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MICROWAVE MEDIATED MILD, ONE-POT SYNTHESIS OF NOVEL PYRAZINOBENZIMIDAZOLE DERIVATIVES AND THEIR ANTICANCER PROPERTIES

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ABSTRACT

Some novel pyrazinobenzimidazole derivatives were synthesized via a microwave-assisted cycloaddition and cyclocondensation heterocyclic reactions using I-(2-aryl-2-oxoethyl)-2-aryloylimidazole as a key intermediate compound and tested their anticancer properties on Leukemia all lines. All the prepared compounds were characterized by FTIR, ^{I}H NMR and mass spectral analysis.

Key words: Pyrazinobenzimidazole, Cycloaddition, Cyclocondensation, Anticancer activity.

INTRODUCTION

Cancer is the worldwide health problem and the most frightening disease of human.[1-4] The importance of imidazo [l,2-a] pyrazines[5] stems especially from their remarkable anticancer[6-10] and antimicrobial activities[11] along with antihypertensive,[12-13] antibroncospastic[14-15] and isotropic activities[16-17] on the cardiovascular system.

Motivated by these observations and as an extension of our previous works on imidazo [l,2-a] pyrazine and pyrazino [l,2-a] benzimidazole,[3-18] exhibiteding remarkable anticancer activities especially on Leukemia, we now report the synthesis of some novel l,3-diary/pyrazino [l,2-a]benzimidazole derivatives via a microwave-assisted cycloaddition and cyclocondensation heterocyclic reactions and tested their anticancer properties on Leukemia all lines.

Microwave heating is very attractive for chemical application and has become widely accepted non-conventional energy source for performing organic synthesis. This statement is supported by the increasing number of related publication in recent years particularly in 21st century with the general availability of new and reliable microwave instrumentation. A large number of heterocyclic compound are synthesized by microwave irradiation with various applications in the field of medical chemistry.

Chemistry

Some novel 1,3-diarylpyrazino [1,2-a] benzimidazole derivatives were synthesized and their structures were elucidated by analytical and spectroscopic methods. 2-Aryloylimidazole derivatives were

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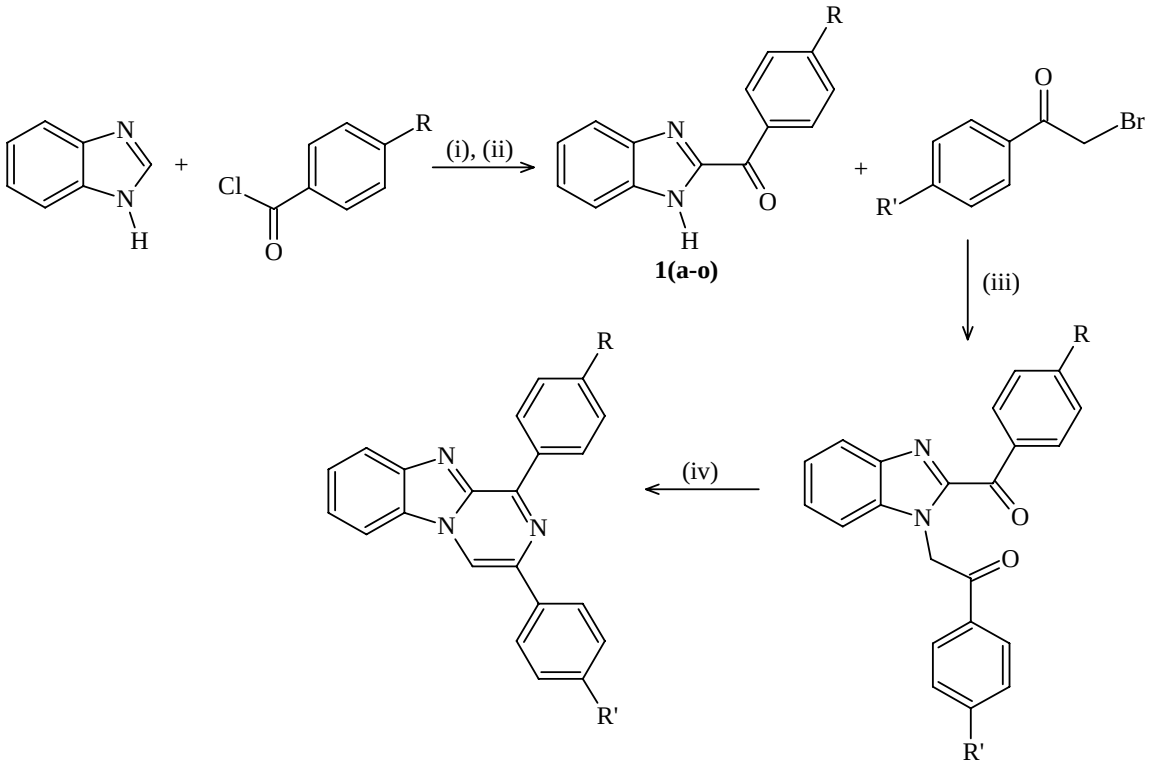
taken as starting materials. These compounds were reacted with 2-bromoacetophenones to afford 1-(2-aryl-2-oxoethyl)-2-arylolbenzimidazoles **(2a-o)**. 1,3-Diarylpyrazino [1,2-a] benzimidazole derivatives, **(3a-o)** were obtained by treating the diketone derivatives **(2a-o)** with ammonium acetate in a minimum amount of acetic acid by using microwave irradiation[1], which is a facile synthetic method[1,2]. The synthesis pathway of compounds have been outlined in **Scheme 1**. It was demonstrated that many organic reactions can be conducted very rapidly under microwave irradiation. This method was preferred due to high reaction rates, pure products and operational simplicity. In this alternative reaction condition, no product could be obtained in the absence of solvent. Thus, a small amount of acetic acid was used for dissolving the substrates and microwave energy transfer.



1	R
a	H
b	OCH_3
c	Cl

2, 3	R	R′
a	H	H
b	H	CH_3
c	H	OCH_3
d	H	F
e	H	Cl
f	OCH_3	H
g	OCH_3	CH_3
h	OCH_3	OCH_3

2, 3	R	R′
i	OCH_3	F
j	OCH_3	Cl
k	Cl	H
l	Cl	CH_3
m	Cl	OCH_3
n	Cl	F
o	Cl	Cl

Scheme 1

Reagents and conditions; (i) $(C_2H_5)_3N$, pyridine, stirring at RT; (ii) NaOH, H_2O, reflux' (iii) K_2CO_3, CH_3COCH_3, stirring at RT; (iv) CH_3COONH_4, CH_3COOH, MW irradiation, 2 min.

In the IR spectra of compounds **(2a-p)** were observed at about 1708-1685 cm^{-1} and 1645-163 cm^{-1} regions, which are characteristic for carbonyl stretching bands. These carbonyl stretching bands disappeared after cyclization to give pyrazino [1,2-a] banzimidazole ring system. Methylene protons resonated in aliphatic area at 6.3 ppm for **(2a-o)** in the NMR spectra. After cyclization; however, the corresponding protons were shifted to the aromatic area in **3a-o** and observed at 9.6 ppm as singlets. Other characteristic peaks due to the aromatic protons were detected as base peak.

EXPERIMENTAL

Melting points were determined by using an Electrothermal 9100 digital melting point apparatus. Spectroscopic data was recorded on the following instruments: IR, Shimadzu, 8400 FTIR Spectrophotometer; ^{1}H NMR; Bruker DPX 500 Mh_z nmr SPECTOMETER. Microwave irradiated reactions were performed by using a milestone Microsynth apparatus.

Compound **(1a)**, **(2a)** and **(3a)** were synthesized by using the reported literature methods.

General procedure for 2-aryloybenzimidazoles (1b,c)

Benzimidazole (100 mmol) was completely dissolved in pyridine (30 mL) and then triethylamine (28.4 mL) was added. Benzoylchloride (20 mmol) was gently and slowly dropped to the reaction media in the solution during stirring in ice bath under atmosphere with nitrogen gas. Then the mixture was stirred at room temperature without nitrogen atmosphere for a day. NaOH solution (7.5N, 6 g NaOH and 20 mL water) was added to the mixture and refluxed for an hour. The reaction media was poured into ice water and kept in a refrigerator for two days. The residue was filtered and washed with water. The raw product was recystallized from ethanol.

General procedure for 1-(2-aryl-2-oxoethyl)-2-aryloylbenzimidazoles (2a-o)

A mixture of the suitable 2-aryloylbenimidazole (5 mmol), 2-bromoacetophenone (5 mmol) and potassium carbonate (5 mmol) in acetone (50 mL) was stirred at room temperature. Stirring was continued at room temperature until the disappearance of the starting material (4-6 h, TLC analyses).

The solvent was evaporated at low temperature. The residue was washed with water and then ethanol. The raw product was recrystallized from ethanol.

1-(2-4-Methoxyphenyl)-2-oxoethyl)-2-(4-methoxybenzoyl) benzimidazole (2h)

Yield: 66% m.p. 180-181^{o}C. IR (KBr)^v maks (cm^{-1}): 1690, 1635 (CO), 1597-1495 (CN and CC), 1290, 1232 (C-O-Ar) ^{1}H NMR (500 MHz) (DMSO-d_6) (ppm): 3.89 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 6.23 (2H, s, CH_2CO), 7.14 (4H, t, J:9.1 Hz) and 9.13 Hz, Ar-H), 7.40 (1H, t, J: 7.28H, Ar-H), 7.46 (1H, t, J:7.81 HZ and 7.80 Hz, Ar-H), 7.82 (1H, d, J:8.19 HZ), 7.92 (1H, d, J:8.07Hz, Ar-H) 8.11 (2H, d, J: 8.78 Hz Ar-H), 8.36 (2H, d, J:8.78 Hz, Ar-H). Anal. Calcd. for $C_{24}H_{20}N_2O_4{\cdot}2H_2O$:C:66.04, H:5.54, N:6.42. Found: C:65.88, H:5.45, N:6.77.

General procedure for 1,3-dairylpyrazino[1,2-a]benzimidazoles (3a-o)

A mixture of suitable **(2a-o)** (1 mmol) and ammonium acetate (10 mmol) in 0.5 mL of acetic acid was irradiated at power 600 W in a Microwave organic synthesis apparatus for 2 min. The solution was cooled, poured into ice water and neutralized with sodium carbonate. The precipitate formed was filtered and crystallized in ethanol.

RESULTS AND DISCUSSION

In the first step, the compounds **(2a-e)**, **(2h-j)**, **(2m)**, **(2o)**, **(3a-f)**, **(3j)** and **(3l)** were selected by NCI for the anticancer tests. The selected compounds were tested *in vitro* against sixty human tumor cell lines derived from nine neoplastic diseases and the test results were determined as growth percent values for 10^{-5} M concentration.

Table 1: Anticancer activity of the compounds as % growth

Compds.	L	NSCLC	CC	CNSC	M	OC	RC	PC	BC	Mean
2a	52.17	82.55	68.71	82.33	75.38	87.00	86.38	86.50	58.75	71.95
2b	70.50	91.01	72.24	101.15	72.07	80.45	85.16	91.45	80.51	82.72
2c	-3.26	36.00	22.43	25.38	31.26	30.25	38.60	33.20	23.32	28.08
2d	29.98	70.39	48.96	65.49	46.46	65.25	71.58	87.42	48.89	48.89
2e	21.67	52.44	40.14	37.17	34.13	44.33	45.13	34.00	9.00	35.87
2h	-2.92	38.33	19.69	23.85	26.74	27.87	35.98	44.39	18.28	25.51
2i	71.48	101.01	71.24	106.10	68.06	84.42	97.16	93.45	81.51	85.89
2j	3.41	51.54	33.14	23.44	24.15	29.51	43.77	35.08	28.82	31.68

The remarkable low growth percent values were obtained for the compounds **(2c)** and **(2h)** against leukaemia cell lines as -3.06 and -2.92%, respectively. With respect to mean values, the compound **(2h)** exhibited the lowest growth percent values with 25.51%. The compounds **(2c), (2e)** and **(2j)** also possessed remarkable growth values. As the test method requires, the compounds having growth percent lower than 75% were accepted for the further screening test. Thus, **(2a), (2c-e), (2h), (2j)** and **(2m)**, which are diketone compounds were taken into the second stage. In this step, the selected compounds were tested at 10-fold dilutions of five concentrations (100, 10, 1, 0.1 and 0.01 uM). The results are given as $\log_{10}$ GI_{50} (GI_{50}: growth inhibition of 50%). The detailed test results are given in Table 2.

Table2: $\log_{10}$ GI_{50} values of the selected compounds

Compds.	L	NSCLC	CC	CNSC	M	OC	RC	PC	BC	MG_MID
2a	-4.28	-4.16	-4.2	-4.08	-4	-4	-4.06	-4.14	-5.03	-4.15
2c	-6.14	-5.03	-5.82	-5.40	-5.53	-5.44	-5.49	-5.49	-5.65	-5.54
2d	-5.85	-4.73	-5.45	-5.07	-5.34	-4.97	-4.56	-4.87	-5.40	-5.27
2e	-5.69	-4.74	-5.33	-5.88	-5.43	-5.05	-4.94	-5.37	-5.80	-5.25
2h	-6.13	-4.82	-5.90	-5.48	-5.49	-5.52	-5.41	-5.53	-5.62	-5.51
2j	-6.14	-4.84	-5.60	-5.47	-5.61	-5.28	-5.43	-5.52	-5.46	-5.46
2m	-5.59	-4.73	-5.30	-4.78	-5.02	-4.75	-4.85	-4.62	-5.21	-5.01
Melphalan	-5.48	-5.17	-5.11	-5.12	-5.08	-5.18	-4.99	-4.49	-4.79	-5.09
Cisplatin	-6.39	-6.20	-6.14	-6.18	-6.08	-6.45	-6.17	-6.41	-6.05	-6.20

The test method states that the compounds having $\log_{10}$ GI_{50} values greater than -4 are considered as inactive. It can be seen that for all compounds the $\log_{10}$ GI_{50}values are smaller than -4. Therefore, we may

conclude that all of our compounds under investigation provide a notable activity level. Melphalan and cisplatin (cis-diaminodichloroplatinum) are two of the commonly used chemotherapeutic agents and used as standard compounds. When the mean graph midpoint (MG-MID) values of the compounds melphalan and cisplatin, i.e. -5.09 and -6.20, respectively are considered, it is observed that our compounds provide high activity levels. The MG-MID values of the compounds **(2c-e)**, **(2h)** and **(2j)** are lower than that of the comtrol compound melphalam. In this respect, **(2c)** and **(2h)** are remarkable compounds with the MG-MID values, -5.54 and -5.46, respectively.

The activity levels of the compounds bearing methoxy or halogen are higher than that of the first member of the series, i.e. **(2a)**. It is intersting to see that the more active compounds **(2c)** and **(2h)** bear methoxy group.

Anticancer activity test

The cytotoxic and/or growth inhibtory effects of the compounds were evaluated *in vitro* aginst approximately sixty human tumour cell lines derived from nine neoplastic diseases namely; Leuukemia (L,4 or 6 cell lines), Non-small Cell Lung Cancer (NSCLC, 9 cell lines), Colon Cancer (CC, 7 cell lines), Central Nervous System Cancer (CNSC, 6 cell lines), Melanoma (M, 8 or 9 cell lines), Ovarian Cancer (OC, 6 or 7 cell lines), Renal Cancer (RC, 8 cell lines), Prostate Cancer (PC, 2 cell lines), Breast Cancer (BC, 6 or 8 cell lines). The evaluation of anticancer activity was performed at the National Cancer Institute (NCI) of Bethesda, USA, following the *in vitro* screening program, which is based upon the use of multiple panels of 60 human tumour cell lines and our compounds were tested 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 (SRB) protein assay was used to estimate cell viability of growth.

CONCLUSION

Some novel 1,3-diarylpyrazino[1,2-a] benzimidazole derivatives were synthesized and their structures were elucidated by analytical and spectroscopic methods. I – (2-Aryl-2-oxoethyl)-2-aryloylimidazoles were reacted with ammonium autate in acetic acid to obtain the aimed compound. In this reaction, microwave irradiation method was applied as the energy source. Anticancer activities of the prepared compounds were investigated. It was observed that some of the compounds showed remarkable anticancer activities.

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