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ORIGINAL ARTICLE

Synthesis and anticancer activity of some 1,2,3-trisubstituted pyrazinobenzimidazole derivatives

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Abstract

The synthesis of some new pyrazino[1,2-*a*]benzimidazole derivatives and investigation of their anticancer activities were aimed in this work. Thus, 2-acetylbenzimidazole was reacted with appropriate α-bromoacetophenones and potassium carbonate in acetone to give 2-(2-acetyl-1*H*-benzimidazol-1-yl)-1-phenylethanone derivatives (**3a**–**d**). These diketone compounds were reacted with varied benzylamines in acetic acid to obtain 2-benzyl-1-methylidene-3-aryl-1,2-dihydropyrazino[1,2-*a*]benzimidazole derivatives (**4a**–**t**). The structures of the obtained compounds were elucidated by using IR, ^{1}H-NMR, ^{13}C-NMR, MS spectral data and elemental analyses results. Anticancer activities of the selected compounds were investigated in National Cancer Institute, Bethesda, MD. **3c** and **4n** showed remarkable anticancer activity comparing with standard drugs, melphalan and cisplatin.

Keywords

1,2-disubstituted benzimidazole, α,β-unsaturated carbonyl, anticancer activity, pyrazino[1,2-*a*]benzimidazole

History

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Introduction

Natural and synthetic α-methylene-γ-lactone and quinone methide containing compounds are known with high anticancer activity by mechanism acting as DNA alkylating agent[1–5]. For these compounds, it was found that one of the structural requirement for significant cytotoxicity was an $O=C–C=CH_2$ system as part of an ester, a ketone or lacton[6]. Natural compounds namely elephantopin, eupatundin, vernolepin and helenalin including α-methylene-γ-lactone moiety in their structure (Figure 1) are some of the compounds that have known with anticancer activities and also high cytoxicities[7–9]. Toxic properties of these natural compounds leaded medicinal chemists to synthesize new derivatives including α,β-unsaturated carbonyl moiety as a pharmacophoric group (Figure 2), which are thought to have high antitumor activity with low cytotoxicity[10].

Quinone methide moiety is also an important anticancer active group, which has α,β-unsaturated carbonyl structure[11–13]. In studies, indole, benzimidazole and quinazoline rings have been used as precursors of quinone methides because of their excessive reactivity[14–17]. Reversibly binding to nucleophilic sites on telomeric DNA *via* Michael addition reaction is thought to be responsible for the anticancer activity of both of α-methylene-γ-lactone and quinone methide chemical groups[18–21].

In the light of these studies and as an extension of our previous works on anticancer active pyrazino[1,2-*a*]benzimidazole derivatives[22–24], we now report on the synthesis and the anticancer activity testing of some 2-benzyl-1-methylidene-3-phenyl-1,2-dihydropyrazino[1,2-*a*]benzimidazole derivatives.

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Experimental section

Chemistry

All chemicals were purchased from Sigma-Aldrich Corp. (St. Louis, MO) and Merck Chemicals (Merck KGaA, Darmstadt, Germany). Melting points were determined using an Electrothermal 9100 digital melting point apparatus (Electrothermal, Essex, UK) and were uncorrected. Spectroscopic data were recorded on the following instruments. IR: Shimadzu 8400 FTIR spectrophotometer (Shimadzu, Tokyo, Japan); ^{1}H-NMR: Bruker DPX 500 NMR spectrometer (Bruker Bioscience, Billerica, MA), in DMSO-d_6, TMS as internal standard; ^{13}C-NMR: Bruker DPX 125 NMR spectrometer (Bruker Bioscience), in DMSO-d6MS: AB SCIEX-3200 Q-TRAP LC/MS/MS MASS spectrometer (Fisons Instruments Vertriebs GmbH, Mainz, Germany). Elemental analyses were performed on a Leco TruSpec Micro CHN/CHNS elemental analyzer (Leco, St. Joseph, MI).

*2-(1-Hydroxyethyl)benzimidazole (**1**)*

o-Phenylenediamine (100 mmol) and lactic acid (100 mmol) were refluxed in 100 mL 4 N HCl solution for 8 h. The solution was cooled, poured into ice water and neutralized with ammonia. The precipitate was filtered and crystallised from ethanol–water. Yield: 71% m.p. 178–180 °C (ref. 178.5–179.5 °C)[25].

*2-Acetylbenzimidazole (**2**)*

2-(1-Hydroxyethyl)benzimidazole (10 mmol) was dissolved in 100 mL acetic acid and heated to 90 °C. The solution of chromium trioxide (7.5 mmol) in 15 mL water was added dropwise to this mixture and the temperature was fixed at 90 °C. After the addition, the mixture was cooled and poured into water. The precipitate was filtered and extracted with chloroform.

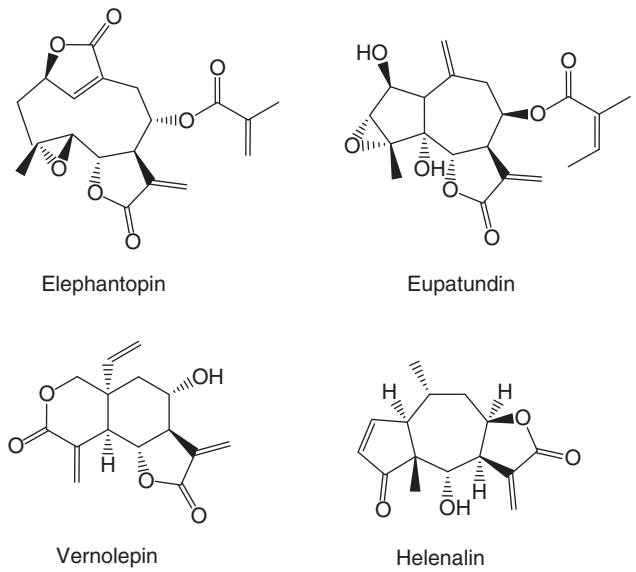


Figure 1. Chemical structures of some natural α-methylene-γ-lactones.

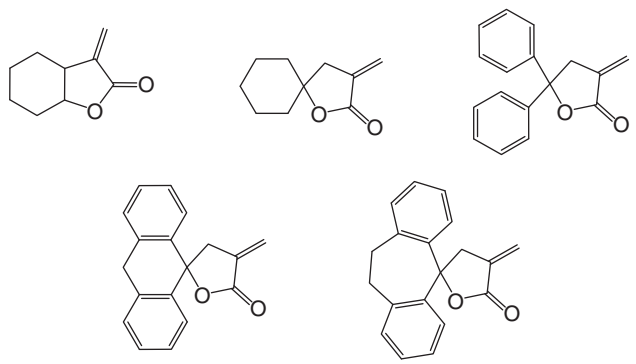


Figure 2. Synthetic derivatives of α-methylene-γ-lactones.

The solvent was evaporated at low pressure and the residue was recrystallized from toluene.

Yield: 76% m.p. 188–190 °C (ref. 188–189 °C)[26]. IR(KBr) ν_{max} (cm^{-1}): 3288–2400 (–N–H), 1674 (–C = O), 1600–1420 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6) δ: (ppm) 2.71 (3H, s, $–COCH_3$), 7.36–7.48 (2H, m, Ar-H), 7.86–8.14 (2H, m, Ar-H), 12.4 (1H, brs, N–H).

*2-(2-Acetyl-1H-benzimidazol-1-yl)-1-arylethanone derivatives (**3a–d**)*

A mixture of the appropriate 2-acetylbenzimidazole (5 mmol), 2-bromoacetophenone (5 mmol) and potassium carbonate (5 mmol) was stirred in acetone (50 mL) at room temperature. Stirring was continued at room temperature until the disappearance of the starting materials by TLC (4–6 h). The solvent was evaporated at low pressure, and the residue was washed with water and then ethanol. The raw product was recrystallized from ethanol.

*2-(2-Acetyl-1H-benzimidazol-1-yl)-1-phenylethanone (**3a**)*

Yield 75%. m.p. 167 °C (ref. 166–168 °C)[27]. IR (KBr) ν_{max} (cm^{-1}): 3132–3061 (aromatic –C–H), 2937–2850 (aliphatic –C–H), 1686 and 1676 (–C = O), 1593–1446 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.71 (3H, s, $–COCH_3$), 6.23 (2H, s, $–CH_2CO$), 7.36–7.48 (2H, m, Ar-H), 7.60 (1H, m, Ar-H), 7.72–7.82 (2H, m, Ar-H), 7.92 (2H, d, *J*: 7.95 Hz) Ar-H), 8.13 (2H, d, *J*: 8.09 Hz, Ar-H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 27.56, 52.13, 112.39, 123.03, 124.57, 126.42, 129.43, 130.36, 134.04, 135.21, 140.12, 143.36, 147.02, 193.82 and 194.10. For $C_{17}H_{14}N_2O_2$ calculated: 73.37% C, 5.07% H, 10.07% N; found: 73.56% C, 5.02% H, 10.21% N. MS: *m/z* 279 (M + 1).

*2-(2-Acetyl-1H-benzimidazol-1-yl)-1-(4-methoxyphenyl)ethanone (**3b**)*

Yield 65%. m.p. 141–142 °C (ref. 141–142 °C)[27] IR (KBr) ν_{max} (cm^{-1}): 3052–3016 (Aromatic –C–H), 2983–2851 (Aliphatic –C–H), 1689 and 1674 (–C = O), 1641–1463 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.71 (3H, s, $–COCH_3$), 3.72 (3H, s, $–COCH_3$), 6.24 (2H, s, $–CH_2CO$), 7.15–7.24 (4H, m, Ar-H), 7.58 (2H, d, *J*: 8.15 Hz, Ar-H), 7.92 (2H, d, *J*: 8.14 Hz, Ar-H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 27.68, 52.94, 56.06, 113.08, 123.44, 124.52, 127.29, 130.07, 132.14, 139.52, 140.63, 142.45, 147.53, 150.12, 193.69 and 194.55. For $C_{18}H_{16}N_2O_3$ calculated: 70.12% C, 5.23% H, 9.09% N; found: 70.16% C, 5. 25% H, 9.11% N. MS: *m/z* 309 (M + 1).

*2-(2-Acetyl-1H-benzimidazol-1-yl)-1-(3-chlorophenyl)ethanone (**3c**)*

Yield 72%. m.p. 151–153 °C. IR (KBr) ν_{max} (cm^{-1}): 3068–3020 (Aromatic –C–H), 2993–2931 (Aliphatic –C–H), 1695 and 1675 (–C = O), 1620–1452 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.71 (3H, s, $–COCH_3$), 6.24 (2H, s, $–CH_2CO$), 7.39–7.42 (1H, m, Ar-H), 7.45–7.49 (1H, m, Ar-H), 7.69 (1H, t, *J*: 7.88 Hz, Ar-H), 7.82 (1H, d, *J*: 8.24 Hz, Ar-H), 7.84–7.87 (1H, m, Ar-H), 7.91 (1H, d, *J*: 8.13 Hz, Ar-H), 8.06–8.09 (1H, m, Ar-H), 8.17 (1H, t, *J*: 1.82 Hz, *J*: 1.77 Hz, *J*: 1.81 Hz, Ar-H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 27.98, 52.78, 112.66, 122.53, 124.90, 127.44, 128.02, 129.15, 132.28, 135.03, 135.17, 137.68, 138.19, 142.35, 147.21, 194.02 and 194.30. For $C_{17}H_{13}ClN_2O_2$ calculated: 65.29% C, 4.17% H, 8.96% N; found: 63.56% C, 4.02% H, 8.11% N. MS: *m/z* 312.9 (M + 1).

*2-(2-Acetyl-1H-benzimidazol-1-yl)-1-(4-chlorophenyl)ethanone (**3d**)*

Yield 71%. m.p. 161–162 °C (ref. 161–162 °C)[22]. IR (KBr) ν_{max} (cm^{-1}): 3045–3025 (Aromatic –C–H), 2933–2833 (Aliphatic –C–H), 1695 and 1674 (–C = O), 1587–1452 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.73 (3H, s, $–COCH_3$), 6.24 (2H, s, $–CH_2CO$), 7.39–7.44 (2H, m, Ar-H), 7.70 (2H, d, *J*: 6.12 Hz, Ar-H), 7.96–7.97 (1H, m, Ar-H), 8.10 (2H, d, *J*: 8.15 Hz, Ar-H), 8.22 (1H, d, *J*: 7.75 Hz, Ar-H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 27.99, 52.62, 112.68, 122.52, 124.87, 127.10, 130.37, 131.35, 134.54, 138.22, 140.29, 142.36, 147.24, 193.97 and 194.31. For $C_{17}H_{13}ClN_2O_2$ calculated: 65.29% C, 4.17% H, 8.96% N; found: 63.66% C, 4.05% H, 8.15% N. MS: *m/z* 313.1 (M + 1).

*1-Methylidene-2-(4-substituted benzyl)-3-(4-substituted phenyl)-pyrazino[1,2-a]benzimidazole (**4a–t**)*

A mixture of the **3a–d** (2 mmol) and an appropriate benzylamine (2 mmol) was refluxed for 10 h in acetic acid (50 mL). At the end of this time after cooling the mixture, ice water was poured into it and the aqueous mixture was neutralized with sodium carbonate. After removal of water, the obtained cohesive precipitate was crystallized from ethanol.

*2-Benzyl-1-methylidene-3-phenyl-1,2-dihydropyrazino[1,2-a] benzimidazole (**4a**)*

Yield 52%. m.p. 82–84 °C. IR (KBr) ν_{max} (cm^{-1}): 3057–3000 (Aromatic –C–H), 2926–2852 (Aliphatic –C–H), 1601–1471

(–C = C, –C = N). ^{1}H-NMR (DMSO-d$_6$): δ (ppm) 4.64 (2H, d, *J*: 6.22 Hz, Ar-CH$_2$–), 6.62 (1H, s, Ar-H), 7.13 (1H, t, *J*: 6.28 Hz, Ar-H), 7.23 (1H, t, *J*: 7.33 Hz, Ar-H), 7.32–7.38 (4H, m, Ar-H), 7.45–7.50 (5H, m, Ar-H), 7.68 (2H, d, *J*: 8.02 Hz, Ar-H), 7.83 (1H, d, *J*: 8.16 Hz, =CH$_2$), 8.35 (1H, d, *J*: 8.14 Hz, =CH$_2$), 8.64 (1H, s, pyrazinobenzimidazole C$_4$–H). ^{13}C NMR (125 MHz, DMSO-d$_6$): δ 46.58, 101.17, 112.33, 113.49, 120.12, 121.89, 126.13, 126.27, 127.75, 128.06, 128.4, 128.66, 129.63, 130.14, 131.40, 137.50, 139.04, 140.72, 143.24 and 144.64. For $C_{24}H_{19}N_3$ calculated: 82.49% C, 5.48% H, 12.03% N; found: 82.44% C, 5.17% H, 12.28% N. MS: *m/z* 350 (M + 1).

*2-(3-Methoxybenzyl)-1-methylidene-3-phenyl-1,2-dihydropyrazino[1,2-a]benzimidazole (**4b**)*

Yield 50%. m.p. 105 °C. IR (KBr) ν_{max} (cm^{-1}): 3052–3024 (Aromatic –C–H), 2985–2886 (Aliphatic –C–H), 1573 – 1482 (–C = C, –C = N). ^{1}H-NMR (DMSO-d$_6$): δ (ppm) 3.72 (3H, s, –CH$_3$), 4.66 (2H, d, *J*: 6.02 Hz, Ar-CH$_2$–), 6.56 (1H, s, Ar-H), 7.10 (1H, t, *J*: 6.33 Hz, Ar-H), 7.18 (1H, t, *J*: 7.22 Hz, Ar-H), 7.28–7.41 (4H, m, Ar-H), 7.45–7.53 (4H, m, Ar-H), 7.62 (1H, d, *J*: 7.82 Hz, Ar-H), 7.78–7.80 (1H, m, Ar-H), 7.83 (1H, d, *J*: 8.21 Hz, =CH$_2$), 8.37 (1H, d, *J*: 8.0 Hz, =CH$_2$), 8.67 (1H, s, pyrazinobenzimidazole C$_4$–H). ^{13}C NMR (125 MHz, DMSO-d$_6$): δ 46.82, 56.28, 102.52, 112.85, 113.68, 114.12, 115.23, 121.23, 121.87, 125.56, 126.47, 128.87, 129.79, 130.74, 131.45, 131.56, 132.74, 137.58, 139.10, 144.32 and 157.52. For $C_{25}H_{21}N_3O$ calculated: 79.13% C, 5.58% H, 11.07% N; found: 79.17% C, 5.61% H, 11.12% N. MS: *m/z* 380 (M + 1).

*2-(3-Chlorobenzyl)-1-methylidene-3-phenyl-1,2-dihydropyrazino[1,2-a]benzimidazole (**4c**)*

Yield 54%. m.p. 74–75 °C. IR (KBr) ν_{max} (cm^{-1}): 3055–3030 (Aromatic –C–H), 2956–2902 (Aliphatic –C–H), 1585–1479 (–C = C, –C = N). ^{1}H-NMR (DMSO-d$_6$): δ (ppm) 4.65 (2H, d, *J*: 6.03 Hz, Ar-CH$_2$–), 6.56 (1H, s, Ar-H), 7.25–7.55 (10H, m, Ar-H), 7.69 (2H, d, *J*: 8.07 Hz, Ar-H), 7.84 (1H, d, *J*: 8.21 Hz, =CH$_2$), 8.36 (1H, d, *J*: 8.0 Hz, =CH$_2$), 8.66 (1H, s, pyrazinobenzimidazole C$_4$–H). ^{13}C NMR (125 MHz, DMSO-d$_6$): δ 46.04, 102.52, 113.26, 114.41, 122.58, 127.23, 128.04, 129.19, 129.78, 130.03, 130.77, 131.48, 132.96, 138.46, 138.96, 139.56, 143.54 and 144.68. For $C_{24}H_{18}ClN_3$ calculated: 75.09% C, 4.73% H, 10.95% N; found: 75.24% C, 4.77% H, 11.10% N. MS: *m/z* 383.6 (M + 1).

*2-(4-Methoxybenzyl)-1-methylidene-3-phenyl-1,2-dihydropyrazino[1,2-a]benzimidazole (**4d**)*

Yield 63%. m.p. 102–103 °C. IR (KBr) ν_{max} (cm^{-1}): 3063–3015 (Aromatic –C–H), 2956–2898 (Aliphatic –C–H), 1554–1469 (–C = C, –C = N). ^{1}H-NMR (DMSO-d$_6$): δ 3.71 (ppm) (3H, s, –CH$_3$), 4.56 (2H, d, *J*: 6.25 Hz, Ar-CH$_2$–), 6.57 (1H, s, Ar-H), 6.90 (2H, d, *J*: 8.72 Hz, Ar-H), 7.02 (1H, t, *J*: 6.28 Hz, Ar-H), 742–7.34 (4H, m, Ar-H), 7.47–7.50 (3H, m, Ar-H), 7.71 (2H, d, *J*: 7.38 Hz, Ar-H), 7.83 (1H, d, *J*: 8.17 Hz, =CH$_2$), 8.35 (1H, d, *J*: 8.16 Hz, =CH$_2$), 8.64 (1H, s, pyrazinobenzimidazole C$_4$–H). ^{13}C NMR (125 MHz, DMSO-d$_6$): 46.06, 56.25, 101.84, 113.48, 114.56, 122.87, 127.48, 128.64, 129.42, 129.89, 130.21, 130.79, 131.86, 132.74, 138.63, 138.97, 139.82, 143.43, 146.54 and 160.10. For $C_{25}H_{21}N_3O$ calculated: 79.13% C, 5.58% H, 11.07% N; found: 79.19% C, 11.03% H, 11.08% N. MS: *m/z* 380 (M + 1).

*2-(4-Chlorobenzyl)-1-methylidene-3-phenyl-1,2-dihydropyrazino[1,2-a]benzimidazole (**4e**)*

Yield 65%. m.p. 104–106 °C. IR (KBr) ν_{max} (cm^{-1}): 3057–3032 (Aromatic –C–H), 2893–2845 (Aliphatic –C–H), 1562–1481 (–C = C, –C = N). ^{1}H-NMR (DMSO-d$_6$): δ (ppm) 4.64 (2H, d, *J*: 6.38 Hz, Ar-CH$_2$–), 6.53 (1H, s, Ar-H), 7.22 (1H, t, *J*: 6.48 Hz, Ar-H), 7.35–7.40 (4H, m, Ar-H), 7.46–7.50 (5H, m, Ar-H), 7.69 (2H, d, *J*: 7.73 Hz, Ar-H), 7.83 (1H, d, *J*: 8.18 Hz, = CH$_2$), 8.35 (1H, d, *J*: 8.10 Hz, = CH$_2$), 8.66 (1H, s, pyrazinobenzimidazole C$_4$–H). ^{13}C NMR (125 MHz, DMSO-d$_6$): δ 45.74, 101.16, 112.47, 113.50, 121.92, 126.15, 127.78, 128.68, 129.56, 130.15, 130.27, 131.39, 132.53, 137.35, 138.98, 139.89, 143.20 and 144.64. For $C_{24}H_{18}ClN_3$ calculated: 75.09% C, 4.73% H, 10.95% N; found: 74.95% C, 5.02% H, 10.68% N. MS: *m/z* 383.7 (M + 1).

*2-Benzyl-1-methylidene-3-(4-methoxyphenyl)-1,2-dihydropyrazino[1,2-a]benzimidazole (**4f**)*

Yield 51%. m.p. 136 °C. IR (KBr) ν_{max} (cm^{-1}): 3063–3012 (Aromatic C–H), 2923–2876 (Aliphatic C–H), 1574–1486 (–C = C, –C = N). ^{1}H-NMR (DMSO-d$_6$): δ (ppm) 3.81 (3H, s, –CH$_3$), 4.64 (2H, d, *J*: 6.20 Hz, Ar-CH$_2$–), 6.50 (1H, s, Ar-H), 7.03 (2H, d, *J*: 8.63 Hz, Ar-H), 7.09 (1H, t, *J*: 6.30 Hz, Ar-H), 7.23 (1H, t, *J*: 7.17 Hz, Ar-H), 7.32–7.37 (3H, m, Ar-H), 7.46–7.49 (3H, m, Ar-H), 7.61 (2H, d, *J*: 8.59 Hz, Ar-H), 7.82 (1H, d, *J*: 8.18 Hz, =CH$_2$), 8.34 (1H, d, *J*: 7.11 Hz, =CH$_2$), 8.57 (1H, s, pyrazinobenzimidazole C$_4$–H). ^{13}C NMR (125 MHz, DMSO-d$_6$): 46.07, 55.66, 102.41, 113.26, 115.23, 122.63, 128.42, 128.76, 129.02, 129.89, 130.22, 130.65, 131.49, 132.46, 136.54, 137.86, 138.72, 139.12, 143.57, 146.86 and 157.46. For $C_{25}H_{21}N_3O$ calculated: 79.13% C, 5.58% H, 11.07% N; found: 79.18% C, 5.59% H, 11.10% N. MS: *m/z* 380 (M + 1).

*2-(3-Methoxybenzyl)-1-methylidene-3-(4-methoxyphenyl)-1,2-dihydropyrazino[1,2-a]benzimidazole (**4g**)*

Yield 61%. m.p. 99 °C. IR (KBr) ν_{max} (cm^{-1}): 3046–3021 (aromatic –C–H), 2964–2859 (aliphatic –C–H), 1565–1446 (–C = C, –C = N). ^{1}H-NMR (DMSO-d$_6$): δ (ppm) 3.71 (3H, s, –CH$_3$), 3.81 (3H, s, –CH$_3$), 4.60 (2H, d, *J*: 6.20 Hz, Ar-CH$_2$–), 6.52 (1H, s, Ar-H), 6.80 (1H, d, *J*: 8.81 Hz, Ar-H), 7.03–7.10 (5H, m, Ar-H), 7.25 (1H, t, *J*: 7.80 Hz, Ar-H), 7.35 (1H, t, *J*: 7.40 Hz, Ar-H), 7.48 (1H, t, *J*: 7.64 Hz, Ar-H), 7.62 (2H, d, *J*: 8.46 Hz, Ar-H), 7.82 (1H, d, *J*: 8.20 Hz, =CH$_2$), 8.33 (1H, d, *J*: 8.00 Hz, = CH$_2$), 8.57 (1H, s, pyrazinobenzimidazole C$_4$–H). ^{13}C NMR (125 MHz, DMSO-d$_6$): δ 46.03, 55.69, 56.33, 102.40, 113.32, 113.46, 114.68, 121.69, 121.95, 123.44, 125.27, 126.75, 127.14, 127.76, 128.72, 130.61, 132.57, 135.24, 137.48, 141.54, 143.66, 145.58, 158.22 and 159.12. For $C_{26}H_{23}N_3O_2$ calculated: 76.26% C, 5.66% H, 10.26% N; found: 76.23% C, 5.69% H, 10.20% N. MS: *m/z* 410 (M + 1).

*2-(3-Chlorobenzyl)-1-methylidene-3-(4-methoxyphenyl)-1,2-dihydropyrazino[1,2-a]benzimidazole (**4h**)*

Yield 61%. m.p. 91–92 °C. IR (KBr) ν_{max} (cm^{-1}): 3061–3028 (Aromatic –C–H), 2961–2877 (Aliphatic –C–H), 1563–1486 (–C = C, –C = N). ^{1}H-NMR (DMSO-d$_6$): δ (ppm) 3.81 (3H, s, –CH$_3$), 4.65 (2H, d, *J*: 6.32 Hz, Ar-CH$_2$–), 6.56 (1H, s, Ar-H), 7.04 (2H, d, *J*: 8.55 Hz, Ar-H), 7.22 (1H, t, *J*: 6.37 Hz, Ar-H), 7.28–7.38 (3H, m, Ar-H), 7.44–7.50 (2H, m, Ar-H), 7.57 (1H, m, Ar-H), 7.62 (2H, d, *J*: 8.55 Hz, Ar-H), 7.83 (1H, d, *J*: 8.18 Hz, =CH$_2$), 8.34 (1H, d, *J*: 8.15 Hz, =CH$_2$), 8.58 (1H, s, pyrazinobenzimidazole C$_4$–H). ^{13}C NMR (125 MHz, DMSO-d$_6$): δ 45.54, 57.45, 101.54, 110.62, 113.49, 115.89, 120.24, 121.46, 125.48, 126.36, 128.42, 129.12, 130.34, 131.45, 131.86, 132.85, 137.69, 139.73, 143.14, 144.37 and 159.46. For $C_{25}H_{20}ClN_3O$ calculated: 72.55% C, 4.87% H, 10.15% N; found: 72.52% C, 4.79% H, 10.10% N. MS: *m/z* 413.6 (M + 1).

*2-(4-Methoxybenzyl)-1-methylidene-3-(4-methoxyphenyl)-1,2-dihydropyrazino[1,2-a]benzimidazole (**4i**)*

Yield 55%. m.p. 148–149 °C. IR (KBr) ν_{max} (cm^{-1}): 3076–3012 (Aromatic –C–H), 2917–2837 (aliphatic –C–H), 1549–1453 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6): δ (ppm) 3.71 (3H, s, $–CH_3$), 3.81 (3H, s, $–CH_3$), 4.55 (2H, d, *J*: 6.10 Hz, Ar-CH_2–), 6.53 (1H, s, Ar-H), 6.90 (2H, d, *J*: 8.40 Hz, Ar-H), 6.98 (1H, t, *J*: 7.60 Hz, Ar-H), 7.05 (2H, d, *J*: 8.49 Hz, Ar-H), 7.35 (1H, t, *J*: 7.33 Hz, Ar-H), 7.40 (2H, d, *J*: 8.17 Hz, Ar-H), 7.47 (1H, t, *J*: 7.58 Hz, Ar-H), 7.64 (2H, d, *J*: 8.51 Hz, Ar-H), 7.81 (1H, d, *J*: 8.19 Hz, $=CH_2$), 8.33 (1H, d, *J*: 8.18 Hz, $=CH_2$), 8.56 (1H, s, pyrazinobenzimidazole C_4–H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 46.01, 55.79, 56.04, 101.26, 111.44, 113.43, 114.94, 115.51, 120.06, 121.73, 126.02, 128.88, 129.71, 131.35, 132.45, 137.38, 143.14, 144.61, 159.62 and 160.29. For $C_{26}H_{23}N_3O_2$ calculated: 76.26% C, 5.66% H, 10.26% N; found: 76.25% C, 5.71% H, 10.22% N. MS: *m/z* 410 (M + 1).

*2-(4-Chlorobenzyl)-1-methylidene-3-(4-methoxyphenyl)-1,2-dihydropyrazino[1,2-a]benzimidazole (**4j**)*

Yield 52%. m.p. 155 °C. IR (KBr) ν_{max} (cm^{-1}): 3063–3016 (aromatic –C–H), 2967–2837 (aliphatic –C–H), 1569–1416 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6): δ (ppm) 3.80 (3H, s, $–CH_3$), 4.63 (2H, d, *J*: 6.33 Hz, Ar-CH_2–), 6.49 (1H, s, Ar-H), 7.04 (2H, d, *J*: 8.58 Hz, Ar-H), 7.17 (1H, t, *J*: 6.34 Hz, Ar-H), 7.34–7.40 (3H, m, Ar-H), 7.46–7.50 (3H, m, Ar-H), 7.61 (2H, d, *J*: 8.62 Hz, Ar-H), 7.82 (1H, d, *J*: 8.22 Hz, $=CH_2$), 8.33 (1H, d, *J*: 8.14 Hz, $=CH_2$), 8.57 (1H, s, pyrazinobenzimidazole C_4–H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 45.77, 56.03, 101.31, 111.65, 113.46, 115.51, 120.07, 121.79, 125.97, 126.04, 128.89, 129.55, 130.26, 131.23, 131.33, 132.52, 137.23, 139.91, 143.07, 144.61 and 160.31. For $C_{25}H_{20}ClN_3O$ calculated: 72.55% C, 4.87% H, 10.15% N; found: 72.53% C, 4.82% H, 10.13% N. MS: *m/z* 413.7 (M + 1).

*2-Benzyl-1-methylidene-3-(3-chlorophenyl)-1,2-dihydropyrazino[1,2-a]benzimidazole (**4k**)*

Yield 55%. m.p. 85–86 °C. IR (KBr) ν_{max} (cm^{-1}): 3059–3035 (Aromatic C–H), 2887 (aliphatic C–H), 1589–1465 (C = C, C = N). ^{1}H-NMR (DMSO-d_6): δ (ppm) 4.66 (2H, d, *J*: 6.28 Hz, Ar-CH_2–), 6.56 (1H, s, Ar-H), 7.13 (1H, t, *J*: 6.34 Hz, Ar-H), 7.23 (1H, t, *J*: 7.49 Hz, Ar-H), 7.32–7.44 (4H, m, Ar-H), 7.46–7.52 (4H, m, Ar-H), 7.65 (1H, d, *J*: 7.74 Hz, Ar-H), 7.78–7.80 (1H, m, Ar-H), 7.84 (1H, d, *J*: 8.23 Hz, $=CH_2$), 8.37 (1H, d, *J*: 8.15 Hz, $=CH_2$), 8.74 (1H, s, pyrazinobenzimidazole C_4–H). ^{13}C NMR (125 MHz, DMSO-d_6): 46.24, 102.48, 113.36, 115.54, 122.28, 128.01, 128.13, 128.51, 129.04, 130.04, 130.47, 131.31, 133.52, 136.45, 137.88, 138.84, 139.17, 143.23 and 146.45. For $C_{24}H_{18}ClN_3$ calculated: 75.09% C, 4.73% H, 10.95% N; found: 75.24% C, 4.97% H, 10.90% N. MS: *m/z* 383.7 (M + 1).

*2-(3-Methoxybenzyl)-1-methylidene-3-(3-chlorophenyl)-1,2-dihydropyrazino[1,2-a]benzimidazole (**4l**)*

Yield 62%. m.p. 115 °C. IR (KBr) ν_{max} (cm^{-1}): 3067–3012 (Aromatic –C–H), 2950–2837 (aliphatic –C–H), 1588–1451 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6): δ (ppm) 3.71 (3H, s, $–CH_3$), 4.62 (2H, d, *J*: 6.23 Hz, Ar-CH_2–), 6.52 (1H, s, Ar-H), 7.35–7.52 (9H, m, Ar-H), 7.69 (1H, d, *J*: 7.68 Hz, Ar-H), 7.82 (1H, s, Ar-H), 7.86 (1H, d, *J*: 8.16 Hz, $=CH_2$), 8.37 (1H, d, *J*: 8.12 Hz, $=CH_2$), 8.74 (1H, s, pyrazinobenzimidazole C_4–H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 46.01, 56.04, 101.26, 111.44, 113.43, 114.95, 115.51, 120.06, 121.73, 126.02, 128.89, 129.72, 131.35, 132.46, 137.37, 143.14, 144.61 and 159.62. For $C_{25}H_{20}ClN_3O$ calculated: 72.55% C, 4.87% H, 10.15% N; found: 72.50% C, 4.83% H, 10.11% N. MS: *m/z* 413.6 (M + 1).

*2-(3-Chlorobenzyl)-1-methylidene-3-(3-chlorophenyl)-1,2-dihydropyrazino[1,2-a]benzimidazole (**4m**)*

Yield 72%. m.p. 105–106 °C. IR (KBr) ν_{max} (cm^{-1}): 3097–3020 (aromatic C–H), 2840 (aliphatic C–H), 1591–1471 (C = C, C = N). ^{1}H-NMR (DMSO-d_6): δ (ppm) 4.65 (2H, d, *J*: 6.03 Hz, Ar-CH_2–), 6.56 (1H, s, Ar-H), 7.28–7.58 (9H, m, Ar-H), 7.67 (1H, d, *J*: 7.76 Hz, Ar-H), 7.80 (1H, s, Ar-H), 7.84 (1H, d, *J*: 8.26 Hz, $=CH_2$), 8.38 (1H, d, *J*: 8.12 Hz, $=CH_2$), 8.76 (1H, s, pyrazinobenzimidazole C_4–H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 45.76, 101.66, 113.18, 113.60, 120.17, 122.07, 124.69, 126.27, 126.36, 127.18, 127.45, 128.01, 128.33, 128.47, 131.45, 131.49, 131.94, 134.35, 135.02, 137.37, 141.15, 143.18, 143.62 and 144.65. For $C_{24}H_{17}Cl_2N_3$ calculated: 68.91% C, 4.10% H, 10.05% N; found: 69.22% C, 4.07% H, 10.20% N. MS: *m/z* 419 (M + 1).

*2-(4-Methoxybenzyl)-1-methylidene-3-(3-chlorophenyl)-1,2-dihydropyrazino[1,2-a]benzimidazole (**4n**)*

Yield 64%. m.p. 88–89 °C. IR (KBr) ν_{max} (cm^{-1}): 3069–3029 (Aromatic –C–H), 2837 (aliphatic –C–H), 1567–1459 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6): δ (ppm) 3.72 (3H, s, $–CH_3$), 4.62 (2H, d, *J*: 6.20 Hz, Ar-CH_2–), 6.54 (1H, s, Ar-H), 7.27–7.48 (9H, m, Ar-H), 7.69 (2H, d, *J*: 8.12 Hz, Ar-H), 7.85 (1H, d, *J*: 8.11 Hz, $=CH_2$), 8.35 (1H, d, *J*: 8.05 Hz, $=CH_2$), 8.65 (1H, s, pyrazinobenzimidazole C_4–H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 45.96, 55.79, 101.62, 112.87, 113.55, 114.93, 120.14, 121.98, 124.76, 126.21, 126.34, 126.43, 127.44, 128.43, 129.79, 131.45, 131.96, 132.09, 132.39, 135.02, 137.55, 141.26, 143.27, 144.64 and 159.65. For $C_{25}H_{20}ClN_3O$ calculated: 72.55% C, 4.87% H, 10.15% N; found: 72.51% C, 4.82% H, 10.14% N. MS: *m/z* 414.5 (M + 1).

*2-(4-Chlorobenzyl)-1-methylidene-3-(3-chlorophenyl)-1,2-dihydropyrazino[1,2-a]benzimidazole (**4o**)*

Yield 60%. m.p. 106–108 °C. IR (KBr) ν_{max} (cm^{-1}): 3052–3016 (Aromatic –C–H), 2963–2841 (Aliphatic –C–H), 1590–1433 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6): δ (ppm) 4.65 (2H, d, *J*: 6.39 Hz, Ar-CH_2–), 6.55 (1H, s, Ar-H), 7.21 (1H, t, *J*: 6.41 Hz, Ar-H), 7.38–7.44 (4H, m, Ar-H), 7.47–7.52 (4H, m, Ar-H), 7.67 (1H, d, *J*: 7.74 Hz, Ar-H), 7.80 (1H, s, Ar-H), 7.84 (1H, d, *J*: 8.18 Hz, $=CH_2$), 8.36 (1H, d, *J*: 8.17 Hz, $=CH_2$), 8.75 (1H, s, pyrazinobenzimidazole C_4–H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 46.52, 101.25, 112.68, 113.96, 121.36, 123.16, 124.75, 126.37, 126.89, 127.63, 128.29, 128.86, 128.94, 130.23, 131.53, 131.84, 133.28, 135.69, 138.21, 140.25, 142.69, 143.58 and 144.85. For $C_{24}H_{17}Cl_2N_3$ calculated: 68.91% C, 4.10% H, 10.05% N; found: 69.10% C, 4.27% H, 10.10% N. MS: *m/z* 419 (M + 1).

*2-Benzyl-1-methylidene-3-(4-chlorophenyl)-1,2-dihydropyrazino[1,2-a]benzimidazole (**4p**)*

Yield 54%. m.p. 174–176 °C. IR (KBr) ν_{max} (cm^{-1}): 3051–3012 (Aromatic –C–H), 2973–2850 (aliphatic –C–H), 1564–1469 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6): δ (ppm) 4.64 (2H, d, *J*: 6.30 Hz, Ar-CH_2–), 6.54 (1H, s, Ar-H), 7.14 (1H, t, *J*: 6.40 Hz, Ar-H), 7.23 (1H, t, *J*: 7.33 Hz, Ar-H), 7.33–7.39 (3H, m, Ar-H), 7.46–7.50 (3H, m, Ar-H), 7.53 (2H, d, *J*: 9.63 Hz, Ar-H), 7.73 (2H, d, *J*: 8.58 Hz, Ar-H), 7.83 (1H, d, *J*: 8.16 Hz, $=CH_2$), 8.33 (1H, d, *J*: 8.18 Hz, $=CH_2$), 8.70 (1H, s, pyrazinobenzimidazole C_4–H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 45.69, 102.12, 113.54, 113.97, 122.89, 123.46, 124.15, 126.79, 126.87, 127.19, 128.47, 128.84, 129.04, 130.47, 131.24, 131.87, 133.47, 135.59, 138.76,

140.33, 142.72, 143.14 and 144.46. For $C_{24}H_{18}ClN_3$ calculated: 75.09% C, 4.73% H, 10.95% N; found: 75.20% C, 4.57% H, 10.78% N. MS: *m/z* 383.7 (M + 1).

*2-(3-Methoxybenzyl)-1-methylidene-3-(4-chlorophenyl)-1,2-dihydropyrazino[1,2-a]benzimidazole (**4q**)*

Yield 59%. m.p. 118 °C. IR (KBr) ν_{max} (cm^{-1}): 3058–3029 (aromatic –C–H), 2964–2863 (aliphatic –C–H), 1586–1451 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6): δ (ppm) 3.72 (3H, s, $–CH_3$), 4.64 (2H, d, *J*: 6.72 Hz, Ar-CH_2–), 6.52 (1H, s, Ar-H), 7.17 (2H, d, *J*: 8.45 Hz, Ar-H), 7.26 (1H, t, *J*: 6.75 Hz, Ar-H), 7.25–7.34 (3H, m, Ar-H), 7.48–7.54 (2H, m, Ar-H), 7.59 (1H, m, Ar-H), 7.64 (2H, d, *J*: 8.45 Hz, Ar-H), 7.88 (1H, d, *J*: 8.13 Hz, $=CH_2$), 8.37 (1H, d, *J*: 8.19 Hz, $=CH_2$), 8.71 (1H, s, pyrazinobenzimidazole C_4–H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 45.93, 56.08, 101.22, 113.12, 113.85, 114.76, 121.25, 121.89, 123.65, 125.47, 126.89, 127.12, 127.82, 128.13, 129.65, 130.79, 132.02, 132.53, 135.22, 137.14, 141.13, 143.56, 144.48 and 158.74. For $C_{25}H_{20}ClN_3O$ calculated: 72.55% C, 4.87% H, 10.15% N; found: 72.49% C, 4.83% H, 10.17% N. MS: *m/z* 413.6 (M + 1).

*2-(3-Chlorobenzyl)-1-methylidene-3-(4-chlorophenyl)-1,2-dihydropyrazino[1,2-a]benzimidazole (**4r**)*

Yield 62%. m.p. 191–193 °C. IR (KBr) ν_{max} (cm^{-1}): 3056–3021 (aromatic –C–H), 2937–2864 (aliphatic C–H), 1557–1450 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6): δ (ppm) 4.66 (2H, d, *J*: 6.42 Hz, Ar-CH_2–), 6.53 (1H, s, Ar-H), 7.24 (1H, t, *J*: 6.51 Hz, Ar-H), 7.36–7.40 (3H, m, Ar-H), 7.48–7.55 (3H, m, Ar-H), 7.54 (2H, d, *J*: 8.51 Hz, Ar-H), 7.74 (2H, d, *J*: 8.63 Hz, Ar-H), 7.84 (1H, d, *J*: 8.19 Hz, $=CH_2$), 8.35 (1H, d, *J*: 8.18 Hz, $=CH_2$), 8.70 (1H, s, pyrazinobenzimidazole C_4–H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 46.22, 101.54, 112.75, 113.41, 121.24, 122.52, 124.46, 126.78, 127.13, 128.28, 128.76, 129.38, 131.89, 132.67, 132.83, 134.52, 135.29, 137.58, 141.29, 143.69, 143.92 and 144.46. For $C_{24}H_{17}Cl_2N_3$ calculated: 68.91% C, 4.10% H, 10.05% N; found: 68.66% C, 4.35% H, 10.20% N. MS: *m/z* 419 (M + 1).

*2-(4-Methoxybenzyl)-1-methylidene-3-(4-chlorophenyl)-1,2-dihydropyrazino[1,2-a]benzimidazole (**4s**)*

Yield 63%. m.p. 146 °C. IR (KBr) ν_{max} (cm^{-1}): 3059–3012 (Aromatic –C–H), 2954–2897 (aliphatic –C–H), 1565–1413 (–C = C, –C = N). ^{1}H-NMR (DMSO-d_6): δ (ppm) 3.71 (3H, s, $–CH_3$), 4.55 (2H, d, *J*: 8.21 Hz, Ar-CH_2–), 6.56 (1H, s, Ar-H), 6.89 (2H, d, *J*: 8.58 Hz, Ar-H), 7.03 (1H, t, *J*: 8.24 Hz, Ar-H), 7.39–7.41 (3H, m, Ar-H), 7.48 (1H, t, *J*: 7.4 Hz, Ar-H), 7.54 (2H, d, *J*: 8.46 Hz, Ar-H), 7.75 (2H, d, *J*: 8.48 Hz, Ar-H), 7.85 (1H, d, *J*: 8.17 Hz, $=CH_2$), 8.34 (1H, d, *J*: 8.14 Hz, $=CH_2$), 8.68 (1H, s, pyrazinobenzimidazole C_4–H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 45.87, 55.85, 101.54, 113.64, 113.89, 114.77, 120.58, 121.54, 125.63, 126.57, 127.56, 128.94, 129.96, 131.62, 132.05, 132.86, 133.29, 135.54, 137.73, 141.69, 143.12, 144.44 and 158.98. For $C_{25}H_{20}ClN_3O$ calculated: 72.55% C, 4.87% H, 10.15% N; found: 72.48% C, 4.85% H, 10.12% N. MS: *m/z* 413.6 (M + 1).

*2-(4-Chlorobenzyl)-1-methylidene-3-(4-chlorophenyl)-1,2-dihydropyrazino[1,2-a]benzimidazole (**4t**)*

Yield 68%. m.p. 213–215 °C. IR (KBr) ν_{max} (cm^{-1}): 3041 (aromatic C–H), 2897 (aliphatic C–H), 1587–1460 (C = C, C = N). ^{1}H-NMR (DMSO-d_6): δ (ppm) 4.64 (2H, d, *J*: 6.42 Hz, Ar-CH_2–), 6.53 (1H, s, Ar-H), 7.24 (1H, t, *J*: 6.51 Hz, Ar-H), 7.36–7.40 (3H, m, Ar-H), 7.47–7.51 (3H, m, Ar-H), 7.54 (2H, d, *J*: 8.76 Hz, Ar-H), 7.74 (2H, d, *J*: 8.63 Hz, Ar-H), 7.84 (1H, d, *J*: 8.19 Hz, $=CH_2$), 8.35 (1H, d, *J*: 8.18 Hz, $=CH_2$), 8.70 (1H, s, pyrazinobenzimidazole C_4–H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 45.69, 101.46, 112.89, 113.45, 120.46, 122.68, 124.69, 127.21, 127.78, 128.57, 128.89, 129.01, 131.63, 131.79, 132.21, 134.46, 135.34, 137.42, 141.63, 143.52, 143.88 and 144.17. For $C_{24}H_{17}Cl_2N_3$ calculated: 68.91% C, 4.10% H, 10.05% N; found: 68.75% C, 3.92% H, 9.90% N. MS: *m/z* 419 (M + 1).

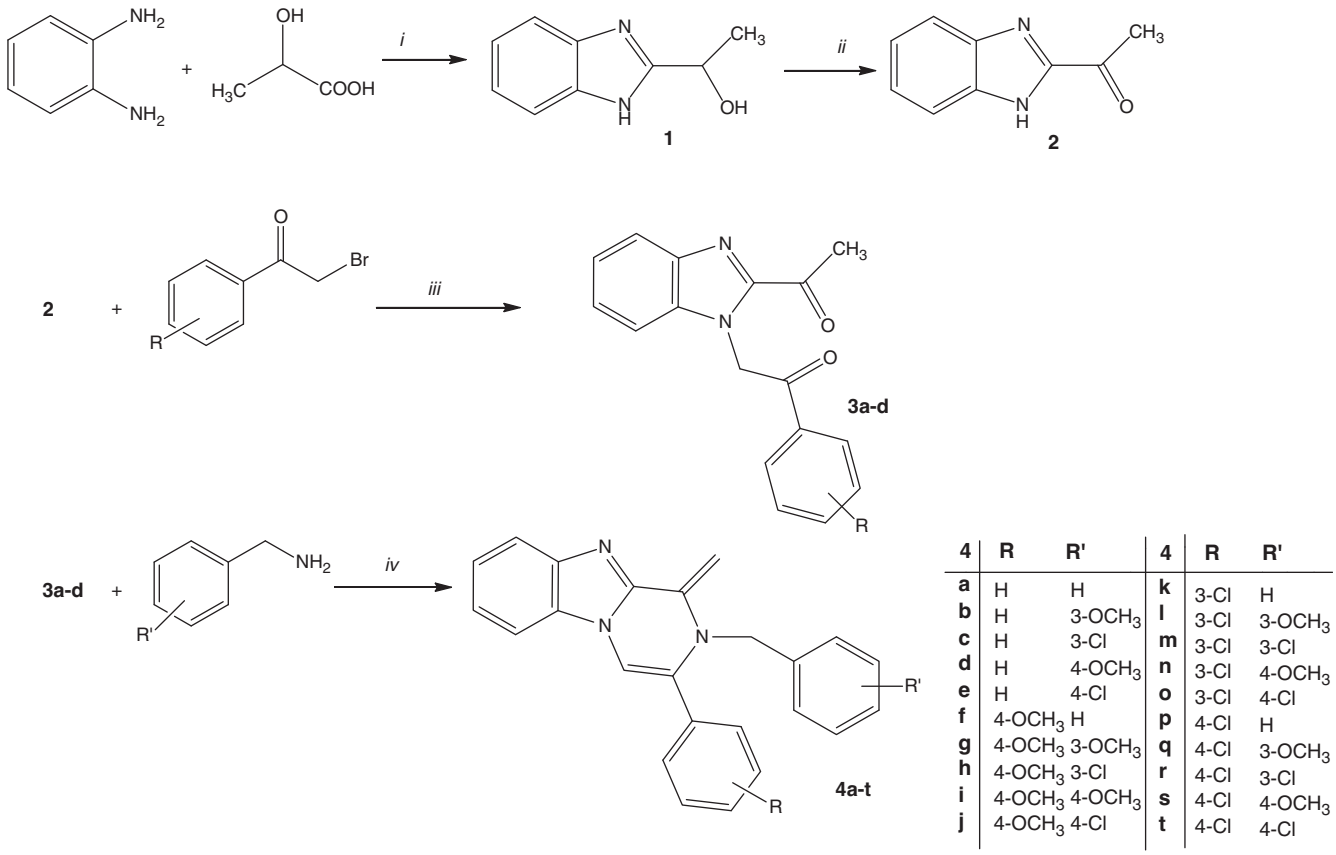
Anticancer activity tests

The cytotoxic and/or growth inhibitory effects of the compounds were evaluated *in vitro* against approximately 60 human tumor cell lines derived from nine neoplastic diseases namely leukemia (L, four or six cell lines), non-small cell lung cancer (NSCLC, nine cell lines), colon cancer (CC, seven cell lines), central nervous system cancer (CNSC, six cell lines), melanoma (M, eight or nine cell lines), ovarian cancer (OC, six or seven cell lines), renal cancer (RC, eight cell lines), prostate cancer (PC, two cell lines) and breast cancer (BC, six or eight cell lines). The evaluation of anticancer activity was performed at the National Cancer Institute (NCI) of Bethesda, MD, USA, following the *in vitro* screening program at 10-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents. A 48 h continuous drug exposure protocol was followed and a sulforhodamine B protein assay was used to estimate cell viability of growth. Three dose response parameters (GI_{50}, TGI and LC_{50}) were calculated for each experimental agent[28–31].

Results and discussions

Chemistry

In this study, the syntheses of the new pyrazinobenzimidazoles (**4a–t**), have been carried out as shown in Scheme 1. First, *o*-phenylenediamine and lactic acid were reacted with 4 N HCl solution according to Phillips method to give 2-(1-hydroxyethyl)-benzimidazole. The obtained compound (**1**) was oxidized with chromium trioxide to give 2-acetyl benzimidazole (**2**) compound, which was then reacted with appropriate α-bromoacetophenones in the presence of potassium carbonate. IR, ^{1}H-NMR and ^{13}C-NMR spectra were measured for the compounds **2** and **3a–d**. In the spectrum of the compound **2**, bands were observed at about 3288–2400 cm^{-1} and 1674 cm^{-1} belonging to –N–H and –C = O bonds, respectively. Proton of the benzimidazole nitrogen was observed at 12.4 ppm as broad singlet peak as expected. In the spectrum of the compounds **3a–d**, carbonyl bands were observed 1695–1674 cm^{-1} and methylene protons were observed at about 6.23–6.24 ppm. At the final step, 2-(2-acetyl-1*H*-benzimidazol-1-yl)-1-(subtituted phenyl)ethanone derivatives (**3a–d**) and varied benzylamines were refluxed in acetic acid to afford corresponding 2-(4-substituted benzyl)-1-methylidene-3-(4-substituted phenyl)-pyrazino[1,2-*a*]benzimidazole (**4a–t**) derivatives. The resulting products were yielded in the range of 50–72%. In the IR spectra of the final compounds (**4a–t**), all bands were observed in expected areas and in addition disappearance of stretching bands about 1695–1674 cm^{-1} belonging to ketone carbonyl was an evidence for ring closure analyzed by IR spectroscopy. In the ^{1}H-NMR spectra of the final compounds, doublet peak was observed at 4.64–4.66 ppm belonging to –N–CH_2 protons and the resonating of these protons as doublet was thought to be due to magnetic anisotropy. The peaks seen at 7.83–8.38 ppm were assigned for methylene protons in the first position of pyrazinobenzimidazole ring system. The signal belonging to the C_4–H proton of the pyrazinobenzimidazole condensed ring system was observed much further downfield at about 8.64–8.75 ppm according to the other aromatic protons. The other characteristic aromatic



4	R	R'	4	R	R'
a	H	H	k	3-Cl	H
b	H	3-OCH_3	l	3-Cl	3-OCH_3
c	H	3-Cl	m	3-Cl	3-Cl
d	H	4-OCH_3	n	3-Cl	4-OCH_3
e	H	4-Cl	o	3-Cl	4-Cl
f	4-OCH_3	H	p	4-Cl	H
g	4-OCH_3	3-OCH_3	q	4-Cl	3-OCH_3
h	4-OCH_3	3-Cl	r	4-Cl	3-Cl
i	4-OCH_3	4-OCH_3	s	4-Cl	4-OCH_3
j	4-OCH_3	4-Cl	t	4-Cl	4-Cl

Scheme 1. The synthetic protocol of the compounds **3a–d** and **4a–t**.
The synthetic protocol of the compounds (**4a–t**). Reagents: (i) 4N HCl, reflux, 8 h, 71%; (ii) CrO_3, CH_3COOH, 90 °C during the addition of chromium trioxide solution addition then cooled, 76%; (iii) K_2CO_3, acetone, rt, 4–6 h, 65–75%; (iv) CH_3COOH, reflux, 10 h, 50–72%.

protons were observed at expected areas, about 6.52–7.80 ppm. In the ^{13}C NMR spectra of the compounds **3a–d**, the characteristic peaks were seen at about 193.82–194.55 ppm and 27.56–27.99 ppm belonging to –C = O and $–CH_3$ groups carbon. Peaks at about 45.54–46.82 ppm and 101.16–102.52 ppm belonging to $–CH_2–$ and =CH– carbon atoms were also seen in spectra of the final compounds (**4a–t**). The mass spectra of the compounds showed [M + 1] peaks, in agreement with their molecular weight. All compounds gave satisfactory elemental analysis results in correlation with the calculations. During the laboratory work, another similar compound 2-(2-acetyl-1*H*-benzimidazol-1-yl)-1-(3-methoxyphenyl)ethanone was synthesized but 2-(substituted benzyl)-1-methylidene-3-(3-methoxyphenyl)-1,2-dihydropyrazino[1,2-*a*]benzimidazole derivatives which are planned to synthesize from this diketone compound could not be obtained purely. So, the indicated compounds did not include to this study.

Anticancer activity evaluation

All final compounds (**4a–t**) and new diketone compound **3c** were submitted to NCI for testing their anticancer activity according to *in vitro* drug screening protocol of the institute. The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose. **3c** (NSC 748533), **4d** (NSC 748537), **4e** (NSC 748536), **4f** (NSC 748535), **4i** (NSC 748540), **4j** (NSC 748539), **4n** (NSC 748541), **4p** (NSC 748534) and **4s** (NSC 748538) were selected by NCI for the anticancer tests for single dose testing. *In vitro* single dose anticancer assay was performed in full NCI 60 cell panel representing L, NSCLC, CC, CNSC, M, OC, RC, PC and BC. In accordance with the protocol of the NCI, test results were determined as growth percent values at 10^{-5} M concentration of the tested compounds. Results for each compound were reported as a mean graph of the percent growth of the treated cells when compared to the untreated control cells. The obtained growth percent values of the selected nine compounds were depicted in Table 1.

As can be seen from the Table 1, according to the mean values **3c** including 3-chloro phenyl moiety and **4n** derived from **3c** including 3-chloro phenyl and 4-methoxy benzyl moieties possessed significant low growth percentages among the other tested compounds, which were found 24.53% and 22.62% (mean values), respectively. Among all of the cell lines, L cells were the most susceptible cells against all tested compounds. Compounds **3c** and **4n** had the lowest growth percentages, which were −7.27% and −0.37% (mean values) against L cells. Among the L cells, HL-60 (TB), SR and RPMI-8236 cell lines were defined as the most susceptible cell lines against the compounds **3c** and **4n**. NCI-H522 was found to be the most sensitive cell line through NSCLC cells with the −15.98% and −10.93% growth percentages against **3c** and **4n** compounds. The pointed two compounds induced growth percentage below 13% on HCT-116 and KM12 cell lines through CC cells. Against CNSC cell lines, **3c** and **4n** caused 8.27% and 10.72% growth percentages, and among these cell lines and among CNSC cancer cell lines and also all cell lines SF-539 was found to be one of most sensitive cell line with a growth value of −31.29%. The active two compounds were brought about −32.33 to 90.58% growth percentage against M cell lines. The highest activity was seen against MDA-MB-43 cell line, which was a M cancer cell line by compound **4n** with a value of −32.33%. Compounds **3c** and **4n** caused −31.30, −28.13% growth percentage against OVCAR-3 and **4p** caused −8.06%

Table 1. Sixty human tumor cell lines' anticancer screening data at single dose assay as percent cell growth promotion of selected compounds.

	Compounds								
Cell lines	**3c**	**4d**	**4e**	**4f**	**4i**	**4j**	**4n**	**4p**	**4s**
L									
CCRF-CEM	5.08	82.04	99.07	78.95	80.25	90.00	47.21	78.93	86.32
HL-60(TB)	–28.87	61.87	43.76	80.04	62.86	67.68	–25.03	60.15	53.04
K-562	–0.21	70.42	60.26	73.50	47.48	78.29	0.53	59.12	59.25
MOLT-4	4.87	70.01	67.96	71.48	57.36	77.49	8.13	58.46	64.80
RPMI-8236	–11.83	73.24	76.68	39.44	48.43	50.64	–15.64	35.25	30.93
SR	–12.63	75.83	42.75	47.29	52.13	72.43	–17.39	23.70	–2.20
NSCLC									
A549/ATCC	15.25	84.92	87.16	96.00	79.22	87.43	14.32	70.56	84.54
EKVX	17.06	75.16	81.17	62.59	73.42	80.76	8.80	70.79	71.27
HOP-62	55.75	134.64	125.50	136.46	130.11	142.98	41.19	105.64	118.31
HOP-92	75.62	86.79	75.61	95.87	58.30	77.68	77.90	73.65	77.44
NCI-H226	104.70	97.32	92.71	102.38	96.17	98.52	94.08	76.86	80.83
NCI-H23	36.61	63.37	80.87	81.31	78.37	85.05	29.39	73.41	54.18
NCI-H322M	48.22	97.04	96.97	102.47	113.70	117.07	44.64	96.01	100.79
NCI-H460	4.22	90.34	91.76	90.84	88.60	88.76	4.58	88.22	81.32
NCI-H522	–15.98	72.68	73.54	64.47	72.77	68.19	–10.93	66.35	57.28
CC									
COLO 205	116.88	103.00	104.39	92.78	84.08	72.28	95.04	104.70	71.05
HCC-2998	29.91	77.52	92.03	82.11	86.84	101.52	25.70	89.23	80.42
HCT-116	5.82	72.49	79.97	73.68	64.02	73.08	12.82	63.02	63.48
HCT-15	27.32	78.21	82.16	73.78	75.21	89.15	25.66	78.20	67.35
HT29	93.95	73.99	91.79	73.24	77.55	96.51	99.11	77.45	88.80
KM12	8.72	100.32	103.84	98.46	93.62	101.38	12.95	86.88	95.44
SW-620	23.03	95.39	127.83	88.71	81.97	95.69	20.62	86.94	92.66
CNSC									
SF-268	13.16	96.96	124.80	89.24	84.58	104.73	21.75	90.33	96.09
SF-295	8.35	83.39	100.83	102.67	85.19	107.37	2.35	89.78	94.91
SF-539	–31.29	105.36	104.20	115.47	107.64	114.42	–14.95	96.39	104.61
SNB-19	24.10	82.42	85.11	79.32	73.40	73.82	20.37	85.27	79.14
SNB-75	20.99	97.82	120.62	109.33	105.08	108.24	16.46	–	103.24
U-251	14.31	83.52	91.67	80.55	85.09	91.11	18.35	90.92	92.84
M									
LOX IMVI	36.89	96.59	104.80	91.07	94.21	101.33	37.18	90.54	87.54
MALME-3M	90.58	107.73	59.59	109.27	108.81	105.75	76.81	94.53	99.30
M14	16.58	101.51	94.15	98.08	99.69	103.25	22.27	85.22	81.15
MDA-MB-43	–27.42	97.54	94.07	95.05	96.58	106.51	–32.33	98.10	97.89
SK-MEL-2	61.92	74.75	71.80	57.68	42.17	102.15	28.21	69.73	70.43
SK-MEL-28	67.72	121.53	126.92	118.52	100.22	119.44	67.67	147.42	88.91
SK-MEL-5	–13.23	88.53	111.93	87.13	89.96	94.32	–31.06	79.06	72.35
UACC-257	36.52	95.94	91.88	97.11	94.84	99.44	34.38	75.41	89.97
UACC-62	31.46	95.75	90.88	88.62	93.19	86.13	44.71	71.03	68.02
OC									
IGROV1	–	69.76	75.59	96.65	74.40	80.39	–	106.30	55.23
OVCAR-3	–31.30	94.76	111.35	85.39	93.80	97.63	–28.13	92.16	85.41
OVCAR-4	22.93	79.86	103.76	80.07	91.53	101.10	32.45	–8.06	71.56
OVCAR-5	47.52	77.82	101.70	100.51	86.75	103.90	35.26	99.92	72.08
OVCAR-8	10.07	95.19	92.27	88.25	79.22	91.41	9.77	75.61	91.12
NCI/ADR-RES	13.86	69.61	84.12	77.38	68.58	83.74	10.19	79.44	62.68
SK-OV-3	17.49	105.52	107.66	107.39	107.63	89.61	5.13	83.45	85.83
RC									
786-0	35.81	83.91	85.38	83.60	84.84	82.90	35.47	89.51	94.03
A498	88.52	91.41	108.49	69.71	84.40	100.22	91.68	84.69	90.46
ACHN	51.27	95.02	94.72	96.15	88.85	97.47	49.20	81.38	89.53
CAKI-1	5.79	91.64	88.09	100.92	95.53	114.08	3.02	79.01	85.71
RXF 393	2.00	71.73	86.88	100.79	76.37	82.42	4.81	65.65	77.53
SN12C	31.88	101.32	105.78	98.69	109.51	99.82	36.99	95.15	92.21
TK-10	34.34	95.93	103.27	118.19	121.76	155.99	30.82	119.12	137.90
UO-31	28.28	65.28	66.41	54.57	80.35	54.94	9.80	48.24	52.18
PC									
DU-145	5.27	93.14	132.14	102.71	94.60	92.76	15.29	103.20	103.14
BC									
MCF7	1.85	82.25	85.59	86.26	79.30	77.25	4.64	62.95	53.99
MDA-MB-231[a]	28.06	109.80	106.86	99.97	94.86	101.40	36.28	85.37	90.09
HS 578T	18.99	108.22	196.08	116.05	91.32	105.86	15.47	87.01	103.87
BT-549	34.85	119.02	117.67	–	143.00	97.11	–1.26	142.39	97.82
T-47D	–11.32	58.31	67.12	59.87	46.13	57.03	–22.52	66.04	48.05
MDA-MB468	32.66	91.60	107.90	–	89.00	97.02	32.02	59.06	80.79

(*continued*)

Table 1. Continued

Cell lines	Compounds								
	3c	**4d**	**4e**	**4f**	**4i**	**4j**	**4n**	**4p**	**4s**
Mean	24.53	88.43	94.57	88.56	85.51	93.13	22.62	80.77	80.08
Delta	55.83	30.12	51.82	49.12	43.34	42.49	54.95	88.83	82.28
Range	148.18	76.33	153.33	97.02	100.83	105.35	131.44	155.48	140.10

[a]MDA-MB-231/ATCC.

growth percentage against OVCAR-4, which were OC cell lines. Tested compounds showed the lowest activity to the RC cell lines with the growth percentages between 2 and 155.99% among all tested cancer cell lines. As well as other types of cancer, compounds **3c** and **4n** was found to be the most active compounds with the respective values of 5.27 and 15.29% against PC. BT-549 and T-47D cell lines were found to be the most sensitive cells through BC.

Compounds **3a**, **3b** and **3d** were synthesized, and **3a** and **3d** were investigated for their anticancer activities in our previous study[22]. In addition similar diketone compounds and ring closure products obtained from these diketone compounds were studied and anticancer activities of diketone compounds were found to be higher than final compounds. This situation supports the finding that intermediate diketone compound **3b** had significant activity compared with final products in this study. But increasingly, final compound **4n** synthesized from **3c** also showed as higher activity as **3c**, in contrast to other studies[22–24]. Other final products were found to be inactive with growth percentages greater than 80%. Trying to explain the situation of the substituent effect is needed but anticancer test assay was not performed for all compounds due to NCI protocol. However, according to obtained test results, 4-methoxybenzyl moiety on the second position pyrazinobenzimidazole condensed ring system has increased activity a little bit with respect to chloro substitution. Another explanation is needed for the lack of the activity of the final compounds except **4n** when evaluated older studies. According to earlier studies, 2-phenyl-attached pyrazinobenzimidazole compounds showed good anticancer activity, but in this study, the new compounds reported to have a benzyl group attached at the same position but the activity shown is less than the former compounds. This situation can be related with disconnecting of methylene group (=CH–) at the first position of the pyrazinobenzimidazole, which is considered to be the active part of the molecule and binds to biological receptor. Because of the molecular structure of benzyl residue (conformations); alkylation reaction of the methylene group with the nucleophilic sites of DNA can be avoided and so activity is lacked.

In the second stage of the anticancer screening tests, five-dose assay was performed to the selected compounds against same 60 cell line at five different concentrations. The compounds that reduced the growth of the cell lines to 32% or less (negative number indicate kills) were considered *in vitro* active[32]. Compounds **3c** and **4n** satisfied pre-determined threshold growth inhibition criteria and further selected for NCI full panel five-dose assay at 10-fold dilutions of five different concentrations (0.01, 0.1, 1, 10 and 100 μM) Dose response curves obtained from the NCI's *in vitro* disease-oriented human tumor cells line of compounds **3c** and **4n** on nine cancer disease at five concentrations were shown in Figures 3 and 4. The results of tested compounds were also given by three response parameters (GI_{50}, TGI and LC_{50}) for each cell line from log concentration of % growth inhibition curves on nine cancer diseases (Table 2). The GI_{50} value (growth inhibitory activity) corresponds to the concentration of the compound causing 50% decrease in net cell growth, the TGI value (cytostatic activity) is the concentration of the compound resulting in total growth inhibition and LC_{50} value (cytotoxic activity) is the concentration of the compound causing net 50% loss of initial cells at the end of the incubation period of 48 h. A mean graph midpoint (MG-MID) was calculated for each subpanels of cancer types for the tested compounds and standard drugs cisplatin and melphalan, which are two of the commonly used chemotherapeutic agents by giving $\log_{10}GI_{50}$, $\log_{10}TGI$ and $\log_{10}LC_{50}$ (Table 3). The test method states that the compounds having $\log_{10}GI_{50}$ values greater than -4 were considered as inactive. It can be seen that both of the compounds $\log_{10}GI_{50}$ values are smaller than -4. The title compounds under investigation (**3c** and **4n**) exhibited remarkable anticancer activity against all the tested cell lines representing nine different subpanels with $\log_{10}GI_{50}$ values between -7.61 and -5.18 for **3c** and -6.70 and -4.81 for **4n**. MDA-MB-43 (M cell line) and HL-60 (TB) (L cell line) were found to be the most sensitive cell lines against compounds **3c** and **4n** with $\log_{10}GI_{50}$ values of -7.61 and -6.70, respectively. According to subpanel average $\log_{10}GI_{50}$ values, L cancer was found to be the most sensitive cancer type against both of the tested compounds **3c** and **4n**. Compound **3c** possessed a value of $\log_{10}GI_{50}$ -6.64, which was smaller than standard drugs values ($\log_{10}GI_{50}$-melphalan: -5.48, $\log_{10}GI_{50}$-cisplatin: -6.39) against L. Furthermore, according to (all cancer types) subpanel average $\log_{10}GI_{50}$ values, both of the two compounds possessed smaller values than melphalan. Therefore, we may conclude that both of our compounds under investigation provide a notable activity level compared with standard drugs.

Conclusions

Twenty 2-benzyl-1-methylidene-3-aryl-1,2-dihydropyrazino[1,2-*a*]benzimidazole derivatives (**4a–t**) were synthesized from four different diketone compounds (**3a–d**). The obtained final compounds (**4a–t**) and diketone compounds (**3a–d**) were offered to American NCI. Anticancer activities of the selected compounds were evaluated *in vitro* against approximately 60 human cell lines derived from nine neoplastic diseases, and the results were given as growth percentage values. Compound **3c** namely 2-(2-acetyl-1*H*-benzimidazol-1-yl)-1-(3chlorophenyl)ethanone and compound **4n** derived from **3c** namely 2-(4-methoxybenzyl)-1-methylidene-3-(3-chlorophenyl)-1,2-dihydropyrazino[1,2-*a*]benzimidazole were found to be as the most active compounds with the lowest growth percentages 24.53 and 22.62%, respectively. In addition, $\log_{10}GI_{50}$ value ($\log_{10}$ of molar sample concentration resulting in 50% growth inhibition) of the (MG-MID) compound **3c** was found to be -6.32, which was smaller than anticancer drug cisplatin ($\log_{10}GI_{50}$: -6.20) and $\log_{10}GI_{50}$ value of the compound **4n** was found to be -5.87, which was smaller than anticancer drug melphalan ($\log_{10}GI_{50}$: -5.09). In connection with the result of this work and earlier works, we plan to synthesize novel compounds including diketone

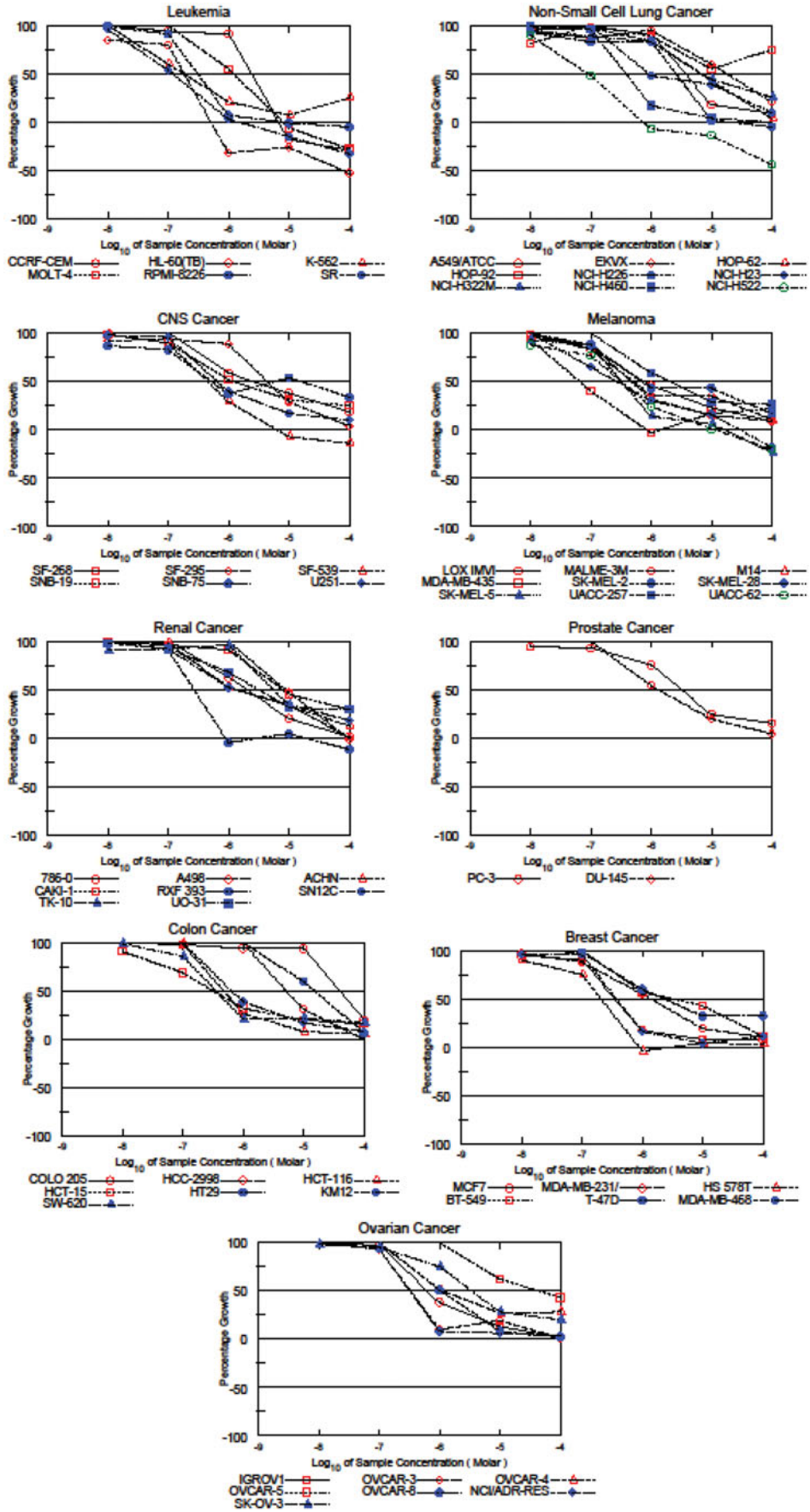


Figure 3. Dose response curves (% growth verses sample concentration at NCI fixed protocol, μM) obtained from the NCI's *in vitro* disease-oriented human tumor cells line of compound (**3c**) on nine cancer diseases.

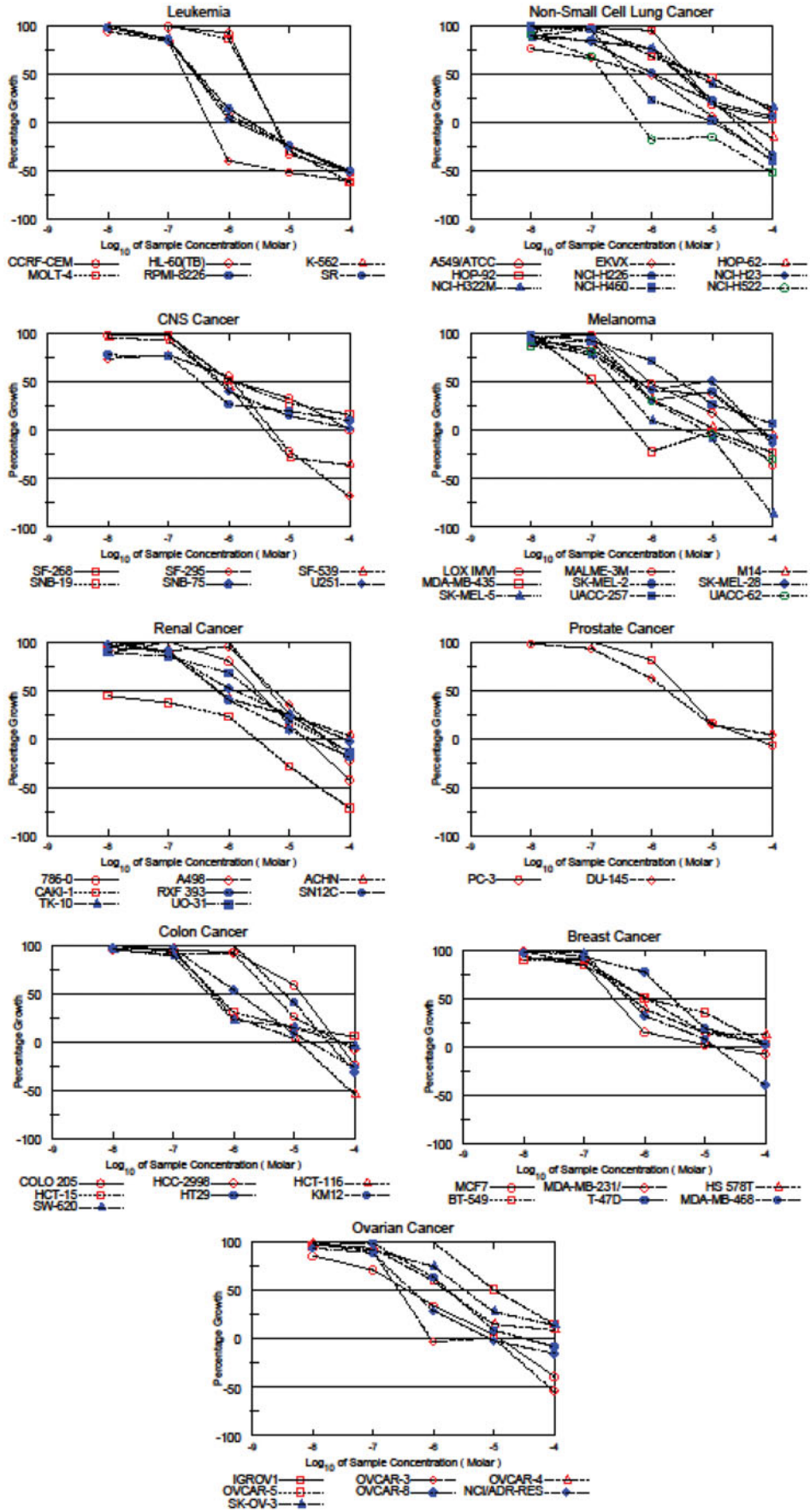


Figure 4. Dose response curves (% growth verses sample concentration at NCI fixed protocol, μM) obtained from the NCI's *in vitro* disease-oriented human tumor cells line of compound (**4n**) on nine cancer diseases.

Table 2. NCI DTP *in vitro* testing results of compounds **3c** and **4n** at five-dose assay.

	3c values (μM)			**4n** values (μM)		
Cell lines	$Log_{10}GI_{50}$	$Log_{10}TGI$	$Log_{10}LC_{50}$	$Log_{10}GI_{50}$	$Log_{10}TGI$	$Log_{10}LC_{50}$
L						
CCRF-CEM	–6.24	>–4.00	>–4.00	–5.75	>–4.00	>–4.00
HL-60(TB)	–6.95	–6.14	>–4.00	–6.70	–6.07	>–4.00
K-562	–7.15	–4.71	>–4.00	–6.54	–4.80	>–4.00
MOLT-4	–6.18	–4.05	>–4.00	–5.49	–4.65	>–4.00
RPMI-8236	–6.68	–4.56	>–4.00	–6.38	–4.85	>–4.00
NSCLC						
A549/ATCC	–5.71	>–4.00	>–4.00	–5.38	>–4.00	>–4.00
EKVX	–5.99	>–4.00	>–4.00	–5.91	–4.91	>–4.00
HOP-62	–6.38	–4.50	>–4.00	–5.71	–4.36	>–4.00
HOP-92	–5.42	–4.13	>–4.00	–6.07	–4.17	>–4.00
NCI-H226	–5.35	>–4.00	>–4.00	–5.18	–4.45	>–4.00
NCI-H23	–6.53	–4.65	>–4.00	–6.25	–4.89	>–4.00
NCI-H322M	–5.79	>–4.00	>–4.00	–5.06	>–4.00	>–4.00
NCI-H460	–6.47	–5.74	>–4.00	–6.39	–4.99	>–4.00
NCI-H522	–7.11	–6.16	>–4.00	–6.52	–5.71	>–4.00
CC						
COLO 205	–5.18	–4.20	>–4.00	–5.19	–4.61	–4.14
HCC-2998	–6.38	–5.78	–4.87	–5.91	–5.49	–5.08
HCT-116	–6.47	>–4.00	>–4.00	–6.27	–4.94	>–4.00
HCT-15	–6.74	>–4.00	>–4.00	–6.24	>–4.00	>–4.00
HT29	–5.06	>–4.00	>–4.00	–5.16	–4.12	>–4.00
KM12	–6.05	–4.73	>–4.00	–5.60	–4.83	>–4.00
CNSC						
SF-268	–6.35	–4.29	>–4.00	–6.08	–4.49	>–4.00
SF-295	–6.44	–5.27	>–4.00	–5.77	–5.05	>–4.00
SF-539	–6.62	–6.11	>–4.00	–6.15	–5.30	>–4.00
SNB-19	–6.28	>–4.00	>–4.00	–6.03	>–4.00	>–4.00
SNB-75	–6.50	–4.22	>–4.00	–6.23	>–4.00	>–4.00
U-251	–6.37	>–4.00	>–4.00	–6.07	>–4.00	>–4.00
M						
LOX IMVI	–6.35	>–4.00	>–4.00	–6.07	4.67	>–4.00
MALME-3M	–6.27	>–4.00	>–4.00	–6.08	–4.09	>–4.00
M14	–6.84	>–4.00	>–4.00	–6.16	>–4.00	>–4.00
MDA-MB-43	–7.61	–	>–4.00	–6.65	>–4.00	>–4.00
SK-MEL-2	–6.45	–4.02	>–4.00	–6.11	–4.16	>–4.00
SK-MEL-28	–6.14	>–4.00	>–4.00	–4.98	–4.26	>–4.00
SK-MEL-5	–7.02	–4.93	>–4.00	–6.53	–5.46	–4.46
UACC-257	–5.83	>–4.00	>–4.00	–4.98	>–4.00	>–4.00
UACC-62	–6.27	–5.33	>–4.00	–5.69	–4.32	>–4.00
OC						
IGROV1	–6.33	>–4.00	>–4.00	–6.22	–4.70	>–4.00
OVCAR-3	–6.76	>–4.00	>–4.00	–6.53	–	>–4.00
OVCAR-4	–6.28	>–4.00	>–4.00	–5.69	>–4.00	>–4.00
OVCAR-5	–6.56	–4.29	>–4.00	–5.50	–5.02	>–4.00
OVCAR-8	–6.13	>–4.00	>–4.00	–5.48	>–4.00	>–4.00
NCI/ADR-RES	–6.79	–6.19	>–4.00	–6.36	–5.47	>–4.00
SK-OV-3	–6.32	>–4.00	>–4.00	–5.61	>–4.00	>–4.00
RC						
786-0	–6.39	–4.77	>–4.00	–5.47	–4.77	>–4.00
A498	–5.31	–4.08	>–4.00	–4.81	–4.04	>–4.00
ACHN	–6.04	>–4.00	>–4.00	–5.74	>–4.00	>–4.00
CAKI-1	–6.00	–5.39	>–4.00	–5.82	–5.10	>–4.00
RXF 393	–6.58	–6.06	>–4.00	–5.68	–5.09	>–4.00
SN12C	–6.29	>–4.00	>–4.00	–5.87	–4.50	>–4.00
TK-10	–5.72	>–4.00	>–4.00	–5.03	>–4.00	>–4.00
UO-31	–6.27	>–4.00	>–4.00	–5.80	–4.52	>–4.00
PC						
PC-3	–6.39	>–4.00	>–4.00	–5.79	>–4.00	>–4.00
DU-145	–6.49	–5.00	>–4.00	–5.74	–4.54	>–4.00
BC						
MCF7	–6.50	>–4.00	>–4.00	–6.45	>–4.00	>–4.00
MDA-MB-231[a]	–6.09	>–4.00	>–4.00	–6.06	>–4.00	>–4.00
HS 578T	–6.53	–	>–4.00	–6.27	>–4.00	>–4.00
BT-549	–6.38	>–4.00	>–4.00	–5.55	>–4.00	>–4.00
T-47D	–6.46	>–4.00	>–4.00	–5.66	–4.19	>–4.00
MDA-MB468	–6.55	–5.56	>–4.00	–5.93	–4.70	>–4.00

[a]MDA-MB-231/ATCC.

Table 3. Antiproliferative activities of the compounds ($\log_{10}GI_{50}$).

Comp	L	NSCLC	CC	CNSC	M	OC	RC	PC	BC	MG-MID
3c	–6.64	–6.08	–6.08	–6.43	–6.53	–6.45	–6.08	–6.44	–6.42	–6.32
4n	–6.17	–5.83	–5.81	–6.06	–5.92	–5.91	–5.53	–5.77	–5.99	–5.87
A	–5.48	–5.17	–5.11	–5.12	–5.08	–5.18	–4.99	–4.49	–4.79	–5.09
B	–6.39	–6.20	–6.14	–6.18	–6.08	–6.45	–6.17	–6.41	–6.05	–6.20

Growth inhibition dose (GI_{50}; M) represents the concentration required for 50% inhibition of growth of the treated cells. The compounds, whose $\log_{10}GI_{50}$ was higher than -4 were considered as not active.
A: melphalan, **B:** cisplatin, L: leukemia, NSCLC: non-small cell lung cancer, CC: colon cancer, CNSC: central nervous system cancer, M: melanoma, OC: ovarian cancer, RC: renal cancer, PC: prostate cancer, BC: breast cancer, MG-MID: mean-graph midpoint.

substructure on the first and/or second position of benzimidazole ring in further anticancer drug design studies.

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Declaration of interest

The authors report no conflicts of interest.

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