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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Efficient synthesis of piperazine-2,6-dione and 4-(1*H*-indole-2-carbonyl)piperazine-2,6-dione derivatives and their evaluation for anticancer activity

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Abstract Condensation of iminodiacetic acid **1** with various amines i.e., cyclohexanamine, 1-(3-aminopropyl)imidazole, pyridin-2-ylmethanamine, pyridin-3-ylmethanamine, pyridin-4-ylmethanamine, 2-morpholinoethanamine, thiophen-2-ylmethanamine, 2-(thiophen-2-yl)ethanamine, furan-2-ylmethanamine, 2-(pyrrolidin-1-yl)ethanamine, and 1-(3-aminopropyl) pyrrolidin-2-one **2a–k** under microwave irradiation gave the corresponding piperazine-2,6-dione derivatives **3a–k** in quantitative yields. Piperazine-2,6-dione derivatives **3a–k** on condensation with 1*H*-indole-2-carboxylic acid under microwave irradiation gave the corresponding 4-(1*H*-indole-2-carbonyl)piperazine-2,6-dione derivatives **4a–k** in quantitative yields. All the synthesized compounds (**3a–k** & **4a–k**) were purified by crystallization and characterized by spectroscopic means. On screening at a concentration of 10 μM, compounds **3k**, **4e**, **4i** breast (T47D), **4j** lung (NCI H-522), **3i** colon (HCT-15), **4e** ovary (PA-1), and **4g** liver (HepG-2) exhibited good anticancer activity.

Keywords Microwave synthesis · Piperazine dione derivatives · Anticancer activity

Introduction

Diketopiperazine derivatives form an important class of heterocyclic compounds due to their biological activities and have attracted attention of the researchers working in the area of medicinal chemistry (Poster *et al.*, 1981; Toshiharu *et al.*, 1990; Folkes *et al.*, 2001; Ong *et al.*, 2003; Martins and Carvalho, 2007). Diketopiperazine derivatives exhibiting antitumor (Boger *et al.*, 2000, Loughlin *et al.*, 2000), antiviral (Sinha *et al.*, 2004), antifungal (Houston *et al.*, 2004; Tuntiwachwuttikul *et al.*, 2008), antibacterial (Jhaumeer-Laulloo *et al.*, 2003; Abraham, 2005), antiaggregatory (Pons *et al.*, 1998), antileishmanial (Hazra *et al.*, 2007), and anti-inflammatory (Shvedaite *et al.*, 1999) activities are well documented in literature. Diketopiperazine derivatives also act as plasminogen activator inhibitor-1 (PAI-1) (Bryans *et al.*, 1996), thrombin inhibitor (Cody *et al.*, 1999) and topoisomerase-II inhibitors (Grauslund *et al.*, 2007). Besides, these are known to exhibit phototoxic activity (Molesworth *et al.*, 2010).

In search of potent antitumor molecules we have synthesized and evaluated several molecules containing thiazoline, N-substituted cyclic imides, benzimidazole, acridine, amidine & bis amidine, and pyrimidine moieties (Sondhi *et al.*, 2009a, b, 2010a, b, 2011, 2012). In view of the literature cited above, references cited therein, and our background in this area it was considered worthwhile to synthesize diketopiperazine derivatives using iminodiacetic acid and heterocyclic amines under microwave irradiation followed by evaluation of their antitumor activity. To the best of our knowledge all the derivatives synthesized and reported in this paper are unknown in literature.

Results and discussion

Chemistry

Iminodiacetic acid (**1**; Scheme 1) and cyclohexanamine (**2a**; Scheme 1) were mixed in equimolar proportion and

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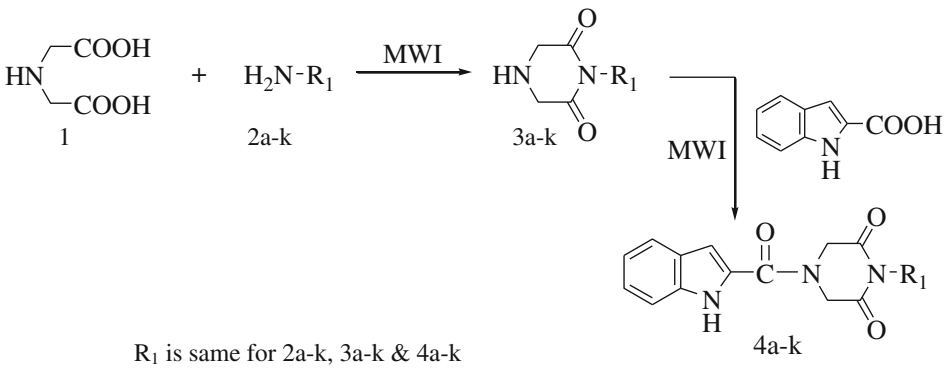
then subjected to microwave irradiation at 85 °C for 3 min. TLC of reaction mixture showed completion of reaction. Crude product, so obtained, was purified by crystallization from methanol to get 1-cyclohexyl-piperazine-2,6-dione (**3a**; Scheme 1) in 89 % yield.

In another experiment the above reaction mixture was subjected to microwave irradiation for 3 min at a power level of 300 W. TLC of reaction mixture showed that the reaction is complete. Crude product, so obtained, was crystallized from methanol to get pure product **3a** in 87 % yield. Yields of product **3a** obtained by the above two methods are comparable. Structure of compound **3a** i.e., 1-cyclohexyl-piperazine-2,6-dione is fully supported by the correct spectral (IR, ^{1}H NMR, ^{13}C NMR, GC–MS) and elemental analysis data reported in the experimental section. Similarly, condensation of 1-(3-aminopropyl)imidazole, pyridin-2-ylmethanamine, pyridin-3-ylmethanamine, pyridin-4-ylmethanamine, 2-morpholinoethanamine, thiophen-2-ylmethanamine, 2-(thiophen-2-yl)ethanamine, furan-2-ylmethanamine, 2-(pyrrolidin-1-yl) ethanamine, and 1-(3-aminopropyl) pyrrolidin-2-one (**2b–k**; Scheme 1) with iminodiacetic acid (**1**; Scheme 1) gave the corresponding piperazine-2,6-dione derivatives **3b–k** (Scheme 1) in quantitative yields. Physical constants, spectral and elemental analysis data of compounds **3b–k** reported in the experimental section are in agreement with the structures assigned to them.

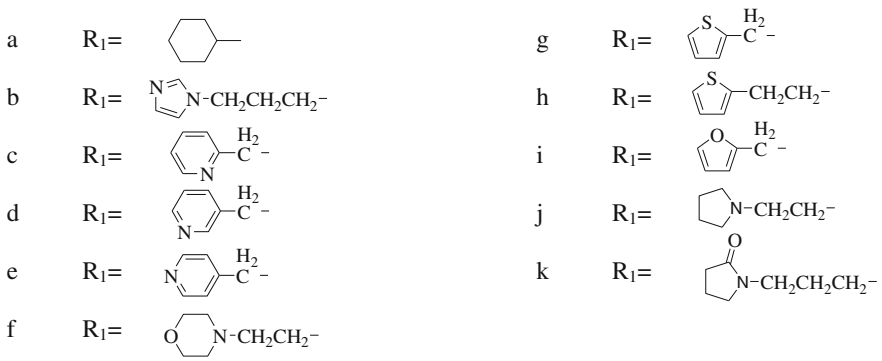
1-Cyclohexyl-piperazine-2,6-dione (**3a**; Scheme 1) and 1*H*-indole-2-carboxylic acid were mixed in equimolar proportion and subjected to microwave irradiation at 150 °C for 7 min. TLC of the reaction mixture showed absence of the starting materials. Crude product so obtained was purified by crystallization from methanol to get pure product 1-cyclohexyl-4-(1*H*-indole-2-carbonyl)-piperazine-2,6-dione (**4a**; Scheme 1) in 86 % yield.

In another experiment the above reaction mixture was subjected to microwave irradiation at a power level of 850 W for 4 min. TLC of the reaction mixture showed absence of the starting materials. Crude product so obtained was purified by crystallization from methanol to get pure product **4a** in 82 % yield. Yields of product **4a** obtained from the above two methods are comparable. Structure assigned to 1-cyclohexyl-4-(1*H*-indole-2-carbonyl)-piperazine-2,6-dione (**4a**; Scheme 1) is fully supported by correct spectral data (IR, ^{1}H NMR, ^{13}C NMR, GC–MS) and elemental analysis. Similarly condensation of piperazine-2,6-dione derivatives **3b–k** (Scheme 1) with 1*H*-indole-2-carboxylic acid gave the corresponding 4-(1*H*-indole-2-carbonyl)piperazine-2,6-dione derivatives **4b–k** (Scheme 1) in quantitative yields. Physical constants, spectral and elemental analysis data of **4b–k** reported in the experimental section fully support the structures assigned to them.

Scheme 1 Synthesis of piperazine-2,6-dione **3a–k** and 4-(1*H*-indole-2-carbonyl)piperazine-2,6-dione **4a–k** derivatives



R_1 is same for 2a-k, 3a-k & 4a-k



Antitumor activity

Purified and fully characterized compounds i.e., piperazine-2,6-dione **3a–k** (Scheme 1) and 4-(1*H*-indole-2-carbonyl)piperazine-2,6-dione derivatives **4a–k** (Scheme 1) were screened in vitro for anticancer activity (Skehan *et al.*, 1990; Monks *et al.*, 1991) against five human cancer cell lines consisting of breast (T47D), lung (NCl H-522), colon (HCT-15), ovary (PA-1), and liver (HepG-2). Percentage (%) growth inhibition of compounds **3a–k** and **4a–k** against various cancer cell lines was determined at a concentration of 1×10^{-5} M solution and results are summarized in Table 1. Compounds **3k**, **4e**, **4i** breast (T47D), **4j** lung (NCl H-522), **3j** colon (HCT-15), **4e** ovary (PA-1), and **4g** liver (HepG-2) exhibited good anticancer activity as compared to standard drugs i.e., 5-fluorouracil, cyclophosphamide, and cycloheximide.

Anticancer activity of two series of compounds i.e., piperazine-2,6-dione **3a–k** and 4-(1*H*-indole-2-carbonyl)piperazine-2,6-dione **4a–k** derivatives is reported in Table 1. From the activity data it is clear that 4-(1*H*-indole-2-carbonyl)piperazine-2,6-dione derivatives **4e**, **4g**, **4i** & **4j** prepared from the corresponding piperazine-2,6-dione derivatives **3e**, **3g**, **3i** & **3j** are useful in increasing the anticancer activity of **4e** (breast T47D, ovary PA-1), **4g** (liver HepG-2), **4i** (breast T47D), and **4j** (lung NCl H-522). Good anticancer activity shown by some of these molecules may be due to the fact that these molecules met the electronic and other stereochemical requirements on the target site in a better way as compared to other molecules which failed to act.

Conclusion

Several new piperazine-2,6-dione **3a–k** and 4-(1*H*-indole-2-carbonyl)piperazine-2,6-dione **4a–k** derivatives have been synthesized in high yields using microwave irradiation technique. These compounds were screened for anticancer activity against five human cancer cell lines. Compounds **4e**, **4g**, **4i**, **4j**, **3j** & **3k** exhibited good anticancer activity.

Experimental

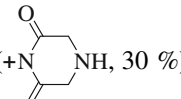
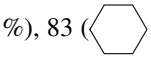
Microwave reactor model CEM DISCOVER model No 908010 and microwave oven model M197DL (Samsung) were used for microwave irradiation. Melting points (mp) were determined on a JSGW apparatus and are uncorrected. IR spectra were recorded using a Perkin Elmer 1600 FT spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker WH-500 spectrometer in a ca 5–15 % (w/v) solution in deuterated solvent (TMS as internal standard). GCMS was recorded on a 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). Compounds **3a–k** and **4a–k** were purified by crystallization from methanol.

General procedure for synthesis of piperazine-2,6-dione derivatives **3a–k**

*Synthesis of 1-cyclohexylpiperazine-2,6-dione (**3a**)*

(i) Iminodiacetic acid **1** (0.133 g; 1 mmol) and cyclohexanamine (0.100 g; 1 mmol) were mixed thoroughly to form a homogeneous mixture. This mixture was subjected to microwave irradiation at 85 °C for 3 min. TLC of the reaction mixture on silica gel using ethyl acetate/methanol (1:1) as mobile phase showed the absence of starting materials. Crude product so obtained was purified by crystallization from methanol to get pure product **3a** in quantitative yield.

(ii) Above experiment was also performed by irradiating homogeneous mixture at a power level of 300 W for 3 min. Completion of the reaction was checked by TLC on silica gel using ethyl acetate/methanol (1:1) as mobile phase. Crude product so obtained was purified by crystallization from methanol to get pure product **3a** in quantitative yield. Yields of product **3a** obtained by method (i) and (ii) are comparable.

Yield-85 %, mp: 118 °C: IR (KBr) ν_{max} 3,146 (NH) 1,669 (>C=O) cm^{-1}. ^{1}H NMR (500 MHz, D_2O) δ: 3.529 (s, 4H, 2× CH_2), 3.024–3.031 (m, 1H, >CH–N–), 1.859–1.873 (d, 2H, J = 7 Hz, CH_2), 1.679–1.693 (t, 2H, J = 7 Hz, CH_2), 1.520–1.546 (d, 1H, J = 13 Hz, one H of CH_2), 1.194–1.270 (m, 4H, 2× CH_2), 1.039–1.070 (m, 1H, one H of CH_2), ^{13}C NMR (125 MHz, D_2O) δ: 171.1, 50.1, 48.8, 30.2, 24.1 and 23.6. GC–MS: *m/z* 197 (M^++1, 10 %), 196 (M^+, 50 %), 113 ( , 30 %), 83 ( , 22 %), 18 ( , 100 %). Anal. calcd for $C_{10}H_{16}N_2O_2$: C 61.20, H 8.22, N 14.27; Found C 61.47, H 8.18, N 14.35.

Similarly, compounds **3b–k** were synthesized.

*1-(3-Imidazol-1-yl-propyl)-piperazine-2,6-dione (**3b**)*

Yield-91 %, mp: 114 °C: IR (KBr) ν_{max} 3,470 (NH), 1,674 (>C=O) cm^{-1}. ^{1}H NMR (500 MHz, D_2O) δ: 7.628 (s, 1H,

Table 1 In vitro anticancer activity of piperazine-2,6-dione **3a–k** and 4-(1*H*-indole-2-carbonyl)piperazine-2,6-dione **4a–k** derivatives

Compd. no	Anticancer activity (% growth inhibition at a concentration of 1×10^{-5} M*				
	Breast T47D	Lung NCI H-522	Colon HCT-15	Ovary PA-1	Liver HepG-2
3a	**15**	11	07	24	38
3b	08	08	32	23	13
3c	16	08	01	17	31
3d	08	04	20	20	21
3e	04	08	33	27	25
3f	01	09	04	37	38
3g	08	15	04	18	29
3h	16	21	25	15	24
3i	04	07	30	26	21
3j	01	00	**49**	26	18
3k	**21**	21	19	25	40
4a	07	06	30	40	25
4b	00	00	07	17	34
4c	20	19	02	23	34
4d	12	04	14	13	17
4e	**21**	05	38	**42**	20
4f	16	08	07	18	03
4g	06	00	15	24	**45**
4h	09	14	04	11	19
4i	**21**	15	24	23	36
4j	16	**31**	17	29	30
4k	14	08	27	08	11
5-FU[a]	15	24	19	22	18
CYC-PHO[b]	26	11	12	12	18
CYC-HEXI[c]	11	15	16	34	26

Bold values represent compounds showing good anticancer activity

* Compounds tested in triplicate, data expressed as mean value of three independent experiments

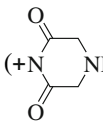
[a] 5-FU 5-Fluorouracil

[b] CYC-PHO Cyclophosphamide

[c] CYC-HEXI Cycloheximide

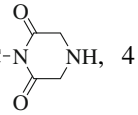
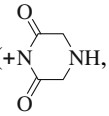
Ar), 7.050 (s, 1H, Ar), 6.909 (s, 1H, Ar), 3.983–4.010 (t, 2H, $J = 6.5$ Hz, CH_2), 3.461 (s, 4H, 2× CH_2), 2.781–2.813 (t, 2H, $J = 7.5$ Hz, CH_2), 2.011–2.140 (m, 2H, CH_2), ^{13}C NMR (125 MHz, D_2O) δ: 171.3, 137.5, 127.3, 120.0, 48.9, 43.9, 36.5 and 28.0. GC–MS: *m/z* 223 (M^++1, 5 %), 222 (M^+, 30 %). Anal. calcd for $C_{10}H_{14}N_4O_2$: C 54.04, H 6.35, N 25.21; Found C 54.13, H 6.30, N 25.27.

*1-(Pyridin-2-ylmethyl)piperazine-2,6-dione (**3c**)*

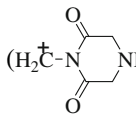
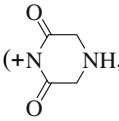
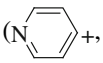
Yield-94 %, mp: 142 °C: IR (KBr) ν_{max} 3,500 (NH) 1,673 (>C=O), 1,473(Ar) cm^{-1}. ^{1}H NMR (500 MHz, D_2O) δ: 8.450–8.460 (d, 1H, $J = 5$ Hz, Ar), 7.779–7.798 (m, 1H, Ar), 7.377–7.393 (d, 1H, $J = 8$ Hz, Ar), 7.329–7.354 (q, 1H, $J = 5$ Hz & 7 Hz, Ar), 4.215 (s, 2H, CH_2), 3.524 (s, 4H, 2× CH_2), ^{13}C NMR (125 MHz, D_2O) δ: 171.2, 151.4, 149.1, 138.5, 124.1, 123.2, 48.9 and 43.2. GC–MS: *m/z* 206 (M^++1, 5 %), 205 (M^+, 50 %), 113 ( , 22 %), 78 (+, 25 %). Anal. calcd for $C_{10}H_{11}N_3O_2$: C 58.53, H 5.40, N 20.48; Found C 58.69, H 5.47, N 20.54.

*1-(Pyridin-3-ylmethyl)piperazine-2,6-dione (**3d**)*

Yield-92 %, mp: 128 °C: IR (KBr) ν_{max} 3,500 (NH) 1,682 (>C=O), 1,474 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, D_2O) δ: 8.457 (s, 1H, Ar), 8.425–8.432 (d, 1H, $J = 3.5$ Hz, Ar), 7.812–7.827 (d, 1H, $J = 7.5$ Hz, Ar), 7.382–7.406 (t, 1H, Ar), 4.092 (s, 2H, CH_2), 3.502 (s, 4H, 2× CH_2), ^{13}C NMR

(125 MHz, D_2O) δ: 171.2, 151.2, 149.9, 135.5, 134.1, 123.2, 48.1 and 43.0. GC–MS: *m/z* 206 (M^++1, 6 %), 205 (M^+, 65 %), 127 ( , 4 %), 113 ( , 24 %), 78 ( , 25 %). Anal. calcd for $C_{10}H_{11}N_3O_2$: C 58.53, H 5.40, N 20.48; Found C 58.71, H 5.45, N 20.55.

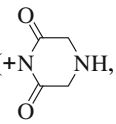
*1-(Pyridin-4-ylmethyl)piperazine-2,6-dione (**3e**)*

Yield-90 %, mp: 133 °C: IR (KBr) ν_{max} 3,500(NH), 1,662(>C= O), 1,474 (Ar) cm^{-1}. ^1H NMR (500 MHz, D_2O) δ: 8.464 (s, 2H, Ar), 7.370 (s, 2H, Ar), 4.116 (s, 2H, CH_2), 3.533 (s, 4H, 2× CH_2), ^{13}C NMR (125 MHz, D_2O) δ: 171.2, 149.9, 143.3, 123.2, 48.1 and 43.0. GC–MS: *m/z* 206 (M^++1, 6 %), 205 (M^+, 53 %), 127 ( ,5 %), 113 ( , 22 %), 78 ( , 20 %). Anal. calcd for $C_{10}H_{11}N_3O_2$: C 58.53, H 5.40, N 20.48; Found C 58.69, H 5.44, N 20.57.

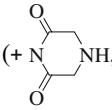
*1-(2-Morpholinoethyl)piperazine-2,6-dione (**3f**)*

Yield-93 %, mp: 123 °C: IR (KBr) ν_{max} 3,500(NH), 1,686(>C=O) cm^{-1}. ^1H NMR (500 MHz, D_2O) δ: 3.641–3.659 (t, 4H, J = 4.5 Hz, 2× CH_2), 3.522 (s, 4H, 2× CH_2), 3.052 (t, 2H, J = 5 Hz, CH_2), 2.624 (t, 2H, J = 5 Hz, CH_2), 2.509 (s, 4H, 2× CH_2), ^{13}C NMR (125 MHz, D_2O) δ: 171.3, 66.0, 54.1, 52.4, 48.9 and 35.5. GC–MS: *m/z* 228 (M^++1, 8 %), 227 (M^+, 45 %), 18 ($H_2O^{\bullet+}$, 100 %). Anal. calcd for $C_{10}H_{17}N_3O_3$: C 52.85, H 7.54, N 18.49; Found C 52.78, H 7.59, N 18.60.

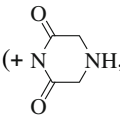
*1-Thiophen-2-ylmethyl-piperazine-2,6-dione (**3g**)*

Yield-87 %, mp: 130 °C: IR (KBr) ν_{max} 3,427(NH), 1,651(>C=O), 1,464 (Ar) cm^{-1}. ^1H NMR (500 MHz, D_2O) δ: 7.348–7.361 (t, 1H, J = 3 Hz, Ar), 7.076 (s, 1H, Ar), 6.934–6.954(q, 1H, J = 2 Hz & 5 Hz, Ar), 4.230–4.236 (d, 2H, J = 3 Hz, CH_2), 3.454–3.468 (q, 4H, J = 4 Hz, 5 Hz, 2× CH_2,), ^{13}C NMR (125 MHz, D_2O) δ: 171.3, 134.0, 129.3, 127.7, 127.6, 48.9 and 37.3. GC–MS: *m/z* 211 (M^++1, 6 %), 210 (M^+, 35 %), 113 ( , 25 %), 83 ( , 20 %). Anal. calcd for $C_9H_{10}N_2O_2S$: C 51.41, H 4.79, N 13.32, S 15.25; Found C 51.47, H 4.82, N 13.38, S 15.31.

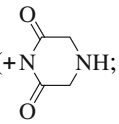
*1-(2-Thiophen-2-yl-ethyl)-piperazine-2,6-dione (**3h**)*

Yield-87 %, mp: 136 °C: IR (KBr) ν_{max} 3,450 (NH), 1,669, 1,647 (>C=O), 1,613 (Ar) cm^{-1}. ^1H NMR (500 MHz, D_2O) δ: 7.257–7.267 (d, 1H, J = 5 Hz, Ar), 6.916–6.946 (m, 2H, Ar), 3.521(s, 4H, 2× CH_2), 3.180–3.206 (t, 2H, J = 6.5 Hz, CH_2), 3.113–3.139 (t, 2H, J = 6.5 Hz, CH_2), ^{13}C NMR (125 MHz, D_2O) δ: 171.5, 138.5, 127.5, 126.6, 125.3, 48.9, 40.7 and 26.9. GC–MS: *m/z* 225 (M^++1, 9 %), 224 (M^+, 50 %), 113 ( , 30 %), 83( , 20 %). Anal. calcd for $C_{10}H_{12}N_2O_2S$: C 53.55, H 5.39,N 12.49, S 14.30; Found C 53.51, H 5.41, N 12.54, S 14.36.

*1-Furan-2-ylmethyl-piperazine-2,6-dione (**3i**)*

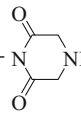
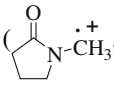
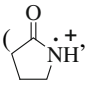
Yield-96 %, mp: 122 °C: IR (KBr) ν_{max} 3,420 (NH), 1,652 (>C=O), 1,593 (Ar) cm^{-1}. ^1H NMR (500 MHz, D_2O) δ: 7.424–7.425 (d, 1H, J = 0.5 Hz, Ar), 6.403–6.409 (d, 1H, J = 3 Hz, Ar), 6.331–6.337 (t, 1H, Ar), 4.070 (s, 2H, CH_2), 3.478 (s, 4H, 2× CH_2), ^{13}C NMR (125 MHz, D_2O) δ: 171.0, 146.1, 144.2, 110.8, 110.7, 48.9 and 35.5. GC–MS: *m/z* 195 (M^++1, 8 %), 194 (M^+, 50 %), 113 ( , 25 %). Anal. calcd for $C_9H_{10}N_2O_3$: C 55.67, H 5.19, N 14.43; Found C 55.75, H 5.23, N 14.37.

*1-(2-Pyrrolidin-1-yl-ethyl)-piperazine-2,6-dione (**3j**)*

Yield-89 %, mp: 117 °C: IR (KBr) ν_{max} 3,440(NH), 1,678 (>C=O) cm^{-1}. ^1H NMR (500 MHz, D_2O) δ: 3.493 (s, 4H, 2× CH_2), 3.003–3.072 (m, 8H, 4× CH_2), 1.859(s, 4H, 2× CH_2), ^{13}C NMR (125 MHz, D_2O) δ: 171.4, 55.9, 53.7, 48.9, 36.5 and 22.5. GC–MS: *m/z* 212 (M^++1, 5 %), 211 (M^+, 30 %), 113 ( ; 25 %). Anal. calcd for $C_{10}H_{17}N_3O_2$: C 56.85, H 8.11, N 19.89; Found C 56.96, H 8.14, N 19.76.

*1-[3-(2-Oxo-pyrrolidin-1-yl)-propyl]-piperazine-2, 6-dione (**3k**)*

Yield-94 %, mp: 109 °C: IR (KBr) ν_{max} 3,432 (NH), 1,668, 1,626 (>C=O) cm^{-1}. ^1H NMR (500 MHz, D_2O) δ: 3.550 (s, 4H, 2× CH_2), 3.384–3.413 (t, 2H, J = 7 Hz, 2× CH_2), 3.246–3.273 (t, 2H, J = 6.5 Hz, CH_2), 2.854–2.884 (t, 2H, J = 7.5 Hz, CH_2), 2.316–2.349(t, 2H, J = 8.5 Hz, CH_2),

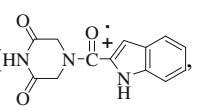
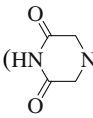
1.928–1.958 (t, 2H, J = 7.5 Hz, CH_2), 1.806–1.835 (t, 2H, J = 7.5 Hz, CH_2), ^{13}C NMR (125 MHz, D_2O) δ: 178.7, 171.1, 48.8, 47.9, 39.4, 36.8, 30.8, 24.4 and 18.2. GC–MS: m/z 240 (M^++1, 5 %), 239 (M^+, 35 %), 113 ( , 25 %), 99 ( , 17 %), 85 ( , 17 %). Anal. calcd for $C_{11}H_{17}N_3O_3$: C 55.22, H 7.16, N 17.56; Found C 55.29, H 7.11, N 17.63.

General procedure for synthesis of 4-(1*H*-indole-2-carbonyl)piperazine-2,6-dione derivatives **4a–k**

*Synthesis of 1-cyclohexyl-4-(1H-indole-2-carbonyl)piperazine-2,6-dione (**4a**)*

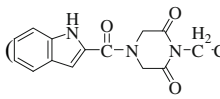
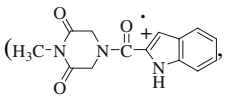
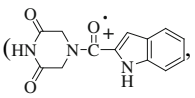
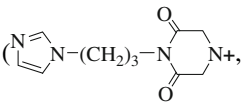
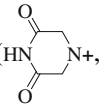
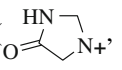
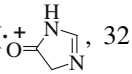
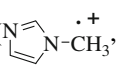
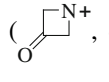
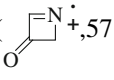
(i) Cyclohexylpiperazine-2,6-dione **3a** (0.196 g; 1 mmol) and 1*H*-indole-2-carboxylic acid (0.161 g; 1 mmol) were mixed thoroughly to form a homogeneous mixture. This reaction mixture was subjected to microwave irradiation at 150 °C for 7 min. TLC of the reaction mixture on silica gel using ethyl acetate/methanol (1:1) as mobile phase showed absence of starting materials. Crude product so obtained was crystallized from methanol to get pure product **4a** in quantitative yield.

(ii) The above experiment was also performed by irradiating the homogeneous mixture for 4 min at a power level of 850 W. Completion of the reaction was checked by TLC on silica gel using ethyl acetate/methanol (1:1) as mobile phase. Crude product so obtained was crystallized from methanol to get pure product **4a** in quantitative yield. Yields of product **4a** obtained by method (i) and (ii) are comparable.

Yield-80 %, mp: 280 °C: IR (KBr) ν_{max} 3,403 (NH), 1,702 (>C=O), 1,519 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, MeOD) δ: 7.614–7.631 (d, 1H, J = 8.5 Hz, Ar), 7.441–7.459 (d, 1H, J = 8.5 Hz, Ar), 7.216–7.249 (dt, 1H, J = 1 & 8 Hz, Ar), 7.108 (s, 1H, Ar), 7.046–7.077 (m, 1H, Ar), 3.575 (s, 4H, 2× CH_2), 3.031–3.074 (m, 1H, CH), 2.003–2.018 (m, 2H, CH_2), 1.798–1.845 (m, 2H, CH_2), 1.677–1.706 (dd, 1H, J = 1.5 Hz & 13.5 Hz, one H of CH_2), 1.312–1.404 (m, 4H, 2× CH_2), 1.221–1.246 (q, 1H, J = 3.5 Hz & 9 Hz, one H of CH_2), ^{13}C NMR (125 MHz, MeOD) δ: 171.2, 166.9, 138.6, 131.7, 128.9, 125.2, 122.9, 120.9, 113.1, 108.0, 51.5, 50.4, 31.9, 25.9 and 25.4. GC–MS: m/z 340 (M^++1, 5 %), 339 (M^+, 35 %), 257 ( , 15 %), 113 ( , 18 %), 83 ( , 30 %), 82 (, 12 %). Anal. calcd for $C_{19}H_{21}N_3O_3$: C 67.24, H 6.24, N 12.38; Found C 67.11, H 6.31, N 12.44.

Similarly compounds **4b–k** were synthesized.

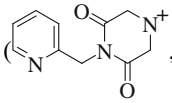
*1-(3-(1H-imidazol-1-yl)propyl)-4-(1H-indole-2-carbonyl)-piperazine-2,6-dione (**4b**)*

Yield-86 %, mp: >300 °C: IR (KBr) ν_{max} 3,470 (NH), 1,694, 1,635 (>C=O), 1,515 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, MeOD) δ: 7.813 (s, 1H, Ar), 7.593–7.609 (d, 1H, J = 8 Hz, Ar), 7.434–7.450 (d, 1H, J = 8 Hz Ar), 7.188–7.235 (m, 2H, Ar), 7.040–7.085 (m, 3H, Ar), 4.079–4.159 (t, 2H, J = 7 Hz, CH_2), 3.582 (s, 4H, 2× C_2), 2.900–2.930 (t, 2H, J = 7.5 Hz, CH_2), 2.130–2.188 (m, 2H, CH_2), ^{13}C NMR (125 MHz, MeOD) δ: 171.4, 168.6, 138.3, 138.3, 133.8, 129.2, 128.6, 124.7, 122.7, 120.8, 120.8, 113.0, 106.9, 50.4, 45.2, 37.9 and 30.0. GC–MS: m/z 366 (M^++1, 5 %), 365 (M^+, 20 %), 284 ( , 11 %), 271 ( , 8 %), 257 ( , 20 %), 221 ( , 6 %), 113 ( , 20 %), 85 ( , 6 %), 84 ( , 32 %), 82 ( , 12 %), 70 ( , 40 %), 69 ( , 57 %).

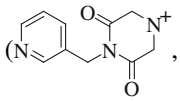
Anal. calcd for $C_{19}H_{19}N_5O_3$: C 62.46, H 5.24, N 19.17; Found C 62.62, H 5.19, N 19.35.

*4-(1H-indole-2-carbonyl)-1-pyridin-2-ylmethy lpiperazine-2,6-dione (**4c**)*

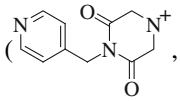
Yield-88 %, mp: >300 °C: IR (KBr) ν_{max} 3,470 (NH), 1,692 (>C=O), 1,528 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, MeOD) δ: 8.622–8.632 (d, 1H, J = 5 Hz, Ar), 7.827–7.862 (m, 1H, Ar), 7.617–7.634 (d, 1H, J = 8.5 Hz, Ar), 7.440–7.458 (dd, 2H, J = 1 Hz & 8.5 Hz Ar), 7.375–7.400 (m, 1H, Ar), 7.223–7.256 (m, 1H, Ar), 7.118–7.120 (d, 1H, J = 1 Hz), 7.067–7.083 (m, 1H, Ar), 4.289 (s, 2H, CH_2), 3.511(s, 4H,2× CH_2), ^{13}C NMR (125 MHz, MeOD) δ: 171.1, 166.4, 153.8, 150.5, 138.7, 138.6, 130.9, 128.9, 125.3, 124.7, 123.5, 122.9, 121.0, 113.1, 108.4, 50.4 and 44.1. GC–MS: m/z 349 (M^++1, 7 %), 348 (M^+, 35 %),

204 ( , 50 %). Anal. calcd for $C_{19}H_{16}N_4O_3$: C 65.51, H 4.63, N 16.08; Found C 65.69, H 4.70, N 16.31.

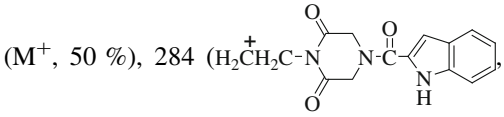
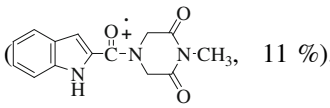
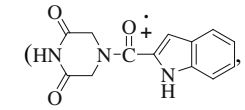
*4-(1H-indole-2-carbonyl)-1-pyridin-3-ylmethyl piperazine-2,6-dione (**4d**)*

Yield-82 %, mp: 276 °C: IR (KBr) ν_{max} 3,444 (NH), 1,691, 1,624 (>C=O), 1,511 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO+D_2O) δ: 8.267–8.270 (d, 1H, J = 1.5 Hz, Ar), 8.133–8.140 (d, 1H, J = 3.5 Hz, Ar), 7.507–7.522 (d, 1H, J = 7.5 Hz, Ar), 7.166–7.182 (d, 1H, J = 8.0 Hz, Ar), 7.021–7.041 (m, 2H, Ar), 6.719–6.749 (t, 1H, J = 7.5 Hz, Ar), 6.589–6.619 (t, 1H, J = 7.5 Hz, Ar), 6.358 (s, 1H, Ar), 3.917 (s, 2H, CH_2), 3.590 (s, 4H, 2× CH_2), ^{13}C NMR (125 MHz, DMSO+D_2O) δ: 172.1, 166.4, 149.1, 148.5, 136.5, 136.3, 135.6, 133.4, 127.5, 123.7, 122.2, 120.9, 118.9, 111.9, 103.2, 48.9 and 40.7. GC–MS: *m/z* 349 (M^++1, 6 %), 348 (M^+, 30 %), 204 ( , 30 %). Anal. calcd for $C_{19}H_{16}N_4O_3$: C 65.51, H 4.63, N 16.08; Found C 65.72, H 4.57, N 16.27.

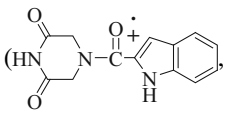
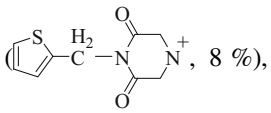
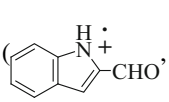
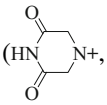
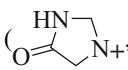
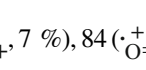
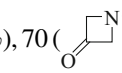
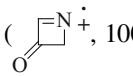
*4-(1H-indole-2-carbonyl)-1-pyridin-4-ylmethyl piperazine-2,6-dione (**4e**)*

Yield-86 %, mp: >300 °C: IR (KBr) ν_{max} 3,452 (NH), 1,684, 1,624 (>C=O), 1,515 (Ar) cm^{-1}. ^1H NMR (500 MHz, DMSO+D_2O) δ: 8.534–8.545 (d, 2H, J = 5 Hz, Ar), 7.549–7.372 (m, 4H, Ar), 7.090–7.120 (t, 1H, J = 7.5 Hz, Ar), 6.955–6.985 (t, 1H, J = 7.5 Hz, Ar), 6.750 (s, 1H, Ar), 3.982 (s, 2H, CH_2), 3.505 (s, 4H, 2× CH_2), ^{13}C NMR (125 MHz, DMSO+D_2O) δ: 171.9, 166.7, 149.2, 145.6, 135.8, 135.6, 127.4, 123.1, 122.4, 121.1, 119.1, 111.9, 103.5, 48.1, 41.7. GC–MS: *m/z* 349 (M^++1, 5 %), 348 (M^+, 50 %), 204 ( , 40 %). Anal. calcd for $C_{19}H_{16}N_4O_3$: C 65.51, H 4.63, N 16.08; Found C 65.69, H 4.69, N 16.01.

*4-(1H-indole-2-carbonyl)-1-2-morpholinoethy lpiperazine-2,6-dione (**4f**)*

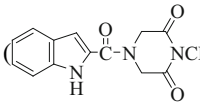
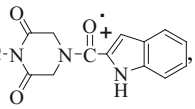
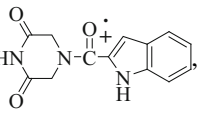
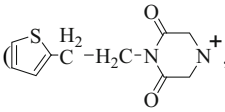
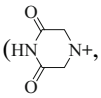
Yield-81 %, mp: >300 °C: IR (KBr) ν_{max} 3,426 (NH), 1,706 (>C=O), 1,594 & 1,523 (Ar) cm^{-1}. ^1H NMR (500 MHz, MeOD) δ: 7.615–7.632 (d, 1H, J = 8.5 Hz, Ar), 7.442–7.460 (d, 1H, J = 9 Hz, Ar), 7.217–7.250 (dt, 1H, J = 1 & 7.5 Hz, Ar), 7.107(s, 1H, Ar), 7.045–7.076 (t, 1H, J = 7.5 Hz, Ar), 3.641–3.659 (t, 4H, J = 4.5 Hz, 2× CH_2), 3.522 (s, 4H, 2× CH_2), 3.038–3.066 (t, 2H, J = 7 Hz, CH_2), 2.610–2.638 (t, 2H, J = 7 Hz, CH_2), 2.509 (s, 4H, 2× CH_2), ^{13}C NMR (125 MHz, DMSO+D_2O) δ: 171.7, 167.3, 138.6, 131.7, 128.9, 125.2, 122.9, 121.0, 113.1, 108.0, 55.1, 53.2, 50.3, 37.4 and 24.1. GC–MS: *m/z* 371 (M^++1, 10 %), 370 (M^+, 50 %), 284 ( , 11 %), 271 ( , 11 %), 257 ( , 16 %), 85 ( , 5 %), 84 (, 35 %). Anal. calcd for $C_{19}H_{22}N_4O_4$: C 61.61, H 5.99, N, 15.13; Found C 61.79, H 5.88, N 15.32.

*4-(1H-indole-2-carbonyl)-1-(thiophen-2-ylmethyl)piperazine-2,6-dione (**4g**)*

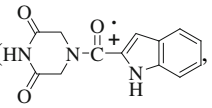
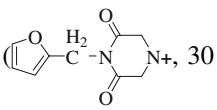
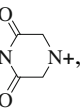
Yield-85 %, mp: >300 °C: IR (KBr) ν_{max} 3,390 (NH), 1,683(>C=O), 1,476, (Ar) cm^{-1}. ^1H NMR (500 MHz, D_2O) δ: 7.615–7.632 (d, 1H, J = 8.5 Hz, Ar), 7.443–7.460 (d, 1H, J = 8.5 Hz, Ar), 7.257–7.267 (d, 1H, J = 5 Hz Ar), 7.217–7.248 (dt, 1H, J = 1 Hz & 7.5 Hz, Ar), 7.108 (s, 1H, Ar), 7.046–7.077 (t, 1H, J = 7.5 Hz, Ar), 6.918–6.988 (m, 2H, Ar), 4.182 (s, 2H, CH_2), 3.524 (s, 4H, 2× CH_2), ^{13}C NMR (125 MHz, MeOD) δ: 171.2, 166.7, 138.7, 134.1, 131.3, 129.3, 128.9, 127.7, 127.6, 125.2, 122.9, 120.9, 113.1, 108.2, 48.1 and 40.9. GC–MS: *m/z* 354 (M^++1, 10 %), 353 (M^+, 78 %), 257 ( , 25 %), 209 ( , 8 %), 145 ( , 10 %), 113 ( , 25 %), 85 ( , 7 %), 84 ( , 32 %), 70 ( , 40 %), 69 ( , 100 %), Anal. calcd for $C_{18}H_{15}N_3O_3S$; C 61.18, H 4.28, N 11.89; Found C 61.39, H 4.21, N 12.07.

*4-(1H-indole-2-carbonyl)-1-(2-(thiophen-2-yl)ethyl)piperazine-2,6-dione (**4h**)*

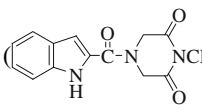
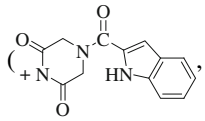
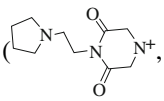
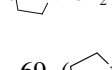
Yield-87 %, mp: >300 °C: IR (KBr) ν_{max} 3,403 (NH), 1,701 (>C=O), 1,615 (Ar) cm^{-1}. ^1H NMR (500 MHz, MeOD) δ: 7.614–7.631 (d, 1H, J = 8.5 Hz, Ar), 7.441–7.459 (d, 1H, J = 8.5 Hz, Ar), 7.257–7.267 (d, 1H, J = 5 Hz, Ar), 7.216–7.249 (dt, 1H, J = 1 Hz & 8 Hz, Ar), 7.108 (s, 1H, Ar), 7.046–7.077 (t, 1H, J = 7.5 Hz, Ar), 6.917–6.947 (m,

2H, Ar), 3.522 (s, 4H, 2× CH_2), 3.181–3.207 (t, 2H, J = 6.5 Hz, CH_2), 3.114–3.140 (t, 2H, J = 6.5 Hz, CH_2), ^{13}C NMR (125 MHz, DMSO+D_2O) δ: 171.2, 166.7, 138.7, 134.1, 131.3, 129.3, 128.9, 127.7, 127.6, 125.2, 122.9, 120.9, 113.1, 108.2, 48.1, 40.7 and 26.9. GC–MS: m/z 368 (M^++1, 10 %), 367 (M^+, 55 %), 284 ( , 12 %), 271 ( , 11 %), 257 ( , 16 %), 223 ( , 5 %), 113 ( , 18 %), 84 ( , 32 %). Anal. calcd for $C_{19}H_{17}N_3O_3S$: C 62.11, H 4.66, N 11.44; Found C 62.28, H 4.71, N 11.35.

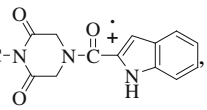
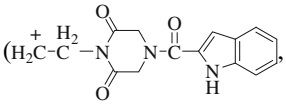
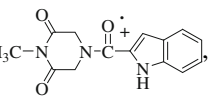
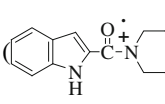
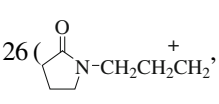
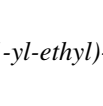
*1-(Furan-2-ylmethyl)-4-(1H-indole-2-carbonyl)-piperazine-2,6-dione (**4i**)*

Yield-87 %, mp: 281 °C: IR (KBr) ν_{max} 3,450 (NH), 1,692 (>C=O), 1,536 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, MeOD) δ: 7.596–7.632 (m, 2H, Ar), 7.439–7.458 (dd, 1H, J = 1 Hz & 8.5 Hz, Ar), 7.221–7.252 (m, 1H, Ar), 7.109–7.110 (d, 1H, J = 0.5 Hz, Ar), 7.048–7.080 (m, 1H, Ar), 6.554–6.561 (d, 1H, J = 3.5 Hz, Ar), 6.468–6.479 (q, 1H, J = 2 & 3.5 Hz, Ar), 4.183 (s, 2H, CH_2), 3.568 (s, 4H, 2× CH_2), ^{13}C NMR (125 MHz, MeOD) δ: 171.2, 166.7, 148.3, 145.3, 138.7, 131.3, 128.9, 125.2, 122.9, 120.9, 113.1, 111.9, 111.7, 108.2, 50.4 and 36.8. GC–MS: m/z 338 (M^++1, 8 %), 337(M^+, 30 %), 257 ( , 16 %), 193 ( , 30 %), 113 ( , 18 %), 85 ( , 6 %), 84 ( , 75 %), 70 (, 58 %), 69. ( , 70 %). Anal. calcd for $C_{18}H_{15}N_3O_4$: C 64.09, H 4.48, N 12.46; Found C 64.34, H 4.55, N 12.34.

*4-(1H-indole-2-carbonyl)-1-(2-pyrrolidin-1-yl-ethyl)-piperazine-2,6-dione (**4j**)*

Yield-83 %, mp: 276 °C: IR (KBr) ν_{max} 3,450 (NH), 1,691, 1,624 (>C=O), 1,541 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, MeOD) δ: 7.626–7.643 (d, 1H, J = 8.5 Hz, Ar), 7.449–7.467 (dd, 1H, J = 1 Hz & 8.5 Hz, Ar), 7.233–7.266 (m, 1H, Ar), 7.138–7.139 (d, 1H, J = 0.5 Hz Ar), 7.058–7.090 (dt, 1H, 1 Hz & 8.5 Hz, Ar), 3.711–3.730 (t, 4H, J = 5 Hz, 2× CH_2), 3.601 (s, 4H, 2× CH_2), 3.064–3.088 (q, 2H, J = 6 Hz & 7 Hz, CH_2), 2.628–2.652 (t, 2H, J = 6 Hz, CH_2), 2.528 (s, 4H, 2× CH_2), ^{13}C NMR (125 MHz, MeOD) δ: 171.2, 166.1, 138.8, 130.5, 128.9, 125.5, 123.0, 121.1, 113.2, 108.6, 67.7, 56.0, 54.4, 50.3 and 36.9. GC–MS: m/z 355 (M^++1, 7 %), 354 (M^+, 25 %), 284 ( , 10 %), 256 ( , 12 %), 210 ( , 50 %), 84 ( , 100 %), 71 ( , 25 %), 70 ( , 55 %), 69 ( , 87 %). Anal. calcd for $C_{19}H_{22}N_4O_3$: C 64.39, H 6.26, N 15.81; Found C 64.51, H 6.32, N 15.97.

*4-(1H-indole-2-carbonyl)-1-[3-(2-oxopyrrolidin-1-yl)-propyl]-piperazine-2,6-dione (**4k**)*

Yield-83 %, mp: >300 °C: IR (KBr) ν_{max} 3,426 (NH), 1,702 (>C=O), 1,591 & 1,532 (Ar) cm^{-1}. ^{1}H NMR (500 MHz, MeOD) δ: 7.624–7.640 (d, 1H, J = 8 Hz, Ar), 7.447–7.465 (dd, 1H, J = 1 Hz & 8.5 Hz, Ar), 7.229–7.261 (dt, 1H, J = 0.5 Hz & 7.0 Hz Ar), 7.127 (s, 1H, Ar), 7.056-7.086 (m, 1H, Ar), 3.583 (s, 4H, 2× CH_2), 3.448–3.477 (t, 2H, 7.5 Hz, Ar), 3.369–3.396 (t, 2H, J = 7 Hz, CH_2), 2.911–2.941 (t, 2H, J = 7.5 Hz, CH_2), 2.387–2.419 (t, 2H, J = 8 Hz, CH_2), 2.057–2.072 (m, 2H, CH_2), 1.888–1.917 (m, 2H, CH_2), ^{13}C NMR (125 MHz, MeOD) δ: 209.8, 178.6, 171.2, 138.7, 128.9, 125.4, 122.9, 121.1, 113.1, 108.4, 54.6, 50.3, 40.4, 38.0, 31.8, 26.2 and 18.8. GC–MS: m/z 383 (M^++1, 7 %), 382 (M^+, 25 %), 299 ( , 5 %), 284 ( , 10 %), 271 ( , 10 %), 257 ( , 22 %), 126 ( , 5 %), 113 ( , 18 %), 85 ( , 5 %), 84 ( , 60 %), 70 ( , 58 %), 69 (, 70 %), Anal. calcd for

$C_{20}H_{22}N_4O_4$: C 62.82, H 5.80, N 14.65; Found C 62.98, H 5.87, N 14.49.

Pharmacology

In vitro *cytotoxicity against human cancer cell lines* (Skehan *et al.*, 1990; Monks *et al.*, 1991) The human cancer cell lines procured from the National Cancer Institute, Frederick, U. S. A. were used in this 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 a sub confluent 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 obtain working test solution of required concentrations.

In vitro cytotoxicity against various human cancer cell lines was determined (Monks *et al.*, 1991) 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 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. were air dried. Cell growth was measured by staining with sulforhodamine B dye (Skehan *et al.*, 1990). 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 using a mechanical stirrer. The optical density (OD) was recorded on ELISA reader at 540 nm.

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