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Synthesis of some 2,3,6,8-tetraarylimidazo[1,2-a]pyrazine derivatives by using either reflux or microwave irradiation method, and investigation their anticancer activities

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In this study, some 2,3,6,8-tetraarylimidazo[1,2-a]pyrazine derivatives were synthesized by reacting 1-(2-aryl-2-oxoethyl)-2-aryloyl-4,5-diarylimidazoles from 2-aryloyl-4,5-diarylimidazole and 2-bromoacetophenone derivatives with ammonium acetate in acetic acid by using the method that was previously developed and repeatedly tested in our studies. Structural elucidation of the compounds was performed by IR, ^{1}H-NMR, and MASS spectroscopic data and elemental analysis results. Anticancer activities of selected compounds were evaluated and the noticeable activity values were reported.

Key Words: Substituted imidazoles, imidazo[1,2-a]pyrazines, microwave irradiation, anticancer activity

Introduction

Cancer is the biggest health hazard and the most frightening disease for the world. In the course of identifying numerous chemical substances that may serve as leads for designing novel antitumor agents,[1] we were especially interested in the present work, as in some our previous publications, with substituted imidazo[1,2-a]pyrazines.[2]

The importance of imidazo[1,2-a]pyrazines stems especially from their remarkable anticancer and antimicrobial activities, besides antihypertensive,[3−7] antibronchospastic, and inotropic activities on the cardiovascular

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system.[8−11] The great attention paid to these structures can be attributed to the fact that the chemiluminescent compounds, such as luciferin from some *Cypridina*, *Renilla*, *Oplophorus*, and *Watasenia* species, are imidazo[1,2-a]pyrazine derivatives.[12−14]

Motivated by the above observations and as an extension of our previous work on imidazo[1,2-a]pyrazine and pyrazino[1,2-a]benzimidazoles, which show notable anticancer activities, especially on leukemia,[2,15] we report here on the synthesis and anticancer activity testing of some 2,3,6,8-tetraarylimidazo[1,2-a]pyrazine derivatives.

Three general synthetic methods used for the formation of imidazo[1,2-a]pyrazine ring systems in the literature and a fourth method developed by us were summarized in our previous study.[2] The first method involves the reactions between 2-aminopyrazine and α-functional carbonyl compounds. In the second method, the intermediate compounds obtained from 2-halopyrazine and an aminoethanol were first oxidized to give α-(pyrazine-2-ylamino)ethanone. The ring closure was then achieved. In the third method, 2-(aminomethyl)imidazoles were reacted with α-halocarbonyl compounds. In the fourth method, 2-aryloylimidazole derivatives were reacted with 2-bromoacetophenones to give 1-(2-aryl-2-oxoethyl)-2-aryloylimidazoles. These diketo compounds were reacted with ammonium acetate in acetic acid to obtain 6,8-diarylimidazo[1,2-a]pyrazines.

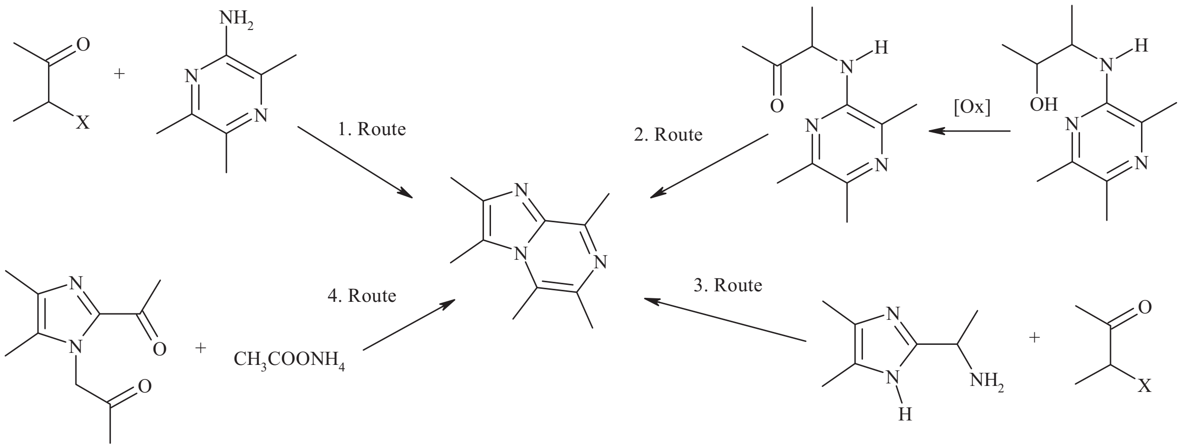


Figure 1. Synthesis of imidazo[1,2-a]pyrazine reported in the literature.

The synthetic studies were performed under green chemistry conditions using a microwave irradiation reaction apparatus and a minimum amount of solvent, in addition to the conventional reflux method.

Experimental

Chemistry

Microwave-irradiated reactions were performed using a Milestone MicroSYNTH apparatus. Melting points were determined using an Electrothermal 9100 digital melting point apparatus and are uncorrected. Spectroscopic data were recorded on the following instruments: IR, Shimadzu 8400 FTIR spectrophotometer; ^{1}H-NMR, Bruker DPX 400 MHz and Bruker 500 MHz NMR spectrometers; MASS, Agilent 1100 MSD mass spectrometer. Analyses for C, H, and N were within 0.4% of the theoretical values. The benzil, 4,5-diarylimidazole, and

2-bromoacetophenone derivatives used as starting materials were prepared according to the methods in the literature.[16−18]

General procedure for the synthesis of 2-aryloyl-4,5-diarylimidazoles (II_{a-l})

A suitable 4,5-diarylimidazole (100 mmol) was completely dissolved in pyridine (30 mL), then added to triethylamine (28.4 mL). A suitable benzoylchloride (200 mmol) was gently and slowly dropped into the reaction medium while the solution was stirred in an ice bath under atmosphere with nitrogen gas. The mixture was then stirred at room temperature without a nitrogen atmosphere for 24 h. A NaOH solution (7.5 N, 6 g NaOH, and 20 mL water) was added to the mixture and refluxed for 1 h. The reaction medium was poured into ice water and kept in a refrigerator for 48 h. The residue was filtered and washed with water. The raw product was recrystallized from ethanol.

2-(4-Methoxybenzoyl)-4,5-diphenylimidazole (II_b): This compound was prepared according to the general procedure above, in a yield of 85%, IR (potassium bromide): 1638 (C=O), 1597-1450 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 3.83 (s, 3H), 7.21-7.43 (m, 10H), 7.49 (d, J = 8.60 Hz, 2H), 7.80 (d, J = 7.46 Hz, 2H), 12.51 (s, 1H).

2-Benzoyl-4,5-di(4-methylphenyl)imidazole (II_d): This compound was prepared according to the general procedure above, in a yield of 86%, IR (potassium bromide): 1642 (C=O), 1595-1448 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 2.34 (s, 6H), 7.22 (d, J = 7.91 Hz, 4H), 7.36 (d, J = 8.12 Hz, 2H), 7.40 (d, J = 8.11 Hz, 2H), 7.61-7.71 (m, 3H), 8.53 (d, J = 7.56 Hz, 2H), 13.73 (s, 1H).

2-(4-Chlorobenzoyl)-4,5-di(4-methylphenyl)imidazole (II_f): This compound was prepared according to the general procedure above, in a yield of 87%, IR (potassium bromide): 1638 (C=O), 1598-1448 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 2.35 (s, 6H), 7.14-7.22 (m, 4H), 7.36 (d, J = 8.44 Hz, 2H), 7.41 (d, J = 8.36 Hz, 2H), 7.68 (d, J = 8.59 Hz, 2H), 8.58 (d, J = 7.64 Hz, 2H), 13.81 (s, 1H).

2-(4-methoxybenzoyl)-4,5-di(4-chlorophenyl)imidazole (II_k): This compound was prepared according to the general procedure above, in a yield of 84%, IR (potassium bromide): 1636 (C=O), 1595-1446 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 3.84 (s, 3H), 7.14-7.23 (m, 4H), 7.53 (d, J = 8.54 Hz, 2H), 7.62-7.71 (m, 4H), 8.52 (d, J = 7.61 Hz, 2H), 13.94 (s, 1H).

General procedure for the synthesis of 1-(2-aryl-2-oxoethyl)-2-aryloyl-4,5-diarylimidazoles (1-36)

A mixture of suitable 2-aryloyl-4,5-diarylimidazole (5 mmol), ω-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 under a vacuum. The residue was washed with water and then ethanol. The raw product was recrystallized from ethanol.[2]

1-(2-Phenyl-2-oxoethyl)-2-benzoyl-4,5-diphenylimidazole (1): This compound was prepared according to the general procedure above, in a yield of 89%, IR (potassium bromide): 1697, 1630 (C=O), 1597-1452 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 5.73 (s, 2H), 7.24-7.31 (m, 3H), 7.37-7.39 (m, 2H), 7.46 (d, J = 7.09 Hz, 2H), 7.53-7.61 (m, 7H), 7.67-7.75 (m, 2H), 8.04 (d, J = 7.31 Hz, 2H), 8.36 (d, J = 7.25 Hz, 2H).

1-(2-(4-Methoxyphenyl)-2-oxoethyl)-2-benzoyl-4,5-diphenylimidazole (2): This compound was prepared according to the general procedure above, in a yield of 88%, IR (potassium bromide): 1698, 1632 (C=O), 1595-1449 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 3.87 (s, 3H), 5.69 (s, 2H), 7.09 (d, J = 8.91 Hz, 2H), 7.23-7.26 (m, 1H), 7.28-7.31 (m, 2H), 7.36-7.38 (m, 2H), 7.46 (d, J = 7.16 Hz, 2H), 7.52-7.55 (m, 3H), 7.58-7.61 (m, 2H), 7.68-7.71 (m, 1H), 8.01 (d, J = 7.04 Hz, 2H), 8.35 (d, J = 7.15 Hz, 2H).

1-(2-(4-Chlorophenyl)-2-oxoethyl)-2-benzoyl-4,5-diphenylimidazole (3): This compound was prepared according to the general procedure above, in a yield of 87%, IR (potassium bromide): 1697, 1639 (C=O), 1597-1452 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 5.72 (s, 2H), 7.23-7.31 (m, 3H), 7.38-7.39 (m, 2H), 7.46 (d, J = 7.07 Hz, 2H), 7.53-7.61 (m, 5H), 7.65-7.71 (m, 3H), 8.06 (d, J = 8.63 Hz, 2H), 8.35 (d, J = 7.13 Hz, 2H).

1-(2-Phenyl-2-oxoethyl)-2-(4-chlorobenzoyl)-4,5-diphenylimidazole (7): This compound was prepared according to the general procedure above, in a yield of 89%, IR (potassium bromide): 1699, 1634 (C=O), 1596-1448 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 5.74 (s, 2H), 7.25-7.32 (m, 3H), 7.36-7.38 (m, 2H), 7.46 (d, J = 7.05 Hz, 2H), 7.53-7.60 (m, 5H), 7.67 (d, J = 8.60 Hz, 2H), 7.71-7.73 (m, 1H), 8.03 (d, J = 8.12 Hz, 2H), 8.40 (d, J = 8.59 Hz, 2H).

1-(2-(4-Methoxyphenyl)-2-oxoethyl)-2-(4-chlorobenzoyl)-4,5-diphenylimidazole (8): This compound was prepared according to the general procedure above, in a yield of 84%, IR (potassium bromide): 1698, 1635 (C=O), 1596-1449 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 3.86 (s, 3H), 5.66 (s, 2H), 7.09 (d, J = 8.93 Hz, 2H), 7.23-7.31 (m, 3H), 7.34-7.36 (m, 2H), 7.45 (d, J = 7.06 Hz, 2H), 7.52-7.55 (m, 3H), 7.67 (d, J = 8.62 Hz, 2H), 8.00 (d, J = 8.90 Hz, 2H), 8.38 (d, J = 8.63 Hz, 2H).

1-(2-Phenyl-2-oxoethyl)-2-benzoyl-4,5-di(4-methylphenyl)imidazole (10): This compound was prepared according to the general procedure above, in a yield of 88%, IR (potassium bromide): 1701, 1637 (C=O), 1595-1454 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 2.27 (s, 3H), 2.36 (s, 3H), 5.72 (s, 2H), 7.11 (d, J = 8.09 Hz, 2H), 7.24 (d, J = 8.02 Hz, 2H), 7.33 (d, J = 8.19 Hz, 2H), 7.36 (d, J = 8.18 Hz, 2H), 7.56-7.60 (m, 4H), 7.66-7.75 (m, 2H), 8.04 (d, J = 7.28 Hz, 2H), 8.34 (d, J = 7.16 Hz, 2H).

1-(2-Phenyl-2-oxoethyl)-2-(4-chlorobenzoyl)-4,5-di(4-methylphenyl)imidazole (16): This compound was prepared according to the general procedure above, in a yield of 85%, IR (potassium bromide): 1699, 1634 (C=O), 1585-1448 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 2.27 (s, 3H), 2.36 (s, 3H), 5.71 (s, 2H), 7.11 (d, J = 7.96 Hz, 2H), 7.23 (d, J = 8.02 Hz, 2H), 7.32-7.37 (m, 4H), 7.57-7.61 (m, 2H), 7.66 (d, J = 8.66 Hz, 2H), 7.72-7.73 (m, 1H), 8.04 (d, J = 7.29 Hz, 2H), 8.38 (d, J = 8.64 Hz, 2H).

1-(2-(4-Chlorophenyl)-2-oxoethyl)-2-(4-chlorobenzoyl)-4,5-di(4-methylphenyl)imidazole (18): This compound was prepared according to the general procedure above, in a yield of 86%, IR (potassium bromide): 1698, 1635 (C=O), 1589-1450 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 3.73 (s, 3H), 3.79 (s, 3H), 5.68 (s, 2H), 6.88 (d, J = 8.91 Hz, 2H), 7.08 (d, J = 8.85 Hz, 2H), 7.26 (d, J = 8.67 Hz, 2H), 7.40 (d, J = 8.86 Hz, 2H), 7.64-7.68 (m, 4H), 8.06 (d, J = 8.62 Hz, 2H), 8.37 (d, J = 8.63 Hz, 2H).

1-(2-Phenyl-2-oxoethyl)-2-benzoyl-4,5-di(4-methoxyphenyl)imidazole (19): This compound was prepared according to the general procedure above, in a yield of 87%, IR (potassium bromide): 1701, 1646 (C=O), 1571-1452 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 3.73 (s, 3H), 3.79 (s, 3H), 5.70 (s, 2H), 6.88 (d, J = 8.62 Hz, 2H), 7.08 (d, J = 8.45 Hz, 2H), 7.28 (d, J = 8.46 Hz, 2H), 7.41 (d, J = 8.57 Hz, 2H), 7.56-7.61 (m, 4H), 7.66-7.73 (m, 2H), 8.05 (d, J = 7.88 Hz, 2H), 8.34 (d, J = 7.81 Hz, 2H).

1-(2-(4-Methoxyphenyl)-2-oxoethyl)-2-benzoyl-4,5-di(4-methoxyphenyl)imidazole (20): This compound was prepared according to the general procedure above, in a yield of 88%, IR (potassium bromide): 1701, 1635 (C=O), 1587-1447 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 3.72 (s, 3H), 3.78 (s, 3H), 3.86 (s, 3H), 5.66 (s, 2H), 6.87 (d, J = 8.85 Hz, 2H), 7.06-7.10 (m, 4H), 7.26 (d, J = 8.60 Hz, 2H), 7.39 (d, J = 8.81 Hz, 2H), 7.55-7.59 (m, 2H), 7.66-7.69 (m, 1H), 8.01 (d, J = 8.86 Hz, 2H), 8.32 (d, J = 7.27 Hz, 2H).

1-(2-(4-Chlorophenyl)-2-oxoethyl)-2-benzoyl-4,5-di(4-methoxyphenyl)imidazole (21): This compound was prepared according to the general procedure above, in a yield of 84%, IR (potassium bromide): 1699, 1638 (C=O), 1585-1449 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 3.73 (s, 3H), 3.80 (s, 3H), 5.68 (s, 2H), 6.88 (d, J = 8.88 Hz, 2H), 7.08 (d, J = 8.82 Hz, 2H), 7.27 (d, J = 8.66 Hz, 2H), 7.40 (d, J = 8.88 Hz, 2H), 7.56-7.59 (m, 2H), 7.66-7.69 (m, 3H), 8.07 (d, J = 8.60 Hz, 2H), 8.33 (d, J = 7.15 Hz, 2H).

1-(2-Phenyl-2-oxoethyl)-2-(4-chlorobenzoyl)-4,5-di(4-methoxyphenyl)imidazole (25): This compound was prepared according to the general procedure above, in a yield of 85%, IR (potassium bromide): 1695, 1651 (C=O), 1614-1448 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 3.74 (s, 3H), 3.79 (s, 3H), 5.70 (s, 2H), 6.88 (d, J = 8.87 Hz, 2H), 7.08 (d, J = 8.78 Hz, 2H), 7.27 (d, J = 8.66 Hz, 2H), 7.41 (d, J = 8.81 Hz, 2H), 7.57-7.61 (m, 2H), 7.65 (d, J = 8.62 Hz, 2H), 7.72-7.75 (m, 1H), 8.04 (d, J = 7.27 Hz, 2H), 8.39 (d, J = 7.62 Hz, 2H).

1-(2-Phenyl-2-oxoethyl)-2-benzoyl-4,5-di(4-chlorophenyl)imidazole (28): This compound was prepared according to the general procedure above, in a yield of 89%, IR (potassium bromide): 1705, 1638 (C=O), 1593-1454 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 5.76 (s, 2H), 7.39-7.46 (m, 6H), 7.56-7.62 (m, 6H), 7.67-7.74 (m, 2H), 8.04 (d, J = 8.12 Hz, 2H), 8.32 (d, J = 7.45 Hz, 2H).

1-(2-Phenyl-2-oxoethyl)-2-(4-chlorobenzoyl)-4,5-di(4-chlorophenyl)imidazole (34): This compound was prepared according to the general procedure above, in a yield of 88%, IR (potassium bromide): 1695, 1642 (C=O), 1589-1444 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 5.75 (s, 2H), 7.38-7.47 (m, 6H), 7.59-7.61 (m, 6H), 7.63-7.67 (m, 1H), 8.04 (d, 2H, J = 7.25 Hz), 8.36 (d, J = 8.66 Hz, 2H).

General procedure for the synthesis of 2,3,6,8-tetraarylimidazo[1,2-a]pyrazines (37-72)

Method A

A mixture of suitable **1-36** (3 mmol) and ammonium acetate (30 mmol) in 50 mL of acetic acid was refluxed for 3 h. The solution was cooled, poured into ice water, and neutralized with sodium carbonate. The precipitate formed was filtered and crystallized in ethanol.[2]

Method B

A mixture of suitable **1-36** (1 mmol) and ammonium acetate (10 mmol) in 0.5 mL of acetic acid was taken in a 25 mL Erlenmeyer flask covered with a watch glass. The mixture was irradiated at a power of 600 W in the MicroSYNTH oven for 1-2 min. The work-up was as described under Method A.

2,3,6,8-Tetraphenylimidazo[1,2-a]pyrazine (37): This compound was prepared according to the general procedures above, in yields of 77% for Method A and 85% for Method B, IR (potassium bromide): 1601-1471 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δ (ppm): 7.35-7.46 (m, 4H), 7.50-7.54 (m, 2H), 7.63-7.71

(m, 10H), 8.09 (d, J = 7.36 Hz, 2H), 8.38 (s, 1H), 9.02 (d, J = 7.08 Hz, 2H); ms: (35 eV, electron spray) m/z 424 (M+1, 100%), 425 (M+2, 29%), 426 (M+3, 4%).

2,3,8-Triphenyl-6-(4-methylphenyl)imidazo[1,2-a]pyrazine (38): This compound was prepared according to the general procedures above, in yields of 79% for Method A and 86% for Method B, IR (potassium bromide): 1602-1473 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δg ppm): 3.82 (s, 3H), 7.07 (d, J = 8.87 Hz, 2H), 7.33-7.41 (m, 3H), 7.62-7.70 (m, 10H), 8.02 (d, J = 8.83 Hz, 2H), 8.28 (s, 1H), 9.00 (d, J = 7.17 Hz, 2H).

2,3,8-Triphenyl-6-(4-chlorophenyl)imidazo[1,2-a]pyrazine (39): This compound was prepared according to the general procedures above, in yields of 77% for Method A and 87% for Method B, IR (potassium bromide): 1601-1472 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δg ppm): 7.37-7.41 (m, 3H), 7.60 (d, J = 8.61 Hz, 2H), 7.62-7.70 (m, 10H), 8.15 (d, J = 8.61 Hz, 2H), 8.44 (s, 1H), 9.01 (d, J = 7.10 Hz, 2H).

2,3,6-Triphenyl-8-(4-chlorophenyl)imidazo[1,2-a]pyrazine (43): This compound was prepared according to the general procedures above, in yields of 78% for Method A and 86% for Method B, IR (potassium bromide): 1600-1471 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δg ppm): 7.36-7.46 (m, 4H), 7.50-7.54 (m, 2H), 7.67-7.70 (m, 7H), 7.76 (d, J = 8.53 Hz, 2H), 8.08 (d, J = 7.50 Hz, 2H), 8.39 (s, 1H), 9.09 (d, J = 8.47 Hz, 2H).

2,3-Diphenyl-6-(4-methoxyphenyl)-8-(4-chlorophenyl)imidazo[1,2-a]pyrazine (44): This compound was prepared according to the general procedures above, in yields of 78% for Method A and 87% for Method B, IR (potassium bromide): 1602-1473 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δg ppm): 3.82 (s, 3H), 7.07 (d, J = 8.83 Hz, 2H), 7.36-7.41 (m, 3H), 7.64-7.70 (m, 7H), 7.75 (d, J = 8.66 Hz, 2H), 8.02 (d, J = 8.79 Hz, 2H), 8.30 (s, 1H), 9.08 (d, J = 8.67 Hz, 2H).

2,3-Diphenyl-6-(4-chlorophenyl)-8-(4-chlorophenyl)imidazo[1,2-a]pyrazine (45): This compound was prepared according to the general procedures above, in yields of 79% for Method A and 91% for Method B, IR (potassium bromide): 1601-1471 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δg ppm): 7.36-7.41 (m, 3H), 7.57 (d, J = 8.58 Hz, 2H), 7.64-7.69 (m, 7H), 7.76 (d, J = 8.66 Hz, 2H), 8.14 (d, J = 8.58 Hz, 2H), 8.45 (s, 1H), 9.08 (d, J = 8.64 Hz, 2H).

2,3-Di(4-methylphenyl)-6,8-diphenylimidazo[1,2-a]pyrazine (46): This compound was prepared according to the general procedures above, in yields of 77% for Method A and 85% for Method B, IR (potassium bromide): 1602-1479 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δg ppm): 2.32 (s, 3H), 2.48 (s, 3H), 7.21 (d, J = 8.08 Hz, 2H), 7.43-7.69 (m, 12H), 8.08 (d, J = 7.37 Hz, 2H), 8.36 (s, 1H), 9.01 (d, J = 7.22 Hz, 2H).

2,3-Di(4-methylphenyl)-6-phenyl-8-(4-chlorophenyl)imidazo[1,2-a]pyrazine (52): This compound was prepared according to the general procedures above, in yields of 79% for Method A and 88% for Method B, IR (potassium bromide): 1599-1475 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δg ppm): 2.32 (s, 3H), 2.48 (s, 3H), 7.21 (d, J = 8.02 Hz, 2H), 7.44-7.54 (m, 7H), 7.59 (d, J = 8.13 Hz, 2H), 7.75 (d, J = 8.71 Hz, 2H), 8.08 (d, J = 7.21 Hz, 2H), 8.38 (s, 1H), 9.08 (d, J = 8.71 Hz, 2H); ms: (35 eV, electron spray) m/z 486 (M+1, 100%), 487 (M+2, 35%), 488 (M+3, 32%), 489 (M+4, 14%).

2,3-Di(4-methoxyphenyl)-6,8-diphenylimidazo[1,2-a]pyrazine (55): This compound was prepared according to the general procedures above, in yields of 76% for Method A and 85% for Method B, IR (potassium bromide): 1608-1477 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δg ppm): z.78 (s, 3H), 3.90 (s, 3H), 6.97 (d, J = 8.94 Hz, 2H), 7.22 (d, J = 8.79 Hz, 2H), 7.43-7.53 (m, 3H), 7.60 (d, J = 8.76 Hz, 2H), 7.61-7.69 (m, 5H), 8.08 (d, J = 7.18 Hz, 2H), 8.33 (s, 1H), 9.01 (d, J = 7.06 Hz, 2H); ms: (35 eV, electron spray) m/z 484 (M+1, 100%), 485 (M+2, 32%), 486 (M+3, 6%).

2,3,6-Tri(4-methoxyphenyl)-8-phenylimidazo[1,2-a]pyrazine (56): This compound was prepared according to the general procedures above, in yields of 75% for Method A and 87% for Method B, IR (potassium bromide): 1608-1477 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δg ppm): z.77 (s, 3H), 3.82 (s, 3H), 3.90 (s, 3H), 6.97 (d, J = 8.87 Hz, 2H), 7.06 (d, J = 8.86 Hz, 2H), 7.22 (d, J = 8.72 Hz, 2H), 7.56 (d, J = 8.67 Hz, 2H), 7.61-7.68 (m, 5H), 8.01 (d, J = 8.81 Hz, 2H), 8.23 (s, 1H), 8.99 (d, J = 7.20 Hz, 2H).

2,3-Di(4-methoxyphenyl)-6-phenyl-8-(4-chlorophenyl)imidazo[1,2-a]pyrazine (61): This compound was prepared according to the general procedures above, in yields of 79% for Method A and 91% for Method B, IR (potassium bromide): 1608-1479 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δg ppm): z.78 (s, 3H), 3.90 (s, 3H), 6.98 (d, J = 8.83 Hz, 2H), 7.23 (d, J = 8.67 Hz, 2H), 7.42-7.45 (m, 2H), 7.50-7.53 (m, 1H), 7.57 (d, J = 8.64 Hz, 2H), 7.66 (d, J = 8.78 Hz, 2H), 7.75 (d, J = 8.72 Hz, 2H), 8.08 (d, J = 7.29 Hz, 2H), 8.35 (s, 1H), 9.09 (d, J = 8.70 Hz, 2H).

2,3-Di(4-methoxyphenyl)-6-(4-methoxyphenyl)-8-(4-chlorophenyl)imidazo[1,2-a]pyrazine (62): This compound was prepared according to the general procedures above, in yields of 74% for Method A and 86% for Method B, IR (potassium bromide): 1606-1478 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δg ppm): z.77 (s, 3H), 3.81 (s, 3H), 3.90 (s, 3H), 6.95 (d, J = 8.86 Hz, 2H), 7.04 (d, J = 8.83 Hz, 2H), 7.21 (d, J = 8.69 Hz, 2H), 7.54 (d, J = 8.66 Hz, 2H), 7.63 (d, J = 8.82 Hz, 2H), 7.72 (d, J = 8.65 Hz, 2H), 7.99 (d, J = 8.78 Hz, 2H), 8.22 (s, 1H), 9.05 (d, J = 8.64 Hz, 2H).

2,3-Di(4-chlorophenyl)-6,8-diphenylimidazo[1,2-a]pyrazine (64): This compound was prepared according to the general procedures above, in yields of 78% for Method A and 89% for Method B, IR (potassium bromide): 1601-1471 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δg ppm): 7.44-7.54 (m, 5H), 7.63-7.75 (m, 9H), 8.14 (d, J = 7.28 Hz, 2H), 8.46 (s, 1H), 8.99 (d, J = 7.06 Hz, 2H).

2,3-Di(4-chlorophenyl)-6-phenyl-8-(4-chlorophenyl)imidazo[1,2-a]pyrazine (70): This compound was prepared according to the general procedures above, in yields of 78% for Method A and 88% for Method B, IR (potassium bromide): 1598-1469 (C=C, C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6)δg ppm): 7.43-7.54 (m, 5H), 7.67-7.76 (m, 8H), 8.13 (d, J = 7.56 Hz, 2H), 8.47 (s, 1H), 9.06 (d, J = 8.51 Hz, 2H); ms: (35 eV, electron spray) m/z 526 (M+1, 100%), 527 (M+2, 31%), 528 (M+3, 93%), 529 (M+4, 31%), 530 (M+5, 32%), 531 (M+6, 10%), 532 (M+7, 5%).

Anticancer activity tests

The cytotoxic and/or growth inhibitory effects of the compounds were evaluated in vitro against approximately 66 human tumor cell lines derived from 9 neoplastic diseases, namely leukemia (L), non-small cell lung cancer (NSCLC), colon cancer (CC), central nervous system cancer (CNSC), melanoma (M), ovarian cancer (OC), renal cancer (RC), prostate cancer (PC), and breast cancer (BC). The evaluation of anticancer activity was performed at the National Cancer Institute (NCI) of Bethesda, USA, following the in vitro screening program, which was based on the use of multiple panels of 66 human tumor cell lines against which our compounds were tested at 10-fold dilutions of 5 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.[19] Activity test results are given in Table 2.

Table 1. The synthesized compounds and their properties.

Comp.	R	R'	R"	Molecular Formula	MP (°C)
II_a	H	H	-	$C_{22}H_{16}N_2O$	Ref. 21*
II_b	H	OCH_3	-	$C_{23}H_{18}N_2O_2$	190-192
II_c	H	Cl	-	$C_{22}H_{15}ClN_2O$	Ref. 21*
II_d	CH_3	H	-	$C_{24}H_{20}N_2O$	212-213
II_e	CH_3	OCH_3	-	$C_{25}H_{22}N_2O_2$	252-253
II_f	CH_3	Cl	-	$C_{24}H_{19}ClN_2O$	238-239
II_g	OCH_3	H	-	$C_{24}H_{20}N_2O_3$	Ref. 22*
II_h	OCH_3	OCH_3	-	$C_{25}H_{22}N_2O_4$	Ref. 22*
II_i	OCH_3	Cl	-	$C_{24}H_{19}ClN_2O_3$	Ref. 22*
II_j	Cl	H	-	$C_{22}H_{14}Cl_2N_2O$	Ref. 23*
II_k	Cl	OCH_3	-	$C_{23}H_{16}Cl_2N_2O_2$	215-216
II_l	Cl	Cl	-	$C_{22}H_{13}Cl_3N_2O$	260-261
1, 37	H	H	H	$C_{30}H_{22}N_2O_2$, $C_{30}H_{21}N_3$	152-153, 277-278
2, 38	H	H	OCH_3	$C_{31}H_{24}N_2O_3$, $C_{31}H_{23}N_3O$	142-144, 260-261
3, 39	H	H	Cl	$C_{30}H_{21}ClN_2O_2$, $C_{30}H_{20}ClN_3$	146-148, 277-278
4, 40	H	OCH_3	H	$C_{31}H_{24}N_2O_3$, $C_{31}H_{23}N_3O$	128-129, 258-261
5, 41	H	OCH_3	OCH_3	$C_{32}H_{26}N_2O_4$, $C_{32}H_{25}N_3O_2$	149-150, 272-273
6, 42	H	OCH_3	Cl	$C_{31}H_{23}ClN_2O_3$, $C_{31}H_{22}ClN_3O$	113-114, 251-252
7, 43	H	Cl	H	$C_{30}H_{21}ClN_2O_2$, $C_{30}H_{20}ClN_3$	156-158, 237-239
8, 44	H	Cl	OCH_3	$C_{31}H_{23}ClN_2O_3$, $C_{31}H_{22}ClN_3O$	176-177, 248-249
9, 45	H	Cl	Cl	$C_{30}H_{20}Cl_2N_2O_2$, $C_{30}H_{19}Cl_2N_3$	178-179, >300
10, 46	CH_3	H	H	$C_{32}H_{26}N_2O_2$, $C_{32}H_{25}N_3$	176-177, 260-261
11, 47	CH_3	H	OCH_3	$C_{33}H_{28}N_2O_3$, $C_{33}H_{27}N_3O$	201-202, 296-297
12, 48	CH_3	H	Cl	$C_{32}H_{25}ClN_2O_2$, $C_{32}H_{24}ClN_3$	223-224, 283-284
13, 49	CH_3	OCH_3	H	$C_{33}H_{28}N_2O_3$, $C_{33}H_{27}N_3O$	160-162, 234-235
14, 50	CH_3	OCH_3	OCH_3	$C_{34}H_{30}N_2O_4$, $C_{34}H_{29}N_3O_2$	177-178, 268-270
15, 51	CH_3	OCH_3	Cl	$C_{33}H_{27}ClN_2O_3$, $C_{33}H_{26}ClN_3O$	156-157, 201-204
16, 52	CH_3	Cl	H	$C_{32}H_{25}ClN_2O_2$, $C_{32}H_{24}ClN_3$	187-188, 210-212
17, 53	CH_3	Cl	OCH_3	$C_{33}H_{27}ClN_2O_3$, $C_{33}H_{26}ClN_3O$	161-163, 231-237
18, 54	CH_3	Cl	Cl	$C_{32}H_{24}Cl_2N_2O_2$, $C_{32}H_{23}Cl_2N_3$	182-183, 272-273
19, 55	OCH_3	H	H	$C_{32}H_{26}N_2O_4$, $C_{32}H_{25}N_3O_2$	191-193, 255-257
20, 56	OCH_3	H	OCH_3	$C_{33}H_{28}N_2O_5$, $C_{33}H_{27}N_3O_3$	188-189, 255-256
21, 57	OCH_3	H	Cl	$C_{32}H_{25}ClN_2O_4$, $C_{32}H_{24}ClN_3O_2$	195-196, 244-245
22 ,58	OCH_3	OCH_3	H	$C_{33}H_{28}N_2O_5$, $C_{33}H_{27}N_3O_3$	189-190, >300
23, 59	OCH_3	OCH_3	OCH_3	$C_{34}H_{30}N_2O_6$, $C_{34}H_{29}N_3O_4$	181-182, >300
24, 60	OCH_3	OCH_3	Cl	$C_{33}H_{27}ClN_2O_5$, $C_{33}H_{26}ClN_3O_3$	172-173, 263-265
25, 61	OCH_3	Cl	H	$C_{32}H_{25}ClN_2O_4$, $C_{32}H_{24}ClN_3O_2$	162-164, 254-255

Table 1. Continued.

Comp.	R	R'	R"	Molecular Formula	MP (°C)
26, 62	OCH_3	Cl	OCH_3	$C_{33}H_{27}ClN_2O_5$, $C_{33}H_{26}ClN_3O_3$	> 300, > 300
27, 63	OCH_3	Cl	Cl	$C_{32}H_{24}Cl_2N_2O_4$, $C_{32}H_{23}Cl_2N_3O_2$	187-188, >300
28, 64	Cl	H	H	$C_{30}H_{20}Cl_2N_2O_2$, $C_{30}H_{19}Cl_2N_3$	182-184, 245-246
29, 65	Cl	H	OCH_3	$C_{31}H_{22}Cl_2N_2O_3$, $C_{31}H_{21}Cl_2N_3O$	183-184, >300
30, 66	Cl	H	Cl	$C_{30}H_{19}Cl_3N_2O_2$, $C_{30}H_{18}Cl_3N_3$	225-226, >300
31, 67	Cl	OCH_3	H	$C_{31}H_{22}Cl_2N_2O_3$, $C_{31}H_{21}Cl_2N_3O$	> 300, > 300
32, 68	Cl	OCH_3	OCH_3	$C_{32}H_{24}Cl_2N_2O_4$, $C_{32}H_{23}Cl_2N_3O_2$	155-156, 242-253
33, 69	Cl	OCH_3	Cl	$C_{31}H_{21}Cl_3N_2O_3$, $C_{31}H_{20}Cl_3N_3O$	163-164, 286-287
34, 70	Cl	Cl	H	$C_{30}H_{19}Cl_3N_2O_2$, $C_{30}H_{18}Cl_3N_3$	212-213, 250-252
35, 71	Cl	Cl	OCH_3	$C_{31}H_{21}Cl_3N_2O_3$, $C_{31}H_{20}Cl_3N_3O$	193-194, >300
36, 72	Cl	Cl	Cl	$C_{30}H_{18}Cl_4N_2O_2$, $C_{31}H_{22}ClN_3O$	> 300, > 300

* Only references were given, instead of melting points, for the compounds that were not original.

Table 2. Log_{10} GI_{50} values.

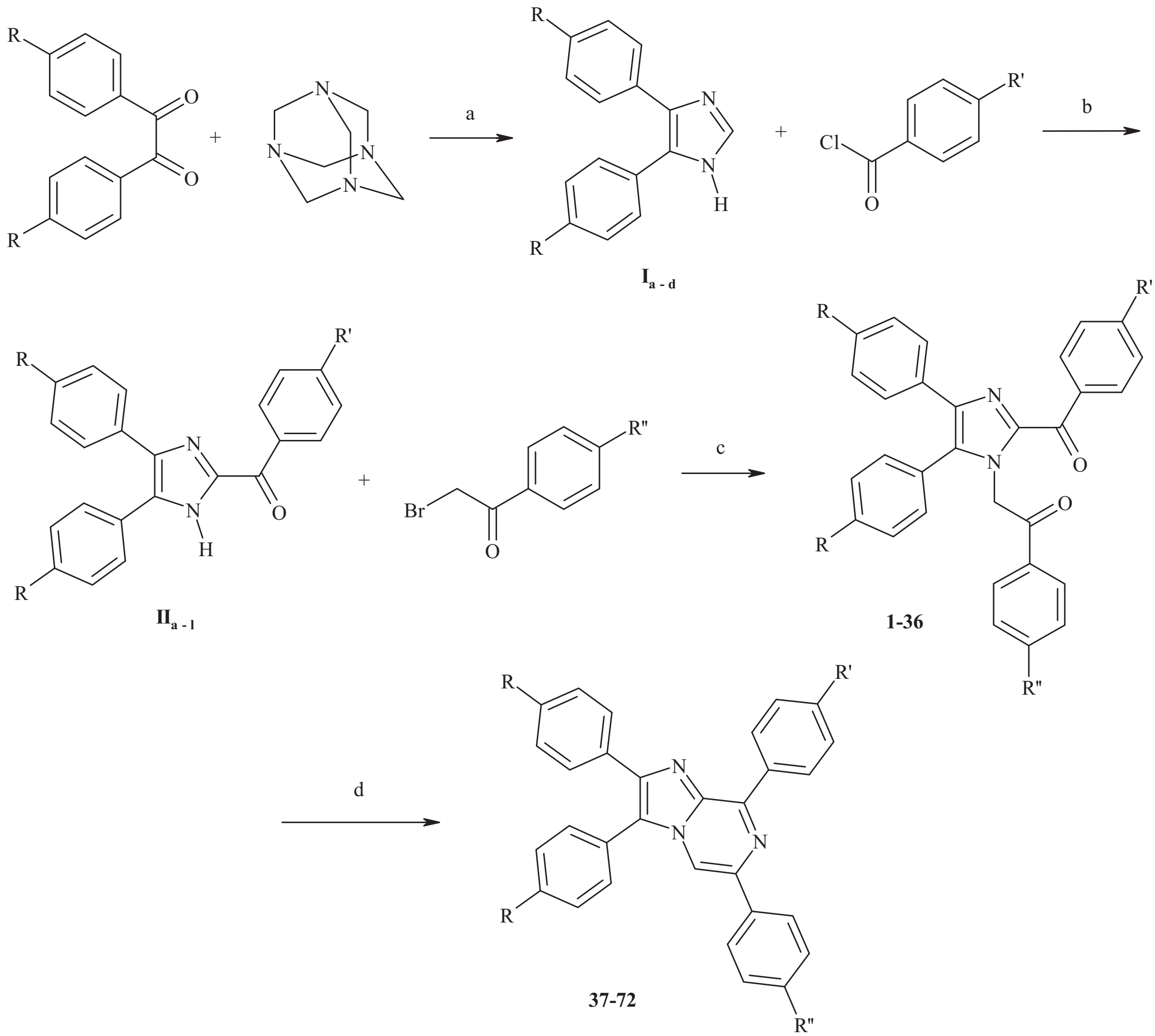
Compounds	L	NSCLC	CC	CNSC	M	OC	RC	PC	BC	MG-MID
11	-4.00	-4.15	-4.00	-4.11	-4.00	-4.07	-4.31	-4.00	-4.10	-4.10
12	-4.00	-4.07	-4.00	-4.00	-4.00	-4.00	-4.00	-4.00	-4.00	-4.01
19	-4.00	-4.10	-4.00	-4.00	-4.00	-4.00	-4.00	-4.00	-4.00	-4.02
20	-4.00	-4.08	-4.00	-4.37	-4.02	-4.03	-4.17	-4.00	-4.08	-4.09
21	-4.07	-4.00	-4.00	-4.00	-4.00	-4.00	-4.00	-4.00	-4.01	-4.01
34	-4.00	-4.10	-4.00	-4.00	-4.00	-4.00	-4.00	-4.00	-4.00	-4.02
55	-4.49	-4.09	-4.00	-4.10	-4.01	-4.02	-4.07	-4.00	-4.10	-4.08
64	-4.00	-4.00	-4.00	-4.01	-4.00	-4.00	-4.00	-4.00	-4.00	-4.00
70	-4.30	-4.42	-4.30	-4.62	-4.37	-4.42	-4.44	-4.30	-4.51	-4.42
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

A: Melphalan, **B**: Cisplatin

Results and discussion

Chemistry

In this study, the fourth method mentioned above was used for the formation of an imidazo[1,2-a]pyrazine ring system. To obtain 4,5-diarylimidazoles, $\mathbf{I}_{a-d}$, benzoyls were taken as starting materials and reacted with urotropine and ammonium acetate in acetic acid. Imidazole derivatives were reacted with suitable benzoylchlorides in pyridine and triethylamine to give 2-aryloyl-4,5-diarylimidazole derivatives, $\mathbf{II}_{a-l}$. Those were then reacted with suitable 2-bromoacetophenones to give 1-(2-aryl-2-oxoethyl)-2-aryloyl-4,5-diarylimidazoles **1-36**. To obtain the target compounds, 2,3,6,8-tetraarylimidazo[1,2-a]pyrazines **37-72**, the diketo compounds **1-36** were reacted with ammonium acetate in acetic acid. The reaction was carried out by using either the classical reflux or microwave irradiation method as a facile synthetic method.[2,17] The synthetic sequences of the compounds are outlined in Figure 2, and some of their characteristics are given in Table 1.



Ia - d, **II**a - l, **1-36**, **37-72**

Reagents: a: CH_3COONH_4, CH_3COOH; b: triethylamine, pyridine;
c: K_2CO_3, CH_3COCH_3; d: CH_3COONH_4, CH_3COOH

Figure 2. Synthesis of the compounds.

It was demonstrated that many organic reactions can be conducted very rapidly under microwave irradiation. This method has been preferred due to high reaction rates, cleaner 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 solving the substrates and microwave energy transfer. The combination of solvent-free procedures with microwave irradiation is an interesting and well-accepted approach within the concepts of green chemistry. This combination takes advantage of both the absence of solvent and of microwave technology under economical, efficient, and safe conditions with a minimization of waste and pollution.[20] In this case, to use a small amount of solvent in any reaction is suitable in terms of environmental pollution. In this sense, it can be reported that microwave synthetic methods are environmentally safe.

The structural elucidation of the compounds was achieved by using spectral data. In the IR spectra, the carbonyl stretching bands, which were characteristic for compounds **1-36**, were observed at about the 1700 cm^{-1} and 1640 cm^{-1} regions. These 2 groups of carbonyl stretching bands were not observed after cyclization to give the imidazopyrazine ring system.

In the NMR spectra, methylene protons resonated in the aliphatic area at about 6 ppm for **1-36.** After cyclization, however, the corresponding proton resonances were shifted to the aromatic area at about 9.2 ppm as singlets for compounds **37-72**. Other characteristic peaks due to the aromatic protons were observed as expected.

Anticancer activity

According to the test method, it can be stated that the compounds having $\log_{10} GI_{50}$ (GI_{50}: growth inhibition of 50%) values greater than -4 are considered inactive. Therefore, we may conclude that compounds **55** and **70** provide a notable activity level. The highest activity value (i.e. -4.62) was obtained for compound **70** against CNSC. The other higher values were -4.52 and -4.50, due to compounds **70** and **55** against BC and L, respectively. Melphalan and cisplatin (cis-diaminodichloroplatinum), 2 of the commonly used chemotherapeutic agents, were 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, were considered, it was observed that compound **70** provided an acceptable activity level (MG-MID -4.42). With regard to all of these data, in the 2 groups of newly synthesized compounds, no significant difference was observed among substituents.

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References

1. Adjei, A. A.; Buolamwini, J. K. *Novel Anticancer Agents, Strategies for Discovery and Clinical Testing*, Elsevier Academic Press, New York, 2006.
2. Demirayak, S.; Kayagil, I. *J. Heterocycl. Chem.* **2005**, *42*, 319-325.
3. Contour-Galcera, M. O.; Poitout, L.; Moinet, C.; Morgan, B.; Gordon, T.; Roubert, P.; Thurieau, C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 741-745.
4. Prevost, C.; Coulomb, H.; Lavergne, O.; Lanco, C.; Teng, B. P. World Office Patent WO 02 96,348, **2003**.
5. Miyake, A.; Yoshimura, Y. Japan Patent JP 01,203,383 [89,203,383], **1990**.

6. Lumma, W. C.; Randall, W. C.; Cresson, E. L.; Huff, J. R.; Hartman, R. D.; Lyon, T. F. *J. Med. Chem.* **1983**, *26*, 357-363.
7. Muler-Schweinitzer, E.; Fozard, J. R. *Br. J. Pharmacol.* **1997**, *120*, 1241-1248.
8. Bonnet, P. A.; Michel, A.; Laurent, F.; Sablayrolles, C.; Rechencq, E.; Mani, J. C.; Boucard, M.; Chapat, J. P. *J. Med. Chem.* **1992**, *35*, 3353-3358.
9. Naline, E.; Cui, Y. Y.; Michel, A.; Bonnet, P. A.; Bakdach, H.; Advenier, C. *Fundam. Clin. Pharmacol.* **1996**, *10*, 368-378.
10. Vitse, O.; Laurent, F.; Pocock, T. M.; Benezech, V.; Zanik, L.; Elliott, K. R. F.; Subra, G.; Portet, K.; Bompart, J.; Chapat, J. P.; Small, R. C.; Michel, A.; Bonnet, P. A. *Bioorg. Med. Chem.* **1999**, *7*, 1059-1065.
11. Spitzer, W. A.; Victor, F.; Don Pollock, G.; Hayes, J. S. *J. Med. Chem.* **1988**, *31*, 1590-1595.
12. Yoshito, K.; Sumi, S.; Shoji, I.; Toshio, G. *Yakugaku Zasshi* **1969**, *89*, 1657-1660.
13. McCapra, F.; Roth, M. *J. Chem. Soc. Chem. Commun.* **1972**, *15*, 894-895.
14. Adamczyk, M.; Akireddy, S. R.; Johnson, D. D.; Mattingly, P. G.; Pan, Y.; Reddy, R. E. *Tetrahedron* **2003**, *59*, 8129-8142.
15. Demirayak, S.; Mohsen, U. A.; Karaburun, A. C. *Eur. J. Med. Chem.* **2002**, *37*, 255-260.
16. Clarke, H. T.; Dreger, E. E. *Org. Syn. Coll. Vol.* **1941**, *1*, 87-89.
17. Lombardino, J. G.; Wiseman, E. H. *J. Med. Chem.* **1974**, *17*, 1182-1188.
18. Cawper, R. N.; Davidson, L. H. *Org. Syn. Coll. Vol.* **1943**, *2*, 480.
19. Boyd, M. R. *Principles and Practice of Oncology* **1989**, *3*, 1-12.
20. Loupy, A. *Microwaves in Organic Synthesis,* Second Ed., Vol. 1, John Wiley & Sons, New York, 2006.
21. Dora, E. K.; Dash, B.; Panda, C. S. *J. Indian Chem. Soc.* **1988**, *65*, 784-786.
22. Chosaku, Y.; Takihiro, I.; Keiichi, T.; Junichi, Y.; Takatsune, T.; Shinji, M.; Kenji, O.; Shuntaro, T.; Isamu, S. Japan Patent JP 63107963, **1988**.
23. Toshio, H.; Takanori, O.; Terasawa, M. Japan Patent JP 62093278, **1987**.

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