Bioorganic & Medicinal Chemistry Letters 20 (2010) 2306–2310

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Solvent free synthesis, anti-inflammatory and anticancer activity evaluation of tricyclic and tetracyclic benzimidazole derivatives

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ARTICLE INFO

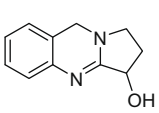
Article history:
Received 8 November 2009
Revised 5 January 2010
Accepted 29 January 2010
Available online 2 February 2010

Keywords:
Benzimidazole
Heterocyclic
Anti-inflammatory
Anticancer
Microwave

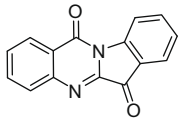
ABSTRACT

Heterocyclic benzimidazole derivatives **3a–h**, **5a–c** and **7a–d** have been synthesized by condensation of succinic acid (**1**) homophthalic acid (**4**) and 2,3-pyrazinedicarboxlic acid (**6**) with various substituted diamines under microwave irradiation in good yields. Structures assigned to **3a–h**, **5a–c** and **7a–d** are fully supported by spectral data. All these compounds were screened for anti-inflammatory and anticancer activities. At a dose of 50 mg/kg po compounds **3b** (39.4%) and **3c** (39.2%) exhibited anti-inflammatory activity, comparable to standard ibuprofen which showed 39% activity at 50 mg/kg po and compound **7c** exhibit good anticancer activity against ovary (IGR-OV-1), breast (MCF-7) and CNS(SF-295) human cancer cell lines.

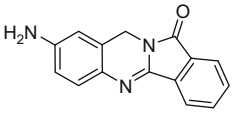
Synthesis and biological evaluation of heterocyclic compounds containing benzimidazole and isoquinoline moieties is an area of current research.[1] Benzimidazole derivatives are reported to exhibit antitumor[2a,b] and anti-inflammatory[3] activities. These compounds also act as COX-2 and LOX-5[4] and topoisomerase inhibitors.[5] Isoquinoline derivatives exhibiting anti-inflammatory,[6a,b] anticancer[7a,b] and other pharmacological activities[8a–d] are also described in literature. Natural products vasicine,[9a] and tryptanthrin[9b] exhibited potent anti-inflammatory activity where as synthetic heterocyclic compound batracyclin[9c] exhibited antitumor activity.



Vasicine



Tryptanthrin



Batracyclin

In recent years scientists have shown great interest in microwave irradiation technology because of their unique features, that is, solvent free reactions, low waste, energy efficiency, high yield and short reaction time. Use of microwave chemistry and solvent free reaction conditions allow us to synthesize a large number of compounds in a very short period of time. With this hypothesis in mind and in continuation of our work[10a–c] in search of potent molecules exhibiting anti-inflammatory and anticancer activities, we have synthesized a number of tricyclic and tetracyclic benzimidazole derivatives under solvent free condition and screened them for anti-inflammatory and anticancer activity, which we wish to report in this Letter.

A number of tricyclic benzimidazole derivatives **3a–h** have been synthesized by following reaction Scheme 1. Condensation of succinic acid **1** (Scheme 1) with various diamines **2a–h** (Scheme 1) was carried out by mixing both the reactants in 1:1 molar ratio, and irradiating them in microwave oven for 4–8 min at a power level of 850 W. Condensed product so obtained was purified by crystallization from methanol to give pure product. Irradiation time and percentage yield for compounds **3a–h** are summarized in Table 1. Formation of tricyclic system occur via an intermediate **3′**, in this reaction more nucleophilic amino group of diamines[11] will react first giving rise to formation of N-substituted cyclic imide[12a–c] **3′** which then undergoes further cyclization to give tricyclic product **3**. In case of **2a** and **2b** diamines are symmetrical in structure and hence there is formation of only product **3a** and **3b**. In case of **2c** and **2g** amino group ortho to –OH group and $-CH_3$ group are more nucleophilic (due to activating effect of –OH and $-CH_3$ group) as compared to amino group at *meta* position of –OH and $-CH_3$ group hence N-substituted cyclic imide **3′** is formed with the amino group ortho to –OH and $-CH_3$ group which after cyclization gave **3c** and **3g**, respectively. In case of dimines **2d**, **2e**, **2f** and **2h** deactivating group, that is, –Br, $-NO_2$, –CN and –Cl

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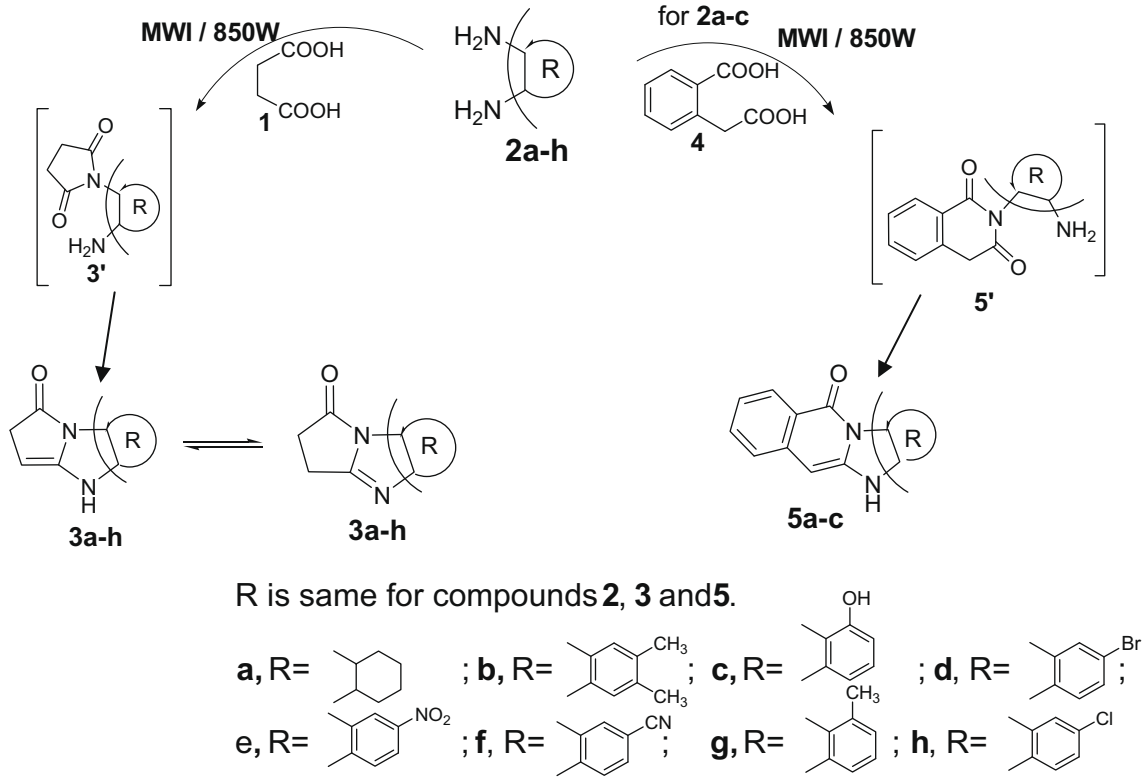
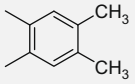
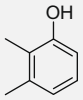
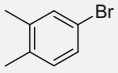
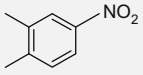
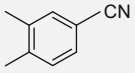
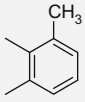
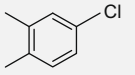
doi:10.1016/j.bmcl.2010.01.147



Scheme 1. synthesis of tricyclic **3a**–**h** and tetracyclic **5a**–**c** benzimidazole derivatives.

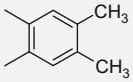
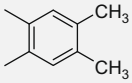
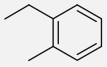
Table 1
Irradiation time, percentage yield and melting point of compounds **3a–h**

Compd no.	R	Irradiation time (min)	Yield (%)	Melting point (°C)
3a		4	98	136
3b		6	93	215
3c		5	85	255
3d		8	90	212–218
3e		8	92	165–169
3f		5	89	185
3g		5	92	158–160
3h		6	95	164–167

are present which makes amino group at *meta* position more nucleophilic than amino group at *para* position. In these cases cyclic imide **3′** is formed by amino group present at *meta* position and then undergoing cyclization to give **3d**, **3e**, **3f** and **3h**, respectively. Physical constants, spectral and analytical data of compounds **3a–h** reported in this Letter[13] fully support the structures assigned to them.

A series of tetracyclic benzimidazole derivatives **5a–c** have been synthesized by condensation of homophthalic acid **4** (Scheme 1), with various diamines **2a–c** (Scheme 1). Condensation was carried

Table 2
Irradiation time, percentage yield and melting point of compounds **5a–c** and **7a–d**

Compd no.	R	Time (min)	Yield (%)	Melting point
5a		5	98	235
5b		6	93	268–270
5c		5	95	290–292
7a		4	95	237
7b		5	85	210–212
7c		6	80	198
7d		5	90	215

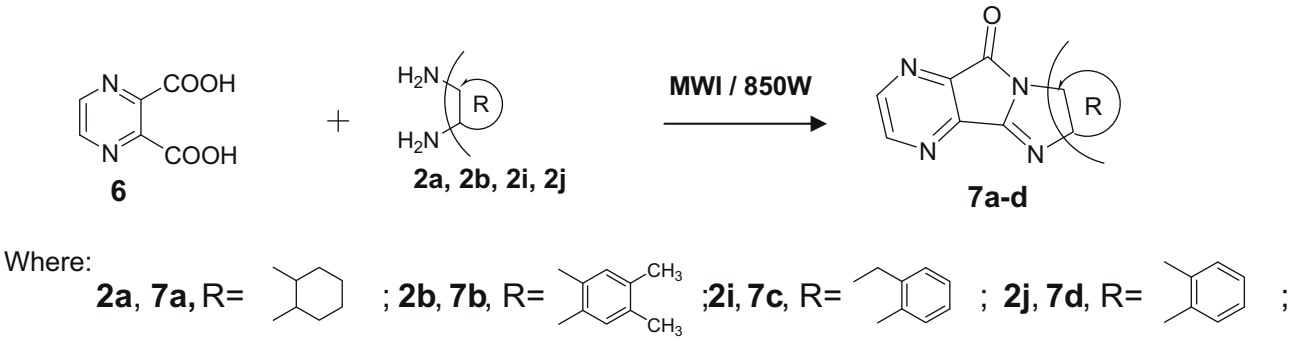
out by mixing both the reactants in 1:1 molar ratio and irradiating them in microwave oven for 4–8 min at a power level of 850 W. Condensed products so obtained were purified by crystallization from DMF to give pure products. Irradiation time and percentage yield for compounds **5a–c** are summarized in Table 2. Diamines **2a** and **2b** react with homophthalic acid **4** to form tetracyclic heterocyclic compounds **5a** and **5b**, respectively, in this reaction formation of **5a** and **5b** occur via formation of N-substituted cyclic imide **5′**. In case of **2c** strong activating effect of –OH makes amino group at *ortho* position a strong nucleophile and only one tetracyclic product, that is, **5c** is formed. Physical constants, spectral and analytical data of compounds **5a–c** reported in this Letter[13] fully support the structures assigned to them.

More complex tetracyclic benzimidazole derivatives **7a–d** (Scheme 2) were synthesized by condensation of 2,3-pyrazinedicarboxlic acid **6** with diamines **2a**, **2b**, **2i** and **2j**. Condensation was carried out by mixing both the reactants in 1:1 molar ratio and irradiating them in microwave oven for 4–6 min at a power level of 850 W. Condensed products so obtained were purified by crystallization from DMF to give pure product **7a–d**. Irradiation time and percentage yield of **7a–d** are summarized in Table 2. Physical constants, spectral and analytical data of compounds **7a–d** reported in this Letter[13] fully support the structures assigned to them.

Anti-inflammatory activity[14] evaluation of **3a–h**, **5a–c** and **7a–d** was carried out using carrageenan induced paw oedema assay and results are summarized in Table 3. Groups of five animals of both sexes (body weight 120–160 g), excluding pregnant females, were given a dose (50 mg/kg po) of test compound. Thirty minute 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 was calculated. A look at Table 3 indicates that compounds **3b**, **3c**, **5a** and **5c** exhibited interesting, that is, 39.4%, 39.2%, 34.7% and 31.8% anti-inflammatory activity, respectively, whereas standard drug ibuprofen exhibited 39% anti-inflammatory activity at 50 mg/kg po. Compounds **3b** and **3c** exhibited anti-inflammatory activity comparable to standard drug ibuprofen.

In vitro anticancer activity[15a,b] evaluation of compounds **3a–h**, **5a–c** and **7a–d** was carried out against various human cancer cell lines consisting of lung (A-549, HOP-62), prostate (PC-3), ovary (IGR-OV-1), breast (MCF-7) and CNS (SF-295). The human cancer cell lines procured from National Cancer Institute, Frederick, USA were used in present study. Cells were grown in tissue culture flasks 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 units/mL penicillin) in a carbon dioxide incubator (37 °C, 5% CO_2, 90% RH). The cells at subconfluent stage were harvested from the flask by treatment with trypsin (0.05% in PBS (pH 7.4) containing 0.02% EDTA). Cells with viability of more than 98%, as determined by trypan blue exclusion, were used for determination of cytotoxicity. The cell suspension of 1×10^5 cells/mL was prepared in complete growth medium. Stock 4×10^{-2} M compound solutions were prepared in DMSO. The stock solutions were serially diluted with complete growth medium containing 50 μg/mL of gentamycin to obtained working test solution of required concentrations.

In vitro cytotoxicity against various human cancer cell lines was determined (Monks et al.)[15a] using 96-well tissue culture plates. The 100 μL of cell suspension was added to each well of the 96-well tissue culture plates. The cells were allowed to grow in CO_2 incubator (37 °C, 5% CO_2, 90% RH) for 24 h. The test materials in complete growth medium (100 μL) were added after 24 h incubation to the wells containing cell suspension. The plates were further incubated for 48 h (37 °C in an atmosphere of 5% CO_2 and 90% relative humidity) in a carbon dioxide incubator after addition of test material and then the cell growth was stopped by gently layering trichloroacetic acid (50% TCA, 50 μL) on top of the medium in all the wells. The plates were incubated at 4 °C for 1 h to fix the cells attached to the bottom of the wells. The liquid of all the wells was gently pipetted out and discarded. The plates were washed five times with distilled water to remove TCA, growth medium low molecular weight metabolites, serum proteins etc. and air-



Scheme 2. Synthesis of tetracyclic compounds **7a–d**.

Table 3
Anti-inflammatory and anticancer activity of compounds **3a–h**, **5a–c** and **7a–d**

Compd no.	Anti-inflammatory activity (%) at 50 mg/kg po	Anticancer activity at a concentration of 1×10^{-5} M% growth inhibition					
		Lung		Prostate	Ovary	Breast	CNS
		A-549	HOP-62	PC-3	IGR-OV-1	MCF-7	SF-295
3a	19.7	2	0	16	2	13	38
3b	**39.4**	0	4	7	26	8	**54**
3c	**39.2**	0	9	13	20	22	**53**
3d	10.0	12	16	40	**50**	31	0
3e	0.0	5	0	8	0	12	38
3f	9.0	6	0	3	21	25	**52**
3g	29.9	2	0	14	3	08	38
3h	27.6	3	11	14	0	**52**	37
5a	**34.7**	10	12	6	28	6	0
5b	29.6	41	0	39	7	22	13
5c	**31.8**	0	0	13	15	20	**55**
7a	25.6	10	6	15	28	48	37
7b	15.9	25	0	39	7	22	13
7c	28.6	48	23	46	**55**	**56**	**66**
7d	10.0	0	10	13	15	20	25
Ibuprofen	39.0	—	—	—	—	—	—
Mito-C	—	—	—	53	—	—	43
Adri.	—	—	—	—	—	72	69
Pact.	—	50	42	—	43	—	—

dried. Cell growth was measured by staining with sulforhodamine B dye (Skehan et al.).[15b] The adsorbed dye was dissolved in Tris–HCl Buffer (100 μL, 0.01 M, pH 10.4) and plates were gently stirred for 10 min on a mechanical stirrer. The optical density (OD) was recorded on ELISA reader at 540 nm.

Percentage (%) growth inhibition of cancer cell lines was determined at a concentration of 10 μM and results are summarized in Table 3. A look at Table 3 indicates that compounds **3b**, **3c**, **3f**, **5c** and **7c** exhibited good anticancer activity against CNS (SF-295) cancer cell lines, that is, 54%, 53%, 52%, 55% and 66%, respectively. Compounds **3d** and **7c** showed good anticancer activity against ovary (IGR-OV-1) cancer cell line , that is, 50% and 55%, respectively, and compounds **3h** and **7c** possess good anticancer activity against breast (MCF-7) cancer cell line , that is, 52% and 56%, respectively.

In summary a number of tricyclic and tetracyclic benzimidazole derivatives have been synthesized in high yields in a very short time period using microwave irradiation technique. These heterocyclic compounds were screened for anti-inflammatory and anticancer activities. Compounds **3b** and **3c** exhibited anti-inflammatory activity comparable to standard drug ibuprofen and compounds **7c** exhibited good anticancer activity against ovary (IGROV-1), breast (MCF-7) and CNS (SF-295) human cancer cell lines.

Acknowledgements

We are thankful to technical staff of the Chemistry Department, I. I. T. Roorkee, for spectroscopic studies and elemental analysis. One of the authors Ms. Reshma Rani (SRF-NET) is thankful to CSIR, New Delhi, for financial assistance.

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13. *General*: Microwave oven model M197DL (Samsung) was used for microwave irradiation. Compounds **3a–h** were purified by crystallization from methanol whereas all other compounds **5a–c** and **7a–d** were purified by crystallization from DMF. 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 NMR spectra were recorded on a Bruker WH-500 spectrometer at a ca. 5–15% (w/v) solution in DMSO-d_6 (TMS as internal standard). GC–MS was recorded on Perkin Elmer Clarus 500 gas chromatograph where built in MS detector was used. 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). *Reaction procedure for synthesis of* **3a**: Succinic acid (0.250 g, 2.1 mmol) and *o*-cyclohexane-1,2-diamine (0.240 g, 2.1 mmol) were mixed together thoroughly to form fine powder. This fine powder was subjected to microwave irradiation for 5 min at a power level of 850 W. Completion of reaction was checked by TLC. Crude reaction product was washed with water. The product so obtained was further purified by crystallization from methanol. Yield 358 mg (95%) mp 136 °C. Similarly compounds **3b–h**, **5a–c** and **7a–d** were synthesized. *Spectral and analytical data of tricyclic and tetracyclic compounds* **3a–h**, **5a–c** and **7a–d**; (**3a**) IR(KBr) ν_{max}: 3252 (NH), 1656 (–CO–), 1598 (–C=C–) cm^{-1}. ^{1}H NMR (500 MHz; DMSO-d_6) δ: 1.19–1.31 (m, 4H, aliphatic), 1.55–1.66 (m, 5H, aliphatic), 1.902–1.922 (d, 2H, J = 10 Hz, aliphatic), 2.630–2.645 (d, 2H, J = 7.5 Hz, aliphatic), 3.102–3.112 (d, 1H, J = 5 Hz). GC–MS m/z 178 ($M^{+\cdot}$, 34%). Anal. Calcd for $C_{10}H_{14}N_2O$: C, 67.42; H, 7.86; N, 15.73. Found: C, 67.40; H, 7.81; N, 15.71. Compound **3b** IR(KBr) ν_{max}: 3129 (NH), 1703 (–CO–), 1630 (–C=C–), 1579, 1472, 1415 (Ar) cm^{-1}. ^{1}H NMR (500 MHz; DMSO-d_6) δ: 2.269 (s, 6H, 2 × CH_3), 2.741–2.771 (t, 1H, J = 7.5 Hz, one H of CH_2), 2.991–3.001 (t, 1H, J = 7.5 Hz, one

H of CH_2), 3.485 (s, 1H, –CH–), 7.24 (s, 2H, Ar), 12.11 (s, 1H, NH exch.). GC–MS *m*/*z* 200 ($M^{+\cdot}$, 87%). Anal. Calcd for $C_{12}H_{12}N_2O$: C, 72.00; H, 6.00; N, 14.00. Found: C, 71.98; H, 6.00; N, 13.5. Compound **3c** IR(KBr) ν_{max}: 3410, 3068 (NH & OH), 1705 (–CO–), 1631 (–C=C–), 1597, 1486, 1408 (Ar) cm^{-1}. ^1H NMR (500 MHz; DMSO-d_6) δ: 2.764–2.793 (t, *J* = 7.0 Hz, 2H, CH_2), 2.989–3.018 (t, *J* = 7.0 Hz, 2H, CH_2), 6.50–6.53 (m, 1H, Ar), 6.87–6.91 (m, 2H, Ar), 9.674 (s, 1H, OH exch.). GC–MS *m*/*z* 188 ($M^{+\cdot}$, 55%). Anal. Calcd for $C_{10}H_8N_2O_2$: C, 63.82; H, 4.25; N, 14.89. Found: C, 63.80; H, 4.21; N, 14.85. Compound (**3d**) IR(KBr) ν_{max}: 3427 (NH),1707 (–CO–), 1631 (–C=C–), 1583, 1491, 1459 (Ar) cm^{-1}. ^1H NMR (500 MHz; DMSO-d_6) δ: 2.77–2.79 (t, 1H, one H of CH_2), 3.001–3.032 (t, 1H, one H of CH_2), 3.30 (s, 1H, –CH–), 6.93–6.95 (d, 1H, Ar), 7.25–7.26 (d, 1H, Ar), 7.33–7.36 (m, 1H, Ar), 12.10 (s, 1H, NH exch.). GC–MS *m*/*z* 253 ($M+1_{Br81}^{+\cdot}$, 23%), 252 ($M_{Br81}^{+\cdot}$, 17%), 251 ($M+1_{Br79}^{+\cdot}$, 64%), 250 ($M+1_{Br79}^{+\cdot}$, 19%). Anal. Calcd for $C_{10}H_7N_2OBr$: C, 70.97; H, 5.38; N, 15.05. Found: C, 70.95; H, 5.30; N, 15.00. Compound **3e** IR(KBr) ν_{max}: 3112 (NH), 1703 (–CO–), 1626 (–C=C–), 1516 & 1472 (Ar) cm^{-1}. ^1H NMR (500 MHz; DMSO-d_6) δ: 2.814–2.844 (t, *J* = 7.5 Hz, 1H, one H of CH_2), 3.106–3.134 (t, *J* = 7.0 Hz, 1H, one H of CH_2), 3.51 (s, 1H, –CH=), 7.705–7.722 (d, *J* = 8.5 Hz, 1H, Ar), 8.071–8.089 (d, *J* = 9.0 Hz, 1H, Ar), 8.54 (s, 1H, Ar), 13.1 (s, 1H, NH exch.). GC–MS *m*/*z* 217 ($M^{+\cdot}$, 100%). Anal. Calcd for $C_{10}H_7N_3O_3$: C, 50.30; H, 3.22; N, 19.35. Found: C, 50.28; H, 3.20; N, 19.30. Compound **3f** IR(KBr) ν_{max}: 3111 (NH), 2218 (–CN), 1703 (–CO–), 1627 (–C=C–), 1517, 1471 (Ar) cm^{-1}. ^1H NMR (500 MHz; DMSO-d_6) δ: 2.80–2.82 (m, 1H, one H of CH_2), 3.04–3.09 (m, 1H, one H of CH_2), 3.46 (s, 1H, –CH=), 7.51–7.52 (m, 1H, Ar), 7.71–7.19 (m, 1H, Ar), 8.25 (s, 1H, Ar), 12.55 (s, 1H, NH exch.). GC–MS *m*/*z* 197 ($M^{+\cdot}$, 45%). Anal. Calcd for $C_{11}H_7N_3O$: C, 67.00; H, 3.55; N, 21.32. Found: C, 66.97; H, 3.51; N, 21.32. Compound **3g** IR(KBr) ν_{max}: 3427 (NH), 1707 (–CO–), 1631 (–C=C–), 1583, 1491, 1459 (Ar) cm^{-1}. ^1H NMR (500 MHz; DMSO-d_6) δ: 2.33 (s, 3H, CH_3), 2.766–2.795 (t, *J* = 7.5 Hz, 1H, one H of CH_2), 3.000–3.029 (t, *J* = 7.5 Hz, 1H, one H of CH_2), 3.30 (s, 1H, –CH–), 6.930–6.947(d, *J* = 8.5 Hz, 1H, Ar), 7.248–7.259 (d, *J* = 5.5 Hz, 1H, Ar), 7.33–7.36 (m, 1H, Ar), 12.05 (s, 1H, NH exch.). GC–MS *m*/*z* 186 ($M^{+\cdot}$, 63%). Anal. Calcd for $C_{11}H_{10}N_2O$: C, 70.97; H, 5.38; N, 15.05. Found: C, 70.95; H, 5.30; N, 15.00. Compound **3h** MeOH; IR(KBr) ν_{max}: 3114 (NH),1698 (–CO–), 1627 (–C=C–), 1516, 1471, 1417 (Ar) cm^{-1}. ^1H NMR (500 MHz; DMSO-d_6) δ: 2.777–2.806 (t, *J* = 7.5 Hz, 1H, one H of CH_2), 3.026–3.055 (t, *J* = 7.5 Hz, 1H, one H of CH_2), 3.30 (s, 1H, -CH-), 7.14–7.16 (m, 1H, Ar), 7.467–7.484 (d, *J* = 8.5 Hz, 1H, Ar), 7.532–7.535 (d, *J* = 1.5 Hz, 1H, Ar), 12.26 (s, 1H, NH exch.). GC–MS *m*/*z* 208 ($M_{Cl37}^{+\cdot}$, 15%), 206 ($M_{Cl35}^{+\cdot}$, 43%). Anal. Calcd for $C_{10}H_7N_2OCl$: C, 58.25; H, 5.398; N, 13.59. Found: C, 58.20; H, 5.36; N, 13.55. Compound **5a** IR(KBr) ν_{max}: 3144 (NH), 1650 (–CO–), 1535 & 1446 (Ar) cm^{-1}. ^1H NMR (500 MHz; DMSO-d_6) δ: 1.44–2.03 (m, 8H, aliphatic), 3.11–3.15 (m, 1H, aliphatic), 3.53–3.54 (m, 1H, aliphatic), 5.67 (s, 1H, CH), 7.02–7.09 (s + m, 2H, NH one H exch., one H Ar), 7.28- 7.32 (m, 1H, Ar), 7.43–746 (m, 1H, Ar), 7.951–7.966 (d, *J* = 7.5 Hz, 1H, Ar). GC–MS *m*/*z* 240 ($M^{+\cdot}$, 100%). Anal. Calcd for $C_{15}H_{16}N_2O$: C, 75.00; H, 6.67; N, 11.67. Found: C, 74.98; H, 6.64; N, 11.65. Compound **5b** IR(KBr) ν_{max}: 3138 (NH), 1677 (–CO–), 1618, 1576, 1498, 1471 (Ar) cm^{-1}. ^1H NMR (500 MHz; DMSO-d_6) δ: 2.32–2.33 (2s look like doublet, 6H, 2 × CH_3), 6.32 (s, 1H, =CH–), 7.09 (s, 1H, Ar), 7.19–7.22 (m, 1H, Ar), 7.55–7.61 (m, 2H, Ar), 8.247–8.263 (d, *J* = 8.0 Hz, 1H, Ar), 8.41 (s, 1H, Ar), 11.66 (s, 1H, NH, exch.). GC–MS *m*/*z* 262 ($M^{+\cdot}$, 100%). Anal. Calcd for $C_{17}H_{14}N_2O$: C, 77.86; H, 5.34; N, 10.69. Found: C, 77.83; H, 5.32; N, 10.65. Compound **5c** IR(KBr) ν_{max}: 3138 (NH), 1677 (–CO–), 1576, 1491, 1472 (Ar) cm^{-1}. ^1H NMR (500 MHz; DMSO-d_6) δ: 6.65 (s, 1H, =CH–), 6.655–6.673 (dd, *J* = 0.5 Hz, *J* = 8.0 Hz, 1H, Ar), 6.824–6.841 (dd, *J* = 0.5 Hz, *J* = 8.0 Hz, 1H, Ar), 7.30–7.34 (m, 2H, Ar), 7.66–7.70 (m, 1H, Ar), 7.722–7.738 (d, *J* = 8.0 Hz, 1H, Ar), 8.325–8.342 (d, *J* = 8.5 Hz, 1H, Ar), 12.10 (s, 1H, NH exch.) 13.23 (s, 1H, OH, exch.). GC–MS *m*/*z* 250 (M^{+}, 100%). Anal. Calcd for $C_{15}H_{10}N_2O_2$ C: 72.00; H, 4.00; N, 11.20%. Found C: 72.00; H, 3.98; N, 11.13%. Compound **7a** IR (KBr) ν_{max}: 1679 (–CO–), 1638 (>C=N–), 1588 & 1446 (Ar) cm^{-1}. ^1H NMR (500 MHz; DMSO-d_6) δ: 1.10–1.41 (m, 3H, aliphatic), 1.56–1.66 (m, 4H, aliphatic), 1.936–1.959 (d, *J* = 11.5 Hz 1H, aliphatic), 2.917–2.935 (d, *J* = 9.0 Hz 1H, aliphatic), 3.252–3.271 (d, *J* = 9.5 Hz 1H, aliphatic), 8.55 (s, 2H, pyrazine). GC–MS *m*/*z* 228 ($M^{+\cdot}$, 2%). Anal. Calcd for $C_{12}H_{12}N_4O$ C: 63.16; H, 5.26; N, 24.56 found 63.12; H, 5.24; N, 24.50. **(7b)** IR (KBr) ν_{max}: 1712 (–CO–), 1635 (>C=N–), 1554 & 1497 (Ar) cm^{-1}. ^1H NMR (500 MHz; DMSO-d_6) δ: 2.84 (s, 6H, 2 × CH_3), 6.45–6.47 (s, 2H, Ar), 8.84 (s, 2H, Ar). GC–MS *m*/*z* 250 ($M^{+\cdot}$, 13%). Anal. Calcd for $C_{14}H_{10}N_4O$: C, 67.19; H, 4.03; N, 22.39. Found: C, 67.17; H, 4.00; N, 22.35. Compound **7c** IR (KBr) ν_{max}: 1650 (–CO–), 1604, 1543, 1502, 1449 (Ar) cm^{-1}. ^1H NMR (500 MHz; DMSO-d_6) δ: 3.92 (s, 2H, CH_2), 6.59–6.62 (m, 1H, Ar), 6.705–6.721 (d, *J* = 8.0 Hz 1H, Ar), 7.06–7.10 (m, 1H, Ar), 7.117-7.133 (d, *J* = 8.0 Hz 1H, Ar), 8.80 (s, 2H, pyrazine). GC–MS *m*/*z* 236 ($M^{+\cdot}$, 29%). Anal. Calcd for $C_{13}H_8N_4O$: C, 66.10; H, 3.39; N, 23.73. Found: C, 66.10; H, 3.34; N, 23.70. Compound **7d** IR (KBr) ν_{max}: 1711 (–CO–), 1638(>C=N–), 1558 & 1397 (Ar) cm^{-1}. ^1H NMR (500 MHz; DMSO-d_6) δ: 6.45–6.47 (m, 2H, Ar), 6.58–6.59 (m, 2H, Ar), 8.84–8.86 (m, 2H, Ar). GC–MS *m*/*z* 222 ($M^{+\cdot}$, 7%). Anal. Calcd for $C_{12}H_6N_4O$: C, 64.86; H, 2.70; N, 25.23. Found: C, 64.83; H, 2.70; N, 25.20.

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