Chemistry & Biology Interface, 2021, 11, 5, 175-181

RESEARCH PAPER ISSN: 2249 –4820

CHEMISTRY & BIOLOGY INTERFACE

An official Journal of ISCB, Journal homepage; www.cbijournal.com

Synthesis and evaluation of in vitro anti-inflammatory activity of pyrazole derivative in presence of molecular sieve is an efficient and reusable catalyst

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Received; 3 September 2020, Accepted; 14 April 2021

Abstract: An easy and convenient one-pot three component synthesis of pyrazole derivatives prepared by using molecular sieve catalyzed in ethanol under reflux condition. The efficiency of catalyst has been compared with other aluminates and silicate based catalyst in order to optimize this organic transformation. This protocol has several advantages of easy recovery, reusability of catalyst, short reaction time, good yield and avoid the use of hazardous solvents and chemicals. The synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectroscopy. This moiety also screened for in-vitro anti-inflammatory activity.

Keywords: Aromatic aldehyde, Malononitrile, Phenyl hydrazine, Pyrazole, Molecular sieve

1. INTRODUCTION

The multi-component reactions are very important for the synthesis of heterocyclic compounds containing nitrogen linkage which exhibit more importance due to its application in synthetic organic chemistry [1]. Pyrazole is characterized by a 5-membered ring of three carbon atom and two adjacent nitrogen atom[2]. Pyrazole moiety exhibit wide variety of applications in the field of medicinal and pharmaceutical chemistry[3].

In the last decades it has been used as antibacterial[4], antifungal [5], antioxidant [6], antitumor[7], anti-inflammatory[8], anticancer[9], antimalerial[10], protein kinase inhibitor[11], as well as pyrazole ring can be found in variety of pesticides[12], herbicides[13], insecticides[14].

The drug containing pyrazole moiety are Celecoxib, Tepoxalin and Difenamizole are constitute in non steroidal anti-inflammatory (NSAID) drug class[15] **(Fig.1.)**

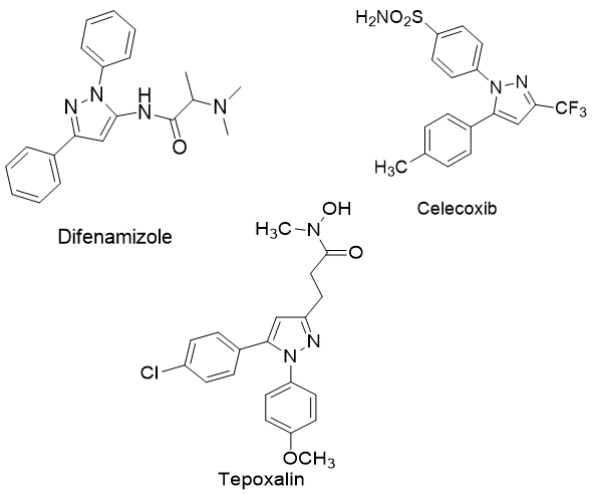


Fig.1. Anti-inflammatory drug contain pyrazole moiety.

Literature survey reveals that many synthetic methods were reported for the synthesis of pyrazole derivatives. The 5-amino-pyrazole-4-carbonitrile derivative has been by three component reaction of aromatic aldehyde, malononitrile and phenyl hydrazine in presence of various catalysts like L-Proline[16], Iodine[17], Urea[18], sodium chloride[19], Ionic liquids[20], silica coated magnetite nanoparticals[21], dodecylbenzene sulphonic acid[22], $AlCl_3$[23], non conventional method like ultrasonic in water-PEG media[24], and simple grinding method[25].

Table 1 : Reported methods for synthesis of Pyrazole 4-Carbonitrile.

Although these methods are exist, each method has its own merits and demerits.

Entry	Catalyst	Time (Min)	Yield (%)	Ref
1	No catalyst	65	91	Reported [26]
2	Cuo/ZrO_2	90	90	Reported [27]
3	$AlCl_3$	82	60	Reported [23]
5	MS 4A	25	94	This work

From the above data current method used Molecular sieve as a catalyst which is more superior than reported method.

Molecular sieves (MS 4A) have been reported as eco-friendly catalyst and its utility has been demonstrated by its applications. Molecular sieves is nothing but zeolites which represent a group of more than soft aluminosilicate minerals i.e a three dimensional framework of interconnected tetrahedral aluminium, silicone and oxygen atom. They consist of SiO_4^- and AlO_4^- bonded together in such a way that all four oxygen atoms located at corners of each tetrahedron are shared with adjacent tetrahedral crystals. Zeolites consists of aluminium oxide, calcium oxide, sodium oxide, iron oxide, silicon oxide[28]. Zeolites have wide applications as a catalyst in the pharmaceutical and petrochemical industry. In the petrochemical industry, they act as 'catalytic crackers' to break large hydrocarbon molecules into diesel, kerosene, gasoline, waxes and other petroleum by-products[29]. Molecular sieves having acidic and basic sites present over its surface as well as at the aperture of pores present on the molecular sieve. Organic materials get firmly adsorbed over these active sites, increasing their reactivity and causing favourable reaction condition. The ability of ethanol to form hydrogen bonding due to polar characteristics might have a main role in activation of acidic and basic sites over MS 4Å. The framework of Al^{3+} present in MS 4Å acts as Lewis acidic sites and the framework of oxygen^{O2-} located at Al-O-Al bridging present in MS 4Å acts as basic sites. The presence of Lewis acidic sites and basic sites over MS 4Å and interaction between surface of molecular sieves and organic molecules shows the combined effect and create the required driving forces for the completion of reaction. Along with that molecular sieve are stable at higher temperature. Moreover it act as a heterogeneous catalyst as they provide large surface area and reproducibility is the key aspects for this catalyst[30]. It exhibits lots of advantages like long lifetime, stable at any environmental conditions, remain unaffected

when used in organic transformation and reused, it is inexpensive and suitable in both aqueous and organic solvents. In view of this advantages we have used molecular sieves to synthesized biologically active series of pyrazole derivative by using substituted aromatic aldehyde, malononitrile and phenyl hydrazine in ethanol as a solvent.

2. EXPERIMENTALS

All chemicals were used of laboratory grade and used without purification. Reactions were monitored by thin layer chromatography (TLC), visualizing with ultraviolet cabinet. Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded on FT IR Shimadzu IRAffinity-1 KBr pellets with absorptions in cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were recorded on a BRUKER AVANCE 400 MHz spectrometer using DMSO-d_6 as solvent and TMS as an internal standard and given in δ units. Mass spectra were recorded on **Thermo Finnigan LCQ Advantage Max Ion Trap Mass Spectrometer Hyphenated with Thermo finnigan Surveyor HPLC system** using the EI technique at 70 eV.

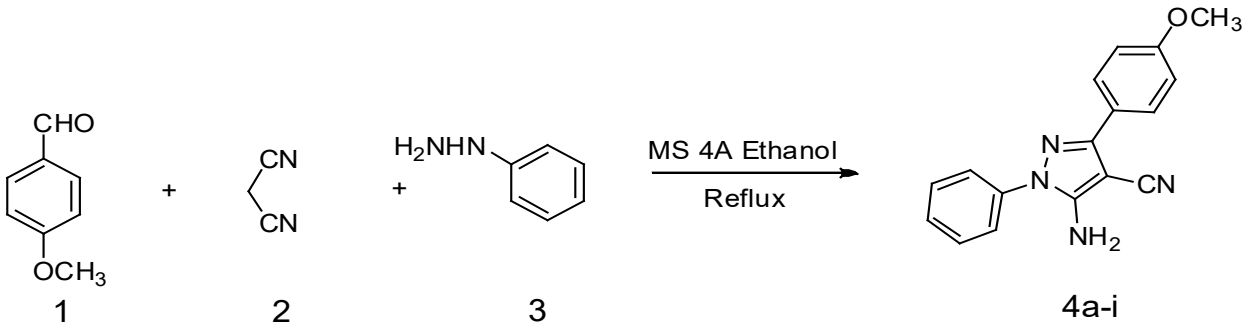
General Procedure for Synthesis of Substituted 5-amino-1,3-diphenyl-1H-pyrazole-4-carbonitrile.

The mixture of aldehyde (1mmol) and malononitrile (1mmol) were taken in round bottom flask to that 100 mg of molecular sieve and 10 mL of ethanol was added. The reaction mixture was stirred at room temperature till the white precipitate was obtained which indicate the formation of arylidine malononitriles by knoevenagel condensation. Afterwords phenylhydrazine (mmol) was added to the reaction mass reflux the reaction mixture for appropriate time.As mention in **(Table 5)**. The progress of reaction was monitored by TLC (hexane: ethyl acetate, 7:3). After completion of reaction, the reaction mixture was diluted with ice cold water. The solid crude product filtered off, wash with water and dried under reduced pressure. The crude product was purified by recrystalization by using ethanol as a solvent.

Selected spectral data5-Amino-3-(4-methoxyphenyl)-1-phenyl-1H-pyrazole-4-carbonitrile (4f):

IR (KBr) cm^{-1}: 3313, 3022, 2835, 1589, 1364, 1292 ^{1}H NMR (400MHz,$CDCl_3$): δ 7.82-7.57(m,3H), 7.20 (merged dd, J1=J2=8.1 Hz, 2H), 6.96 (d, J=8.7Hz, 2H), 3.79 (s, 3H) ppm ^{13}C NMR (100 MHz,$CDCl_3$): δ 159.8, 160.1, 146.0,137.0, 129.5, 129.0 ,118.8, 114.7, 114.2, 112.2, 55.7 ppm; MS (ESI) (m/z): found 291.0(M+1).

3. RESULTS AND DISCUSSION:



Scheme 1: Model reaction for synthesis of pyrazole 4-carbonitriles

The optimization of reaction conditions and performance of catalyst was observed by using the model reaction of p-methoxy benzaldehyde, malononitrile and phenyl hydrazine to gave 5-Amino-1, 3-diphenyl-1*H*-pyrazole-4-carbonitriles by using 100 mg of different molecular sieve in different types of solvents **(Scheme 1)**.

Table 2. Effect of catalyst and solvent.[a]

Entry	Catalyst	Solvent	Yield[b] (%)
1	MS 4A	Ethanol	93
2	MS 3A	Ethanol	85
3	MS 5A	Ethanol	81
4	MS 4A	DMF	56
5	MS 4A	Toluene	60
6	MS 4A	Water	64
7	No catalyst	Ethanol	Trace
8	MS 4A	Neat	32

[a]**Reaction conditions**: Aromatic aldehyde (1 mmol), malononitrile (1 mmol), phenyl hydrazine (1 mmol), catalyst (100 mg) were reflux in 10 ml of solvent. [b]Isolated yields.

In initial study, we investigated model reaction by catalyst such as molecular sieves of different types like MS 3A, MS 4A, MS 5A in presence of ethanol as a solvent. Among these catalysts MS 4A exhibit excellent catalytic activity. MS 5A and MS 3A also gave desired product in good yield. (Table2. Entry2,3) Trace amount of product was formed in absence of catalyst. (Table 2, Entry 7) which indicate the need of catalyst for the present reaction. Further to know the role of solvents the model reaction was carried out under solvent free condition at reflux temperature which yield 32% of product. (Table 2, Entry 8) The study of different solvent like DMF, Toluene, water gave the moderate amount of yield but fail to achieve atom economy of reaction. (Table 2 , Entry 4, 5 & 6).

The solubility of reactants in ethanol reflects the good amount of yield as compared to water because of homogeneous solution of reactant is formed to give the desired product.

With the above optimize condition the loading of catslyst is further studied.In presence of MS 4A (50 mg) product yield was 61% (Table 3, Entry1). However, when reaction carried out in presence of 60 mg and 85 mg the yield were 75 % and 81% respectively.(Table3,Entry2,3). From above study shows that 100 mg of catalyst is sufficient for carried out the reaction with excellent yield of 93% because further increase the concentration of catalyst upto 130 mg has no remarkable variation in the yield of product(Table 3, Entry 5). In the study of temperature conditions for the present reaction the room temperature gave the 40% of yield only (Table 3,Entry 6). Therefore, to attained the desired yield we have enhanced the temperature at 40°C, 60°C and reflux temperature, the yield was improved 62%, 75% and 93% respectively. (Table 3, Entry 7,8 & 4).

Table 3: Effect of catalyst and temperature

Enrty	Temperature(°C)	Catalyst (mg)	Yield[b] (%)
1	Reflux	50	61
2	Reflux	60	75
3	Reflux	85	81
4	Reflux	100	93
5	Reflux	130	91
6	RT	100	40
7	40	100	62
8	60	100	75

[a]**Reaction conditions**: Aromatic aldehyde (1 mmol), malononitrile (1 mmol), phenyl hydrazine (1 mmol), catalyst (100 mg) in 10 mL of solvent. [b]Isolated yields.

Finally to prove the efficacy of catalyst reusability of catalyst is important criteria for which we have carried out the reaction with same catalyst for 3 to 5 run in which it observed that catalyst work efficiently upto 3rd run.(Table 4, Entry1-3). While in 4th and 5th run efficiency of catalyst reduce to some extent (Table 4, Entry 4-5).

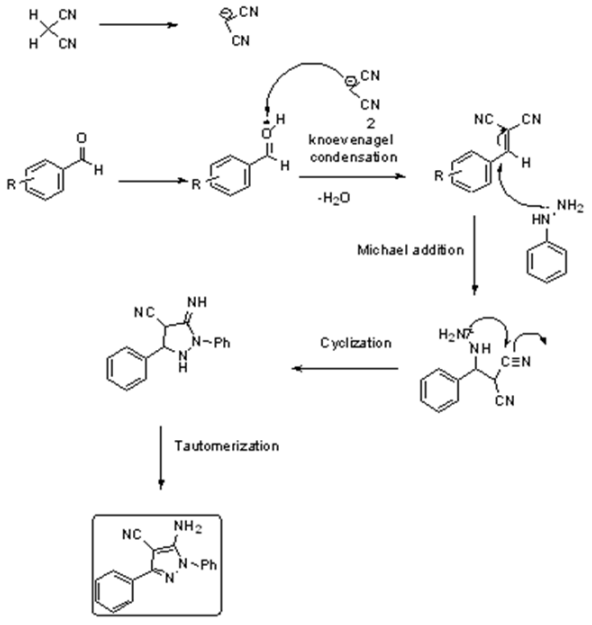
Table 4: Reusability of catalyst

Entry	Run	Time	Yield[b](%)
1	1st	35	93
2	2nd	35	93
3	3rd	38	90
4	4th	35	91
5	5th	35	87

Plausible reaction mechanism for the synthesis of pyrazole 4-carbonitriles depicted in scheme 2.

In the first step enolization of malononitrile and formation of arylidine malononitrile through the condensation reaction of aromatic aldehyde with malononitrile.

The molecular sieve and ethanol helped the enolization of malononitrile and increased the nucleophilic character of active methylene carbon through hydrogen bonds. As a result, the Knoevenagel condensation and Michael addition produced intermediates A and B, respectively. consequently, annulation, tautomerization and aromatization of the intermediate B yielded the final product.



Scheme 2 : A Plausible Mechanism

In general, aromatic aldehydes with electron donating or electron- withdrawing groups gave the pyrazole derivative in good to excellent yield. However, as depicted in **Table 5,** the aldehydes bearing electron-withdrawing functional groups required shorter reaction time in comparison to those having an electron-donating group.

Table 5: Synthesis of pyrazole-4 carbonitrile derivatives[a].

Entry	R-CHO	Yield[b] (%)	Time (min)	Melting point (°C)	Reported mp (°C)
4a	C_6H_5	93	20	158	160-164
4b	3-NO_2 C_6H4	90	15	130	130-133
4c	4-HO C_6H_4	93	30	208	213-215
4d	4- Cl C_6H_4	96	18	128	125-129
4e	2- Cl C_6H_4	91	20	138	141-144
4f	4-OCH_3 C_6H_4	93	35	118	112-115
4g	4-NO_2 C_6H_4	92	22	163	164-166
4h	4-Bromo C_6H_4	85	22	171	165-169
4i	2-furyl	89	20	173	170-172

[a]**Reaction condition:** aromatic aldehyde (1mmol), malononitrile (1mmol), phenylhydrazine (1mmol), catalyst (100 mg) were reflux in 10 ml of ethanol.
[b] isolated yield.

Biological Activity:

In-vitro anti-inflammatory activity:

The standard drug and synthesized derivatives **(5a-k)** were dissolve in minimum amount of dimethylformamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solutions was less than 2.0%. Test solution (1 ml) containing different concentration of drugs was mixed with 1 ml of 1% mM albumin solution in phosphate buffer and incubated at 27±1°C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60±1 0C in water bath for 10 min. After cooling, the

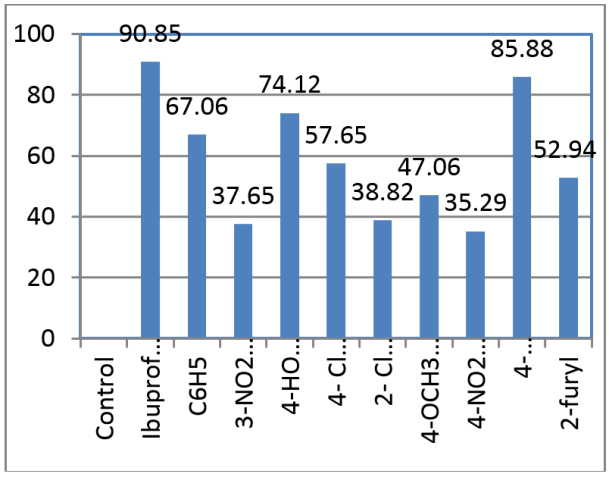
turbidity was measured at 660nm (UV-Visible Elico Spectrophotometer. SL-159). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken. The ibuprofen was used as standard drug. Results are tabulated in (Table no. 5.)

$$\% \text{ of inhibition} = \left(\frac{Vt}{Vc} - 1 \right) \times 100$$

Where,
Vt = Mean absorbance value of test group.
Vc = Mean absorbance value of control group.

Table 5. Antiinflammatory activity of synthesized compouns. (5a-k)

Sr No	Derivatives	Mean absorbance value ±SEM	Inhibition of denaturation (in%)
5a	Control	0.0850	-
5b	Ibuprofen	0.162 ± 0.008	90.58
5c	C_6H_5	0.142 ± 0.005	67.06
5d	3-NO_2 C_6H_4	0.117 ± 0.003	37.65
5e	4-HO C_6H_4	0.148 ± 0.006	74.12
5f	4- Cl C_6H_4	0.134 ± 0.003	57.65
5g	2- Cl C_6H_4	0.118 ± 0.005	38.82
5h	4-OCH_3 C_6H_4	0.125 ± 0.002	47.06
5i	4-NO_2 C_6H_4	0.115 ± 0.007	35.29
5j	4-Bromo C_6H_4	0.158 ± 0.006	85.88



From the above data, it can be concluded that compounds **5c, 5e and 5j** shows significant activity, while compounds (**5f & 5k**) moderate activity as compare with standard drug Ibuprofen.

4. CONCLUSION:

In Conclusion, We have developed a novel efficient and eco-friendly synthesis for the preparation of pyrazoles derivatives by one-pot three component cyclocondensation reaction of aldehyde, malononitrile and phenylhydrazine at reflux temperature by using molecular sieve (MS 4A) as a catalyst in ethanol as a reaction media. All the pyrazoles derivatives were evaluated for (*in-vitro*) anti-inflammatory activity. The screening of anti-inflimmatory data revealed that most of the compounds shows good anti-inflimmatory activity.

5. ACKNOWLEDGEMENT:

The authors are thankful to Director, SAIF, vellore University, vellore for providing spectral analysis facility. Authors are also thankful to Principal, Saraswati bhuwan college of science, Aurangabad for providing laboratory facilities.

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