Synthesis, heterocyclization and anti-tumour activity evaluation of some benzimidazole derivatives

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Methylbenzimidazole 1 is converted to imidazole acrylic acid 3 via cyclo condensation with chloral followed by hydrolysis. Compound 3 also obtained from the reaction of o-phenylenediamine with maleic anhydride. Treatment of 1 with SeO₂ yielded the oxidized product 4 (Aldehyde 4) which undergoes Wittig reaction using 1,3-dinitrogen (0.01 mol), maleic anhydride (0.01 mol) and DMF (50 ml) was stirred at room temperature for one hour. The solid that separated upon dilution with water was crystallized from acetic acid.

Method B: To a solution of 2-methylbenzimidazole (0.01 mol) in (20 ml) anhydrous toluene, 0.3–0.5 g anhydrous zinc chloride (4 g) and chloral (0.01 mol; 9.6 ml in 20 ml toluene) were added. The mixture was heated for 3 h on a water bath at 90–95°C and then, cooled, filtered and washed with ethanol. The mixture was treated with (80 ml) ethanol and (100 ml, 25% sodium hydroxide, heated gently and then cooled in ice. The mixture was boiled for 2–3 h and cooled again, ethanol was removed by vacuum distillation during cooling and then (50–60 ml) conc. HC1 was added. The hydrochloride of β-(2-benzimidazolyl) acrylic acid was formed (soluble in water). The unreacted substances were filtered out, and the filtrate was neutralized by 10% sodium hydroxide to obtain the free acid which was crystallized from acetic acid.

(1): A pale yellow crystal with yield = 70%, m.p. = 197–198°C, IR (KBr): revealed peaks at 3250–3850 cm⁻¹, 3350 cm⁻¹ and 1715 cm –1 for OH, NH and C=O groups respectively. ¹HNMR (DMSO-d₆): 4.2 (2H, dd –CH=CH–), 8.20–8.33 (4H, m), 11.0–12.5 (1H, s, NH), 12.80 (1H, s, –COOH). Anal. Calc. for C₁₁H₁₀N₂O₂ (202.21): %C; 63.82, %H; 4.28, %N; 14.89 and found: %C; 63.70, %H; 4.22, %N; 14.75.

Methyl or ethyl-β-(2-benzimidazolyl) acrylic esters (5a, b) – Method A: A mixture of o-phenylenediamine (0.01 mol), dimethyl or diethylmaleate (0.01 mol) and few drops of piperidine were fused at 120°C (oil bath) for 3 h. The solid product was extracted by benzene after cooling and was crystallized from the proper solvent.

Method A: Treatment of 2-methylbenzimidazole (0.01 mol) by SeO₂ (1.5 g), gives 2-formylbenzimidazole, which was added to methoxy and ethoxy carbonyl methyl triphenyl phosphorus yield (0.01 mol) in (15 ml) benzene and the mixture was refluxed for 5 h, cooled and 50 ml (10%) HC1 was added. The solid obtained was crystallized from benzene.

(5a): A yellowish crystal yield = 80%, m.p. = 116–118°C, IR (KBr): 3350 (NH) cyclic, 1750 (C=O), 1630 (C=N cyclic). ¹HNMR (DMSO-d₆): 3.83 (3H, s, OCH₃), 4.9 (2H, dd –CH=CH–), 8.32–8.43 (4H, m, ar.), 12.93 (1H, br(s), NH). Anal. Calc. for C₁₁H₁₀N₂O₂ (202.21): %C; 65.34, %H; 4.98, %N; 13.85 and found: %C; 65.24, %H; 4.88, %N; 13.80.

(5b): A colourless crystal with yield = 80%, m.p. = 118–121°C, IR (KBr): 3350 (NH) cyclic, 1750
(C=O), 1630 (C=N cyclic). 1H NMR (DMSO-d6): 1.36 (3H, t, CH3), 4.2 (2H, q, CH2), 4.9 (dd, 2H, –CH=CH–), 8.32–8.43 (4H, m, ar.), 12.93 (br(s), 1H, NH). Anal. Calc. for C13H12N2O2 (216.24): %C; 66.65, %H; 5.59, %N; 17.42 (4H, ar.), 7.82–8.25 (4H, cyclic, 8.23–8.38 (4H, Methyl- (170.17): %C; 70.58, %H; 3.55, %N; 16.46 and found: %C; 64.11, %H; 4.75, %N; 22.33. 

Benzimidazol[1,5-a]3-pyrrolin-2-one (1) (0.01 mol) in ethanol. 

(7a): A pale yellow crystal with yield = 90%, m.p. = 160–162°C, IR (KBr): 3370 (NH) cyclic, 3250 (NH) amide, 1700–1680 (C=O) amide. 1H NMR (DMSO-d6): 5.2 (1H, s, CONH), 4.9 (2H, dd, –CH=CH–), 7.31–7.42 (4H, m, ar.), 10.71 (1H, br(s), NH). Calc. for C9H9N2O3 (187.07): %C; 64.16, %H; 4.85, %N; 22.45 and Found: %C; 64.11, %H; 4.75, %N; 22.33. 

(7b): A yellow crystal with yield = 88%, m.p. = 165–166°C, IR (KBr): 3370 (NH) cyclic, 3250 (NH) amide, 1700–1680 (C=O) amide. 1H NMR (DMSO-d6): 5.39 (br, 1H, CONH), 4.89 (2H, dd, –CH=CH–), 7.31–7.42 (4H, m, ar.), 7.5–7.63 (5H, m, ar.), 10.11 (1H, br(s), NH). Calc. for C10H10N2O5 (263.29): %C; 72.99, %H; 4.98, %N; 15.96 and found: %C; 72.80, %H; 4.89, %N; 15.90. 

(E)-1,2-bis(1H-benzo[d]imidazol-2-yl)ethene (8): A mixture of compound (7) (0.01 mol) and o-phenylenediamine (0.1 mol) in dil HCl (3–4 drops) in ethanol was refluxed for 3 h. The solid product was crystallized from ethanol. A brownish crystal with yield = 80%, m.p. = 152–154°C, IR (KBr): 3350 (NH) cyclic, 3250 (NH) amide and 1630 (C=N) cyclic. 1H NMR (DMSO-d6): 4.8 (2H, dd, –CH=CH–), 7.22–7.5 (8H, m, ar.). Calc. for C13H16N4O2 (260.29): %C; 73.83, %H; 4.65, %N; 21.52 and found: %C; 73.75, %H; 4.60, %N; 21.44.

Synthesis of anilide (benzimidazole acrylamide derivatives) (9a–c): A mixture of acid 3 (0.01 mol) and appropriate amine (0.01 mol) in ethanol (20 ml) was refluxed for 2 h. The solid product obtained upon cooling was separated by filtration and crystallized from butanol to give (9a–c) respectively. 

(9a): A colourless crystal with yield = 80%, m.p. = 145–147°C, IR (KBr): 3370 (NH) cyclic, 3250 (NH) amide, 1700–1680 (C=O) amide, 1630 (C=N) cyclic. 1H NMR (DMSO-d6): 4.8 (2H, dd, –CH=CH–), 7.22–7.5 (4H, m, ar.), 7.82–8.25 (4H, m, ar.), 10.22 (1H, br(s), NH). Calc.
Scheme 2. Synthetic approaches of benzimidazole 3, 7a, b and 8 by ring opening of 6.

Scheme 1. Acrolein (1) reacts with phenylglyoxal (2) in the presence of piperidine (3) to give the Schiff-base (4). The reaction of compound 4 with hydroxylamine hydrochloride (5) yields the amide 6. Methylimidazole with its activated methyl group was reacted with chloral hydrate to give the acrylic derivative 3 presumably via the non-isolable aldol intermediate 2 that undergo basic hydrolysis (Scheme 1). Compound 3 was also obtained as a result of condensation reaction between o-phenylenediamine and maleic anhydride (Scheme 1). Structure of compound 3 was confirmed from spectral data, thus its IR revealed peaks at 3250–3850 cm⁻¹, 3350 cm⁻¹ and 1715 cm⁻¹ for OH, NH and C=O groups respectively. Also ¹H NMR displayed the trans-protons of CH=CH as doublet at 4.8 ppm, aromatic protons at 8.33 as multiplet while the deshielded NH and COOH protons were confirmed at 11–12.5 ppm and 12.8 ppm respectively.

SRB assay of cytotoxic activity: It was carried out according to the previously reported work nineteen using human tumour cell lines, breast carcinoma cell line (MCF7) and liver carcinoma cell line (HEPG2). Cell lines were obtained from National Cancer Institute, Cairo, Egypt.

Benzimidazole acrylic acid seemed to be of suitable functionality for further heteroannelation using simple available laboratory reagent, providing imidazole derivative of potential biological activities.

Methylimidazole with its activated methyl group was reacted with chloral hydrate to give the acrylic derivative 3 presumably via the non-isolable aldol intermediate 2 that undergo basic hydrolysis (Scheme 1). Compound 3 was also obtained as a result of condensation reaction between o-phenylenediamine and maleic anhydride (Scheme 1). Structure of compound 3 was confirmed from spectral data, thus its IR revealed peaks at 3250–3850 cm⁻¹, 3350 cm⁻¹ and 1715 cm⁻¹ for OH, NH and C=O groups respectively. Also ¹H NMR displayed the trans-protons of CH=CH as doublet at 4.8 ppm, aromatic protons at 8.33 as multiplet while the deshielded NH and COOH protons were confirmed at 11–12.5 ppm and 12.8 ppm respectively.

The aldehyde 4 and corresponding ester under witting reaction afforded the ester 5, which in turn was obtained from the nucleophilic attack of amino group of o-phenylenediamine to the electrophilic carbon of maleiate (Scheme 1). Structure of compound 5a was elucidated from analytical in addition to IR and ¹H NMR. Thus, IR spectrum showed NH, C=O and C=N absorption bands at 3350 cm⁻¹, 1750 cm⁻¹ and/or 1630 cm⁻¹ for C=N. ¹H NMR spectrum of 5a showed the methoxy protons as a singlet at 3.83 ppm, olefenic protons as double doublet at 4.9 ppm, aromatic protons at 8.32–8.43 ppm as doublet at 4.9 ppm, aromatic protons at 8.32–8.43 ppm as multiplet in addition to NH at 12.93 ppm. ¹H NMR spectrum of 5b showed the ethoxy protons as 1.36 (3H, t, CH₃), 4.2 (2H, q, CH₂) (Scheme 1).

Upon refluxing of 3 with AC₂O as acylating agent resulted in pyrrole cyclization affording the condensed pyrroloimidazole (6) (Scheme 2). The structure of compound 6 was confirmed from the absence of OH absorption band in IR spectrum in addition to the disappearance of cyclic NH in IR and ¹H NMR.

The behaviour of 6 towards nucleophilic reagent was examined; thus ammonolysis of 6 using ammonium...
hydroxide and aniline resulted in ring cleavage affording imidazole derivative 7 which was confirmed from the presence of NH in IR and $^1$H NMR. Treatment of compound 6 with o-phenylenediamine leads to ring opening followed by intramolecular cyclodehydration affording benzimidazole derivative 8. The structure of biscompound 8 was confirmed from $^1$H NMR spectrum which showed the olefenchromatic protons as double doublet at 4.9 ppm in addition to the deshielded NH protons at 11.9 ppm (Scheme 2).

The attack of nucleophilic nitrogen of amines to the electrophilic carbon of carboxylic group of 3 provided the anilide derivative 9 and none of the addition products 10 were obtained.

Structure of compound 9 was elucidated from analytical in addition to IR and $^1$H NMR. Thus, IR spectrum showed 3370 (NH) cyclic, 3250 (NH) amide, 1700–1680 (C=O) amide, 1630 (C=N) cyclic, $^1$HNMR (DMSO-$d_6$): 4.8 (2H, $dd$, –CH=CH–), 7.22–7.5 (4H, $m$, ar.), 7.82–8.25 (4H, $m$, ar.), 10.22 (1H, br($s$), NH) (Scheme 3).
The anilides of type 10 was obtained through 1,4-addition of the aromatic amines to the imidazole derivative 5 (Scheme 3). The α-amino acid structure of 10 was elucidated from spectral analysis, thus IR spectrum showed NH around 3340 cm\(^{-1}\), C=O in the region of 1760–1770 cm\(^{-1}\). \(^1\)H NMR of the same compound provided (CH\(_2\)–CH–) structure as doublet at 1.3 ppm, triplet at 1.4 ppm in addition to aliphatic NH as a broad signal at 4.3 ppm. The benzimidazole derivative 11 was obtained as a result of attack of hydroxyl amine to the ester carbonyl carbon followed by intramolecular cyclodehydration. The pyridone structure 11 was shown from the carbonyl absorption at 1670 cm\(^{-1}\) and NH at 3390 cm\(^{-1}\). Also \(^1\)H NMR of 11 displayed NH signal at 12.89 pm as a broad signal (Schemes 3 and 4).

The potential cytotoxicity activity of compound 11 was tested against two human cell lines (MCF7 breast carcinoma cell line and HEPG2 liver carcinoma cell line) by SRB method. The results of antitumour activity showed that compound 11 has strong activity against all cell lines tested. The antitumour activity of compound 11 is summarized in Figure 1 and Table 1. The IC\(_{50}\) values of compound 11 against each cell lines were 30.1 mg/ml and 3.31 mg/ml for MCF7 and HEPG2 respectively.

Figure 1. Percentage of survival fraction of breast and liver carcinoma cell lines against concentration (μg/ml) compound 11.