Asian Journal of Chemistry Vol. 21, No. 7 (2009), 5207-5211

Synthesis and Biological Evaluation of Mannich Bases of 2-Substituted Benzimidazoles

R. KALIRAJAN*, M. CHITRA, S. JUBIE, B. GOWRAMMA and B. SURESH
Department of Pharmaceutical Chemistry,
J.S.S. College of Pharmacy, Ootacamund-643 001, India
E-mail: rkrmani@yahoo.co.in

Some new 2-substituted benzimidazoles like 2-(1*H*-benzo[d]imidazol-2-yl)benzoic acid (**I**) and 2-methyl benzimidazole (**II**) were synthesized. Then the Mannich bases of the above derivatives (**I** and **II**) were synthesized with formaldehyde and various secondary amines. The synthesized compounds have been characterized by TLC, elemental analysis, IR and ^{1}H NMR spectroscopy. These compounds were screened for their anti-inflammatory, antibacterial and antifungal activities.

Key Words: Benzimidazole, Mannich base, Antiinflammatory, Antibacterial and Antifungal activities.

INTRODUCTION

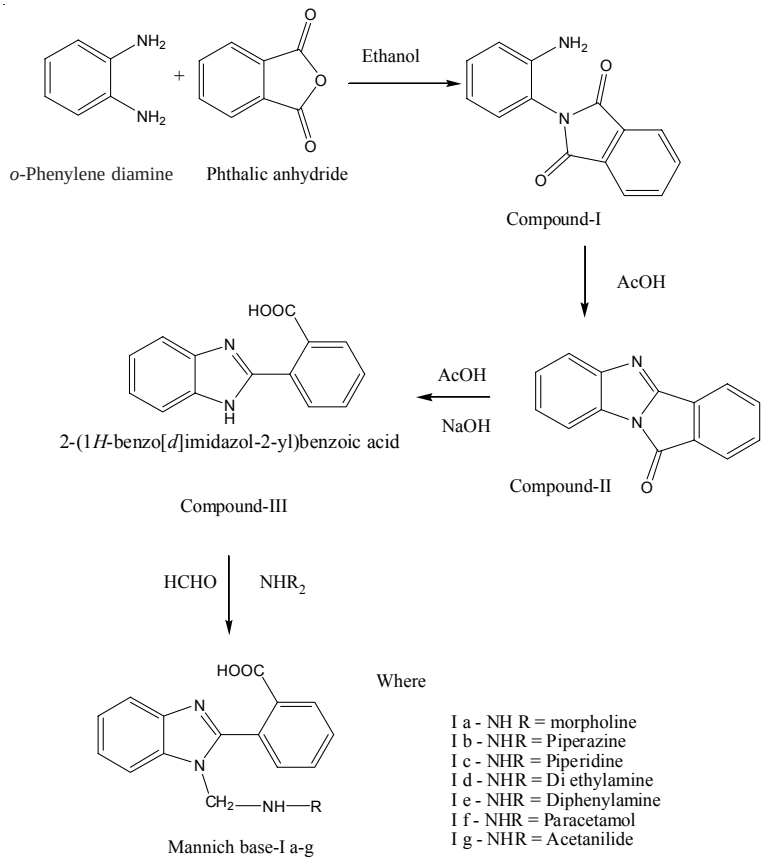
The benzimidazole ring is an important pharmacophore in modern drug discovery. Literature review reveals that benzimidazole derivatives exhibit diverse pharmacological activities[1-5] such as potential antitumor agent, antimicrobial agents, anthelmintics, antiviral, antiinflammatory, *etc*. In addition benzimidazoles are very important intermediates in organic reaction. Based on the above observation it is worthwhile to prepare newer compounds for their antimicrobial and antiinflammatory activities.

In the view of the varied biological and pharmacological application, some new 2-substituted benzimidazoles and their Mannich bases by treated with formaldehyde and various secondary amines (**Ia-g**, **IIa-g**, **Scheme-I** and **II**) were synthesized.

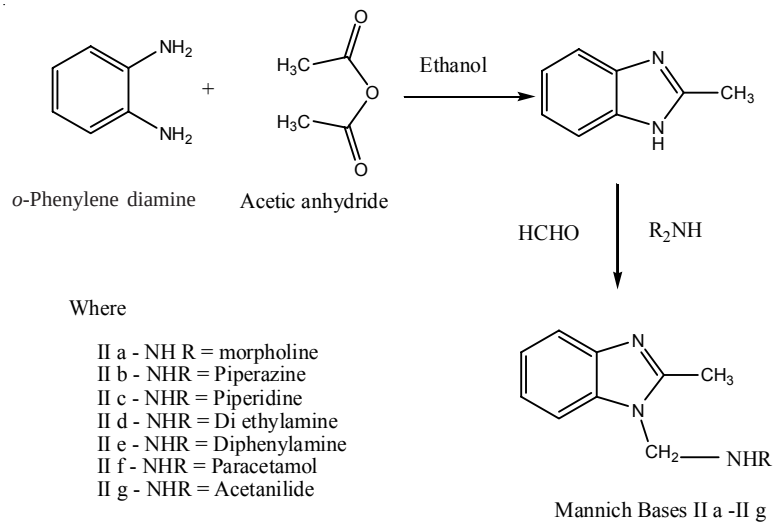
EXPERIMENTAL

Melting points were determined in open capillary tubes and were found uncorrected. IR spectra were recorded on FT-IR spectrometer (Perkin-Elmer) using KBr disc method. ^{1}H NMR spectra were recorded on ^{1}H FT-NMR (Brucker AMX 400 MHz) spectrometer in DMSO. The compounds were analyzed for C, H and N analysis and the percentage of elements were found to be very near that of the calculated values. Physical data of the compounds are recorded in Table-1 and the spectral data are recorded in Table-2.

Synthesis of 2-(1*H*-benzo[d]imidazol-2-yl)benzoic acid (I): A mixture of *o*-phenylene diamine (0.01 mol) phthalic anhydride (0.01mol) was refluxed for 8 h, cooled and diluted with cold water till the solution becomes turbid. It was filtered



Scheme-I



Where

II a - NH R = morpholine
II b - NHR = Piperazine
II c - NHR = Piperidine
II d - NHR = Di ethylamine
II e - NHR = Diphenylamine
II f - NHR = Paracetamol
II g - NHR = Acetanilide

Scheme-II

TABLE-1
PHYSICAL PARAMETERS AND ELEMENTAL ANALYSIS OF SYNTHESIZED COMPOUNDS

Compd.	m.f.	m.w. (g/m)	m.p. (ºC)	Yield (%)	Elemental analysis (%): Calculated		
					C	H	N
Ia	$C_{19}H_{19}N_3O_3$	337	120	84	67.64	5.68	12.46
Ib	$C_{19}H_{20}N_4O_2$	326	246	72	67.84	5.99	16.66
Ic	$C_{20}H_{21}N_3O_2$	335	211	64	70.57	6.55	12.99
Id	$C_{19}H_{21}N_3O_2$	323	122	56	63.14	6.93	16.99
Ie	$C_{27}H_{21}N_3O_2$	419	128	58	77.31	5.05;	10.02
If	$C_{23}H_{19}N_3O_4$	401	132	54	71.67	4.97	10.90
Ig	$C_{23}H_{19}N_3O_3$	385	182	59	66.01	4.89	13.58
IIa	$C_{23}H_{19}N_3O_3$	279	192	82	67.51	7.41	18.17
IIb	$C_{13}H_{27}N_3O_4$	230	182	72	67.80	7.88	24.33
IIc	$C_{13}H_{18}N_4$	229	170	84	73.33	8.35	18.32
IId	$C_{14}H_{19}N_3$	217	174	48	71.85	8.81	19.34
IIe	$C_{13}H_{19}N_3$	313	175	45	80.48	6.11	13.41
IIf	$C_{21}H_{19}N_3$	295	182	58	69.14	5.80	14.23
IIg	$C_{17}H_{17}N_3O_2$	281	188	55	73.10	6.13	15.04

TABLE-2
SPECTRAL ANALYSIS OF SYNTHESIZED COMPOUNDS

Comp.	IR (KBr, ν_{max}, cm^{-1})	^1H NMR (DMSO-d_6) δ (ppm)
Ia	2720, 2969 (OH-COOH), 1133 (C=O, C-N)	2.37 (d, CH_2), 3.65 (s, CH_3), 7.67 (d, Ar-H), 7.28 (m, Ar-H)
Ib	2764, 2972 (OH-COOH), 1704 (C=O), 1132 (C-N)	2.47 (NH), 2.64 (s, CH_2), 7.41 (Ar-CH), 7.68 (t, Ar-H)
Ic	2637 (OH-COOH), 1717 (C=O), 1601 (C=C) 1132 (C-N)	2.25 (d, CH_2), 1.51 (t, CH_2), 7.29 (d, CH), 7.5 (m, Ar-H)
Id	2732 (OH-COOH), 1715 (C=O), 1621 (C=C) 1119 (C-N)	2.24 (d, CH_2), 3.65 (s, CH_3), 7.25 (d, Ar-CH), 7.63 (m, Ar-H)
Ie	2719, 2875 (OH-COOH), 1589 (C=O), 1133 (C-N)	2.07 (s, CH_2), 1.89 (s, CH_3), 7.65 (t, Ar-H), 7.82 (d, Ar-H)
If	3108, 1072 (OH), 1654 (N-C-O), 1699 (C=O)	6.92 (d, Ar-CH), 4.9 (s, Ar-OH), 2.21 (CH_3), 6.77 (d, Ar-H)
Ig	1664 (N-C=O), 1597.91 (C=C), 1179 (C-N)	7.30 (d, Ar-CH), 7.71 (d, Ar-CH), 2.01 (CH_3), 7.11 (d, Ar-H)
IIa	1594 (C=N), 1118 (C-N, C-O)	2.37 (d, CH_2), 3.65 (d, CH_2), 2.42 (s, CH_3), 4.81 (s, CH_2)
IIb	3085 (CH-Stretching), 1622 (C=N), 1591 (C=C)	2.07 (s, CH_2), 1.12 (s, CH_3), 7.2 (d, Ar-H), 7.54 (t, Ar-H)
IIc	3061 (C-H), 1270 (C-N) 1622 (C=C, C=N),	2.49 (s, CH_2), 7.28 (d, CH- (C_2H_5)$_2$), 1.08 (s, CH_3), 7.52 (m, Ar-H)
IId	3046 (CH-Stretching), 1591 (C=N), 1117 (C-N)	2.84 (s, CH_2), 1.12 (s, CH_3), 7.19 (t, Ar-H), 7.48 (d, Ar-H)
IIe	1594 (C=C, C=N), 1178 (C-N)	5.51 (s, CH_2), 2.42 (s, CH_3), 6.43 (t, Ar-H), 7.05 (m, Ar-H)
IIf	1109 (OH). 1650 (N-C=O)	6.94 (d, Ar-CH), 5.1 (s, Ar-OH), 2.41 (CH_3), 6.77 (d, Ar-H)
IIg	1179 (C-N), 1667 (N-C=O), 1598 (C=C, C=N)	7.08 (d, Ar-CH), 2.43 (s, CH_3), 2.01 (CH_3), 7.30 (d, Ar-H)

and recrystallized from ethanol. This compound (0.01 mol) was refluxed with glacial acetic acid (20 mL) for 7 h. The solvent was distilled off under reduced pressure and the residue was taken in *n*-hexane, filtered and recrystallized from chloroform. Then the above compound (0.01 mol) was added to 10 mL of 2 N. NaOH and the reaction mixture was stirred at room temperature for 7 h and acidified with acetic acid. The solid that separated was filtered and recrystallized from ethanol (compound **I**).

Synthesis of 2-methyl benzimidazole (II): A mixture of *o*-phenylene diamine (0.0l mol), acetic anhydride and 15 % hydrochloric acid (15 mL) was refluxed for 6 h. The hot reaction mixture was filtered, cooled and the excess of acid was neutralized with saturated solution of $NaHCO_3$. The reaction mixture was basified with ammonia solution; the separated solid was filtered and recrystallized with hot water (compound **II**).

General method of synthesis of Mannich bases (Ia-g, IIa-g): Equimolar quantities of (0.08 mol) of compound **I** or **II** and respective secondary amines were dissolved in methanol in a beaker under ice-cold condition and stirred constantly. Then formaldehyde (0.08 mol) was added slowly and stirring was continued for *ca*. 10 h. The content of beaker was kept 2-3 d in deep freeze. Then the compound separated was filtered, dried and recrystallized.

***In vitro* antimicrobial screening[6]:** The synthesized compounds were subjected to antimicrobial screening by cup plate method for zone of inhibition. The antibacterial activity was tested against various Gram positive and Gram negative bacteria and antifungal activity against various fungal stains compared with standard drug (ampicillin and ketoconazole) using solvent control. The results were described in the Table-3.

***In vitro* antiinflammatory activity[7]:** All the newly synthesized compounds were tested for antiinflammatory activity by *in vitro* HRBC membrane stabilization method.

The reaction mixtures (4.5 mL) consisted of 2 mL hypotonic saline solution (0.25 % NaCl), 1 mL (0.15 M) phosphate buffer (pH 7.4) and 1 mL test solution in normal saline. 0.5 mL of 10 % rabbit RBC in normal saline was added. For control tests, 1 mL of isotonic solution was used instead of test solution while product control tests lacked RBC. The mixtures were incubated at 56 ºC for 0.5 h. The mixtures were cooled under running water for 20 min and centrifuged and the absorbance of the supernatants were read at 560 nm. Percentage membrane stabilizing activity was calculated as follows:

$$\text{Percentage stabilization} = 100 - \frac{(\text{O.D. of test} - \text{O.D. of product control})}{\text{O.D. of control}} \times 100$$

The control represents 100 % lysis. The result was compared with STD (1000 µg/mL) treated samples.

TABLE-3
ANTIMICROBIAL ACTIVITY OF SYNTHESIZED COMPOUNDS

Compd. (100 µg/mL)	Zone of inhibition (mm)					
	Gram positive		Gram negative		Antifungal	
	B. subtilis	*S. aureus*	*K. pneumonia*	*E. coli*	*C. albicans*	*A. niger*
Ia	22	–	18	–	–	–
Ib	–	–	–	–	–	13
Ic	21	17	19	–	11	12
Id	–	18	–	18	–	–
Ie	–	–	–	–	18	12
If	20	18	–	19	–	-
Ig	18	–	19		19	19
IIa	21	–	18	12	18	17
IIb	–	–	–	–	–	-
IIc	8	–	19		18	19
IId	–	18	–	–	–	–
IIe	–	–	–	–	–	19
IIf	12	–	18	–	12	12
Iig	–	12	20	–	18	13
Standard drug	24	20	21	20	21	21
Solvent control (DMSO)	–	–	–	–	–	–

– = Indicates no activity.

RESULTS AND DISCUSSION

All the synthesized compounds were characterized by TLC, Melting point, elemental analysis, IR and ^{1}H NMR. Analysis indicated by the symbols of the elements is very close to the theoretical values. The compounds were evaluated for their antimicrobial activity by cup-plate method against various Gram positive, Gram negative bacteria and various fungal stains. Many compounds show comparable activity with that of standard (ampicillin and ketoconazole). The compounds were also evaluated for their *in vitro* antiinflammatory activity by HRBC membrane stabilization method. All the compounds have highly significant activity when compared with standard drug Ibuprofen, with percentage of inhibition to the inflammatory response ranging from 64 to 77 %.

REFERENCES

1. S. Magdumchandrakant amd P.Y. Shirodkar, *Indian Drugs*, **11**, 528 (2000).
2. Syedabuzar and Satyavansharma, *Indian J. Chem.*, **24B**, 338 (1985).
3. N.M. Goudgaon, V. Dhondida and A. Vijayalaxmi, *Indian J. Heterocycl. Chem.*, **13**, 271 (2004).
4. O. Algul, N. Duran and G. Gulbol, *Asian J. Chem.*, **19**, 3085 (2007).
5. M.N. Ibrahim, *Asian J. Chem.*, **19**, 2419 (2007).
6. Pharmacopoeia of India, II, A-100, A-108 (1996).
7. S. Chatterjee and S.N. Das, *Indian J. Pharmacol.*, **28**, 116 (1996).

(*Received*: 5 July 2008; *Accepted*: 29 April 2009) AJC-7456