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# Green Chemical Approach to Synthesize 1-(N-Substituted Aniline Malonyl)-3,5-Dimethyl-4-(3,4-Difluoro Phenyl Azo) Pyrazoles and Their Antimicrobial Evaluation

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## ABSTRACT

*Green synthesis of 1-(N-substituted aniline malonyl)-3,5-dimethyl-4-(3,4-difluoro phenyl azo) pyrazole by condensing 2,4-diketo-3-(3,4-difluoro phenyl azo) pentane with a number of N-(substituted) phenyl malonamic acid hydrazides under microwave irradiation conditions compared to the classical heating. Anti-microbial studies of synthesize pyrazoles were also carried out.*

**Keywords:** Green chemistry, Microwave irradiation, Classical heating, Substituted pyrazoles,Substituted malonamic acid hydrazides, Anti-microbial activity.

## INTRODUCTION

Pyrazoles are an important heterocyclic compounds and pyrazoles are being used as psycho-pharmacological agents, pain relief agents (e.g., Celecoxib) and cholesterol lowering agent[1]. Substituted pyrazoles have pronounced sedative action on the central nervous system[2]. Benzo-pyrazoles and their derivatives posses a variety of activities including anti-microbial, anti-tubercular[3] and anti-inflammatory[4]. Fluorine containing pyrazole derivatives are reported to possess anti-cancer and anti-viral activities[5]. Anti-diuretic[6], Anti-helmintic[7], hypoglycaemic[8] and fungicidal activities[9] are associated with aryl azo pyrazoles. One of the most attractive concepts in chemistry for sustainability is Green Chemistry, which is the utilization of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture, and applications of chemical products. Microwave heating has attracted the attention of investigators in that it makes it possible to shorten the length of reactions significantly, to increase their selectivity, and to increase the product yields, which is particularly important in the case of high-temperature processes that take a long time.

## MATERIALS AND METHODS

N-(substituted) phenyl malonamic acid hydrazide was prepared from various substituted aromatic amines and 2, 4-diketo-3-(3,4-difluoro phenyl azo) pentane was prepared from 3,4-difluoro aniline. These were prepared from aromatic amines following the reported procedure[10,11] and then by their condensation

pryazoles were prepared. The chemicals employed were of A. R. grade from Sigma Aldrich. Ethanol and other chemicals of A.R. grade were used as received.

**Experimental:** Two different methodologies were adopted and studied for preparing pyrazoles are described below (table 1) and synthesis of the compound shown in figure 1.

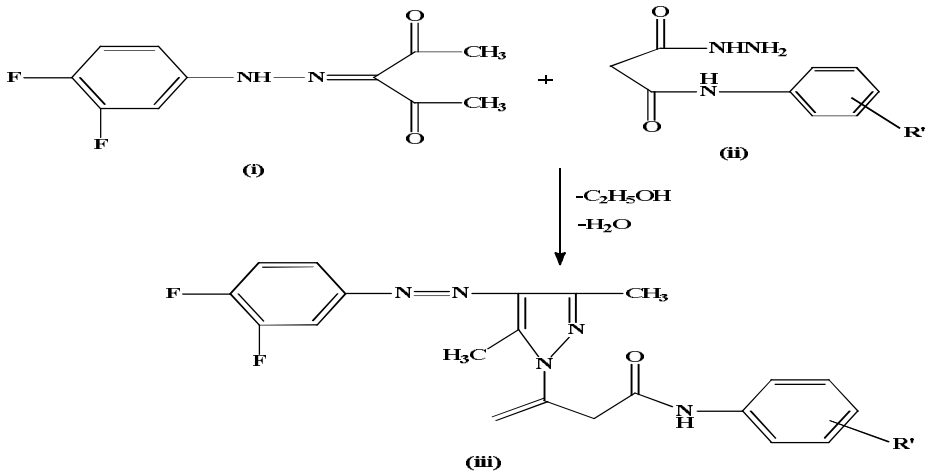
**Classical heating based synthesis (Method A)** : 2,4-diketo-3-(3,4-difluoro phenyl azo) pentane was dissolved in ethanolic solution of N-(substituted) phenyl malonamic acid hydrazide (A-D) and refluxed for 70-120 min. in presence of few drops of glacial acetic acid. A coloured solid was separated after cooling the solution. It was then filtered and purified by washing several times with absolute ethanol. The resulting products were dried over anhydrous calcium chloride in desiccator.

**Microwave "jump start" synthesis (Method B)** : In microwave assisted synthesis, 2,4-diketo-3-(3,4-difluoro phenyl azo) pentane was dissolved in ethanolic solution of N-(substituted) phenyl malonamic acid hydrazide (A-D) in a open beaker. The reaction mixture was irradiated inside a microwave oven until completion of the reaction in presence of few drops of glacial acetic acid. A coloured solid was separated after cooling the solution. It was then filtered and purified by washing several times with absolute ethanol. The resulting products were dried over anhydrous calcium chloride in desiccator.

**Physical measurements and analytical data** : The melting points were determined in open capillaries on Electro thermal apparatus and were uncorrected. Completion of the reaction was monitored by TLC silica-gel-coated Al-Plates (Merck). All the transformations were carried out in a Unmodified IFB domestic microwave oven. All the compounds gave satisfactory microanalysis of C, H and N of the compounds were carried on a Heraeus Carlo Erba 1108 elemental analyzer. IR spectra were recorded on Perkin Elmer RX-1 using KBr Wafers. 1H NMR spectra of the compounds were recorded on a Bruker 300 MHz. The ESI mass spectra were recorded on a JEOL-Accu TOF JMS-100LC Mass spectrometer.

**Table 1.** Synthesis of 1-(N-substituted aniline malonyl)-3,5-dimethyl-4-(3,4-difluoro phenyl azo) pyrazole by conventional and microwave irradiation method

| Entry | Reaction time | | Yield (%) | |
|---|---|---|---|---|
| Compound | Conventional method (A) | Microwave irradiated method (B) | Conventional method A) | Microwave irradiated method (B) |
| **A** | 120 mins. | 3mins | 68.50% | 81.18% |
| **B** | 80 mins. | 2mins. | 59.21% | 92.38% |
| **C** | 80 mins. | 2 mins. | 57.26% | 74.15% |
| **D** | 70 mins. | 1.30 mins. | 67.08% | 82.59% |




R'= a = 3, 4-di f, b = p-Cl, c = p-Br, d = 3-Cl-2-$CH_3$.

**Figure 1.** Synthesis of 1-(N-substituted aniline malonyl)-3,5-dimethyl-4-(3,4-difluoro phenyl azo) pyrazole

**1-(3,4-di fluoro aniline malonyl)-3,5-dimethyl-4-(3,4-di fluoro phenyl azo) pyrazole (a):** Brownish yellow solid; Yield: A:68.50%, B:81.18%; mp: 216˚C; Anal. Calcd. for $C_{20}H_{15}N_5O_2F_4$: C 55.42; H 3.46; N 16.16 (%); found: C 55.39; H 3.42; N 16.24 (%); IR ($cm^{-1}$): 3429 (NH), 1678 (C=O), 1468 (N=N), 1117 (C-F), 1652 (>C=N), 1021 (N-N); $^1$H NMR (300 MHz, DMSO-$d_6$): δ (in ppm) =2.50 (DMSO), 3.06 (s,3H,$CH_3$ pyrazole ring), 7.40-7.96 (m,6H,Ar-H), 10.07 (s,1H,CONH); MS *m/z* 433 (M+).

**1-(p-chloro aniline malonyl)-3,5-dimethyl-4-(3,4-di fluoro phenyl azo) pyrazole (b):** Light yellow solid; Yield: A:59.21%, B:92.38%; mp: 204˚C; Anal. Calcd. for $C_{20}H_{16}N_5O_2F_2Cl$: C 55.62; H 3.70; N 16.22 (%); found: C 55.59; H 3.64; N 16.21 (%); IR ($cm^{-1}$): 3050 (NH), 1674 (C=O), 1465 (N=N), 891 (C-Cl), 1636 (>C=N), 1022 (N-N); $^1$H NMR (300 MHz, DMSO-$d_6$): δ (in ppm) =2.50 (DMSO), 3.14 (s,3H,$CH_3$ pyrazole ring), 7.77-7.82 (m,6H,Ar-H), 10.13 (s,1H,CONH); MS *m/z* 431 (M+).

**1-(p-bromo aniline malonyl)-3,5-dimethyl-4-(3,4-di fluoro phenyl azo) pyrazole (c):** Shiny yellow solid; Yield: A:57.26%, B:74.15%; mp: 225˚C; Anal. Calcd. for $C_{20}H_{16}N_5O_2F_2Br$: C 50.43; H 3.36; N 14.70 (%); found: C 50.46; H 3.30; N 14.68 (%); IR ($cm^{-1}$): 3436 (NH), 1666 (C=O), 1460 (N=N), 1032 (C-Br), 1640 (>C=N), 1018 (N-N); $^1$H NMR (300 MHz, DMSO-$d_6$): δ (in ppm) =2.50 (DMSO), 3.16 (s,3H,$CH_3$ pyrazole ring), 7.38-7.51 (m,6H,Ar-H), 10.09 (s,1H,CONH); MS *m/z* 475.90 (M+).

**1-(3-chloro-2-methyl aniline malonyl)-3,5-dimethyl-4-(3,4-di fluoro phenyl azo) pyrazole (d):** Light yellow solid; Yield: A:67.08%, B:82.59%; mp: 234˚C; Anal. Calcd. for $C_{21}H_{18}N_5O_2F_2Cl$: C 56.57; H 4.04; N 15.71 (%); found: C 56.52; H 4.00; N 15.65 (%); IR ($cm^{-1}$): 3419 (NH), 1672 (C=O), 1458 (N=N), 830 (C-Cl), 1655 (>C=N), 1015 (N-N); $^1$H NMR (300 MHz, DMSO-$d_6$): δ (in ppm) =2.50 (DMSO), 3.07 (s,3H,$CH_3$ pyrazole ring), 7.40-7.90 (m,6H,Ar-H), 10.11 (s,1H,CONH); MS *m/z* 445.45 (M+).

**Antibacterial activity:** Antibacterial activity was evaluated by the paper disc method. The Müller-Hinton agar (beef infusion, casein hydrolysate, starch, and agar) and 5 mm diameter paper discs of Whatman No. 1 were used. The compounds were dissolved in DMSO. The filter paper discs were soaked in different solutions of the compounds, dried and then placed in the petriplates previously seeded with the test organisms *E.coli* and *S.aureus*. The plates were incubated for 24-30 hours at 28±2 $^o$C and the inhibition zone around each disc was measured.

**Antifungal screening:** The antifungal activity of the compounds was evaluated against *Aspergillus niger* by the agar plate technique. The sabouraud dextrose agar (dextrose, peptone, and agar) and 5 mm diameter

paper discs of Whatman No. 1 were used. The compounds were dissolved in DMSO and then were mixed with in the medium. These petriplates were wrapped in the polythene bags containing a few drops of alcohol and were placed in an incubator at 25±2$^{o}$C. The activity was determined after 96 hours of incubation at room temperature (25$^{o}$C).

## RESULTS AND DISCUSSION

IR spectras of substituted pyrazoles showed medium intensity bands at 3436-3050 $cm^{-1}$ due to υ NH vibrations. A sharp bands found at 1678-1666 $cm^{-1}$ due to υ C=O. υ N-N stretching bands in the pyrazole appeared at 1022-1015 $cm^{-1}$. In the IR spectra of substituted pyrazoles, the bands appeared at 1468-1458 $cm^{-1}$ due to the υ N=N. υ >C=N bands appeared at 1655-1636 $cm^{-1}$ in the compounds. A sharp and medium bands of υ C-F, υ C-Cl and υ C-Br showed at 1117 $cm^{-1}$, 891-830 $cm^{-1}$ and 1032 $cm^{-1}$ respectively.

In NMR spectras, the bonding patterns of these compounds are further supported by proton magnetic resonance spectral studies in DMSO-$d_6$. The compounds exhibit a singlet at δ 3.16-3.06 ppm due to pyrazole ring. Compounds showed multiplet in the region at δ 7.96-7.38 ppm attributable to the aromatic protons. Another singlet appeared at δ 10.13-10.07 due to the CONH.
In Mass spectras, the structure further confirmed by appearance of peak at m/z 433, 431, 475.90, 445.45 (M+) for A, B, C, D pyrazole compounds respectively.

## APPLICATIONS

Antimicrobial activity, the data in table 2 showing zone of inhibition against the bacterium *S.aureus, E.coli* and fungus *A.niger* due to different substituted pyrazoles. Compound C was found to be weak in activity against *E.coli* and compound B against *S.aureus*. Highest antibacterial potential was observed with compound A & D against *E.Coli* and compound A against *S.aureus*. Compounds A & C showed highest antifungal potential against *A.niger*.

**Table 2.** Antimicrobial activity of 1-(N-substituted aniline malonyl)-3,5-dimethyl-4-(3,4-difluoro phenyl azo) pyrazole

| Entry Compound | Zone of inhibition (in mm) | | | |
|---|---|---|---|---|
| | Positive control (Amikacin) | *E.coli (Bacteria)* | *S.aureus (Bacteria)* | *Aspergillus niger (Fungus)* |
| **A** | 25 | 22 | 24 | 28 |
| **B** | 24 | 17 | 10 | 25 |
| **C** | 25 | 11 | 14 | 28 |
| **D** | 22 | 19 | 23 | 27 |

## CONCLUSIONS

Microwave irradiation is an efficient and environmentally benign method to accomplish various organic synthesize to afford products in higher yields in shorter reaction periods. Antimicrobial activity showed that the 1-(N-substituted aniline malonyl)-3,5-dimethyl-4-(3,4-difluoro phenyl azo) pyrazoles were proved to have excellent antibacterial ability against Gram-negative *E.coli* & Gram-positive *Staphylococcus aureus* bacteria and these compound also showed highly antifungal activity against *Aspergillus niger.*

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