, H-6Ar), 10.72 (s, 1H, NH, exchanged with D_2O), 11.22 (s, 2H, NH_2, exchanged with D_2O), 11.50 (s, 1H, NH, exchanged with D_2O). Anal. Calcd. for $C_8H_9N_5O$ (191.19): C, 50.26; H, 4.74; N, 36.63. Found: C, 50.41; H, 4.73; N, 37.01.

S4.12. General procedure for synthesis of 3-(2-(un)substituted benzylidene)hydrazinomethylpyrido[2,3-b]pyrazin-2(1H)-ones (series E, 9a-e):

Appropriate aromatic aldehyde or ketone (0.01 mol) was added to a solution of compound **VIII** (1.91 g, 0.01 mol) in glacial acetic acid (10 ml) and refluxed for 8 h. The mixture was allowed to cool then poured onto crushed ice (30 g). The precipitate was filtered, washed with water (5 ml) and dried. The crude product was crystallized from ethanol.

S4.12.1. 3-(2-Benzylidene)hydrazinomethylpyrido[2,3-b]pyrazin-2(1H)-one (9a):

Yield 62%, mp 234-236°C, IR (KBr) [υ/cm^{-1}]: 3398, 3364 (2NH), 3061 (CH Ar), 2901, 2829 (CH Aliph), 1676 (C=O), 1624, 1600, 1583, 1544, 1508 (C=N, NH, C=C). ^{1}HNMR (400 MHz, DMSO-*d*$_6$): δ= 2.32 (s, 2H, CH_2), 7.21 (d, *J*= 7.2 Hz, 1H, H-8 Ar), 7.31-7.42 (m, 3H, H-3′,4′,5′ Ar), 7.45 (t, 1H, H-7 Ar), 7.62 (d, *J*= 6.7 Hz, 2H, H-2′,6′ Ar), 8.11 (s, 1H, N=CH), 8.22 (d, *J*= 6.2 Hz, 1H, H-6 Ar), 11.32 (s, 1H, NH, exchanged with D_2O), 11.54 (s, 1H, NH, exchanged with D_2O). Anal. Calcd. for $C_{15}H_{13}N_5O$ (279.30): C, 64.51; H, 4.69; N, 25.07. Found: C, 64.67; H, 4.73; N, 25.19.

S4.12.2. 3-[2-(4-Chlorobenzylidene)]hydrazinomethylpyrido[2,3-b]pyrazin-2(1H)-one (9b):

Yield 69%, mp 237-239°C, IR (KBr) [υ/cm^{-1}]: 3421, 3387 (2NH), 3012 (CH Ar), 2920, 2837 (CH Aliph), 1683 (C=O), 1616, 1593, 1558, 1541, 1521 (C=N, NH, C=C). ^{1}HNMR (400 MHz, DMSO-*d*$_6$): δ= 2.42 (s, 2H, CH_2), 7.22 (d, *J*= 7.3 Hz, 1H, H-8 Ar), 7.34 (d, *J*= 6.1 Hz, 2H, H-3′,5′ Ar), 7.46 (t, 1H, H-7 Ar), 7.59 (d, *J*= 7.0 Hz, 2H, H-2′,6′ Ar), 8.11 (s, 1H, N=CH), 8.22 (d, *J*= 6.3 Hz, 1H, H-6 Ar), 11.30 (s, 1H, NH, exchanged with D_2O), 11.51 (s, 1H, NH, exchanged with D_2O). MS (*m*/*z*)%; 313 (M^+) 2.63%, 315 (M^++2) 2.77%. Anal. Calcd. for $C_{15}H_{12}ClN_5O$ (313.74): C, 57.42; H, 3.86; N, 22.32. Found: C, 57.52; H, 3.91;N, 22.48.

S4.12.3. 3-[2-(4-Nitrobenzylidene)]hydrazinomethylpyrido[2,3-b]pyrazin-2(1H)-one (9c):

Yield 70%, mp 238-240 °C, IR (KBr) [υ/cm^{-1}]: 3444, 3365 (2NH), 3021 (CH Ar), 2926, 2850 (CH Aliph), 1677 (C=O), 1653, 1614, 1597, 1558 (C=N, NH, C=C), 1519, 1344 (NO_2). ^{1}HNMR (400 MHz, DMSO-*d*$_6$): δ= 2.33 (s, 2H, CH_2), 7.21 (d, *J*= 6.4 Hz, 1H, H-8 Ar), 7.66 (t, 1H, H-7 Ar), 7.91-8.10 (m, 4H, H-2′, 3′, 5′, 6′ Ar), 8.19 (s, 1H, N=CH), 8.23 (d, *J*= 7.2 Hz, 1H, H-6 Ar), 11.32 (s, 1H, NH, exchanged with D_2O), 11.50 (s, 1H, NH, exchanged with D_2O). Anal. Calcd. for $C_{15}H_{12}N_6O_3$ (324.29): C, 55.55; H, 3.73; N, 25.91. Found: C, 55.74; H, 3.74; N, 26.07.

S4.12.4. 3-[2-(4-Methoxybenzylidene)]hydrazinomethylpyrido[2,3-b]pyrazin-2(1H)-one (9d):

Yield 63%, mp 256-258 °C, IR (KBr) [υ/cm^{-1}]: 3417, 3392 (2NH), 3039 (CH Ar), 2933, 2837 (CH Aliph), 1670 (C=O), 1598, 1575, 1558, 1541, 1508 (C=N, NH, C=C). ^{1}HNMR *(300 MHz, DMSO-*d*$_6$): δ= 2.12 (s, 2H, CH_2), 3.52 (s, 3H, OCH_3), 6.76 (d, *J*= 8.2 Hz, 2H, H-3′,5′ Ar), 7.21 (d, *J*= 7.1 Hz, 1H, H-8 Ar), 7.43 (t, 1H, H-7 Ar), 7.66 (d, *J*= 7.4 Hz, 2H, H-2′,6′ Ar), 8.10 (s, 1H, N=CH), 8.22 (d, *J*= 7.6 Hz, 1H, H-6 Ar), 11.30 (s, 1H, NH, exchanged with D_2O), 11.45 (s, 1H, NH, exchanged with D_2O). Anal. Calcd. for $C_{16}H_{15}N_5O_2$ (309.32): C, 62.13; H, 4.89; N, 22.64. Found: C, 62.26; H, 4.87; N, 22.72.

S4.12.5. 3-(2-Methyl-2-phenyl)hydrazinomethylpyrido[2,3-b]pyrazin-2(1H)-one (9e):

Yield 69%, mp 230-233°C, IR (KBr) [υ/cm^{-1}]: 3446, 3363 (2NH), 3032 (CH Ar), 2929, 2856 (CH Aliph), 1670 (C=O), 1645, 1616, 1570, 1558, 1521, (C=N, NH, C=C). ^{1}HNMR (400 MHz, DMSO-*d*$_6$): δ= 1.42 (s, 3H, CH_3),

2.50 (s, 2H, CH_2), 7.22 (d, *J*= 6.2 Hz, 1H, H-8 Ar), 7.30-7.43 (m, 3H, H-3′,4′,5′ Ar), 7.49 (t, 1H, H-7 Ar), 7.63 (d, *J*= 8.1 Hz, 2H, H-2′,6′ Ar), 8.22 (d, *J*= 7.2 Hz, 1H, H-6 Ar), 11.31 (s, 1H, NH, exchanged with D_2O), 11.53 (s, 1H, NH, exchanged with D_2O). Anal. Calcd. for $C_{16}H_{15}N_5O$ (293.32): C, 65.52; H, 5.15; N, 23.88. Found: C, 65.59; H, 5.12; N, 23.98.

S4.13. General procedure for synthesis of 4-(un)substituted phenyl-2,5,10,11-tetrahydropyrido[2,3-g]pyrazino[4,3-e]1,2,4-triazin-11-ones (series H, 12a-d):

Bromine (1.60 g, 0.53 ml, 0.01 mol) in glacial acetic acid (0.6 ml) was added dropwise to solution of compound **9a-d** (0.01 mol) in glacial acetic acid (9 ml) containing sodium acetate (2.46 g, 0.03 mol) at room temperature. The mixture was stirred for 3 h at room temperature then poured onto cold water (50 ml). The formed precipitate was filtered, washed with water (10 ml) and dried. The crude product was crystallized from acetic acid.

S4.13.1. 4-Phenyl-2,5,10,11-tetrahydropyrido[2,3-g]pyrazino[4,3-e]1,2,4-triazin-11-one (12a):

Yield 80%, mp 219-221°C, IR (KBr) [υ/cm^{-1}]: 3365, 3355 (2NH), 3035 (CH Ar), 1760 (C=O), 1608, 1558, 1541, 1521, 1508 (C=N, NH, C=C). ^{1}HNMR (400 MHz, DMSO-d_6): δ= 6.01 (s, 1H, H-1 Ar), 6.99 (t, 1H, H-8 Ar), 7.01 (d, *J*= 6.4 Hz, 1H, H-9 Ar), 7.29-7.41 (m, 3H, H-3′,4′,5′ Ar), 7.63 (d, *J*= 7.2 Hz, 2H, H-2′,6′ Ar), 7.91 (d, *J*= 6.0 Hz, 1H, H-7 Ar), 11.29 (s, 1H, NH, , exchanged with D_2O), 11.52 (s, 1H, exchanged with D_2O). Anal. Calcd. for $C_{15}H_{11}N_5O$ (277.28): C, 64.97; H, 4.00; N, 25.26. Found: C, 65.13; H, 4.06; N, 25.49.

S4.13.2. 4-(4-Chlorophenyl)-2,5,10,11-tetrahydropyrido[2,3-g]pyrazino[4,3-e]1,2,4-triazin-11-one (12b):

Yield 75%, mp 222-225 °C, IR (KBr) [υ/cm^{-1}]: 3390, 3385 (2NH), 3032 (CH Ar), 1680 (C=O), 1635, 1608, 1593, 1570, 1558 (C=N, NH, C=C). ^{1}HNMR (400 MHz, DMSO-d_6): δ= 5.99 (s, 1H, H-1 Ar), 6.86 (t, 1H, H-8 Ar), 7.02 (d, *J*= 8.1 Hz, 1H, H-9 Ar), 7.33 (d, *J*= 7.0 Hz, 2H, H-3′,5′ Ar), 7.62 (d, *J*= 6.5 Hz, 2H, H-2′,6′ Ar), 8.22 (d, *J*= 6.3 Hz, 1H, H-7 Ar), 11.31 (s, 1H, NH, exchanged with D_2O), 11.53 (s, 1H, NH, exchanged with D_2O). Anal. Calcd. for $C_{15}H_{10}ClN_5O$ (311.73): C, 57.79; H, 3.23; N, 22.47. Found: C, 57.96; H, 3.19;N, 22.63.

S4.13.3. 4-(4-Nitrophenyl)-2,5,10,11-tetrahydropyrido[2,3-g]pyrazino[4,3-e]1,2,4-triazin-11-one (12c):

Yield 86%, mp 222-224°C, IR (KBr) [υ/cm^{-1}]: 3410, 3393 (2NH), 3041 (CH Ar), 1674 (C=O), 1654, 1635, 1600, 1568, 1558 (C=N, NH, C=C), 1521, 1346 (NO_2). ^{1}HNMR (400 MHz, DMSO-d_6): δ= 6.21 (s, 1H, H-1 Ar), 6.62 (t, 1H, H-8 Ar), 6.84 (d, *J*= 7.2 Hz, 1H, H-9 Ar), 7.56 (d, *J*= 8.1 Hz, 1H, H-7 Ar), 7.91-8.10 (m, 4H, H-2′, 3′, 5′, 6′ Ar), 11.33 (s, 1H, NH, exchanged with D_2O), 11.50 (s, 1H, NH, exchanged with D_2O). Anal. Calcd. for $C_{15}H_{10}N_6O_3$ (322.28): C, 55.90; H, 3.13;N, 26.08. Found: C, 56.03; H, 3.17; N, 26.32.

S4.13.4. 4-(4-Methoxyphenyl)-2,5,10,11-tetrahydropyrido[2,3-g]pyrazino[4,3-e]1,2,4-triazin-11-one (12d):

Yield 66%, mp 229-231°C, IR (KBr) [υ/cm^{-1}]: 3415, 3388 (2NH), 3020 (CH Ar), 2933, 2841 (CH Aliph), 1674 (C=O), 1633, 1597, 1575, 1558, 1508 (C=N, NH, C=C). ^{1}HNMR *(300 MHz, DMSO-d_6): δ= 3.72 (s, 3H, OCH_3), 5.82 (s, 1H, H-1 Ar), 6.54 (t, 1H, H-8 Ar), 6.96 (d, *J*= 6.0 Hz, 1H, H-9 Ar), 7.03-7.40 (m, 4H, H-2′, 3′, 5′, 6′ Ar), 7.67 (d, *J*= 7.3 Hz, 1H, H-7 Ar), 11.33 (s, 1H, NH, exchanged with D_2O), 11.51 (s, 1H, NH, exchanged with D_2O). Anal. Calcd. for $C_{16}H_{13}N_5O_2$ (307.31): C, 62.53; H, 4.26; N, 22.79. Found: C, 62.64; H, 4.32; N, 22.94.

S5. Molecular docking

S5.1. Preparing the PDE5 protein structure for molecular docking

The PDB file (PDB ID: 3HC8) [2] was visualized by MOE software. The hydrogen atoms were placed and the overall lowest potential configuration energy was determined and identified. Both ligand and the active site pocket were isolated and visualized through molecular surface tool.

S5.2. Validation of docking protocol of reference ligand with the active site

The reference ligand (**t48**) was considered in the validation of docking process with the active site of phosphodiesterase enzyme. The Rigid Protein Docking protocol was used in the validation step. Triangle Matcher method (bond rotation method) was used in order to generate the reference ligand conformations. The produced conformations were ranked with the London dG scoring function. The minimization of conformations energies was accomplished with Forcefield functional form. The identified conformations were rescored with GBVI/WSA dG scoring function (S, kcal/mol).

S5.3. Docking of test set

The test set (series **A–H**) databases were selected as ligand atoms in the molecular docking procedure using the same setting for the reference ligand (**t48**, PDB ID: PD4).

S5.4. Analyzing the docking results

The best conformers for test set (lowest binding score and RMSD values) were selected to be visualized in the PDE5 active site pocket. The 2D and 3D interactions of each conformer were pictured in order to identify the binding modes with the possible interactions.

S6. Molecular dynamic (MD) simulation

Protein-ligand complex MD simulation was conducted to validate the molecular docking results. The binding complex of the most potent derivatives among the tested compounds (**11b**) was selected for molecular dynamic analysis. The crystal structure of the human PDE5 in complex with **t48** (PDB ID: 3HC8 [2]) was used as a control. The entire co-crystallized protein-ligand complex was used as a positive control of a true active binding mode, whereas the unbound protein without co-crystallized ligand – negative control. A total of six MD simulations, 50 ns each, were conducted. GROningen MAchine for Chemical Simulations (GROMACS 2016.4) was used to carry out the MD simulations [12]. The MD systems for each simulation were set up using the CHARMM22 forcefield [13-15] and the SwissParam web service was used to generate the ligand topology and parameters compatible with CHARMM and GROMACS [16]. The full system consisted of a dodecahedral box, solvated with TIP3P water molecules at a 10 Å edge distance and neutralized with Na^+ and Cl^- ions.

To remove any steric clashes, energy minimization was carried out using the steepest descent minimization algorithm with a maximum of 50,000 steps and a target F_{max} of no greater than 100 kJ mol^{-1} nm^{-1}. These energy minimized systems were equilibrated in two steps: thermalization (in the NVT ensemble) at 300 K and pressurization (in the NPT ensemble) at 1 bar each for 50 ns. Throughout both equilibrations, only the solvent molecules were permitted to freely move to ensure proper equilibration in the system while all other atoms were restrained. The particle mesh Eshwald method with a 12 Å cut-off and 16 Å Fourier spacing method were used to obtain the long-range electrostatics. The six equilibrated systems (one unbound protein and five protein-ligand complexes) were subjected to 50 ns unrestrained production runs. The output trajectories were re-centered and analyzed using built-in GROMACS commands and the VMD software (University of Illinois at Urbana-Champaign, Urbana, IL, USA) [17].

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