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FULL-LENGTH PAPER

Synthesis, anti-inflammatory, and antiproliferative activity evaluation of isoindole, pyrrolopyrazine, benzimidazoisoindole, and benzimidazopyrrolopyrazine derivatives

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Abstract A number of isoindole (**3x**, **3y**, **6xa**–**6ye**), pyrrolopyrazine (**3z**, **6za**–**6ze**), benzimidazoisoindole (**4x**, **4y**, **7xa**–**7ye**), and benzimidazopyrrolopyrazine (**4z**, **7za**–**7ze**) derivatives has been synthesized in excellent yields. All these compounds were fully characterized and evaluated against five human cancer cell lines for their anti-inflammatory and antiproliferative activity. Compounds **6yc** and **7zd** exhibited good anti-inflammatory activity whereas compounds **6zc**, **7zd** (lung NCl H-522), **6ye**, **7xd**, **7yd**, **7zc**, **7zd** (colon HCT-15), **6xc**, **7zc** (ovary PA-1), **6xc**, **6yb**, **6zc** (liver HepG-2) exhibited good antiproliferative activity.

Keywords Isoindole · Pyrrolopyrazine · Benzimidazoisoindole · Benzimidazopyrrolopyrazine · Anti-inflammatory · Antiproliferative

Introduction

The synthesis of biologically active heterocyclic molecules is a challenging area of research. Isoindole, pyrrolopyrazine, and benzimidazole derivatives exhibiting antifungal [1–3], antibacterial [4–6], anti-inflammatory [7–13], and anticancer [14–22] activities are well documented in the literature. These molecules also possess antiasthmatic [23,24], antimicrobial [25,26], antiviral [27], and antiarrythmic [28] activities. Pyrrolopyrazine derivatives also act as inhibitors of human N-myristoyltransferase-1 [29] and vasopressin$_{1b}$ receptor antagonists [30].

Since isoindole, pyrrolopyrazine, and benzimidazole derivatives possess a variety of biological activities, it was considered worthwhile to synthesize hybrid molecules of isoindole and benzimidazole (i.e., benzimidazoisoindole), and of pyrrolopyrazine and benzimidazole (i.e., benzimidazopyrrolopyrazine). In continuation of our research in the search of biologically active molecules [31–35], we have synthesized several derivatives of isoindole, pyrrolopyrazine, benzimidazoisoindole, and benzimidazopyrrolopyrazine using microwave irradiation and simple grinding methods and screened them for anti-inflammatory and antiproliferative activity, which are reported in this paper. To the best of our knowledge, all the compounds synthesized and reported in this paper are new to the literature.

Results and discussion

Chemistry

Cis-1,2-Cyclohexane dicarboxylic acid (**1x**; Fig. 1) and 3,4-diaminobenzoic acid (**2**; Fig. 1) were mixed in a 1:1 molar ratio and the reaction mixture was irradiated [34] at either 600 W or at 90 °C for 5 min where TLC indicated no starting materials were present. The final crude material was recrystallized from methanol to give pure product 4-amino-3-(1,3-dioxo-hexahydro-1*H*-isoindol-2(3*H*)-yl)benzoic acid (**3x**; Fig. 1) in 86 % yield.

According to the observations by Cul et al. [36] from the 2 amino groups in 3,4-diaminobenzoic acid, the meta amino group is expected to be more nucleophilic and undergo a

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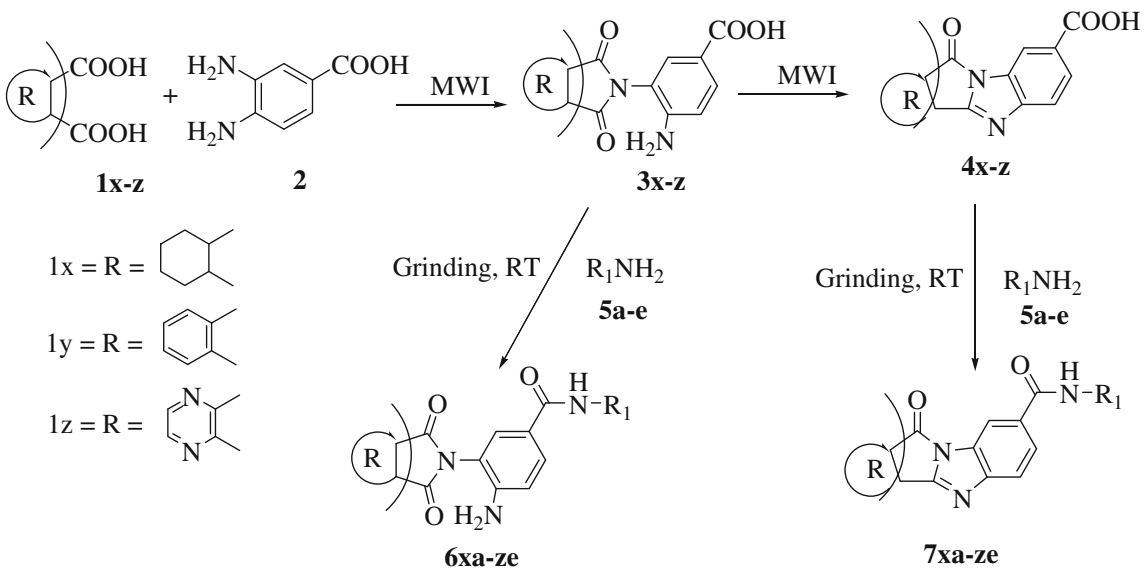

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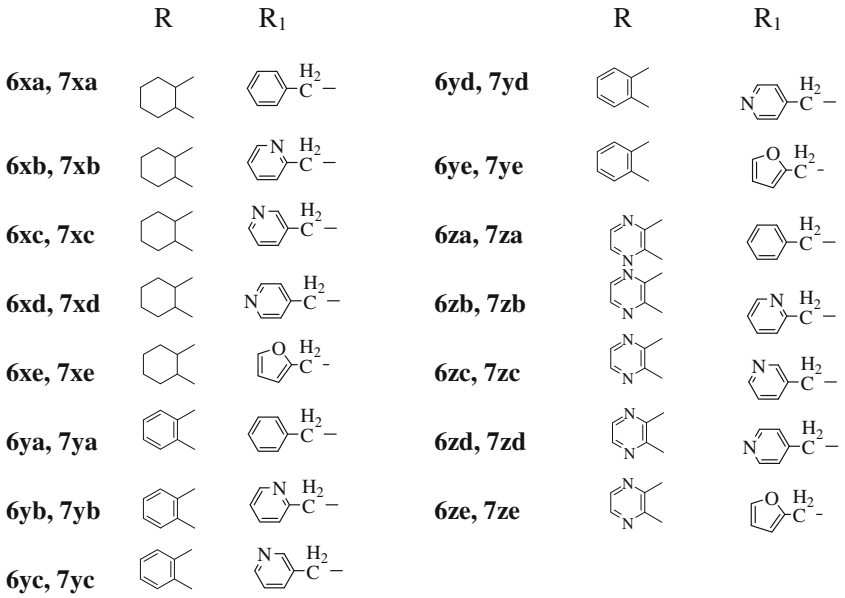


Fig. 1 Synthesis of **3x**–**3z**, **4x**–**4z**, **6xa**–**6ze**, **7xa**–**7ze** derivatives

condensation reaction preferably over the para amino group, and product **3x** (Fig. 1) confirmed this expectation.

In order to further support the structure assigned to **3x**, nuclear overhauser effect (NOE) experiments were carried out. Irradiation of $-NH_2$ at δ 5.051 showed a correlation with aromatic proton at δ 6.486–6.501 (d, 1H, Ar, $J = 7.5$ Hz), whereas irradiation of aromatic proton at δ 6.486–6.501 showed correlation with one aromatic proton δ 7.059–7.079 (dd, 1H, Ar, $J = 2.5$ and 7.5 Hz) and one $-NH_2$ group at δ 5.051 (bs, 2H, $-NH_2$, exch). From these NOE experiments, it is clear that the $-NH_2$ group meta to –COOH group reacts first to give product **3x** (Fig. 1). IR, ^{1}H NMR, ^{13}C NMR, APCI-MS, and elemental analysis data of **3x** reported in the "Experimental protocols" section fully support the structure assigned to **3x** (Fig. 1). Similarly, condensation of phthalic acid (**1y**; Fig. 1), pyrazine-2,3-dicarboxylic acid (**1z**; Fig. 1) with 3,4-diaminobenzoic acid (**2**; Fig. 1) gave corresponding condensation products **3y** and **3z**, respectively, in good yields. Spectral (IR, ^{1}H NMR, NOE, ^{13}C NMR, APCI-MS) and analytical data of **3y** and **3z** reported in the "Experimental

Table 1 In vivo anti-inflammatory activity of compounds **3x–3z**, **4x–4z**, **6xa–6ze**, **7xa–7ze**

Compound nos.	Anti-inflammatory activity (%) at 50 mg/kg p.o.	Compound nos.	Anti-inflammatory activity (%) at 50 mg/kg p.o.	Compound nos.	Anti-inflammatory activity (%) at 50 mg/kg p.o.
3x	12	**6yb**	28	**7xd**	24
3y	18	**6yc**	**34**	**7xe**	18
3z	18	**6yd**	30	**7ya**	31
4x	18	**6ye**	21	**7yb**	24
4y	18	**6za**	21	**7yc**	29
4z	15	**6zb**	23	**7yd**	32
6xa	21	**6zc**	24	**7ye**	21
6xb	27	**6zd**	26	**7za**	22
6xc	24	**6ze**	21	**7zb**	23
6xd	22	**7xa**	15	**7zc**	33
6xe	18	**7xb**	21	**7zd**	**37**
6ya	27	**7xc**	24	**7ze**	27
Ibuprofen	**39**	–	–		

Bold values represent compounds showing good anti-inflammatory activity

protocols" section fully support the structures assigned to them.

Compound **3x** was irradiated at either 850 W or at 130 °C for 5 min to afford a material that upon recrystallization from methanol afforded pure tetracyclic product **4x** (Fig. 1) in 82 % yield. Similarly, compounds **3y** and **3z** were converted to **4y** and **4z**, respectively, in quantitative yields.

Condensation of **3x** with benzyl amine was done by dry grinding [35] both chemicals in a 1:1 ratio in a small mortar with a pestle for 20 min. Recrystallization of the resulting material from methanol afforded pure product 4-amino-*N*-benzyl-3-(1,3-dioxo-hexahydro-1 *H*-isoindol-2 (3*H*)-yl) benzamide (**6xa**; Fig. 1) in 86 % yield. Spectral (IR, ^{1}H NMR, ^{13}C NMR, and APCI-MS) and elemental analysis of **6xa** reported in the "Experimental protocols" section fully support the structure assigned to it. Similarly, condensation of **3x**, **3y**, and **3z** with benzyl amine (**5a**), pyridin-2-ylmethanamine (**5b**), pyridin-3-ylmethanamine (**5c**), pyridin-4-ylmethanamine (**5d**), and furan-2-ylmethanamine (**5e**) gave corresponding condensation products **6xa–6ze** (Fig. 1) in excellent yields. Physical constants, spectral data, and elemental analysis of **6xa–6ze** reported in the "Experimental protocols" section are in full agreement with the structures assigned to them.

Similarly condensation of **4x**, **4y**, and **4z** with the above said amines gave expected products **7xa–7ze** (Fig. 1) in excellent yields.

Biological results

Fully characterized and purified compounds **3x–3z**, **4x–4z**, **6xa–6ze**, and **7xa–ze** (Fig. 1) were screened for anti-inflammatory activity [37] using the carrageenan-induced paw edema assay and the results are summarized in Table 1. Table 1 indicates that compounds **6yc** and **7zd** exhibited 34 and 37 % anti-inflammatory activity, respectively, at 50 mg/kg p.o. as compared to ibuprofen which showed 39 % activity at 50 mg/kg p.o.

Compounds **3x–3z**, **4x–4z**, **6xa–6ze**, and **7xa–7ze** were screened in vitro for antiproliferative activity [38] against five human cancer cell lines, namely breast (T47D), lung (NCl H-522), colon (HCT-15), ovary (PA-1), and liver (HepG-2) at 10 mM concentration and the results are summarized in Table 2. Table 2 indicates that compounds **6zc**, **7zd** (lung NCI H-522), **6ye**, **7xd**, **7yd**, **7zc**, **7zd** (colon HCT-15), **6xc**, **7zc** (ovary PA-1), **6xc**, **6yb**, **6zc** (liver Hep G-2) exhibited antiproliferative activity comparable to or better than reference drug 5-fluorouracil (5-FU) against various cancer cell lines mentioned above. Compounds **6xc**, **6yb**, **6ye**, **6zc, 7xd**, **7yd**, **7zc**, and **7zd** which showed antiproliferative activity comparable to or better than 5-FU were further studied and their IC_{50} values for various cancer cell lines and normal cell line (COS-1) were determined and reported in Table 3.

Anti-inflammatory activity of isoindole and pyrrolopyrazine derivatives reported in the literature are comparable to ibuprofen [39,40] whereas isoindole derivatives reported in this paper are also comparable to ibuprofen but pyrrolopyrazine derivatives are less active than ibuprofen. Bicyclic compounds **3z**, **6xa**, **6xb**, **6yb**, and **6yc** exhibited 18, 21, 27, 28, and 34 % anti-inflammatory activity whereas corresponding tetracyclic compounds **4z**, **7xa**, **7xb**, **7yb**, and **7yc** exhibited 15, 15, 21, 24, and 29 % anti-inflammatory activity, respectively. Tetracyclic compounds **4x**, **7ya**, **7zc**, **7zd**, and **7ze** showed 18, 31, 33, 37, and 27 % anti-inflammatory activity whereas corresponding bicyclic compounds **3x**, **6ya**, **6zc**, **6zd**, and **6ze** exhibited 12, 27, 24, 26, and 21 %

Table 2 In vitro antiproliferative activity of compounds **3x–3z**, **4x–4z**, **6xa–6ze**, **7xa–7ze**

Compound nos.	Antiproliferative activity (% growth inhibition) at a concentration of 1×10^{-5} M[a]				
	Breast T47D	Lung NCI H-522	Colon HCT-15	Ovary PA-1	Liver HepG-2
3x	09	16	16	06	29
3y	20	30	27	15	25
3z	24	04	18	10	03
4x	11	18	15	19	13
4y	NT	NT	NT	NT	NT
4z	29	13	23	27	08
6xa	32	29	18	23	07
6xb	14	10	09	05	07
6xc	04	23	19	**32**	**47**
6xd	22	14	23	08	20
6xe	08	16	21	14	20
6ya	11	21	18	25	32
6yb	09	29	06	17	**37**
6yc	07	25	23	13	12
6yd	02	16	20	12	19
6ye	08	18	**41**	27	23
6za	15	22	24	08	31
6zb	15	16	01	17	26
6zc	02	**32**	03	11	**33**
6zd	19	28	22	01	27
6ze	14	25	09	09	19
7xa	06	14	10	24	20
7xb	19	15	28	25	19
7xc	13	05	20	06	03
7xd	17	20	**37**	26	30
7xe	22	18	20	08	27
7ya	22	07	27	20	07
7yb	13	19	24	10	26
7yc	19	13	06	08	11
7yd	11	04	**35**	25	03
7ye	18	21	27	11	30
7za	09	28	23	13	19
7zb	23	18	10	12	15
7zc	04	25	**35**	**33**	27
7zd	09	**33**	**30**	13	25
7ze	21	08	20	09	11
FU	18	28	26	25	31
CYC-PHO	29	11	11	22	31
CYC-HEXI	21	17	09	36	32

Bold values represent compounds showing good antiproliferative activity
5-FU 5-fluorouracil, *CYC-PHO* cyclophosphamide, *CYC-HEXI* cycloheximide, *NT* not tested
[a]Compounds tested in triplicate, data expressed as mean value of three independent experiments

anti-inflammatory activity, respectively. From the above results, it can be concluded that in some cases bicyclic compounds possess more anti-inflammatory activity than corresponding tetracyclic compounds whereas in other cases tetracyclic compounds possess more anti-inflammatory activity than corresponding bicyclic compounds.

Isoindole derivatives reported in this paper exhibit more antiproliferative activity against HepG-2 cancer cell line than what is exhibited by isoindole derivatives reported in the literature [15], but in the cases of NCI H-522, HCT-15, and T47D cancer cell lines the antiproliferative activity of the literature reported isoindole derivatives [41] is more than

Table 3 IC_{50} values [a,b] of in vitro antiproliferative activity of active compounds

Compound nos.	IC_{50}(μM)					
	Breast T47D	Lung NCI H-522	Colon HCT-15	Ovary PA-1	Liver HepG-2	Normal cell COS-1
6xc	93.1 ± 4.8	24.4 ± 3.6	24.3 ± 2.8	**17.8 ± 2.3**	**09.1 ± 1.8**	**117.5 ± 3.7**
6yb	47.8 ± 3.7	17.2 ± 2.4	84.8 ± 3.2	28.3 ± 3.0	12 ± 1.7	119.7 ± 3.4
6ye	57.3 ± 4.5	31.3 ± 3.7	**11.2 ± 2.0**	17.3 ± 2.3	20.1 ± 3.5	105.8 ± 3.1
6zc	89.9 ± 4.9	**16.4 ± 2.3**	103.8 ± 4.1	43.7 ± 3.7	**14.6 ± 1.9**	129.7 ± 4.7
7xd	31.8 ± 3.2	29.2 ± 2.2	**13.2 ± 1.8**	18.2 ± 1.7	14.3 ± 2.1	130.8 ± 5.1
7yd	39.2 ± 1.5	89.5 ± 2.1	**13.0 ± 2.3**	19.1 ± 2.8	97 ± 3.1	121.3 ± 2.3
7zc	86.6 ± 2.3	23.3 ± 1.6	**16.2 ± 1.9**	**17.0 ± 2.1**	19.6 ± 2.2	122.5 ± 1.9
7zd	51.3 ± 2.6	**16.7 ± 1.3**	**19.1 ± 3.8**	41.9 ± 2.6	24.5 ± 2.7	125.6 ± 2.3
5-FU	51.8 ± 2.3	56.8 ± 3.4	45.0 ± 1.4	39.5 ± 4.3	29.9 ± 1.8	110 ± 8.9
CYC-PHO	70.1 ± 2.3	67.9 ± 3.1	74.3 ± 4.9	64.1 ± 5.4	55.3 ± 3.6	125.4 ± 9.2
CYC-HEXI	65.1 ± 7.3	60.1 ± 5.3	54.1 ± 4.6	40.6 ± 2.1	57.1 ± 4.6	128.3 ± 7.9

5-FU 5-fluorouracil, *CYC-PHO* cyclophosphamide, *CYC-HEXI* cycloheximide

[a] 50 % growth inhibition as determined by MTT assay (24-h drug exposure)

[b] Compounds tested in triplicate, data expressed as mean value ± SD of three independent experiments

isoindole derivatives reported in this paper. Pyrrolopyrazine derivatives reported in this paper exhibit more antiproliferative activity against the NCI H-522 cancer cell line than the literature reported pyrrolopyrazine derivatives [42]. A comparison between bicyclic compounds **6xc**, **6yb**, and **6ye** with corresponding tetracyclic compounds **7xc**, **7yb**, and **7ye** shows that bicyclic compounds possess more antiproliferative activity than tetracyclic compounds, whereas in case of **6xd**, **6yd**, and **6zd** bicyclic compounds possess less antiproliferative activity than tetracyclic compounds **7xd**, **7yd**, and **7zd**.

Structure–activity relationship

Anti-inflammatory activity of bicyclic compounds **3x**, **3y**, and **3z** (Fig. 1) is 12, 18, and 18 % (Table 1), respectively, whereas anti-inflammatory activity of corresponding tetracyclic compounds, i.e., **4x**, **4y**, and **4z** (Fig. 1) is 18, 18, and 15 % (Table 1). From this data, it is clear that conversion of bicyclic compounds to tetracyclic compounds did not increase anti-inflammatory activity and Table 2 shows that same is true for antiproliferative activity also. Conversion of **3y** and **4z**–**6yc** and **7zd**, respectively, increase their anti-inflammatory activity from 12 and 15 to 34 and 37 %, respectively.

Coupling to **3x**, **3z**, **4z** with pyridin-3-ylmethanamine; **4x**, **4y**, **4z** with pyridin-4-ylmethanamine; and **3y** with pyridin-2-ylmethanamine and furan-2-ylmethanamine to give **6xc**, **6zc**, **7zc**, **7xd**, **7yd**, **7zd**, **6yb**, and **6ye** (Fig. 1) enhanced their antiproliferative activity, i.e., antiproliferative activity of coupled products is more than their starting metarials (Tables 2, 3).

Conclusion

A number of new isoindole, pyrrolopyrazine, benzimidazoisoindole, and benzimidazopyrrolopyrazine derivatives (**3x**–**3z**, **4x**–**4z**, **6xa**–**6ze**, **7xa**–**7ze**) have been synthesized using microwave irradiation and simple grinding methods. The compounds were screened for anti-inflammatory and antiproliferative activity. Compounds **6yc** and **7zd** exhibited good anti-inflammatory and **6xc**, **6yb**, **6ye**, **6zc**, **7xd**, **7yd**, **7zc**, and **7zd** exhibited good antiproliferative activity against various cancer cell lines. From above observations, it can be concluded that synthesis of hybrid molecules exhibited good biological activities in some cases whereas in other cases it exhibited only moderate activities.

Experimental protocols

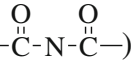
Instruments

Microwave reactor model CEM DISCOVER model no. 908010 and microwave oven model M197DL (Samsung) were used for microwave irradiation. Melting points (mp) were determined on a JSGW apparatus and are uncorrected. IR spectra were recorded using a Perkin Elmer 1600 FT spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker WH-500 spectrometer at a ca. 5–15 % (*w*/*v*) solution in deuterated solvent (TMS as internal standard). APCI mass was recorded using Finnigan Mat LCQ Mass Spectrometer. Elemental analysis was carried out on a Vario EL III elementor. Thin layer chromatography (TLC) was performed on silica gel G for TLC (Merck) and spots were

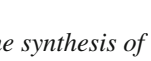
visualized by iodine vapor or by irradiation with ultraviolet light (254 nm).

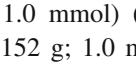
*General procedure for the synthesis of isoindole and pyrrolopyrazine derivatives (**3x–3z**)*

*Synthesis of 4-amino-3-(1,3-dioxo-hexahydro-1H-isoindol-2(3H)-yl)benzoic acid (**3x**)* *Cis*-1,2-Cyclohexane dicarboxylic acid (0.172 g; 1.0 mmol) (**1x**; Fig. 1) and 3,4-diaminobenzoic acid (0.152 g; 1.0 mmol) (**2**; Fig. 1) were mixed thoroughly and this reaction mixture was subjected to microwave irradiation at 600 W for 3 min and the progress of reaction was monitored by TLC on silica gel using ethyl acetate:methanol (2:3) as mobile phase. TLC indicated the presence of starting materials. This reaction mixture was again irradiated for 2 min (600 W) and TLC was performed which showed the absence of starting materials and hence the reaction is complete. The resulting crude material was purified by crystallization from methanol to give pure 4-amino-3-(1,3-dioxo-hexahydro-1*H*-isoindol-2(3*H*)-yl)benzoic acid. Yield: 0.248 g (86 %).

Alternatively a mixture of *cis*-1,2-cyclohexane dicarboxylic acid (0.172 g; 1.0 mmol) (**1x**; Fig. 1) and 3,4-diaminobenzoic acid (0.152 g; 1.0 mmol) (**2**; Fig. 1) was mixed thoroughly and subjected to microwave irradiation at 90 °C for 5 min. TLC of this reaction mixture on silica gel using ethyl acetate:methanol (2:3) as mobile phase showed the absence of starting materials and hence the reaction is complete. This crude product was crystallized from methanol to give pure condensed product 4-amino-3-(1,3-dioxo-hexahydro-1*H*-isoindol-2(3*H*)-yl)benzoic acid. Yield: 0.241 g (84 %). mp: 234 °C. IR (KBr) ν_{max}: 3293 (NH_2), 1680(>C=O), 1621  , 1529, 1426 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 1.377–1.442 (m, 4H, 2× CH_2), 1.622–1.677 (m, 2H, CH_2), 1.766–1.829 (m, 2H, CH_2), 2.659–2.716 (m, 2H, CH+CH), 5.051 (bs, 2H, NH_2, exch), 6.486–6.501 (d, 1H, Ar, J = 7.5 Hz), 7.059–7.079 (dd, 1H, Ar, J = 2.5 and 7.5 Hz), 7.134–7.138 (d, 1H, Ar, J = 2 Hz), 12.548 (bs, 1H, COOH, exch). NOE (500 MHz, DMSO-d_6) Irradiation of –NH_2 at δ 5.051 showed correlation with aromatic proton at δ 6.486–6.501 (d, 1H, Ar, J = 7.5 Hz), whereas irradiation of aromatic proton at δ 6.486–6.501 showed correlation with one aromatic proton δ 7.059–7.079 (dd, 1H, Ar, J = 2.5 and 7.5 Hz) and one –NH_2 group at δ 5.051 (bs, 2H, –NH_2, exch). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 24.265, 26.653, 37.942, 44.390, 116.127, 120.128, 121.941, 126.109, 129.141, 145.207, 169.143, 175.109. APCI-MS: m/z 289.20 (MH^+, 100 %). Anal. Calcd for $C_{15}H_{16}N_2O_4$: C, 62.50; H, 5.55; N, 9.72 %. Found: C, 62.58; H, 5.64; N, 9.81 %.

Compounds **3y** and **3z** were prepared using the above method.

*4-Amino-3-(1,3-dioxoisoindolin-2-yl)benzoic acid (**3y**)* Yield: 86 %. mp: 187 °C. IR (KBr) ν_{max}: 3377 (NH_2), 1682 (>C=O), 1669  , 1612, 1477 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 5.198 (bs, 2H, NH_2, exch), 6.485–6.501 (d, 1H, Ar, J = 8 Hz), 7.059–7.079 (dd, 1H, J = 2 and 8 Hz), 7.132–7.135 (d, 1H, Ar, J = 1.5 Hz), 7.586–7.612 (m, 2H, Ar), 7.659–7.692 (m, 2H, Ar), 12.676 (bs, 1H, COOH, exch). NOE (500 MHz, DMSO-d_6) irradiation of –NH_2 at δ 5.198 showed correlation with aromatic proton at δ 6.485–6.501 (d, 1H, Ar, J = 8 Hz), whereas irradiation of aromatic proton at δ 6.485–6.501 showed correlation with one aromatic proton δ 7.059–7.079 (dd, 1H, Ar, J = 2 and 8 Hz) and one –NH_2 group at δ 5.198 (bs, 2H, –NH_2, exch). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 116.789, 120.630, 121.880, 125.163, 126.460, 127.132, 132.062, 133.263, 147.681, 167.232, 170.125. APCI-MS: m/z 283.33 (MH^+, 100 %). Anal. Calcd for $C_{15}H_{10}N_2O_4$: C, 63.83; H, 3.57; N, 9.92 %. Found: C, 63.77; H, 3.53; N, 9.87 %.

*4-Amino-3-(5, 7-dioxo-5H-pyrrolo [3,4-b] pyrazin-6(7H)-yl) benzoic acid (**3z**)* Yield: 88 %. mp: 247 °C. IR (KBr) ν_{max}: 3215 (NH_2), 1698 (>C=O), 1619  , 1536 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6)δ: 5.198 (bs, 2H, NH_2, exch), 6.486–6.501 (d, 1H, Ar, J = 7.5 Hz), 7.059–7.079 (dd, 1H, Ar, J = 2 and 8 Hz), 7.132–7.136 (d, 1H, Ar, J = 2 Hz), 8.726 (s, 2H, Ar), 12.635 (bs, 1H, COOH, exch). NOE (500 MHz, DMSO-d_6) irradiation of –NH_2 at δ 5.198 showed correlation with aromatic proton at δ 6.486–6.501 (d, 1H, Ar, J = 7.5 Hz), whereas irradiation of aromatic proton at δ 6.486–6.501 showed correlation with one aromatic proton δ 7.059–7.079 (dd, 1H, Ar, J = 2.5 and 7.5 Hz) and one –NH_2 group at δ 5.198 (bs, 2H, –NH_2, exch). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 116.018, 120.529, 122.337, 126.313, 129.634, 143.019, 146.212, 147.875, 163.452, 169.141. APCI-MS: m/z 285.74 (MH^+, 100 %). Anal. Calcd for $C_{13}H_8N_4O_4$: C, 54.93; H, 2.84; N, 19.71 %. Found: C, 54.84; H, 2.88; N, 19.77 %.

*General procedure for the synthesis of benzimidazoisoindole and benzimidazopyrrolopyrazine derivatives (**4x–4z**)*

*Synthesis of (1,2,3,4,4a,11a-hexahydro-11-oxobenzimidazo [2,1-a]isoindol)-8-carboxylic acid (**4x**)* 4-Amino-3-(1,3-dioxo-hexahydro-1*H*-isoindol-2(3*H*)-yl) benzoic acid (0.288 g; 1.0 mmol) (**3x**; Fig. 1) was irradiated at 850 W for 5 min and the progress of reaction was monitored by TLC on silica gel using ethyl acetate:methanol (2:3) as mobile phase. TLC indicated the absence of starting material and hence the reaction is complete. The resulting

material was purified by crystallization from methanol to give pure cyclized product 8-(1,2,3,4,4*a*,11*a*-hexahydro-11-oxobenzimidazo[2,1-*a*]isoindol)-oicacid (**4x**; Fig. 1). Yield: 0.221 g (82 %).

Alternatively the above compound was subjected to microwave irradiation at 130 °C for 5 min. TLC of this reaction mixture on silica gel using ethyl acetate:methanol (2:3) as mobile phase showed the absence of starting material. The resulting material was crystallized from methanol to give pure cyclized product **4x** (Fig. 1). Yield: 0.218 g (81 %). mp: >300 °C. IR (KBr) ν_{max}: 1684 (>C=O), 1624 ($-\overset{O}{\overset{\|}{C}}-N-$), 1513 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 1.372–1.458 (m, 4H, 2× CH_2), 1.623–1.679 (m, 2H, CH_2), 1.765–1.838 (m, 2H, CH_2), 2.649–2.737 (m, 2H, CH+CH), 6.489–6.503 (d, 1H, Ar, J = 7 Hz), 6.864–6.884 (dd, 1H, Ar, J = 2 and 8 Hz), 7.134–7.138 (d, 1H, Ar, J = 2 Hz), 12.516 (bs, 1H, COOH, exch). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 24.105, 25.074, 26.644, 30.303, 30.826, 41.279, 115.777, 119.221, 125.274, 126.619, 130.125, 141.133, 144.949, 169.988, 198.274. APCI-MS: m/z 271.36 (MH^+, 100 %). Anal. Calcd for $C_{15}H_{14}N_2O_3$: C, 66.66; H, 5.18; N, 10.37 %. Found: C, 66.75; H, 5.27; N, 10.43 %.

Compounds **4y** and **4z** were prepared using the above method.

*(11H,11-Oxobenzimidazo[2,1-a]isoindol)-8-carboxylic acid (**4y**)* Yield: 85 %. mp: >300 °C. IR (KBr) ν_{max}: 1686 (>C=O), 1616 ($-\overset{O}{\overset{\|}{C}}-N-$), 1519 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 6.485–6.500 (d, 1H J = 7.5 Hz), 6.854–6.874 (dd, 1H J = 2 and 8 Hz), 7.134–7.139 (d, 1H, J = 2.5 Hz), 7.578–7.619 (m, 1H, Ar), 7.658–7.695 (m, 2H, Ar), 7.840–7.856 (d, 1H, Ar, J = 8 Hz), 12.586 (bs, 1H, COOH, exch). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 115.274, 119.109, 125.216, 126.885, 128.928, 129.280, 130.262, 131.771, 135.004, 135.937, 137.035, 141.374, 144.190, 169.596, 190.280. APCI-MS: m/z 265.86 (MH^+, 100 %). Anal. Calcd for $C_{15}H_8N_2O_3$: C, 68.18; H, 3.05; N, 10.60 %. Found: C, 68.35; H, 3.43; N, 10.80 %.

*(11H,11-Oxobenz[4′,5′]imidazo[1′,2:1,2]pyrrolo[3,4-b]pyrazin)-8-carboxylic acid (**4z**)* Yield: 82 %. mp: >300 °C. IR (KBr) ν_{max}: 1683 (>C=O), 1612 1612 ($-\overset{O}{\overset{\|}{C}}-N-$), 1534 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 6.485–6.500 (d, 1H, Ar, J = 7.5 Hz), 6.854–6.874 (dd, 1H, Ar, J = 2 and 8 Hz), 7.134–7.139 (d, 1H, Ar, J = 2.5 Hz), 8.734 (s, 2H, Ar), 12.548 (bs, 1H, COOH, exch). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 115.337, 119.139, 125.347, 126.529, 131.834, 141.009, 142.285, 144.019, 147.743, 148.435, 149.272, 169.903, 190.088. APCI-MS: m/z 267.63 (MH^+, 100 %). Anal. Calcd for $C_{13}H_6N_4O_3$: C, 58.65; H, 2.27; N, 21.05 %. Found: C, 58.54; H, 2.31; N, 21.14 %.

*General procedure for the synthesis of isoindole and pyrrolopyrazine derivatives (**6xa–6ze**)*

*Synthesis of 4-amino-N-benzyl-3-(1,3-dioxo-hexahydro-1H-isoindol-2(3H)-yl)benzamide (**6xa**)* 4-Amino-3-(1,3-dioxo-hexahydro-1*H*-isoindol-2(3*H*)-yl) benzoic acid (0.288 g; 1.0 mmol) (**3x**; Fig. 1) and benzyl amine (0.107 g; 1.0 mmol) (**5a**; Fig. 1) were grinded together in a small mortar with a pestle for 20 min. TLC of the reaction mixture on silica gel using ethyl acetate:methanol (2:3) mobile phase exhibited that the reaction is complete. The resulting material was purified by crystallization from methanol to give pure product 4-amino-*N*-benzyl-3-(1, 3-dioxo-hexahydro-1 *H*-isoindol-2 (3*H*)-yl)benzamide (**6xa**; Fig. 1). Yield: 0.324 g (86 %). mp: 276 °C. IR (KBr) ν_{max}: 3218 (NH_2), 1695, 1676, 1612 (>C=O), 1478 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 1.377–1.445 (m, 4H, 2× CH_2), 1.623–1.679 (m, 2H, CH_2), 1.763–1.829 (m, 2H, CH_2), 2.657–2.718 (m, 2H, CH+CH), 4.089 (s, 2H, CH_2), 5.051 (bs, 2H, NH_2, exch), 6.485–6.500 (d, 1H, Ar, J = 7.5 Hz), 7.059–7.079 (dd, 1H, Ar, J = 2.5 and 8 Hz), 7.134–7.139 (d, 1H, Ar, J = 2.5 Hz), 7.279–7.341 (m, 5H, Ar), 8.136 (s, 1H, NH, exch). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 24.265, 26.653, 37.942, 44.390, 116.208, 118.442, 123.608, 124.775, 126.109, 127.941, 128.141, 129.109, 141.108, 145.207, 167.143, 174.749. APCI-MS: m/z 378.65 (MH^+, 100 %). Anal. Calcd for $C_{22}H_{23}N_3O_3$: C, 70.02; H, 6.10; N, 11.14 %. Found: C, 70.32; H, 6.29; N, 11.34 %.

Compounds **6xb–6ze** were prepared using the above method.

*4-Amino-3-(1,3-dioxo-hexahydro-1H-isoindol-2(3H)-yl)-N-(pyridin-2-ylmethyl)benzamide (**6xb**)* Yield: 89 %. mp: 285 °C. IR (KBr) ν_{max}: 3399 (NH_2), 1678, 1623 (>C=O), 1530, 1426 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 1.378–1.446 (m, 4H, 2× CH_2), 1.622–1.678 (m, 2H, CH_2), 1.764–1.830 (m, 2H, CH_2), 2.661–2.716 (m, 2H, CH+CH), 4.086 (s, 2H, CH_2), 5.051 (bs, 2H, NH_2, exch), 6.484–6.500 (d, 1H J = 8 Hz), 7.059–7.079 (dd, 1H, J = 2.5 and 8.5 Hz), 7.134–7.138 (d, 1H, Ar, J = 2 Hz), 7.225–7.261 (q, 1H, Ar, J = 8 and 10 Hz), 7.388–7.415 (d, 1H, Ar), 7.752–7.802 (dt, 1H, Ar J = 2.5 and 9.5 Hz), 8.132 (s, 1H, NH, exch), 8.452–8.468 (d, 1H, Ar, J = 8 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 24.123, 26.124, 37.602, 49.309, 116.277, 118.742, 120.608, 123.235, 124.249, 125.541, 129.151, 136.139, 145.108, 148.667, 156.207, 167.233, 174.459. APCI-MS: m/z 379.77 (MH^+, 100 %). Anal. Calcd for $C_{21}H_{22}N_4O_3$: C, 66.66; H, 5.82; N, 14.81 %. Found: C, 66.53; H, 5.97; N, 14.99 %.

*4-Amino-3-(1,3-dioxo-hexahydro-1H-isoindol-2(3H)-yl)-N-(pyridin-3-ylmethyl)benzamide (**6xc**)* Yield: 91 %. mp: 281 °C IR (KBr) ν_{max}: 3317 (NH_2), 1680, 1615 (>C=O), 1533 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 1.378–1.446 (m, 4H, 2× CH_2), 1.622–1.678 (m, 2H, CH_2), 1.763–1.828 (m, 2H, CH_2), 2.659–2.739 (m, 2H, CH+CH), 4.072 (s, 2H, CH_2), 5.051 (bs, 2H, NH_2, exch), 6.488–6.504 (d, 1H, Ar, J = 8 Hz), 7.068–7.084 (dd, 1H, Ar, J = 2 and 6 Hz), 7.134–7.139 (d, 1H, Ar, J = 2.5 Hz), 7.236–7.268 (dt, 1H, Ar, J = 2 and 6 Hz), 7.843–7.859 (d, 1H, Ar, J = 8 Hz), 8.125 (s, 1H, NH, exch), 8.325–8.414 (m, 2H, Ar). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 24.105, 26.263, 37.752, 46.440, 116.277, 118.742, 123.608, 124.235, 125.249, 130.541, 134.151, 135.139, 145.108, 147.667, 150.207, 167.510, 174.444. APCI-MS: *m/z* 379.65 (MH^+, 100 %). Anal. Calcd for $C_{21}H_{22}N_4O_3$: C, 66.66; H, 5.82; N, 14.81 %. Found: C, 66.74; H, 5.75; N, 14.64 %.

*4-Amino-3-(1,3-dioxo-hexahydro-1H-isoindol-2(3H)-yl)-N-(pyridin-4-ylmethyl)benzamide (**6xd**)* Yield: 96 %. mp: >300 °C. IR (KBr) ν_{max}: 3388 (NH_2), 1668, 1654 (>C=O), 1593, 1495 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 1.374–1.446 (m, 4H, 2× CH_2), 1.625–1.681 (m, 2H, CH_2), 1.765–1.828 (m, 2H, CH_2), 2.640–2.737 (m, 2H, CH+CH), 4.085 (s, 2H, CH_2), 5.055 (bs, 2H, NH_2, exch), 6.486–6.502 (d, 1H, Ar, J = 8 Hz), 7.059–7.079 (dd, 1H, Ar, J = 2 and 8 Hz), 7.133–7.136 (d, 1H, Ar, J = 1.5 Hz), 7.327–7.343 (d, 2H, Ar, J = 8 Hz), 8.135 (s, 1H, NH, exch), 8.357–8.373 (d, 2H, Ar, J = 8 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 24.625, 26.273, 37.902, 44.466, 116.108, 118.667, 123.608, 124.035, 124.949, 125.451, 129.151, 145.941, 147.608, 149.775, 167.902, 174.442. APCI-MS: *m/z* 379.39 (MH^+, 100 %). Anal. Calcd for $C_{21}H_{22}N_4O_3$: C, 66.66; H, 5.82; N, 14.81 %. Found: C, 66.55; H, 5.99; N, 14.92 %.

*4-Amino-3-(1,3-dioxo-hexahydro-1H-isoindol-2(3H)-yl)-N-(furan-2-ylmethyl)benzamide (**6xe**)* Yield: 90 %. mp: 271 °C. IR (KBr) ν_{max}: 3313 (NH_2), 1679, 1617 (>C=O), 1533 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 1.381–1.448 (m, 4H, 2× CH_2), 1.623–1.678 (m, 2H, CH_2), 1.763–1.842 (m, 2H, CH_2), 2.639–2.736 (m, 2H, CH+CH), 4.083 (s, 2H, CH_2), 5.053 (bs, 2H, NH_2, exch), 6.181–6.211 (t, 1H, Ar, J = 7.5 Hz), 6.485–6.501 (d, 1H, Ar, J = 8 Hz), 6.726–6.742 (d, 1H, Ar, J = 8 Hz), 7.044–7.060 (d, 1H, Ar, J = 8 Hz), 7.133–7.138 (d, 1H, Ar, J = 2.5 Hz), 7.330–7.346 (d, 1H, Ar, J = 8 Hz), 8.142 (s, 1H, NH, exch). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 24.625, 26.273, 37.902, 38.902, 106.915, 110.152, 116.902, 118.442, 123.949, 125.541, 129.151, 142.940, 145.608, 148.675, 167.608, 174.035. APCI-MS: *m/z* 368.88 (MH^+, 100 %). Anal. Calcd for $C_{20}H_{21}N_3O_4$: C, 65.39; H, 5.72; N, 11.44 %. Found: C, 65.29; H, 5.60; N, 11.63 %.

*4-Amino-N-benzyl-3-(1,3-dioxoisoindolin-2-yl) benzamide (**6ya**)* Yield: 93 %. mp: 267 °C. IR (KBr) ν_{max}: 3380 (NH_2), 1680, 1667 (>C=O), 1611 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 4.089 (s, 2H, CH_2), 5.169 (bs, 2H, NH_2, exch), 6.485–6.502 (d, 1H, J = 8.5 Hz), 7.058–7.079 (dd, 1H, J = 2.5 and 8.5 Hz), 7.134–7.138 (d, 1H, Ar, J = 2 Hz), 7.232–7.397 (m, 5H, Ar), 7.587–7.614 (m, 2H, Ar), 7.658–7.693 (m, 2H, Ar), 8.127 (s, 1H, NH, exch). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 46.942, 118.489, 119.730, 121.880, 123.130, 124.960, 125.133, 126.460, 127.133, 128.760, 132.062, 133.264, 144.444, 146.681, 166.293, 168.039. APCI-MS: *m/z* 372.33 (MH^+, 100 %). Anal. Calcd for $C_{22}H_{17}N_3O_3$: C, 71.15; H, 4.58; N, 11.32 %. Found: C, 71.28; H, 4.65; N, 11.59 %.

*4-Amino-3-(1,3-dioxoisoindolin-2-yl)-N-(pyridin-2-ylmethyl) benzamide (**6yb**)* Yield: 94 %. mp: 283 °C. IR (KBr) ν_{max}: 3424 (NH_2), 1685 (>C=O), 1594, 1492 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 4.089 (s, 2H, CH_2), 5.189 (bs, 2H, NH_2, exch), 6.486–6.502 (d, 1H, J = 8 Hz), 7.059–7.079 (dd, 1H, J = 2 and 8 Hz), 7.135–7.138 (d, 1H, Ar, J = 1.5 Hz), 7.228–7.308 (q, 1H, Ar, J = 6 and 8 Hz), 7.387–7.413 (d, 1H, Ar), 7.588–7.616 (m, 2H, Ar), 7.659–7.685 (m, 2H, Ar), 7.687–7.799 (dt, 1H, Ar, J = 2 and 8 Hz), 8.127 (s, 1H, NH, exch), 8.452–8.462 (d, 1H, Ar, J = 5 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 47.392, 118.562, 119.990, 120.800, 123.190, 123.990, 125.553, 126.230, 127.232, 131.760, 132.062, 134.564, 144.884, 148.671, 156.793, 166.093, 168.333. APCI-MS: *m/z* 373.22 (MH^+, 100 %). Anal. Calcd for $C_{21}H_{16}N_4O_3$: C, 67.74; H, 4.30; N, 15.05 %. Found: C, 67.45; H, 4.37; N, 15.16 %.

*4-Amino-3-(1,3-dioxoisoindolin-2-yl)-N-(pyridin-3-ylmethyl) benzamide (**6yc**)* Yield: 96 %. mp: 274 °C. IR (KBr) ν_{max}: 3318 (NH_2), 1680, 1619 (>C=O), 1538 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 4.074 (s, 2H, CH_2), 5.181 (bs, 2H, NH_2, exch), 6.484–6.500 (d, 1H, Ar, J = 8 Hz), 7.058–7.078 (dd, 1H, Ar, J = 2 and 8 Hz), 7.133–7.137 (d, 1H, Ar, J = 2 Hz), 7.328–7.364 (dt, 1H, Ar, J = 2 and 8 Hz), 7.587–7.614 (m, 2H, Ar), 7.659–7.687 (m, 2H, Ar), 7.840–7.856 (d, 1H, Ar, J = 8 Hz), 8.127 (s, 1H, NH, exch), 8.325–8.412 (m, 2H, Ar). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 47.392, 116.511, 119.990, 123.190, 123.912, 124.553, 126.230, 127.232, 131.760, 132.062, 134.564, 135.564, 144.884, 148.671, 150.793, 166.032, 167.373. APCI-MS: *m/z* 373.28 (MH^+, 100 %). Anal. Calcd for $C_{21}H_{16}N_4O_3$: C, 67.74; H, 4.30; N, 15.05 %. Found: C, 67.81; H, 4.36; N, 15.23 %.

*4-Amino-3-(1,3-dioxoisoindolin-2-yl)-N-(pyridin-4-ylmethyl) benzamide (**6yd**)* Yield: 96 %. mp: >300 °C. IR (KBr) ν_{max}: 3375 (NH_2), 1698, 1619 (>C=O), 1536 (Ar) cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.089 (s, 2H, CH_2), 5.099 (bs, 2H, NH_2, exch), 6.485–6.501 (d, 1H, Ar, J = 8 Hz), 7.060–7.080 (dd, 1H, Ar, J = 2 and 8 Hz), 7.133–7.137 (d, 1H, Ar, J = 2 Hz), 7.323–7.340 (d, 2H, Ar, J = 8.5 Hz), 7.588–7.606 (m, 2H, Ar), 7.616–7.687 (m, 2H, Ar), 8.162 (s, 1H, NH, exch), 8.457–8.474 (d, 2H, Ar, J = 8.5 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 46.412, 116.516, 118.797, 123.929, 124.912, 127.553, 128.100, 129.232, 131.510, 132.192, 145.124, 147.351, 148.993, 166.035, 167.483. APCI-MS: m/z 373.85 (MH^+, 100 %). Anal. Calcd for $C_{21}H_{16}N_4O_3$: C, 67.74; H, 4.30; N, 15.05 %. Found: C, 67.63; H, 4.29; N, 15.11 %.

*4-Amino-3-(1,3-dioxoisoindolin-2-yl)-N-(furan-2-ylmethyl) benzamide (**6ye**)* Yield: 92 %. mp: 262 °C. IR (KBr) ν_{max}: 3378 (NH_2), 1682, 1619 (>C=O), 1535 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.074 (s, 2H, CH_2), 5.193 (bs, 2H, NH_2, exch), 6.182–6.212 (t, 1H, Ar, J = 8 Hz), 6.486–6.501 (d, 1H, Ar, J = 7.5 Hz), 6.731–6.748 (d, 1H, Ar, J = 8.5 Hz), 7.060–7.076 (d, 1H, Ar, J = 8 Hz), 7.134–7.137 (d, 1H, Ar, J = 1.5 Hz), 7.333–7.349 (d, 1H, Ar, J = 8 Hz), 7.588–7.605 (m, 2H, Ar), 7.615–7.687 (m, 2H, Ar), 8.115 (s, 1H, NH, exch). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 38.092, 106.266, 110.527, 116.717, 118.797, 123.292, 124.923, 126.063, 127.553, 131.510, 132.312, 142.454, 145.351, 148.912, 166.265, 167.313. APCI-MS: m/z 362.22 (MH^+, 100 %). Anal. Calcd for $C_{20}H_{15}N_3O_4$: C, 66.48; H, 4.15; N, 11.63 %. Found: C, 66.59; H, 4.21; N, 11.89 %.

*4-Amino-N-benzyl-3-(5,7-dioxo-5H-pyrrolo[3,4-b] pyrazin-6(7H)-yl)benzamide (**6za**)* Yield: 92 %. mp: 289 °C. IR (KBr) ν_{max}: 3317 (NH_2), 1692, 1619 (>C=O), 1536 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.092 (s, 2H, CH_2), 5.051 (bs, 2H, NH_2, exch), 6.486–6.501 (d, 1H, Ar, J = 7.5 Hz), 7.059–7.079 (dd, 1H, Ar, J = 2.5 and 8 Hz), 7.134–7.138 (d, 1H, Ar, J = 2 Hz), 7.279–7.340 (m, 5H, Ar), 8.130 (s, 1H, NH, exch), 8.704 (s, 2H, Ar). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 44.390, 116.023, 118.122, 123.529, 124.337, 125.139, 126.347, 127.834, 128.005, 141.869, 143.019, 145.710, 146.775, 164.272, 168.003. APCI-MS: m/z 374.53 (MH^+, 100 %). Anal. Calcd for $C_{20}H_{15}N_5O_3$: C, 64.34; H, 4.02; N, 18.76 %. Found: C, 64.23; H, 4.19; N, 18.88 %.

*4-Amino-3-(5,7-dioxo-5H-pyrrolo[3,4-b]pyrazin-6(7H)-yl)-N-(pyridin-2-ylmethyl)benzamide (**6zb**)* Yield: 93 %. mp: >300 °C. IR (KBr) ν_{max}: 3363 (NH_2), 1689, 1612 (>C=O), 1520, 1487 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.085 (s, 2H, CH_2), 5.193 (bs, 2H, NH_2, exch), 6.486–6.502 (d, 1H, Ar, J = 8 Hz), 7.058–7.078 (dd, 1H, Ar, J = 2 and 8 Hz), 7.134–7.138 (d, 1H, Ar, J = 2 Hz), 7.266–7.302 (q, 1H, Ar, J = 8 and 10 Hz), 7.388–7.415 (d, 1H, Ar, J = 13.5 Hz), 7.752–7.801 (dt, 1H, Ar, J = 2 and 8.5 Hz), 8.122 (s, 1H, NH, exch), 8.453–8.469 (d, 1H, Ar, J = 8 Hz), 8.776 (s, 2H, Ar). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 49.160, 116.727, 118.372, 120.529, 123.337, 124.139, 125.117, 127.333, 136.834, 143.705, 145.003, 145.869, 148.101, 156.373, 164.233, 167.753. APCI-MS: m/z 375.43 (MH^+, 100 %). Anal. Calcd for $C_{19}H_{14}N_6O_3$: C, 60.96; H, 3.74; N, 22.45 %. Found: C, 60.84; H, 3.92; N, 22.53 %.

*4-Amino-3-(5,7-dioxo-5H-pyrrolo[3,4-b]pyrazin-6(7H)-yl)-N-(pyridin-3-ylmethyl)benzamide (**6zc**)* Yield: 94 %. mp: >300 °C. IR (KBr) ν_{max}: 3283 (NH_2), 1666, 1616 (>C=O), 1519 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.097 (s, 2H, CH_2), 5.183 (bs, 2H, NH_2, exch), 6.483–6.499 (d, 1H, Ar, J = 8 Hz), 7.060–7.080 (dd, 1H, Ar, J = 2 and 8.5 Hz), 7.135–7.139 (d, 1H, Ar, J = 2 Hz), 7.329–7.365 (dt, 1H, Ar, J = 2 and 8 Hz), 7.843–7.859 (d, 1H, Ar, J = 8 Hz), 8.125 (s, 1H, NH, exch), 8.325–8.414 (m, 2H, Ar), 8.778 (s, 2H, Ar). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 46.960, 116.167, 118.242, 123.119, 124.337, 125.139, 127.277, 134.333, 135.834, 143.445, 145.303, 146.369, 147.410, 150.373, 163.953, 167.353. APCI-MS: m/z 375.65 (MH^+, 100 %). Anal. Calcd for $C_{19}H_{14}N_6O_3$: C, 60.96; H, 3.74; N, 22.45 %. Found: C, 60.83; H, 3.81; N, 22.33 %.

*4-Amino-3-(5,7-dioxo-5H-pyrrolo[3,4-b]pyrazin-6(7H)-yl)-N-(pyridin-4-ylmethyl)benzamide (**6zd**)* Yield: 96 %. mp: >300 °C. IR (KBr) ν_{max}: 3368 (NH_2), 1688, 1653 (>C=O), 1594, 1497 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.075 (s, 2H, CH_2), 5.099 (bs, 2H, NH_2, exch), 6.487–6.503 (d, 1H, Ar, J = 8 Hz), 7.060–7.080 (dd, 1H, Ar, J = 2.5 and 8.5 Hz), 7.134–7.138 (d, 1H, Ar, J = 2 Hz), 7.327–7.343 (d, 2H, Ar, J = 8 Hz), 8.134 (s, 1H, NH, exch), 8.458–8.474 (d, 2H, Ar, J = 8 Hz), 8.788 (s, 2H, Ar). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 44.663, 116.567, 118.333, 123.119, 124.337, 125.139, 126.834, 144.745, 145.134, 146.345, 147.310, 149.173, 163.833, 167.413. APCI-MS: m/z 375.65 (MH^+, 100 %). Anal. Calcd for $C_{19}H_{14}N_6O_3$: C, 60.96; H, 3.74; N, 22.45 %. Found: C, 60.86; H, 3.73; N, 22.37 %.

*4-Amino-3-(5,7-dioxo-5H-pyrrolo[3,4-b]pyrazin-6(7H)-yl)-N-(furan-2-ylmethyl)benzamide (**6ze**)* Yield: 93%. mp: 286 °C. IR (KBr) ν_{max}: 3231 (NH_2), 1678, 1624 (>C=O), 1512 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.085 (s, 2H, CH_2), 5.195 (bs, 2H, NH_2, exch), 6.182–6.212 (t, 1H, Ar, J = 7.5 Hz), 6.485–6.501 (d, 1H, Ar, J = 8 Hz), 6.730–6.746 (d, 1H, Ar, J = 8 Hz), 7.064–7.080 (dd, 1H, Ar, J = 8 Hz), 7.135–7.138 (d, 1H, Ar, J = 1.5 Hz), 7.330–7.346 (d, 1H, Ar, J = 8 Hz), 8.128 (s, 1H, NH, exch), 8.782 (s, 2H, Ar). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 38.555, 106.407, 110.303, 116.237, 118.333, 123.119, 124.197, 126.139, 142.774, 144.126, 145.134, 146.345, 148.173, 163.833, 167.413. APCI-MS: m/z 364.48

(MH$^+$, 100 %). Anal. Calcd for $C_{18}H_{13}N_5O_4$: C, 59.50; H, 3.58; N, 19.28 %. Found: C, 59.63; H, 3.67; N, 19.37 %.

*General procedure for the synthesis of benzimidazoisoindole and benzimidazopyrrolopyrazine derivatives (**7xa–7ze**)*

*Synthesis of N-benzyl-8-(1,2,3,4,4a,11a-hexahydro-11-oxobenzimidazo[2,1-a]isoindol)-amide (**7xa**)* 8-(1,2,3,4,4*a*,11*a*-Hexahydrobenzimidazo[2,1-*a*]isoindol-11-one)-oic-acid (0.270 g; 1.0 mmol) (**4x**; Fig. 1) and benzyl amine (0.107 g; 1.0 mmol) (**5a**; Fig. 1) were grinded together in a small mortar with a pestle for 20 min. TLC of the reaction mixture on silica gel using ethyl acetate:methanol (2:3) mobile phase indicated completion of the reaction. The crude product was purified by crystallization from methanol to give pure product *N*-benzyl-8-(1,2,3,4,4*a*,11*a*-hexahydro-11-oxobenzimidazo[2,1-*a*]isoindol)-amide (**7xa**; Fig. 1). Yield: 0.319 g (89 %). mp: >300 °C. IR (KBr) ν_{max}: 3288 (NH), 1697, 1635 (>C=O), 1515 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.374–1.446 (m, 4H, 2× CH_2), 1.626–1.678 (m, 2H, CH_2), 1.764–1.823 (m, 2H, CH_2), 2.647–2.738 (m, 2H, CH+CH), 4.089 (s, 2H, CH_2), 6.489–6.503 (d, 1H, Ar, J = 7 Hz), 6.864–6.884 (dd, 1H, Ar, J = 2 and 8 Hz), 7.134–7.138 (d, 1H, Ar, J = 2 Hz), 7.287–7.354 (m, 5H, Ar), 8.135 (s, 1H, NH, exch). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 24.215, 25.080, 26.657, 30.052, 30.804, 41.390, 44.424, 115.208, 116.442, 122.608, 126.775, 127.109, 127.941, 128.274, 130.941, 141.109, 142.108, 143.227, 167.898, 198.749. APCI-MS: *m/z* 360.87 (MH$^+$, 100 %). Anal. Calcd for $C_{22}H_{21}N_3O_2$: C, 73.52; H, 5.89; N, 11.69 %. Found: C, 73.64; H, 5.83; N, 11.78 %.

Compounds **7xb**–**7ze** were prepared using the above method.

*N-Pyridin-2-ylmethyl-8-(1,2,3,4,4a,11a-hexahydro-11-oxobenzimidazo[2,1-a]isoindol)-amide (**7xb**)* Yield: 93 %. mp: >300 °C. IR (KBr) ν_{max}: 3289 (NH), 1668, 1636 (>C=O), 1515 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.374–1.446 (m, 4H, 2× CH_2), 1.626–1.679 (m, 2H, CH_2), 1.765–1.823 (m, 2H, CH_2), 2.643–2.739 (m, 2H, CH+CH), 4.089 (s, 2H, CH_2), 6.497–6.513 (d, 1H, Ar, J = 8 Hz), 6.854–6.874 (dd, 1H, Ar, J = 2 and 8 Hz), 7.133–7.139 (d, 1H, Ar, J = 3 Hz), 7.233–7.418 (m, 2H, Ar), 7.743–7.779 (m, 1H, Ar), 8.138 (s, 1H, NH, exch), 8.450–8.466 (d, 1H, Ar, J = 8 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 24.215, 25.080, 26.657, 30.052, 30.804, 41.390, 49.024, 115.208, 116.165, 120.149, 122.497, 124.340, 128.024, 130.215, 136.080, 141.657, 142.352, 149.284, 156.524, 167.873, 198.908. APCI-MS: *m/z* 361.69 (MH$^+$, 100 %). Anal. Calcd for $C_{21}H_{20}N_4O_2$: C, 70.00; H, 5.55; N, 15.55 %. Found: C, 69.87; H, 5.50; N, 15.64 %.

*N-Pyridin-3-ylmethyl-8-(1,2,3,4,4a,11a-hexahydro-11-oxobenzimidazo[2,1-a]isoindol)-amide (**7xc**)* Yield: 87 %. mp: >300 °C. IR (KBr) ν_{max}: 3268 (NH), 1682, 1622 (>C=O), 1532, 1422 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.355–1.444 (m, 4H, 2× CH_2), 1.622–1.688 (m, 2H, CH_2), 1.761–1.837 (m, 2H, CH_2), 2.645–2.752 (m, 2H, CH+CH), 4.082 (s, 2H, CH_2), 6.493–6.509 (d, 1H, Ar, J = 8 Hz), 6.849–6.869 (dd, 1H, Ar, J = 2 and 8 Hz), 7.134–7.138 (d, 1H, Ar, J = 2 Hz), 7.333–7.369 (dt, 1H, Ar, J = 2 and 8 Hz), 7.842–7.858 (d, 1H, Ar, J = 8 Hz), 8.140 (s, 1H, NH, exch), 8.321–8.414 (m, 2H, Ar). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 24.134, 25.150, 26.757, 30.162, 30.884, 41.390, 44.344, 115.118, 116.555, 122.149, 124.597, 127.344, 130.524, 134.115, 135.884, 141.757, 142.162, 147.283, 150.723, 167.376, 198.798. APCI-MS: *m/z* 361.55 (MH$^+$, 100 %). Anal. Calcd for $C_{21}H_{20}N_4O_2$: C, 70.00; H, 5.55; N, 15.55 %. Found: C, 69.86; H, 5.64; N, 15.67 %.

*N-Pyridin-4-ylmethyl-8-(1,2,3,4,4a,11a-hexahydro-11-oxobenzimidazo[2,1-a]isoindol)-amide (**7xd**)* Yield: 95 %. mp: >300 °C. IR (KBr) ν_{max}: 3296 (NH), 1680, 1623 (>C=O), 1532, 1425 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.374–1.446 (m, 4H, 2× CH_2), 1.625–1.681 (m, 2H, CH_2), 1.765–1.842 (m, 2H, CH_2), 2.640–2.737 (m, 2H, CH+CH), 4.099 (s, 2H, CH_2), 6.487–6.503 (d, 1H, Ar, J = 8 Hz), 6.844–6.864 (dd, 1H, Ar, J = 2 and 8 Hz), 7.134–7.137 (d, 1H, Ar, J = 1.5 Hz), 7.327–7.343 (d, 2H, Ar, J = 8 Hz), 8.122 (s, 1H, NH, exch), 8.435–8.451 (d, 2H, Ar, J = 8 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 24.230, 25.142, 26.116, 30.076, 30.988, 41.390, 44.772, 115.529, 116.139, 122.106, 124.337, 127.376, 130.524, 140.757, 142.422, 147.130, 149.733, 167.283, 198.742. APCI-MS: *m/z* 361.35 (MH$^+$, 100 %). Anal. Calcd for $C_{21}H_{20}N_4O_2$: C, 70.00; H, 5.55; N, 15.55 %. Found: C, 69.87; H, 5.63; N, 15.44 %.

*N-Furan-2-ylmethyl-8-(1,2,3,4,4a,11a-hexahydro-11-oxobenzimidazo[2,1-a]isoindol)-amide (**7xe**)* Yield: 88 %. mp: >300 °C. IR (KBr) ν_{max}: 3201 (NH), 1688, 1670 (>C=O), 1612, 1478 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.374–1.446 (m, 4H, 2× CH_2), 1.626–1.679 (m, 2H, CH_2), 1.764–1.830 (m, 2H, CH_2), 2.661–2.716 (m, 2H, CH+CH), 4.082 (s, 2H, CH_2), 6.182–6.212 (t, 1H, Ar, J = 7.5 Hz), 6.484–6.500 (d, 1H, Ar, J = 8 Hz), 6.730–6.747 (d, 1H, Ar, J = 8.5 Hz), 6.863–6.879 (d, 1H, Ar, J = 8 Hz), 7.134–7.138 (d, 1H, Ar, J = 2 Hz), 7.330–7.346 (d, 1H, Ar, J = 8 Hz), 8.129 (s, 1H, NH, exch). ^{13}C NMR (125 MHz, DMSO-d_6)δ: 24.262, 25.308, 26.075, 30.041, 30.949, 38.274, 41.390, 106.529, 110.139, 115.529, 116.139, 122.337, 128.366, 130.822, 141.827, 142.495, 143.130, 148.710, 167.143, 199.003. APCI-MS: *m/z* 350.69 (MH$^+$, 100 %). Anal. Calcd for $C_{20}H_{19}N_3O_3$: C, 68.76; H, 5.48; N, 12.03 %. Found: C, 68.87; H, 5.53; N, 12.15 %.

*N-Benzyl-8-(11H,11-oxobenzimidazo[2,1-a]isoindol)-amide (**7ya**)* Yield: 93 %. mp: >300 °C. IR (KBr) ν_{max}: 3267 (NH), 1684, 1623 (>C=O), 1519 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 4.089 (s, 2H, CH_2), 6.486–6.501 (d, 1H, Ar, J = 7.5 Hz), 6.854–6.874 (dd, 1H, Ar, J = 2 and 8 Hz), 7.134–7.138 (d, 1H, Ar, J = 2 Hz), 7.279–7.340 (m, 5H, Ar), 7.579–7.619 (m, 1H, Ar), 7.659–7.695 (m, 2H, Ar), 7.842–7.858 (d, 1H, Ar, J = 8 Hz), 8.136 (s, 1H, NH, exch). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 44.724, 115.138, 116.120, 122.280, 126.165, 127.038, 127.975, 128.128, 128.949, 129.280, 130.861, 131.771, 135.154, 135.938, 137.037, 141.414, 142.131, 142.932, 167.556, 190.133. APCI-MS: *m/z* 354.82 (MH^+, 100 %). Anal. Calcd for $C_{22}H_{15}N_3O_2$: C, 74.78; H, 4.25; N, 11.89 %. Found: C, 74.71; H, 4.31; N, 11.96 %.

*N-Pyridin-2-ylmethyl-8-(11H,11-oxobenzimidazo[2,1-a]isoindol)-amide (**7yb**)* Yield: 94 %. mp: >300 °C. IR (KBr) ν_{max}: 3241 (NH), 1678, 1644 (>C=O), 1582, 1512, 1474 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 4.087 (s, 2H, CH_2), 6.484–6.500 (d, 1H, Ar, J = 8 Hz), 6.829–6.865 (m, 1H, Ar), 7.134–7.138 (d, 1H, Ar, J = 2 Hz), 7.266–7.413 (m, 2H, Ar), 7.587–7.605 (m, 1H, Ar), 7.614–7.685 (m, 2H, Ar), 7.733–7.749 (d, 1H, Ar, J = 8 Hz), 7.755–7.798 (dt, 1H, Ar, J = 1.5 and 8 Hz), 8.122 (s, 1H, NH, exch), 8.451–8.467 (d, 1H, Ar, J = 8 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 49.030, 115.224, 116.878, 120.280, 122.105, 124.065, 128.075, 128.949, 129.262, 130.280, 131.061, 135.001, 135.754, 136.138, 138.077, 141.414, 142.131, 148.262, 156.068, 167.556, 190.133. APCI-MS: *m/z* 355.35 (MH^+, 100 %). Anal. Calcd for $C_{21}H_{14}N_4O_2$: C, 71.18; H, 3.98; N, 15.81 %. Found: C, 71.29; H, 3.94; N, 15.90 %.

*N-Pyridin-3-ylmethyl-8-(11H,11-oxobenzimidazo[2,1-a]isoindol)-amide (**7yc**)* Yield: 91 %. mp: >300 °C. IR (KBr) ν_{max}: 3369 (NH), 1680, 1613 (>C=O), 1512 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 4.089 (s, 2H, CH_2), 6.488–6.503 (d, 1H, Ar, J = 7.5 Hz), 6.848–6.868 (dd, 1H, Ar, J = 2 and 8 Hz), 7.134–7.137 (d, 1H, Ar, J = 1.5 Hz), 7.347–7.387 (dt, 1H, Ar, J = 2 and 9 Hz), 7.587–7.605 (m, 1H, Ar), 7.614–7.677 (m, 2H, Ar), 7.759–7.775 (d, 1H, Ar, J = 8 Hz), 7.840–7.856 (d, 1H, Ar, J = 8 Hz), 8.127 (s, 1H, NH, exch), 8.324–8.413 (m, 2H, Ar). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 44.239, 115.084, 116.877, 122.280, 124.195, 127.165, 128.075, 129.244, 130.262, 131.190, 134.061, 135.106, 135.894, 136.138, 137.077, 141.110, 142.333, 147.060, 150.118, 167.986, 190.435. APCI-MS: *m/z* 355.12 (MH^+, 100 %). Anal. Calcd for $C_{21}H_{14}N_4O_2$: C, 71.18; H, 3.98; N, 15.81 %. Found: C, 71.28; H, 3.95; N, 15.93 %.

*N-Pyridin-4-ylmethyl-8-(11H,11-oxobenzimidazo[2,1-a]isoindol)-amide (**7yd**)* Yield: 96 %. mp: >300 °C. IR (KBr) ν_{max}: 3361 (NH), 1678, 1615 (>C=O), 1512 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 4.094 (s, 2H, CH_2), 6.487–6.503 (d, 1H, Ar, J = 8 Hz), 6.847–6.867 (dd, 1H, Ar, J = 1.5 and 8 Hz), 7.134–7.137 (d, 1H, Ar, J = 1.5 Hz), 7.323–7.340 (d, 2H, Ar, J = 8.5 Hz), 7.589–7.616 (m, 1H, Ar), 7.659–7.685 (m, 2H, Ar), 7.818–7.836 (d, 1H, Ar, J = 9 Hz), 8.118 (s, 1H, NH, exch), 8.457–8.473 (d, 2H, Ar, J = 8 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 44.075, 115.694, 116.599, 122.220, 124.105, 127.535, 128.365, 129.223, 130.061, 131.190, 135.003, 135.988, 137.062, 141.110, 142.949, 147.450, 150.008, 167.535, 190.447. APCI-MS: *m/z* 355.18 (MH^+, 100 %). Anal. Calcd for $C_{21}H_{14}N_4O_2$: C, 71.18; H, 3.98; N, 15.81 %. Found: C, 71.31; H, 3.94; N, 15.76 %.

*N-Furan-2-ylmethyl-8-(11H,11-oxobenzimidazo[2,1-a]isoindol)-amide (**7ye**)* Yield: 93 %. mp: >300 °C. IR (KBr) ν_{max}: 3312 (NH), 1697, 1653 (>C=O), 1594, 1497 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 4.086 (s, 2H, CH_2), 6.181–6.212 (t, 1H, Ar, J = 7.5 Hz), 6.484–6.500 (d, 1H, Ar, J = 8 Hz), 6.730–6.747 (d, 1H, Ar, J = 8.5 Hz), 6.863–6.879 (d, 1H, Ar, J = 8 Hz), 7.134–7.137 (d, 1H, Ar, J = 1.5 Hz), 7.330–7.347 (d, 1H, Ar, J = 8.5 Hz), 7.587–7.614 (m, 1H, Ar), 7.659–7.685 (m, 2H, Ar), 7.801–7.817 (d, 1H, Ar, J = 8 Hz), 8.124 (s, 1H, NH, exch). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 38.392, 105.999, 110.656, 115.559, 116.750, 122.880, 127.530, 128.965, 129.193, 130.061, 131.460, 135.883, 136.360, 137.062, 142.760, 143.441, 145.455, 148.898, 168.039, 190.277. APCI-MS: *m/z* 344.84 (MH^+, 100 %). Anal. Calcd for $C_{20}H_{13}N_3O_3$: C, 69.97; H, 3.78; N, 12.24 %. Found: C, 69.87; H, 3.87; N, 12.32 %.

*N-Benzyl-8-(11H,11-oxobenz[4′,5′]imidazo[1′,2:1,2]pyrrolo[3,4-b]pyrazin)-amide (**7za**)* Yield: 91 %. mp: >300° C. IR (KBr) ν_{max}: 3309 (NH), 1683, 1612 (>C=O), 1544, 1513 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 4.086 (s, 2H, CH_2), 6.486–6.501 (d, 1H, Ar, J = 7.5 Hz), 6.855–6.875 (dd, 1H, Ar, J = 2.5 and 8 Hz), 7.135–7.139 (d, 1H, Ar, J = 2 Hz), 7.279–7.342 (m, 5H, Ar), 8.222 (s, 1H, NH, exch), 8.840 (s, 2H, Ar). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 44.39, 115.155, 116.023, 122.122, 126.529, 127.337, 127.939, 128.347, 130.834, 140.005, 141.869, 142.405, 143.019, 147.710, 148.775, 149.272, 168.003, 191.018. APCI-MS: *m/z* 356.53 (MH^+, 100 %). Anal. Calcd for $C_{20}H_{13}N_5O_2$: C, 67.60; H, 3.66; N, 19.71 %. Found: C, 67.49; H, 3.64; N, 19.79 %.

*N-Pyridin-2-ylmethyl-8-(11H,11-oxobenz[4′,5′]imidazo[1′,2:1,2]pyrrolo[3,4-b]pyrazin)-amide (**7zb**)* Yield: 90 %. mp: >300 °C. IR (KBr) ν_{max}: 3404 (NH), 1664, 1614 (>C=O), 1476 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 4.099 (s, 2H, CH_2), 6.484–6.499 (d, 1H, Ar, J = 7.5 Hz), 7.060–7.078 (dd, 1H, Ar, J = 2.5 and 7 Hz),

7.134–7.139 (d, 1H, Ar, J = 2.5 Hz), 7.225–7.261 (q, 1H, Ar, J = 8 and 10 Hz), 7.388–7.413 (d, 1H, Ar), 7.749–7.799 (dt, 1H, Ar, J = 1.5 and 8.5 Hz), 8.120 (s, 1H, NH, exch), 8.450–8.466 (d, 1H, Ar, J = 8 Hz), 8.846 (s, 2H, Ar). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 49.274, 115.155, 116.023, 121,375, 123.122, 124.529, 128.337, 130.139, 136.347, 140.834, 141.005, 142.869, 147.405, 148.019, 148.710, 150.775, 156.272, 168.011, 191.005. APCI-MS: m/z 357.83 (MH^+, 100 %). Anal. Calcd for $C_{19}H_{12}N_6O_2$: C, 64.04; H, 3.37; N, 23.59 %. Found: C, 64.15; H, 3.43; N, 23.67 %.

*N-Pyridin-3-ylmethyl-8-(11H,11-oxobenz[4′,5′]imidazo[1′,2:1,2]pyrrolo[3,4-b]pyrazin)-amide (**7zc**)* Yield: 88 %. mp: >300 °C. IR (KBr) ν_{max}: 3293 (NH), 1666, 1613 (>C=O), 1474 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.082 (s, 2H, CH_2), 6.486–6.503 (d, 1H, Ar, J = 8.5 Hz), 6.849–6.869 (dd, 1H, Ar, J = 2 and 8 Hz), 7.132–7.137 (d, 1H, Ar, J = 2.5 Hz), 7.339–7.375 (dt, 1H, Ar, J = 2 and 8 Hz), 7.842–7.858 (d, 1H, Ar, J = 8 Hz), 8.128 (s, 1H, NH, exch), 8.324–8.413 (m, 2H, Ar), 8.875 (s, 2H, Ar). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 44.788, 115.155, 116.023, 122.375, 123.122, 127.529, 131.337, 134.139, 135.374, 140.830, 141.001, 142.864, 147.077, 147.999, 148.710, 149.585, 150.222, 167.031, 190.481. APCI-MS: m/z 357.60 (MH^+, 100 %). Anal. Calcd for $C_{19}H_{12}N_6O_2$: C, 64.04; H, 3.37; N, 23.59 %. Found: C, 64.35; H, 3.54; N, 23.72 %.

*N-Pyridin-4-ylmethyl-8-(11H,11-oxobenzo[4′,5′]imidazo[1′,2:1,2]pyrrolo[3,4-b]pyrazin)-amide (**7zd**)* Yield: 96 %. mp: >300 °C. IR (KBr) ν_{max}: 3288 (NH), 1670 (>C=O), 1596, 1532, 1422, (Ar) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.103 (s, 2H, CH_2), 6.487–6.503 (d, 1H, Ar, J = 8 Hz), 6.847–6.867 (dd, 1H, Ar, J = 2 and 8 Hz), 7.133–7.137 (d, 1H, Ar, J = 2 Hz), 7.334–7.350 (d, 2H, Ar, J = 8 Hz), 8.122 (s, 1H, NH, exch), 8.431–8.447 (d, 2H, Ar, J = 8 Hz), 8.912 (s, 2H, Ar). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 44.128, 115.199, 116.022, 122.975, 124.122, 128.929, 131.920, 141.831, 142.001, 142.864, 147.038, 147.997, 148.718, 149.481, 150.112, 167.441, 190.149. APCI-MS: m/z 357.29 (MH^+, 100 %). Anal. Calcd for $C_{19}H_{12}N_6O_2$: C, 64.04; H, 3.37; N, 23.59 %. Found: C, 64.41; H, 3.12; N, 23.73 %.

*N-Furan-2-ylmethyl-8-(11H,11-oxobenzo[4′,5′]imidazo[1′,2:1,2]pyrrolo[3,4-b]pyrazin)-amide (**7ze**)* Yield: 91 %. mp: >300 °C. IR (KBr) ν_{max}: 3317 (NH), 1682, 1619 (>C=O), 1536 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.101 (s, 2H, CH_2), 6.182–6.213 (t, 1H, Ar, J = 7.5 Hz), 6.484–6.500 (d, 1H, Ar, J = 8 Hz), 6.731–6.746 (d, 1H, Ar, J = 7.5 Hz), 6.862–6.878 (d, 1H, Ar, J = 8 Hz), 7.134–7.139 (d, 1H, Ar, J = 2.5 Hz), 7.330–7.346 (d, 1H, Ar, J = 8 Hz), 8.133 (s, 1H, NH, exch), 8.888 (s, 2H, Ar). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 38.724, 106.280, 110.165, 115.038, 116.975, 123.128, 128.949, 130.980, 141.861, 142.071, 142.864, 143.938, 147.037, 148.418, 149.131, 150.032, 167.426, 190.133. APCI-MS: m/z 346.80 (MH^+, 100 %). Anal. Calcd for $C_{18}H_{11}N_5O_3$: C, 62.61; H, 3.18; N, 20.29 %. Found: C, 62.72; H, 3.26; N, 20.37 %.

Anti-inflammatory activity

Paw edema inhibition test was used on albino rats of Charles Foster by adopting the method of Winter et al. [37]. Groups of five animals of both sexes (body weight 120–160 g), excluding pregnant females, were given a dose of test compound. Thirty minutes later, 0.20 mL of 1 % freshly prepared carrageenan suspension in 0.9 % NaCl solution was injected subcutaneously into the planter aponeurosis of the hind paw and the volume was measured by a water plethysmometer apparatus and then measured again 1–3 h later. The mean increase of paw volume at each interval was compared with that of control group (five rats treated with carrageenan but not with test compound) at the same intervals and percent inhibition value calculated by the formula given below

$$\%\ \text{anti-inflammatory activity} = [1 - D_t/D_c] \times 100$$

D_t and D_c are paw volumes of edema in tested and control groups, respectively.

In vitro cytotoxicity against human cancer cell lines

Human breast (T47D), colon (HCT-15), lung (NCI-H522), liver (HepG-2), and ovary (PA-1) cancer cell lines were obtained from National Center for Cell Science (NCCS), Pune, India. Cells were grown in tissue culture flask in complete growth medium (RPMI-1640 medium with 2 mM glutamine, pH 7.4 supplemented with 10 % fetal bovine serum, 100 μg/mL streptomycin, and 100 U/mL penicillin) in a carbon dioxide incubator (37 °C, 5 % CO_2, 90 % RH). All cell culture reagents were from GIBCO (Invitrogen, USA). Penicillin, streptomycin, MTT (3-(4,5-dimethyl-2-thiazolyl)2,5-diphenyl-2H-tetrazoliumbromide), cell culture grade DMSO-d_6, 5-FU, cyclophosphamide, and actidione (cycloheximide) were from Himedia (Mumbai, India).

MTT assay was carried out as described previously [38]. In brief, 5 × 10^3 cells in 200 μL medium were seeded in 96-well plates (Griener, Germany). Serial dilutions of compound initially ranging from 0 to 100 μM in DMSO-d_6 were added to the monolayer. The final DMSO-d_6 concentration for all dilutions was 0.1 % which was used as vehicle control. The cultures were assayed after 24 h by the addition of 50 μL 5 mg/mL MTT and incubating for another 4 h at 37 °C. The MTT-containing medium was aspirated and 200 μL DMSO-d_6 (Himedia, Mumbai, India) and 25 μL Sorensen glycine

buffer (0.1 M glycine and 0.1 M NaCl, pH 10.5) were added to lyse the cells and solubilize the water insoluble formazone. Absorbance of the lysates was determined on a Fluostar optima (BMG Labtech, Germany) microplate reader at 570 nm.

The percentage inhibition was calculated as:

$$\frac{\text{Mean OD of vehicle} - \text{treated cells (negative control)} - \text{mean OD of treated cells} \times 100}{\text{Mean OD of vehicle} - \text{treated cells (negative control)}}$$

The IC_{50} values were calculated using graph pad prism, version 5.02 software (Graph Pad Software Inc., CA, USA).

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