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COMMUNICATION

Synthesis, *in vitro* anticancer activity and SAR studies of arylated imidazo[1,2-*a*]pyrazine-coumarin hybrids

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A new series of imidazo[1,2-*a*]pyrazine-coumarin hybrid has been synthesized by combination of two biological active moieties imidazo[1,2-*a*]pyrazine and coumarin followed by Suzuki-Miyaura cross coupling reaction for monoarylation at C6 position and symmetrical/unsymmetrical diarylation at C3 and C6 positions. These compounds were further screened for their *in vitro* antitumor activities.

Heterocyclic moieties play an important role in pharmaceutical, veterinary and agrochemicals.[1] Among various heterocycles, imidazo[1,2-*a*]pyrazine, a well known privileged fused heterocyclic motif, has attracted its importance in search for anticancer agents by implication of various modifications at different positions of the core.[2] The imidazo[1,2-*a*]pyrazine exhibited prominent anticancer activity with the inhibition of Aurora kinase,[3] phosphoinositide-3-kinase,[4] breast tumor kinase/protein tyrosine kinase 6,[5] check point kinase,[6] spleen tyrosine kinase,[7] topoisomerase-II[8] and cyclin dependent kinase[9] (Figure 1). Coumarin is also a biologically active heterocyclic moiety possessing anticancer,[10] anti HIV,[11] antituberculosis,[12] antihypercholestrolemic activities[13] etc. It is a decade of molecular hybridization to obtain a more active pharmacophore in a single biological molecule by joining two heterocyclic pharmacophores.[14] Concept of hybridization leads to a revolution in field of drug design and development to obtain a hybrid drug with improved characteristics and minimize side effects as compared to parent molecules. Therefore, hybrids of coumarin with various heterocyclic moieties viz., coumarin-benzimidazole,[15] coumarin-benzothiazole[16] and coumarin-chalcone[17] (Figure 2) have been reported in the literature, having great biological significance as anticancer agents.

To the best of my knowledge, no reports for anticancer activity of imidazo[1,2-*a*]pyrazine with biologically active coumarin moiety have been known in literature till now. In view of the previous rationale and in continuation of an ongoing

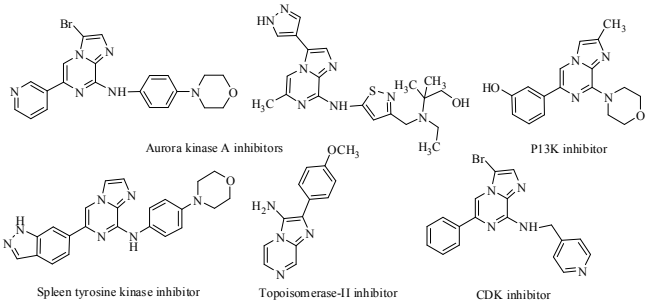

Figure 1. Imidazo[1,2-*a*]pyrazines as anticancer agents

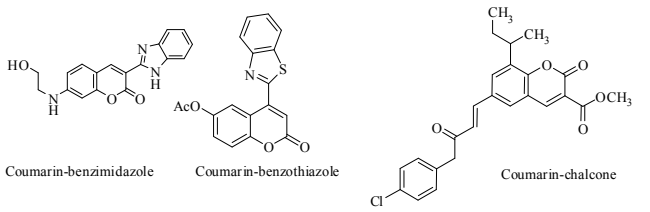

Figure 2. Coumarin based hybrids as anticancer agents

program to find new structural leads with potential chemotherapeutic activities using molecular hybridization, in the present study, a new series of hybrids using imidazo[1,2-*a*]pyrazine and coumarin have been synthesized. These compounds have further been approached for Suzuki-Miyaura cross coupling reaction for monoarylation at C6 position, and symmetrical/unsymmetrical diarylation at C3 and C6 positions. These compounds were further screened for their *in vitro* anticancer activity on a panel of 60 human cancer cell lines viz., leukaemia, non small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast. Molecular docking study has also been performed to support the effective binding of compound at the active site of the enzyme.

2-Aminopyrazine **1** was treated with N-bromosuccinimide (NBS) in DMSO and water (9:1) at room temperature for 6 h to give 2-amino-3,5-dibromopyrazine **2** in 90% yield[18] followed by cyclization with 50% aq. chloroacetaldehyde in isopropyl alcohol at 110 ^{o}C for 12 h to obtain 6,8-dibromoimidazo[1,2-*a*]pyrazine **3**[5] in 80% yield. Compound **3** was again brominated with NBS in acetonitrile (ACN) at room temperature for 2 h to afford 3,6,8-tribromoimidazo[1,2-*a*]pyrazine **4**[19] in 90% yield. 3,6,8-Tribromoimidazo[1,2-*a*]pyrazine **4** was then stirred with 7-

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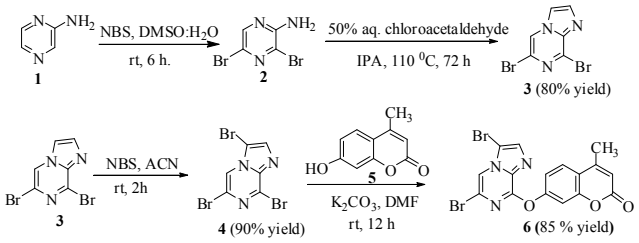
† Electronic Supplementary Information (ESI) available: experimental section, ^{1}H, ^{13}C NMR and Mass spectra of all new compounds **6**, **7a-8a**, **7b-19b**, **20-30,** antitumor methodology and activities of all selected compounds **6**, **7a**, **9b, 10b**, **11b**, **16b**, **28** and **30** DOI: 10.1039/b000000x/



Scheme 1 Synthesis of imidazo[1,2-*a*]pyrazine-coumarin hybrid

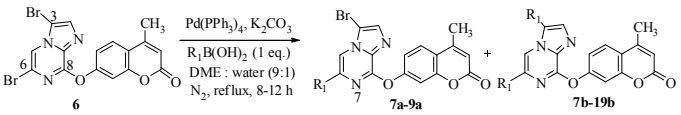
hydroxy-4-methylcoumarin **5** in the presence of K_2CO_3 and DMF at room temperature for 12 h to afford 7'-(3,6-dibromoimidazo[1,2-*a*]pyrazin-8-yloxy)-4'-methyl-2*H*-chromen-2'-one **6** in 85% yield (Scheme 1).

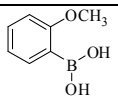
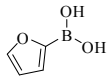
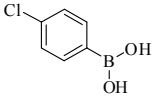
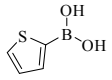
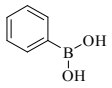
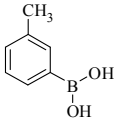
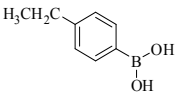
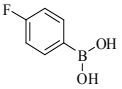
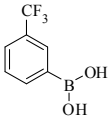
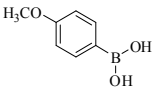
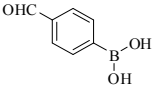
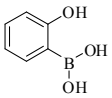
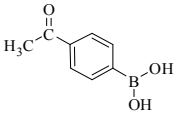
Palladium catalyzed Suzuki-Miyaura cross coupling of compound **6** with 2-methoxyphenyl boronic acid (1 eq.) in the presence of K_2CO_3 (1 eq.) and 5 mol% of $Pd(PPh_3)_4$ in DME:H_2O (9:1) under inert atmosphere afforded C6-monoarylated **7a** and C3, C6 diarylated **7b** products in 61% and 33% yields respectively (Table 1, entry 1). Similarly, compound **6** was also treated with 2-furan boronic acid under same reaction conditions to give compounds **8a** and **8b** in 48% and 25% yields respectively (Table 1, entry 2). Reaction of compound **6** with 4-chlorophenyl boronic acid afforded C6 monoarylated product **9a** and C3, C6 diarylated product **9b** in 40% and 42% GCMS yields respectively. These two compounds could not be separated through column chromatography and used as such without purification (Table 1, entry 3). However, reactions of **6** with other aryl boronic acids under the same reaction conditions gave only C3, C6 diarylated products **10b-19b** with 54-74% yields (Table 1, entries 4-13). Monoarylated products with these boronic acids could not be separated as these were formed only in traces < 5% (determined by GC-MS). Reaction of compound **6** with naphthalene-1-boronic acid could not give a successful attempt. This might be due to steric crowding of the bulky naphthyl ring.

Subsequently use of monoarylated product has been implemented for unsymmetrical diarylation via cross coupling reaction at C3 position. C6 Monoarylated imidazo[1,2-*a*]pyrazine-coumarin hybrid **7a** was further refluxed with variety of aryl boronic acids (1.0 eq.) in the presence of 5 mol% of $Pd(PPh_3)_4$, K_2CO_3 (1.1 eq.) in DME:H_2O (9:1) for 6-8 h to give C3, C6 unsymmetrical diarylated imidazo[1,2-*a*]pyrazine-coumarin hybrids **20**-**29** in 52-75% yields (Table 2). However, Suzuki reaction of mixture of monoarylated and symmetrical diarylated compounds (**9a** and **9b)** with 2-thiophene boronic acid under the same reaction conditions and after column chromatography afforded C3, C6 symmetrical and unsymmetrical disubstituted imidazo[1,2-*a*]pyrazine-coumarin hybrids **9b** and **30** in 42% and 61% isolated yields respectively (Scheme 2). All the synthesized compounds have been well characterized by ^1H and ^{13}C NMR as well as mass spectrometry (Supporting Information).

The assigned regiochemistry of monoarylation at C6 position and unsymmetrical diarylation at C3 and C6 positions of imidazo[1,2-*a*]pyrazine-coumarin hybrid was confirmed by considering 2D NOE difference experiments (SI, Figure S7 and S77). Singlet of C5H of imidazo[1,2- *a*]pyrazine **7a** showed

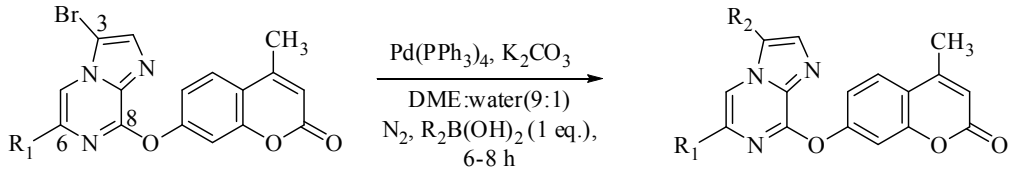
Table 1 Reactions of 3,6-dibromoimidazo[1,2-*a*]pyrazine-coumarin hybrid **6** with aryl boronic acids



Entry	Time	$R_1B(OH)_2$	Products (%)[#]	
1	8		**7a** (61)	**7b** (33)
2	11		**8a** (48)	**8b** (25)
3	10		**9a** (40)*	**9b** (42)
4	10		---	**10b** (66)
5	9		---	**11b** (74)
6	12		---	**12b** (70)
7	12		---	**13b** (69)
8	9		---	**14b** (72)
9	12		---	**15b** (60)
10	8		---	**16b** (73)
11	10		---	**17b** (70)
12	11		---	**18b** (68)
13	12		---	**19b** (54)

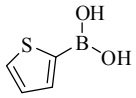
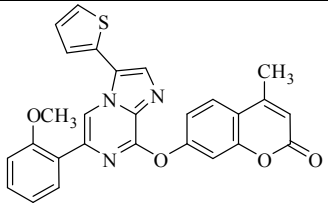
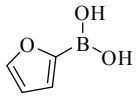
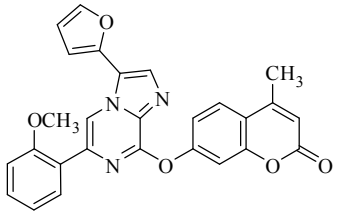
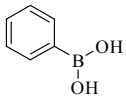
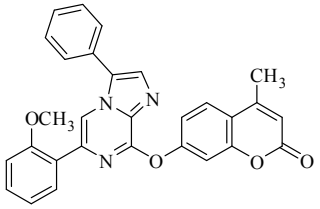
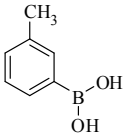
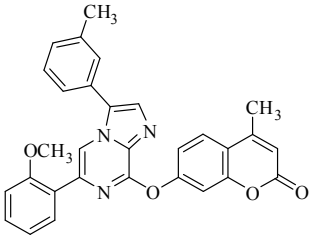
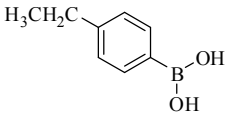
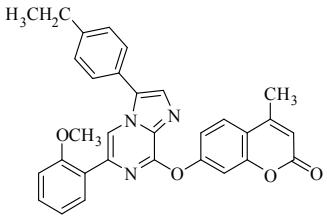
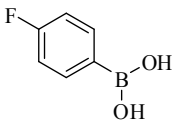
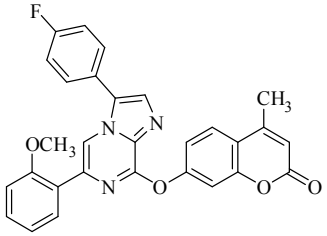
[#] isolated yields, --,*GC-MS yields (< 5%)

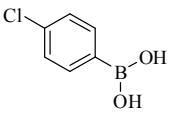
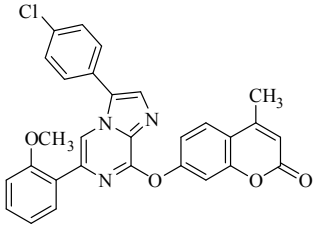
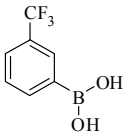
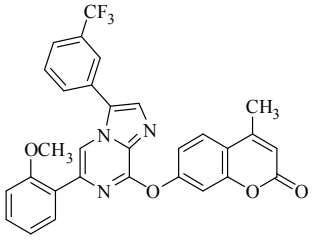
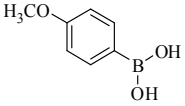
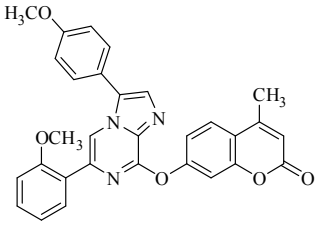
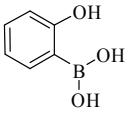
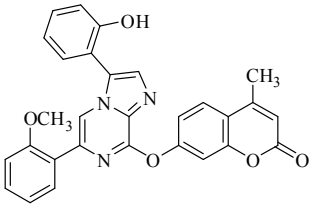
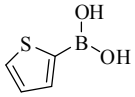
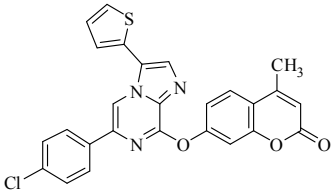
Table 2 Reactions of monoarylated-3-bromoimidazo[1,2-*a*]pyrazine-coumarin hybrids **7a** and **9a** with different aryl boronic acids



7a, R_1 = 2-methoxy phenyl
9a, R_1 = 4-chloro phenyl

20-30

Entry	Starting Material	$R_2B(OH)_2$	Product	Time (h)	Yield (%)[#]	mp (°C)
1	**7a**			6	**20** (64)	221-223
2	**7a**			7	**21** (52)	204-205
3	**7a**			6	**22** (69)	194-195
4	**7a**			8	**23** (72)	202-203
5	**7a**			8	**24** (55)	179-180
6	**7a**			7	**25** (75)	235-237

7	**7a**			7	**26** (65)	243-245
8	**7a**			8	**27** (55)	204-206
9	**7a**			7	**28** (64)	239-241
10	**7a**			8	**29** (53)	>300
11	**9a**			8	**30** (61)	187-189

[#]isolated yields

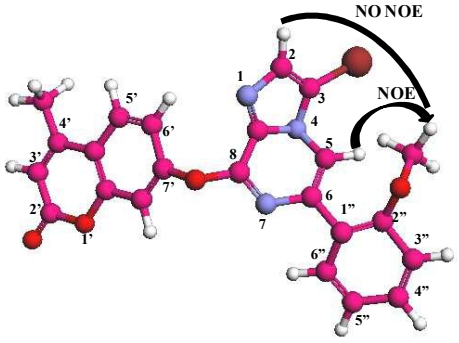


Figure 3. 2D NOEs ^{1}H, ^{1}H correlations used for structural assignment of compound **7a.**

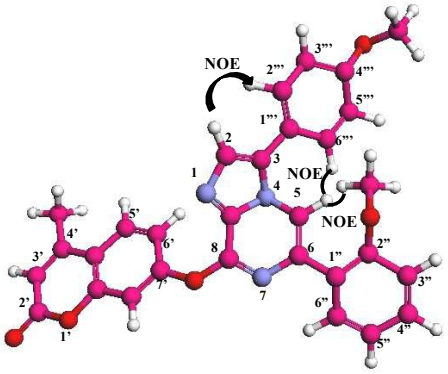


Figure 4. 2D NOEs ^{1}H, ^{1}H correlations used for structural assignment of compound **28.**

positive NOE signal with protons of methoxy group of 2-methoxyphenyl ring while negative NOE signal of singlet of C2H of imidazo[1,2-*a*]pyrazine with protons of methoxy group of 2-methoxyphenyl ring was observed, indicating the monoarylation

primarily at C6 position (Figure 3). On the other hand, singlets of C2H and C5H of imidazo[1,2-*a*]pyrazine ring in compound **28** showed positive NOE signals with protons of C2"' and C6"' of 4-methoxyphenyl ring present at C3 position. Due to negative NOE signal of singlet of C2H at imidazo[1,2-*a*]pyrazine with methoxy protons of 2-methoxy phenyl ring (at C6 position) and positive NOE signal with 4-methoxy phenyl ring (at C3 position) confirmed the second arylation at C3 position of imidazo[1,2-*a*]pyrazine **28** (Figure 4).

Compounds **6**, **7a**, **9b, 10b**, **11b**, **16b**, **28** and **30** were submitted to NCI, USA for *in vitro* anticancer activities against panel of 60 human cancer cell lines at single dose concentration of 10 μM.[20-23] Compound **6** with bromo at C3 and C6 positions showed growth inhibition against renal cancer cell line 786-0 with GI value of 71.02% and breast cancer cell lines MCF-7 and MDA-MB-231/ATCC with respective GI values of 77.75% and 70.43%. But monoarylation with 2-methoxy phenyl at C6 position **7a** displayed poor anticancer activity. Amongst symmetrical diarylated compounds, 4-chlorophenyl ring at both C3 and C6 positions **9b** showed no significant growth inhibition against cancer cell lines while 2-thiophene moieties at C3 and C6 positions **10b** showed excellent inhibition against non small lung cancer cell line HOP-92 with GI value of 92.29%. Replacement with six membered phenyl rings at C3 and C6 positions **11b** exhibited excellent anticancer activity to renal cancer cell line A-498 with GI value of 90.12% while moderate activity against renal cancer RXF-393 and non small lung cancer cell line HOP-92 with respective GI values of 71.67% and 77.65% was observed. Substitution with 4-methoxy group at phenyl rings at C3 and C6 positions of imidazo[1,2-*a*]pyrazine **16b** displayed broad spectrum of anticancer activity towards various cancer cell lines viz., breast cancer cell line MDA-MB-231/ATCC, melanoma cell line LOX IMVI, CNS cancer cell line SNB-75, non small lung cancer cell line A549/ATCC and prostate cancer cell line PC-3 with GI values of 93.71%, 91.98%, 85.69%, 83.52% and 82.27% respectively. It also displayed good inhibition with other cancer cell lines viz., non small lung cancer cell line NCI-H460 (GI: 73.64%), colon cancer cell line HT29 (GI: 74.56%), CNS cancer cell lines U251 (GI: 77.45%) and SF-295 (GI: 76.68%), melanoma cancer cell lines MALME-3M (GI: 73.25%), SK-MEL-5 (GI: 71.37%) and UACC-257 (GI: 70.84%). ovarian cancer cell lines OVCAR-8 (GI: 73.68%) and NCI/ADR-RES (GI: 70.09%), renal cancer cell line SN12C (GI: 75.49%), prostate cancer cell line DU-145 (GI: 70.53%) and breast cancer cell line BT-549 (GI: 70.80%) (Figure 5). Compound **16b** exhibited more than 70% growth inhibition for most of the tumor cell lines and much better than 5-fluorouracil (positive control) in tested derivatives. It showed higher activity than 5-fluorouracil (5-FU) in non-small lung cancer cells (A549/ATCC, HOP-62, NCI-H23 and NCI-H460), colon cancer cells (HCT-116 and HT-29), melanoma (LOX IMVI, MALME-3M, MDA-MB-435, SK-MEL-5 and UACC-257), prostate cancer (PC-3 and DU-145) and breast cancer cells (MCF-7, MDA-MB-231/ATCC and BT-549). In the series of unsymmetrical compounds, imidazo[1,2-*a*]pyrazine with 4-methoxy phenyl at C3 and 2-methoxy phenyl at C6 positions **28** displayed poor anticancer activity while substitution with 2-thiophene at C3 position and 4-chlorophenyl at C6 position **30**, increases the activity with more selectivity towards melanoma cancer cell line MALME-3M with GI value of 77.82% (Table 3).

Compound **16b** was also evaluated for toxicity to Hek293 (human embryonic kidney) cell lines using MTT assay.[24] It has been observed that compound **16b** showed only 17%, 15%, 9%, 5% and 3% cytotoxicity to Hek293 cells at 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M concentrations respectively (Figure S84, Supporting Information). The compound showed only 17% of toxicity to Hek293 cells even at 100 μM concentration. These data indicated that compound **16b** showed potent anticancer activity and low toxicity to normal cells.

The partition coefficient of compounds was also studied in octanol/water system for the determination of log P values by shake-flask method[25] (Supporting information). It has been observed that compounds **11b** and **16b** showed higher log P values (Table 4) that supported the dependency of lipophilicity with higher activity of these compounds towards antitumor activity. Thus, lipophilicity is a crucial factor for the activity amongst the synthesized compounds.

Structure-activity correlation, based on the number of cancer cell lines revealed that the nature of the substituents at C3- and C6-positions of imidazo[1,2-*a*]pyrazine affected the biological activity. Compounds **10b**, **11b** and **16b** showed comparatively higher activity than **6**, **7a**, **9b**, **28** and **30,** suggested that there is much difference in antitumor activity with different substitution of phenyl rings. The antitumor results indicated that compound **6** with coumarin moiety at C8 position and bromo at C3 and C6 positions displayed only moderate anticancer activity. The anticancer activity has been slightly increased with substitution of 2-thiophene rings at C3 and C6 positions, as in the case of compound **10b**. Higher activity has been achieved with substitution of phenyl rings **11b** and 4-methoxyphenyl rings **16b** at C3 and C6 positions. On the other hand, substitution with 4-chlorophenyl at C6 position and 2-thiophene at C3 position as in case of compound **30,** decreased the activity. It has been revealed that symmetrical diarylated imidazo[1,2-*a*]pyrazine-coumarin hybrids **11b** and **16b** showed higher activity than unsymmetrical diarylated hybrids **28** and **30**. These studies indicated that substitution of coumarin heterocycle with various phenyl derivatives on imidazo[1,2-*a*]pyrazine gave highly potent anticancer activities towards 60 human cancer cell lines. Overall, **16b** has been found to be the most effective member amongst these series of compounds and showed broad spectrum of activity against melanoma cancer cell lines.

Table 3 Percentage (%) growth inhibition (GI) of compounds **6**, **7a**, **9b, 10b**, **11b**, **16b**, **28, 30** and **5-FU**over the full panel of 60 tumor cell lines at concentration of 10 μM.

Cell line type	Cell line name	6	7a	9b	10b	11b	16b	28	30	5-FU
Leukemia	CCRF-CEM	-	-	19.41	-	22.39	-	-	14.72	57.13
	HL-60(TB)	**69.19**	-	13.22	32.40	14.82	NT	-	32.62	47.90
	K-562	-	-	14.92	18.35	38.24	NT	-	47.06	42.38
	MOLT-4	12.54	-	25.52	21.25	41.55	NT	-	20.34	43.13
	RPMI-8226	-	-	-	29.69	48.96	36.31	-	17.02	41.41
	SR	30.60	-	49.13	**54.39**	45.46	NT	-	27.81	24.82
Non-Small Cell Lung Cancer	A549/ATCC	22.21	13.25	-	41.45	43.86	**83.52**	-	-	34.25
	EKVX	24.43	24.04	-	35.95	33.20	49.84	-	-	58.40
	HOP-62	48.29	22.74	-	40.98	31.25	**59.14**	-	-	47.89
	HOP-92	**62.81**	-	-	**92.29**	**77.65**	**-13.56**	11.79	15.61	50.64
	NCI-H226	46.40	27.46	-	16.99	**55.35**	29.28	-	-	69.55
	NCI-H23	36.05	25.40	21.91	43.37	37.56	**66.97**	-	28.24	39.01
	NCI-H322M	23.24	18.07	-	10.57	14.03	18.02	-	11.91	59.50
	NCI-H460	26.12	-	-	49.53	36.05	**73.64**	-	-	13.07
	NCI-H522	45.23	36.87	-	25.35	**52.85**	**60.39**	-	-	58.02
Colon Cancer	COLO 205	-	-	-	25.05	29.50	43.89	-	11.30	40.22
	HCC-2998	-	11.61	-	21.86	18.95	22.91	-	-	**L**
	HCT-116	44.49	20.47	-	41.34	**60.66**	**66.14**	-	18.23	17.83
	HCT-15	-	-	36.21	27.11	31.38	36.61	-	29.83	26.56
	HT29	-	-	52.34	**53.36**	46.79	**74.56**	-	49.09	27.19
	KM12	21.08	-	18.81	40.37	24.49	**57.01**	-	23.63	40.70
	SW-620	-	-	33.27	43.33	11.72	**60.98**	-	13.73	50.12
CNS Cancer	SF-268	43.44	22.13	-	23.75	35.64	34.29	-	14.33	59.05
	SF-295	-	11.75	-	**52.52**	45.77	**76.68**	-	-	69.16
	SF-539	**52.78**	18.31	-	40.04	45.41	**-21.15**	-	-	**L**
	SNB-19	25.37	-	NT	NT	35.56	NT	NT	-	65.96
	SNB-75	**-2.07**	68.32	-	**64.44**	**-1.39**	**85.69**	15.18	-	65.93
	U251	**52.35**	17.29	11.95	**52.46**	32.65	**77.45**	-	19.30	50.35
Melanoma	LOX IMVI	25.67	24.60	15.63	**61.63**	43.60	**91.98**	-	21.46	30.40
	MALME-3M	**53.11**	24.30	**56.42**	**60.88**	**-9.77**	**73.25**	-	**77.82**	58.21
	M14	17.22	-	35.77	32.45	32.28	**61.41**	-	**61.10**	NT
	MDA-MB-435	12.82	-	24.52	38.65	27.95	**62.77**	-	20.55	36.66
	SK-MEL-2	22.00	-	-	31.03	48.10	**50.58**	-	-	95.52
	SK-MEL-28	-	-	**61.69**	**76.09**	32.37	**-17.37**	-	40.28	NT
	SK-MEL-5	**59.65**	13.41	15.59	**57.50**	**61.69**	**71.37**	-	30.47	33.75
	UACC-257	10.94	-	11.68	**51.80**	34.28	**70.84**	-	-	19.56
	UACC-62	14.79	-	-	28.89	18.62	49.89	-	-	39.77
Ovarian Cancer	IGROV1	22.57	26.28	-	20.77	16.32	29.38	-	-	51.29
	OVCAR-3	43.29	13.76	-	32.04	23.36	**53.41**	-	-	47.41
	OVCAR-4	**54.55**	-	14.41	45.94	39.32	**-18.64**	-	25.03	59.40
	OVCAR-5	-	-	-	-	---	12.00	-	-	44.34
	OVCAR-8	35.35	-	-	47.45	38.43	**73.68**	-	-	NT
	NCI/ADR-RES	42.47	22.95	21.20	46.63	**54.62**	**70.09**	-	12.56	47.65
	SK-OV-3	14.52	13.64	-	-	36.88	-	-	-	77.56
Renal Cancer	786-0	**71.02**	-	NT	NT	32.72	NT	-	-	48.79
	A498	-	-	-	35.60	**90.12**	29.38	-	-	**L**
	ACHN	34.24	22.25	-	42.72	**51.39**	**53.41**	-	-	39.31
	CAKI-1	-	10.67	-	32.18	14.82	28.33	-	-	39.40
	RXF 393	**55.79**	11.01	29.51	36.41	**71.67**	**-20.76**	-	12.23	34.33
	SN12C	24.19	-	-	26.17	32.41	**75.49**	-	-	54.04
	TK-10	-	-	-	46.11	19.47	**64.26**	-	-	66.98
	UO-31	41.18	37.92	14.45	39.73	**59.18**	25.03	18.30	31.13	41.30
Prostate Cancer	PC-3	17.90	23.95	12.06	47.04	**60.82**	**82.27**	-	19.78	58.26
	DU-145	24.95	12.94	30.37	44.61	31.96	**70.53**	-	18.46	35.52
Breast Cancer	MCF7	**77.75**	24.89	26.92	40.08	49.54	**55.81**	-	44.11	11.55
	MDA-MB-231/ATCC	**70.43**	31.76	26.69	36.78	49.62	**93.71**	24.30	-	78.17
	HS 578T	30.14	15.90	12.25	32.35	41.43	42.41	-	-	**L**
	BT-549	-	-	10.59	**75.01**	17.35	**70.80**	-	-	37.81
	T-47D	46.34	22.05	-	45.27	**61.52**	**54.65**	-	15.70	56.78
	MDA-MB-468	**58.60**	30.06	15.17	45.34	**55.77**	**56.11**	-	17.46	NT

[5] < 50% inhibition, **50-60% inhibition**, **60-70% inhibition**, **70-90% inhibition**, **90-100% growth inhibition**, **highly potent compounds**, NT: not tested

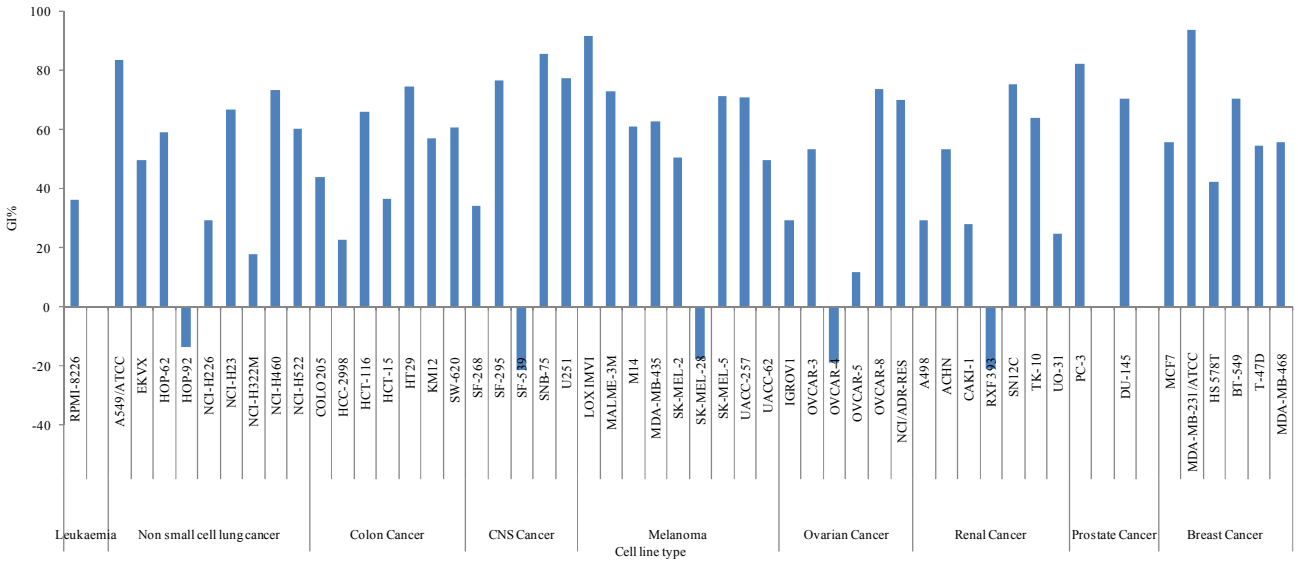


Figure 5. The percentages growth inhibition of compound **16b** over the full panel of tumor cell lines.

Table 4 Experimental determined lipophilicity

Compounds	P	Log P
6	239.88	2.38
7a	489.77	2.69
9b	371.53	2.57
10b	467.73	2.67
11b	2511.88	3.40
16b	758.57	2.88
28	257.03	2.41
30	380.19	2.58

P—partition coefficient; log P—logarithm of the partition coefficient.

Preliminary anticancer screening showed that compound **16b** has been found to be the most active member of imidazo[1,2-*a*]pyrazine-coumarin hybrids and showed excellent inhibition against melanoma than other cancer cell lines. So, in order to observe the molecular interactions of compound in the active site of enzyme for melanoma cancer, docking experiment was performed. The crystal coordinates of enzyme used in melanoma was downloaded from protein data bank (www.rcsb.org)[26] (PDB code: 3OG7).[27] Compound **16b** showed H-bonding interaction of N1 (d = 2.93 Å), N4 (d = 2.72 Å) and N7 (d = 1.75 Å) atoms of imidazo[1,2-*a*]pyrazine with G518 amino acid residue of the coil of enzyme. N7 atom of the pyrazine ring of imidazo[1,2-*a*]pyrazine also showed H-bonding interaction with M517 (d = 2.17 Å) amino acid residue of enzyme. Oxygen atom of the 4-methoxy phenyl at C6 position of imidazo[1,2-*a*]pyrazine showed H-bonding interaction with R781 amino acid residue of enzyme (d = 2.85 Å). Carbonyl group of coumarin ring interacts with W531 amino acid residue of β-strand of enzyme with d = 2.56 Å. Oxygen atom and carbonyl group of coumarin ring also showed H-bonding interactions with Q530 amino acid residues of β-strand of enzyme (d = 1.77 Å and d = 2.31 Å). Therefore, docking of compound **16b** in the active site of this enzyme indicated the probable mode of action for anticancer activities (Figure 6).

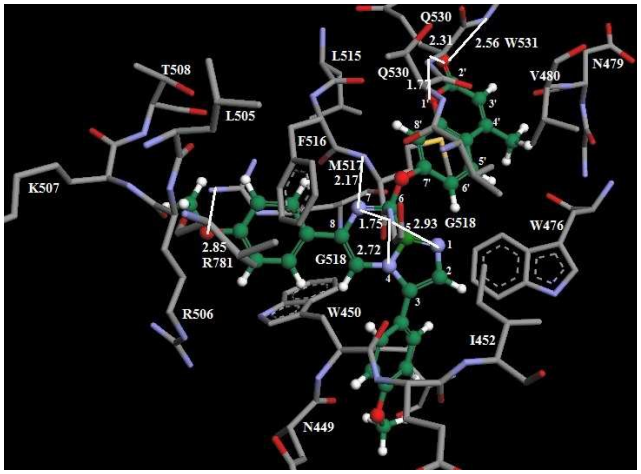


Figure 6 Docking of compound **16b** in the active site of 3OG7. H-bonds of compound **16b** with different amino acid residues are visible. Carbon atoms are given in green colour.

Conclusion

In summary, imidazo[1,2-*a*]pyrazine-coumarin hybrid has been synthesized by nucleophilic substitution approach at C8 position from easily accessible 3,6,8-tribromoimidazo[1,2-*a*]pyrazine and 7-hydroxy-4-methyl coumarin. This compound was further functionalized at C6 position for monoarylation and C3, C6 positions for symmetrical diarylations using palladium catalyzed C-C coupling. Subsequent use of monoarylated products has been implemented for synthesis of unsymmetrical diarylated imidazo[1,2-*a*]pyrazine-coumarin hybrids in moderate to good yields. Evaluation of selected compounds for anticancer activity revealed that symmetrical diarylated hybrids **11b** and **16b** showed broad spectrum of anticancer activity towards most of the cancer cell lines. These compounds also have good lipophilicity that qualifies them to have good pharmacokinetic and drug

bioavailability. Molecular docking study has also further supported the inhibitory activity of **16b** that helped in understanding the interactions between the ligand and enzyme active sites. Further optimizations of anticancer activity and pharmacokinetic profiling of these series of compounds are currently ongoing.

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23. **Antitumor methodology:** The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2.0 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96 well microtiter plates in 100 µl at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 ºC, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line were fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs were solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50µg/ml gentamicin. Aliquot of 100 µl of this drug dilution was added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations. Following drug addition, the plates were incubated for an additional 48 h at 37 ºC, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 µl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 ºC. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing 5 times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

[(Ti - Tz)/(C-Tz)] × 100 for concentrations for which Ti>/= Tz

[(Ti – Tz)/Tz] × 100 for concentrations for which Ti < Tz

24. **MTT assay**: Hek293 (Human embryonic kidney) cells, DMEM with 50mM glutamine, 10% FBS, 100 u/ml pencillin and 100 mg/ml streptomycin. The test was performed against Hek293 (Human embryonic kidney) cells. Cells were seeded in 96 well plates at the density of $1x10^{-5}$ cells/well in DMEM media supplemented with 10% FBS cells. Cells were incubated at 37 ºC in 5% CO_2 incubator. Cells were treated with compound **16b** at five concentrations (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}) in duplicate for 24 h at 37 ºC. 10 µl of MTT (prepared in 1* PBS buffer) from 5 mg/ml stock was added in each well and incubated at 37 ºC for 4 hrs in dark. The formazan crystals were dissolved using 100 µl of DMSO. Further, the amount of formazan crystal formation was measured as difference in absorbance by Bio-

Red ELISA plate reader at 570 nm and 690 nm reference wavelength. The relative cell toxicity (%) related to control wells containing culture medium without test material was calculated by using formula:

$$\% \text{ Cell Toxicity} = 100 - \frac{\text{OD (Compound treated wells)}}{\text{OD (Untreated Wells)}} \text{ X } 100$$

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Synthesis, *in vitro* anticancer activity and SAR studies of arylated imidazo[1,2-*a*]pyrazine-coumarin hybrids

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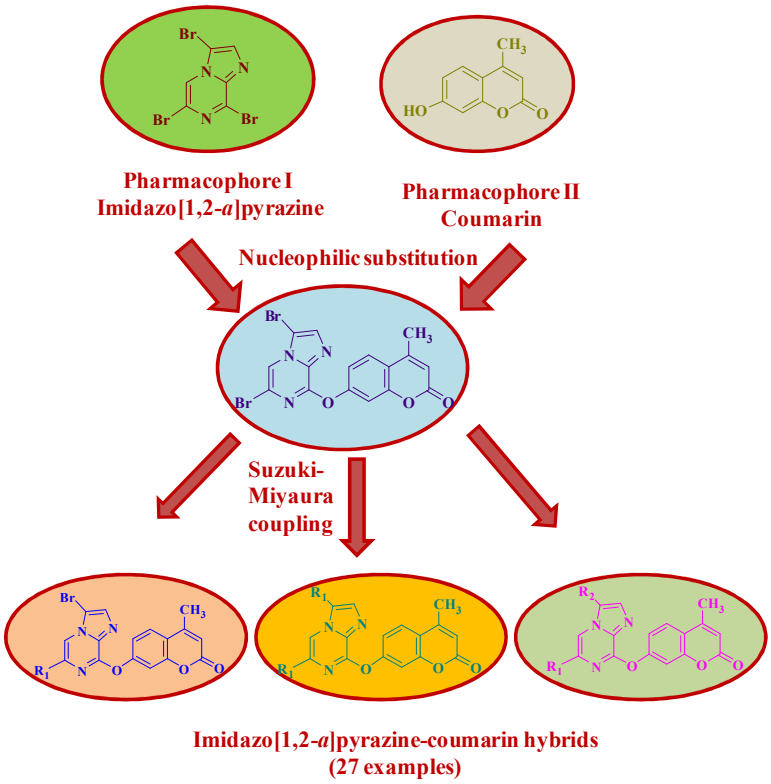

A diverse array of arylated imidazo[1,2-*a*]pyrazine-coumain hybrids have been synthesized for *in vitro* anticancer activities.