Original Article

Bio Catalytic One-Pot Knoevenagel Condensation-Michael Type Addition-Heterocyclization Cascade For The Environmentally Friendly Synthesis Of Pyranopyrazoles In Non-Aqueous Media And Their Pharmacological Studies

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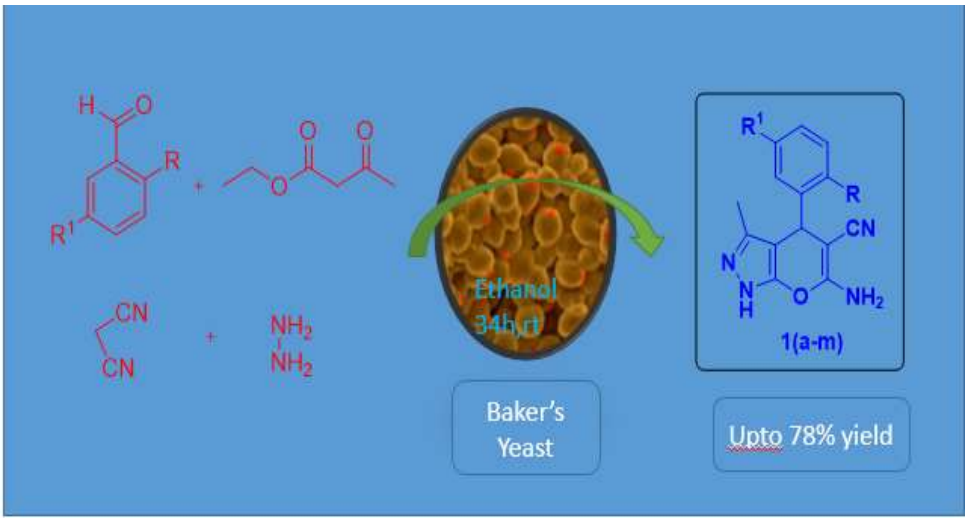
Abstract

Using baker's yeast as a biocatalyst, the condensation of aromatic aldehydes, ethyl acetoacetate, malononitrile, and hydrazine hydrate yields an effective, environmentally friendly, and straightforward one-pot four-component synthesis of pyranopyrazole derivatives. These molecules were screened for pharmacological activities, and among the twelve derivatives, nitro substituent derivatives showed positive results and in docking results also showed more binding activity than all derivatives. Important aspects of the current methodology include the use of an environmentally friendly catalyst, high product yield, and mild reaction conditions, i.e. at neutral pH and ambient temperature.

Keywords:- Baker's yeast · Pyranopyrazoles · Heterocyclization · Biocatalysis · Multicomponent reaction

Graphical abstract



INTRODUCTION

The pyranopyrazole derivatives are fused heterocyclic compounds with antifungal, antibacterial, antimicrobial, analgesic, antipyretic, anti-infammatory, vasodilator, antioxidant, and anticancer properties.[1–8]. They are also excellent agrochemicals and molluscicides, and some of their derivatives are also utilised as cosmetic and pigment precursors. [9–12]. In 1973, the reaction between 3-methyl-1-phenyl-pyrazole-5-one and tetracynomethylene was described as producing the first pyranopyrazole. [13]. The pyrano-[2,3-c] replaced with 2-amino4 By adding malononitrile to 4-arylidene-2-pyrazolin-5-one, pyrazol-3-carbonitrile derivatives were created in 1974. [14]. Later, numerous other synthetic techniques to create these substances were recorded. [15]. One of those methods involves the cyclocondensation of aromatic aldehydes, ethyl acetoacetate, malononitrile, and hydrazine hydrate in a single pot. [16–18] This makes sense over multi-

step reactions. A later development was the prominence of multicomponent reactions (MCR) over conventional multistep synthesis because to atom economy, energy and cost efficiency, and shorter reaction times. As a result, they became a simpler approach for synthesising organic chemicals, and MCR techniques are now used to create heterocyclic compounds. [19–21]. In the recent past, the pyranopyrazoles are synthesized using diferent catalysts including d, l-proline [22], bleaching earth clay (pH 12.5) [23], cetyltrimethylammonium chloride (CTACl) [24], aspirin [25], sulphonic acid-functionalized ionic liquid [26], glycine [27], [bmim]OH [28], meglumine [29], boric acid [30], Ph3CCl [31], choline chloride/thiourea [32], l-proline [33], piperidine [34], maltose [35] and choline chloride–urea deep eutectic solvent-modifed magnetic nanoparticles [36]. Because most of these catalysts have one or more drawbacks, such as the requirement of high energy, waste generation, and time-consuming work setup, and because some catalysts are also expensive and hazardous, the development of a greener method for the synthesis of pyranopyrazoles is still desired. Since many years, the organic transformation aimed at "Greenery" has piqued the public's interest. [37]. Baker's yeast is a readily available, low-cost biocatalyst that can be used without any special microbiology knowledge [38, 39]. This is why baker's yeast has piqued the interest of synthetic organic chemists. Later, it was thought to be a microbial reagent for organic synthesis [40, 41]. The synthesis of alcohols using baker's yeast to reduce a variety of ketone is a well-established organic transformation technology [42]. Commercially, it has also been used to produce bioethanol by immobilising it over sodium alginate gel [43]. In addition to redox reactions, it has been shown to catalyse useful cyclocondensation, resulting in value-added heterocycles [44-46]. The use of baker's yeast for enantioselective synthesis of various organic molecules is a well-established method in synthetic organic chemistry [47-49]. To protect its catalytic activity, the baker's yeast catalyzed organic reactions were mostly carried out in an aqueous medium. Due to the solubility of the substrates, an aqueous medium is not practical for many organic reactions; thus, biocatalysis using an organic solvent is gaining much more attention. Thus, the main benefits of using biocatalyst in an organic medium are higher solubility of organic compounds and easy product recovery [50, 51]. With the a fore mentioned facts in mind, we designed a one-pot four-component synthesis of pyranopyrazole derivatives using aromatic aldehydes, ethyl acetoacetate, malononitrile, hydrazine hydrate, and a readily available, less expensive biocatalyst, namely baker's yeast. This is the first attempt, to the best of our knowledge, to use baker's yeast as a biocatalyst for this multicomponent reaction

Experimental section

General information

All necessary chemicals were obtained from commercial suppliers and used without further purification. A. B. Mauri India Pvt. Ltd. in India supplied the dry baker's yeast. Compounds' 1 H NMR and 13C NMR spectra were determined using a Bruker Avance II spectrometer at 400 MHz and 100 MHz, respectively, with DMSO-d6 as a solvent. Thin-layer chromatography (TLC) was performed on silica gel-precoated aluminum-backed plates and was observed under UV light.

General procedure for the synthesis of 6-amino-4-(2,5 disubstituted phenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (1a-m)

In a round-bottom fask (50 mL), a mixture of various disubstituted aromatic aldehydes (5 mmol), malononitrile (5 mmol), hydrazine hydrrate (5 mmol), and ethyl acetoacetate (5 mmol) was taken with ethanol (20 mL) as a solvent, and then baker's yeast (2 g) was added to the reaction mixture. The resulting reaction mass was stirred at room temperature on a magnetic stirrer, and the reaction progress was monitored using thin-layer chromatography (TLC) in an n-hexane/ethyl acetate solvent system (3:1). The reaction mass was fltered under reduced pressure using a silica bed to remove the catalyst and washed with ethanol after 34 hours of constant stirring at room temperature (50 mL). The crude products were purified by recrystallization in ethanol and column chromatography.

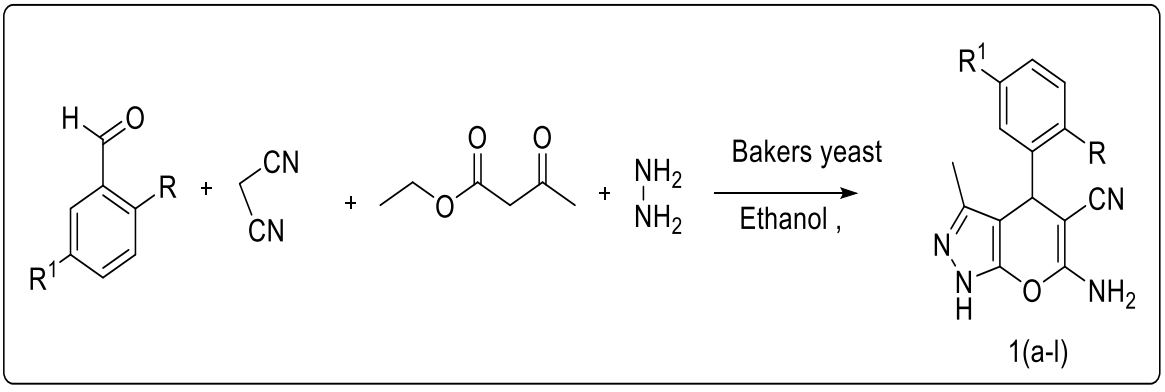


Fig 1: Scheme 1 : Non-aqueous biocatalytic synthesis of pyranopyrazoles via one-pot Knoevenagel condensation–Michael-type addition heterocyclization cascade (1a-m)

Table 1: Optimization of reaction conditions for the nonaqueous biocatalytic synthesis of compound (1a) using a one-pot Knoevenagel condensation-Michael-type addition-hetercyclization cascade

Entry	Solvent	Catalytic amount (g)	Time	Yield (%)[b]
1	Ethanol	2	34	78
2	methanol	2	34	82
3	Acetonitrile	2	34	56
4	DCM	2	34	50
5	DMF	2	50	Trace
6	DMSO	2	50	Trace
7	THF	2	50	-
8	Toluene	2	50	-
9	Water	2	50	Trace
10	Ethanol/Water (3:1)	2	34	75
11	Methanol/Water (3:1)	2	34	70
12	Solvent Free	-	50	No Reaction
13	Solvent Free	2	50	No Reaction
14	Ethanol [c]	-	50	-
15	Ethanol	0.6	50	-
16	Ethanol	1.1	50	Trace
17	Ethanol	1.6	50	30
18	Ethanol	2.6	50	73
19	Ethanol	3.1	50	65

[b] Isolated yield

[c] Without baker's yeast

Table 2 Physical data of 6-amino-4-(2,5 disubstituted phenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitril**e**

Entry	R	R^1	[b] Yield (%)
1a	Br	OH	70
1b	F	NO_2	78
1c	NO_2	F	76
1d	OH	CH_3	66
1e	Cl	NO_2	69
1f	Cl	Cl	70
1g	OH	Cl	68
1h	OH	Br	72
1i	NO_2	Br	71
1j	Cl	CH_3	62
1k	OH	OH	67
1l	F	NO_2	76

[b] Isolated yield

Spectral data of Synthesised Compounds

Compound 1a

^{1}H NMR: δ 2.21 (3H, s), 3.20 (3H, s), 5.23 (1H, s), 6.44 (1H, d, J = 2.9, 0.5 Hz), 6.72 (1H, d, J = 8.6, 2.9 Hz), 7.27 (1H, d, J = 8.6, 0.5 Hz), 7.87 (1H, s).

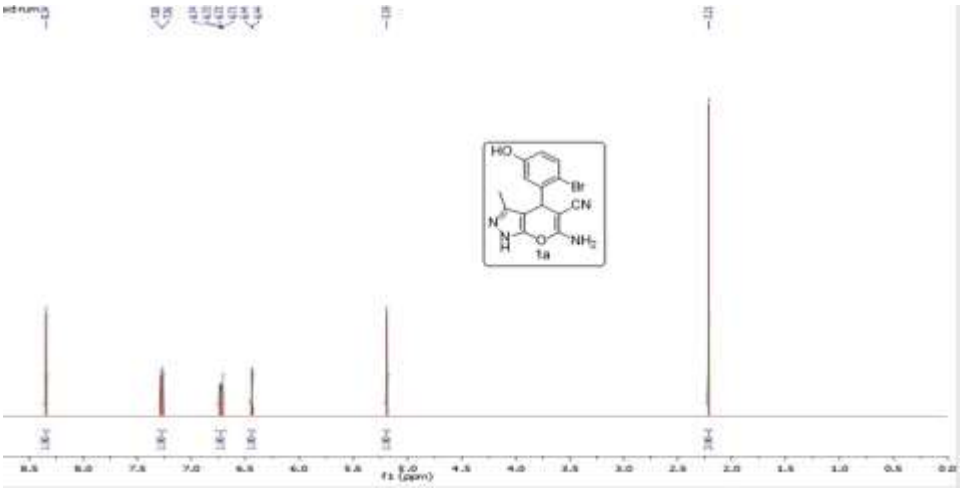


Fig 2: ^{1}H NMR spectra of compound 1a

^{13}C NMR: δ 13.7 (1C, s), 37.7 (1C, s), 48.1 (1C, s), 97.7 (1C, s), 110.6 (1C, s), 114.5 (1C, s), 120.9 (1C, s), 127.3 (1C, s), 133.4 (1C, s), 135.6 (1C, s), 140.0 (1C, s), 143.2 (1C, s), 154.4 (1C, s), 154.8 (1C, s), 159.8 (1C, s).

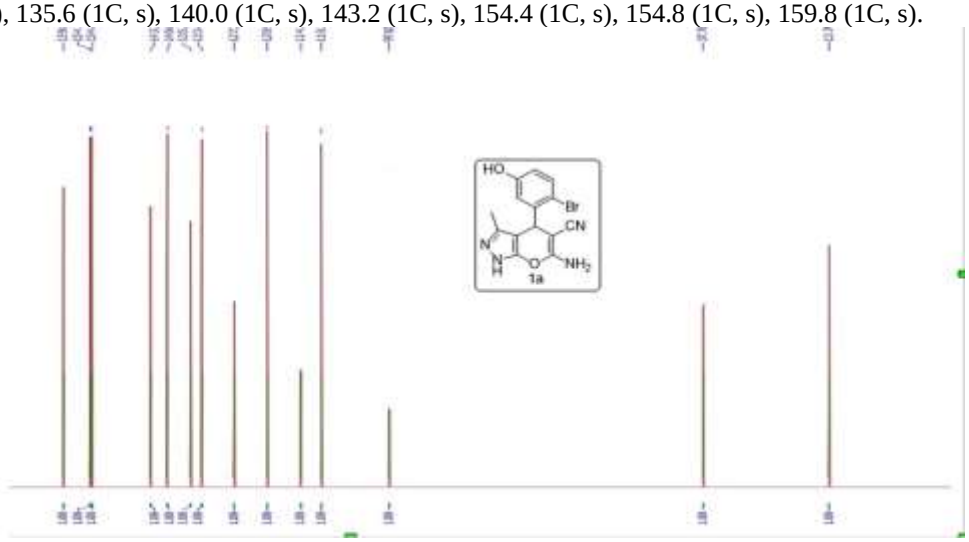


Fig 3: ^{13}C NMR spectra of compound 1a

Mass (m/z):346

IR(Cm^{-1}): 1250-1020(-CN- stretching), 1210-1163(-CO- stretching), 3500 – 3300(-NH- stretching), 1700 – 1500 (-C=C- stretching)

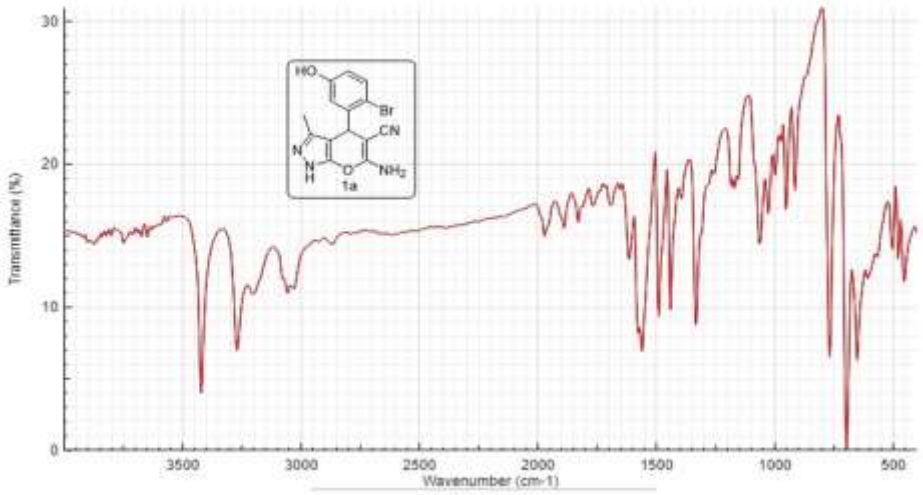


Fig 4: IR spectra of compound 1a

Compound 1b

^{13}C NMR: δ 20.0 (1C, s), 40.7-40.8 (2C, 40.7 (s), 40.7 (s)), 45.9 (1C, s), 55.7 (1C, s), 56.6 (1C, s), 86.0-86.1 (2C, 86.1 (s), 86.1 (s)), 117.6-117.8 (2C, 117.6 (s), 117.7 (s)), 119.3 (1C, s), 137.5 (1C, s), 143.2 (1C, s), 149.5 (1C, s), 159.1 (1C, s).

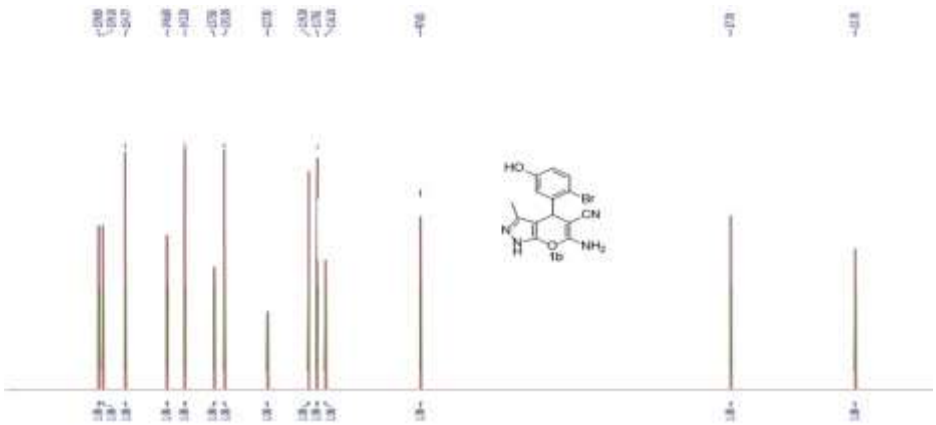


Fig 5 : ^{13}C NMR spectra of compound 1b

^{1}H NMR: δ 1.24-1.36 (3H, 1.30 (d, J = 6.8 Hz), 1.30 (d, J = 6.8 Hz), 1.30 (d, J = 6.8 Hz)), 2.88 (1H, d, J = 6.4, 6.1, 5.8 Hz), 3.03-3.17 (4H, 3.08 (s), 3.08 (s), 3.10 (d, J = 5.5, 4.2, 2.5 Hz)), 3.65 (1H, d, J = 6.4, 5.5 Hz), 4.43 (1H, q, J = 6.8, 6.1 Hz), 5.02 (1H, d, J = 2.5 Hz), 5.72 (1H, d, J = 5.8 Hz), 6.85 (2H, d, J = 8.7, 1.7 Hz), 7.09 (1H, d, J = 2.8, 0.5 Hz), 8.00 (1H, d, J = 4.2 Hz).

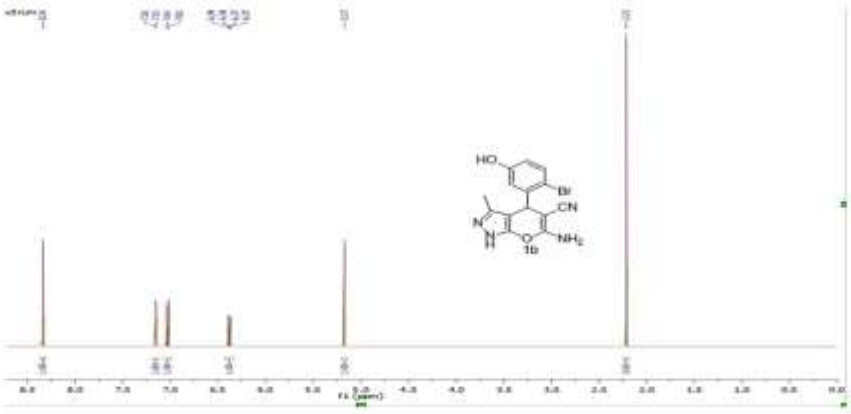


Fig 6: ^{1}H NMR spectra of compound 1b

Mass(m/z):315
Fig: mass spectra of compound 1b
IR(Cm^{-1}): 1251-1023(-CN- stretching), 1211-1160(-CO- stretching), 3501 – 3302(-NH- stretching), 1701 – 1510 (-C=C- stretching)

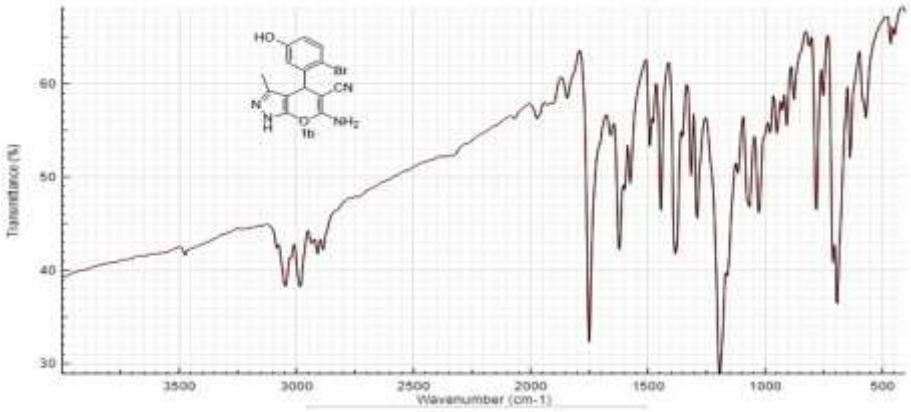


Fig 7: IR spectra of compound 1b

Compound 1c
^{13}C NMR: δ 20.0 (1C, s), 40.7-40.8 (2C, 40.7 (s), 40.7 (s)), 45.9 (1C, s), 55.7 (1C, s), 56.6 (1C, s), 86.0-86.1 (2C, 86.1 (s), 86.1 (s)), 117.6-117.8 (2C, 117.6 (s), 117.7 (s)), 119.3 (1C, s), 137.5 (1C, s), 143.2 (1C, s), 149.5 (1C, s), 159.1 (1C, s).

Fig 8: ^{13}C NMR spectra of compound 1c

^{1}H NMR: δ 1.24-1.36 (3H, 1.30 (d, J = 6.8 Hz), 1.30 (d, J = 6.8 Hz), 1.30 (d, J = 6.8 Hz)), 2.88 (1H, d, J = 6.4, 6.1, 5.8 Hz), 3.03-3.17 (4H, 3.08 (s), 3.08 (s), 3.10 (d, J = 5.5, 4.2, 2.5 Hz)), 3.65 (1H, d, J = 6.4, 5.5 Hz), 4.43 (1H, q, J = 6.8, 6.1 Hz), 5.02 (1H, d, J = 2.5 Hz), 5.72 (1H, d, J = 5.8 Hz), 6.85 (2H, d, J = 8.7, 1.7 Hz), 7.09 (1H, d, J = 2.8, 0.5 Hz), 8.00 (1H, d, J = 4.2 Hz).

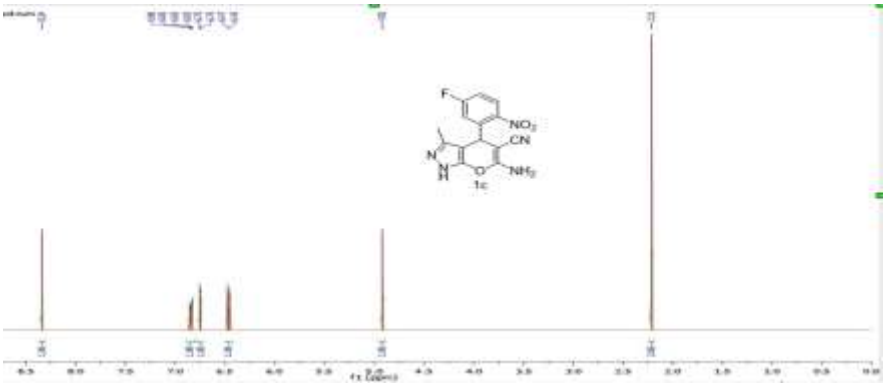


Fig 9: ^{1}H NMR spectra olf compound 1c

Mass(m/z):315

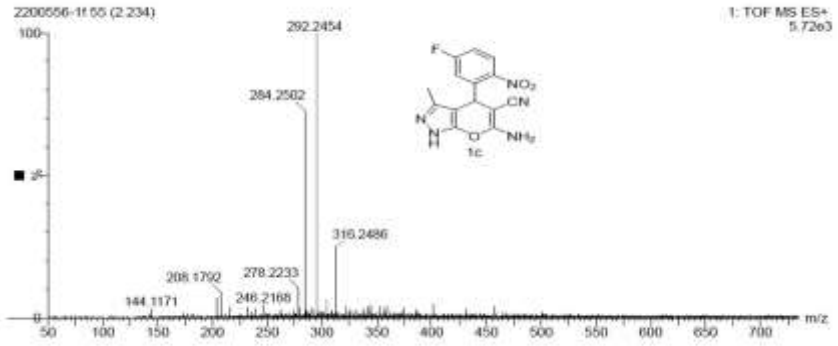


Fig 10 : mass spectra of compound 1c

IR(Cm^{-1}): 1251-1023(-CN- stretching), 1211-1160(-CO- stretching), 3501 – 3302(-NH- stretching), 1701 – 1510 (-C=C- stretching)

Compound 1d

^{1}H NMR: δ 1.30 (3H, d, J = 6.8 Hz), 2.24 (3H, s), 2.85-3.05 (2H, 2.92 (d, J = 6.4, 6.1, 5.8 Hz), 2.98 (ddd, J = 5.5, 4.4, 2.5 Hz)), 3.73 (1H, d, J = 6.4, 5.5 Hz), 4.47 (1H, q, J = 6.8, 6.1 Hz), 5.02 (1H, d, J = 2.5 Hz), 5.71 (1H, d, J = 5.8 Hz), 6.68 (1H, d, J = 8.5, 0.4 Hz), 6.86 (1H, d, J = 2.5, 0.4 Hz), 6.99 (1H, d, J = 8.5, 2.5 Hz), 8.00 (1H, d, J = 4.4 Hz).

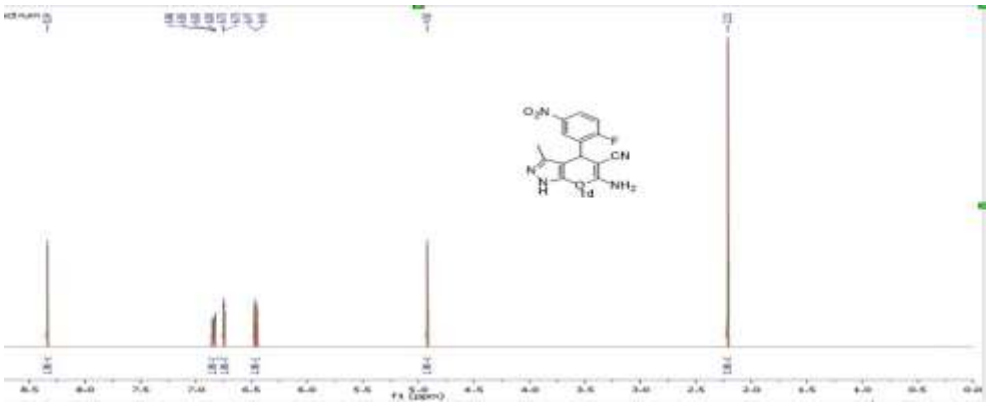


Fig 11: ^{1}H NMR spectra of compound 1d

^{13}C NMR: δ 20.0 (1C, s), 21.3 (1C, s), 40.7-40.8 (2C, 40.7 (s), 40.7 (s)), 45.9 (1C, s), 56.6 (1C, s), 86.0-86.1 (2C, 86.1 (s), 86.1 (s)), 115.0 (1C, s), 124.1 (1C, s), 127.4 (1C, s), 129.9 (1C, s), 134.8 (1C, s), 143.2 (1C, s), 155.9 (1C, s).

Fig 12 : ^{13}C NMR spectra of 1d

Mass (m/z): 282
IR(Cm^{-1}): 1251-1023(-CN- stretching), 1211-1160(-CO- stretching), 3501 – 3302(-NH- stretching), 1701 – 1510 (-C=C- stretching)

Compound 1e

^{13}C NMR: δ 20.0 (1C, s), 40.7-40.8 (2C, 40.7 (s), 40.7 (s)), 45.9 (1C, s), 56.6 (1C, s), 86.0-86.1 (2C, 86.1 (s), 86.1 (s)), 115.0 (1C, s), 119.3 (1C, s), 131.3 (1C, s), 132.9 (1C, s), 143.2 (1C, s), 146.6 (1C, s), 149.4 (1C, s).

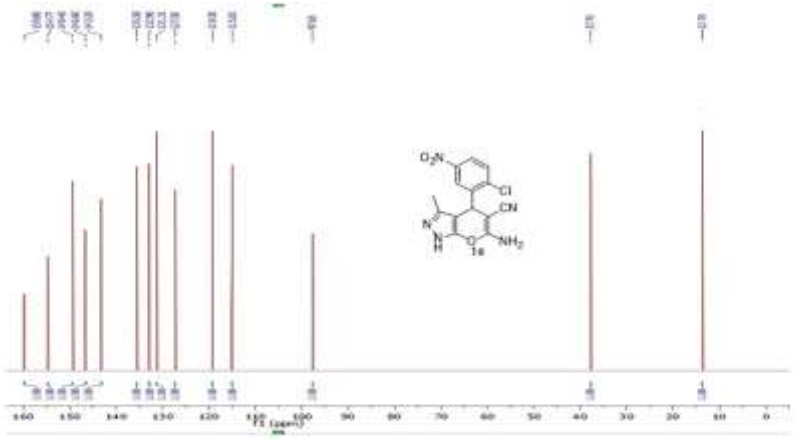


Fig 13: ^{13}C NMR spectra of 1e

^{1}H NMR: δ 1.30 (3H, d, J = 6.8 Hz), 2.89 (1H, d, J = 6.4, 6.1, 5.8 Hz), 3.13 (1H, d, J = 5.5, 3.8, 2.5 Hz), 3.45 (1H, d, J = 6.4, 5.5 Hz), 4.46 (1H, J = 6.8, 6.1 Hz), 5.02 (1H, d, J = 2.5 Hz), 5.72 (1H, d, J = 5.8 Hz), 6.36 (1H, d, J = 8.2, 2.5 Hz), 7.19 (1H, d, J = 2.5, 0.5 Hz), 7.37 (1H, d, J = 8.2, 0.5 Hz), 7.97 (1H, d, J = 3.8 Hz).

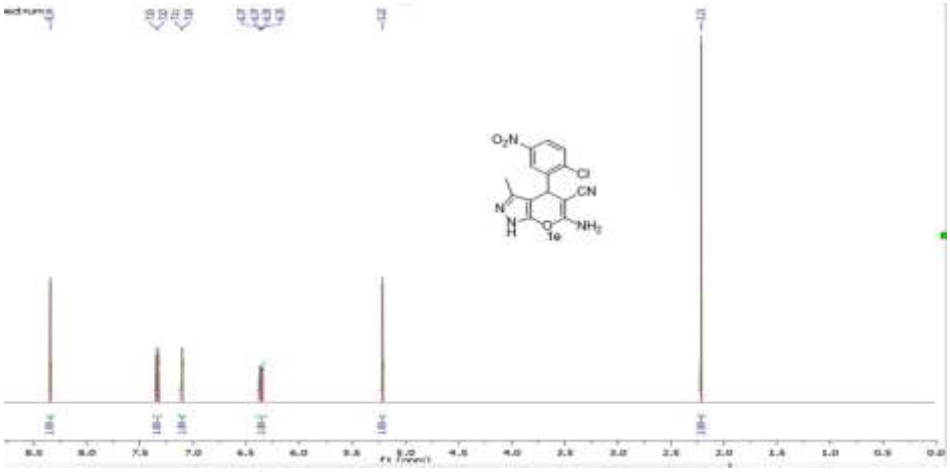


Fig 14 : ^{1}H NMR spectra of compound 1e Mass (m/z):331

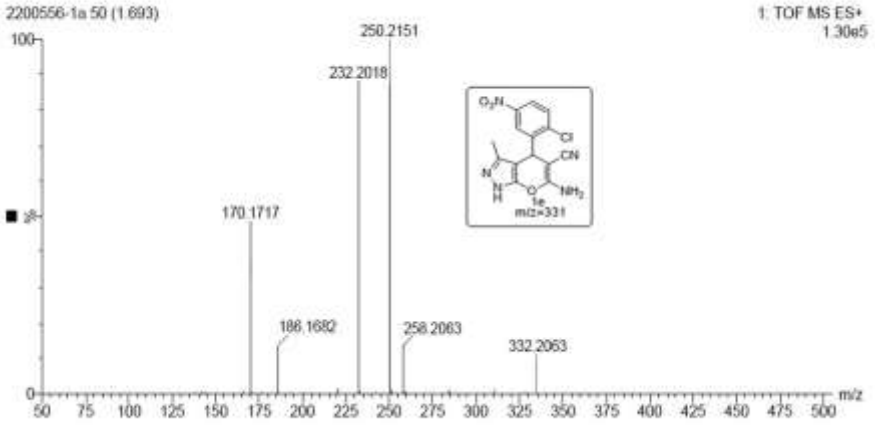


Fig 15: Mass spectra of 1e

IR(Cm^{-1}): 1251-1023(-CN- stretching), 1211-1160(-CO- stretching), 3501 – 3302(-NH- stretching), 1701 – 1510 (-C=C- stretching)

Compound 1f

^{1}H NMR: δ 1.30 (3H, d, J = 6.8 Hz), 2.91 (1H, d, J = 6.4, 6.1, 5.8 Hz), 3.15 (1H, d, J = 5.5, 3.8, 2.5 Hz), 3.43 (1H, d, J = 6.4, 5.5 Hz), 4.46 (1H, J = 6.8, 6.1 Hz), 4.91 (1H, d, J = 2.5 Hz), 5.70 (1H, d, J = 5.8 Hz), 7.30-7.47 (3H, 7.37 (d, J = 8.1, 1.5 Hz), 7.41 (d, J = 8.1, 0.6 Hz), 7.42 (d, J = 1.5, 0.6 Hz)), 7.97 (1H, d, J = 3.8 Hz).

Fig17 : ^{1}H NMR of compounf 1f

C13

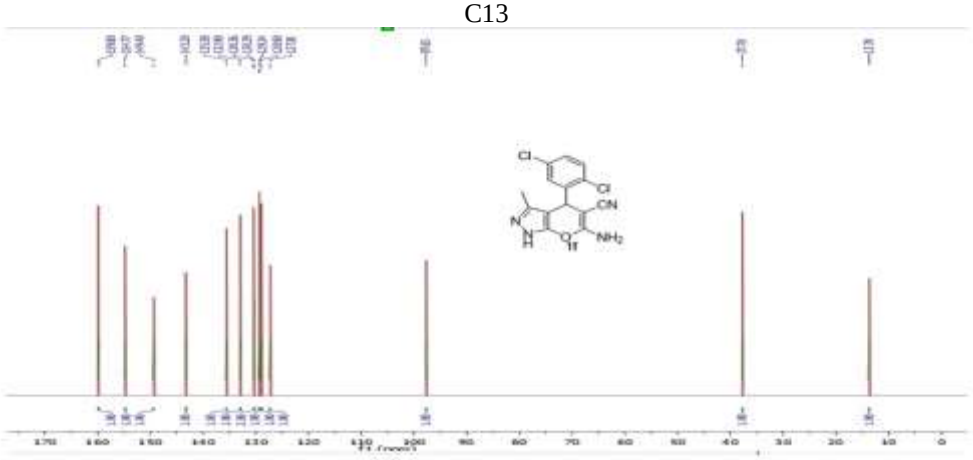


Fig 18 : ^{13}C NMR spectra of compound 1f

Mass(m/z):321

IR(Cm^{-1}): 1251-1023(-CN- stretching), 1211-1160(-CO- stretching), 3501 – 3302(-NH- stretching), 1701 – 1510 (-C=C- stretching)

Fig: IR spectra of compound 1f

Compound 1g

^{13}C NMR: δ 20.0 (1C, s), 40.7-40.8 (2C, 40.7 (s), 40.7 (s)), 45.9 (1C, s), 56.6 (1C, s), 86.0-86.1 (2C, 86.1 (s), 86.1 (s)), 119.0 (1C, s), 124.1 (1C, s), 126.3 (1C, s), 129.3 (1C, s), 130.4 (1C, s), 143.2 (1C, s), 155.9 (1C, s).

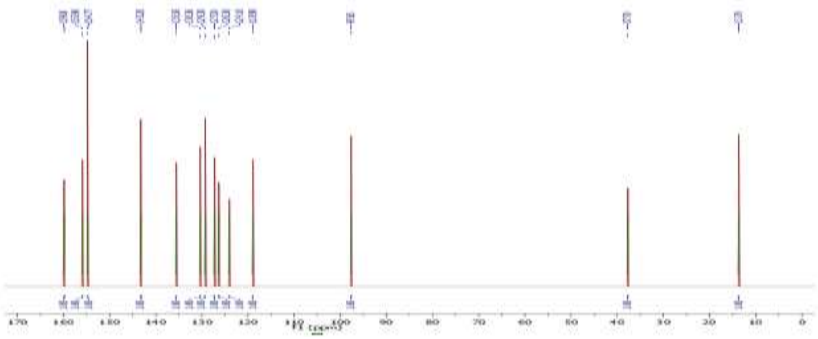


Fig 19: ^{13}C NMR spectra of compound 1g

^{1}H NMR: δ 1.30 (3H, d, J = 6.8 Hz), 2.85-3.05 (2H, 2.92 (d, J = 6.4, 6.1, 5.8 Hz), 2.99 (d, J = 5.5, 4.4, 2.5 Hz)), 3.72 (1H, d, J = 6.4, 5.5 Hz), 4.47 (1H, , J = 6.8, 6.1 Hz), 5.02 (1H, d, J = 2.5 Hz), 5.72 (1H, d, J = 5.8 Hz), 6.74-6.86 (2H, 6.80 (d, J = 8.4, 0.5 Hz), 6.80 (d, J = 1.9, 0.5 Hz)), 7.03 (1H, d, J = 8.4, 1.9 Hz), 7.96 (1H, d, J = 4.4 Hz).

Fig 20 : ^{1}H NMR spectra of compound 1g Mass (m/z):302

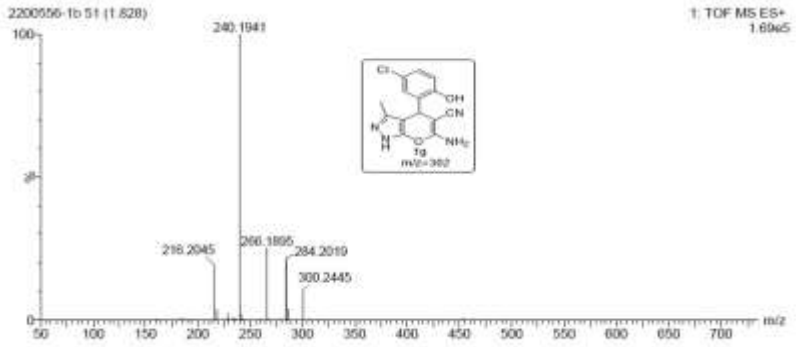


Fig21 : IR spectra of compound 1g

IR(Cm^{-1}): 1251-1023(-CN- stretching), 1211-1160(-CO- stretching), 3501 – 3302(-NH- stretching), 1701 – 1510 (-C=C- stretching)

Compound 1h

^{1}H NMR: δ 1.30 (3H, d, J = 6.8 Hz), 2.85-3.05 (2H, 2.92 (d, J = 6.4, 6.1, 5.8 Hz), 2.98 (d, J = 5.5, 4.4, 2.5 Hz)), 3.75 (1H, d, J = 6.4, 5.5 Hz), 4.47 (1H,d, J = 6.8, 6.1 Hz), 5.02 (1H, d, J = 2.5 Hz), 5.72 (1H, d, J = 5.8 Hz), 6.68-6.84 (2H, 6.73 (d, J = 1.8, 0.5 Hz), 6.78 (d, J = 8.5, 0.5 Hz)), 7.22 (1H, d, J = 8.5, 1.8 Hz), 7.96 (1H, d, J = 4.4 Hz).

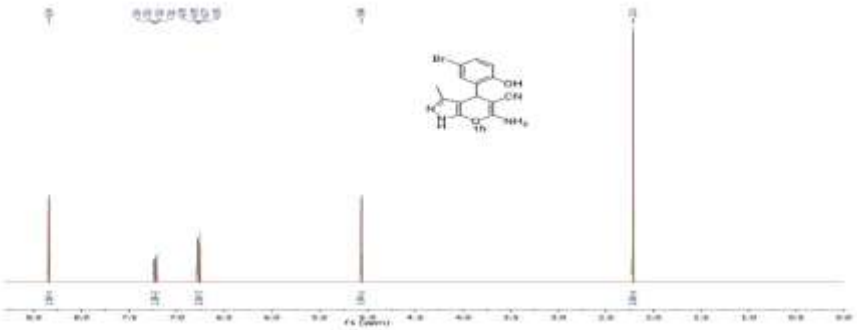


Fig 22: ^{1}H NMR spectra of compound 1h

^{13}C NMR: δ 20.0 (1C, s), 40.7-40.8 (2C, 40.7 (s), 40.7 (s)), 45.9 (1C, s), 56.6 (1C, s), 86.0-86.1 (2C, 86.1 (s), 86.1 (s)), 118.4 (1C, s), 119.9 (1C, s), 124.1 (1C, s), 131.0 (1C, s), 132.5 (1C, s), 143.2 (1C, s), 155.9 (1C, s).

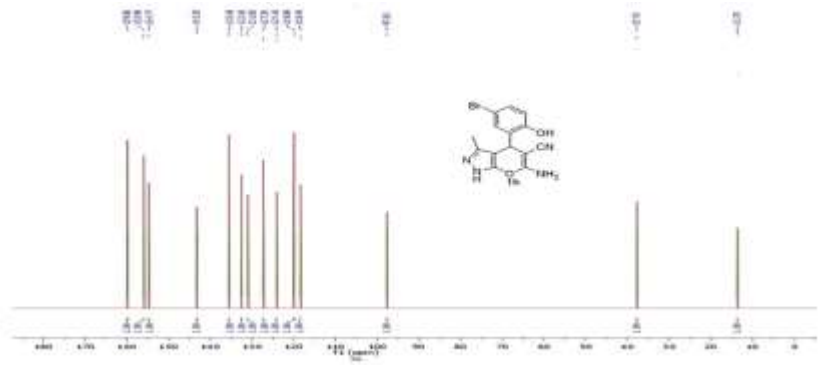


Fig 23 : ^{13}C NMR spectra of compound 1h

Mass (m/z):347

Fig: mass spectra of compound 1h

IR (Cm^{-1}): 1251-1023(-CN- stretching), 1211-1160(-CO- stretching), 3501 – 3302(-NH- stretching), 1701 – 1510 (-C=C- stretching)

Compound 1i

^{13}C NMR: δ 20.0 (1C, s), 40.7-40.8 (2C, 40.7 (s), 40.7 (s)), 45.9 (1C, s), 56.6 (1C, s), 86.0-86.1 (2C, 86.1 (s), 86.1 (s)), 118.4 (1C, s), 118.7 (1C, s), 131.7 (1C, s), 136.1 (1C, s), 136.4 (1C, s), 143.2 (1C, s), 143.4 (1C, s).

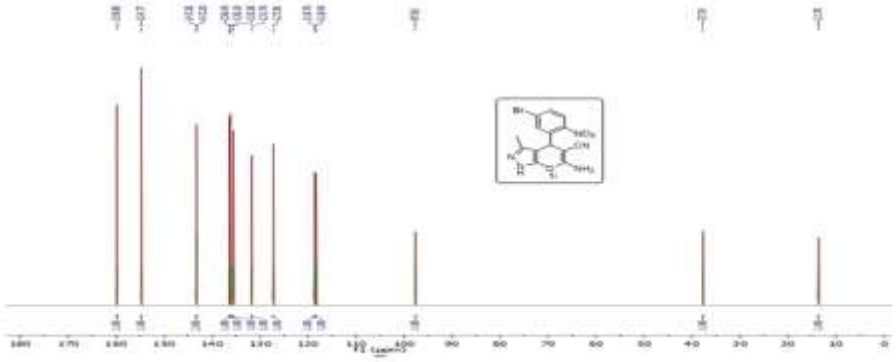


Fig 24: ^{13}C NMR spectra of compound 1i

^{1}H NMR: δ 1.30 (3H, d, J = 6.8 Hz), 2.88-3.07 (2H, 2.95 (d, J = 6.4, 6.1, 5.8 Hz), 3.01 (d, J = 5.5, 3.8, 2.5 Hz)), 3.37 (1H, d, J = 6.4, 5.5 Hz), 4.45 (1H, q, J = 6.8, 6.1 Hz), 4.99 (1H, d, J = 2.5 Hz), 5.68 (1H, d, J = 5.8 Hz), 6.48-6.69 (2H, 6.54 (d, J = 7.7, 0.5 Hz), 6.64 (d, J = 1.8, 0.5 Hz)), 6.91 (1H, d, J = 7.7, 1.8 Hz), 7.97 (1H, d, J = 3.8 Hz).

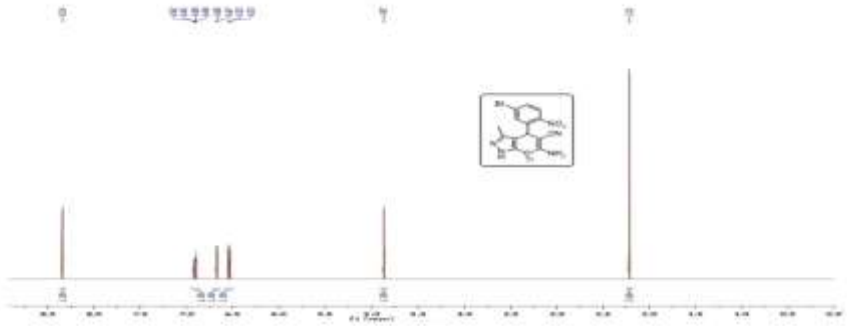


Fig 25: ^{1}H NMR spectra of compound 1i

Mass (m/z):376

IR(Cm^{-1}): 1251-1023(-CN- stretching), 1211-1160(-CO- stretching), 3501 – 3302(-NH- stretching), 1701 – 1510 (-C=C- stretching)

Compound 1k

^{1}H NMR: δ 1.30 (3H, d, J = 6.8 Hz), 2.85-3.08 (2H, 2.92 (d, J = 6.4, 6.1, 5.8 Hz), 3.01 (d, J = 5.5, 4.4, 2.5 Hz)), 3.61 (1H, d, J = 6.4, 5.5 Hz), 4.46 (1H, d, J = 6.8, 6.1 Hz), 5.02 (1H, d, J = 2.5 Hz), 5.71 (1H, d, J = 5.8 Hz), 6.45 (1H, d, J = 2.8, 0.5 Hz), 6.67 (1H, d, J = 8.6, 0.5 Hz), 6.81 (1H, d, J = 8.6, 2.8 Hz), 8.00 (1H, d, J = 4.4 Hz).

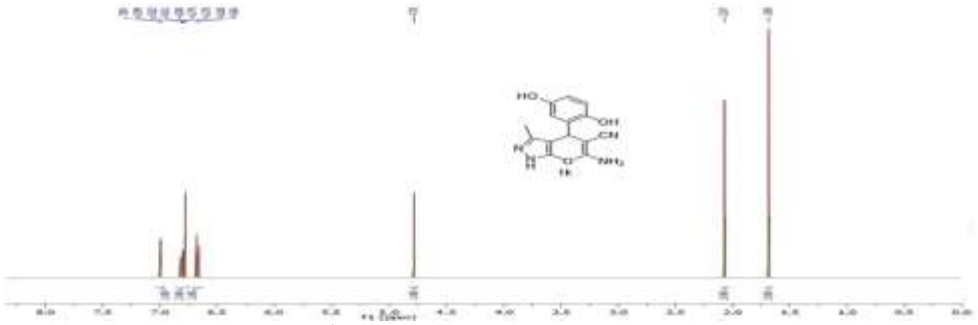


Fig26 : ^{1}H NMR spectra of compound 1 k

^{13}C NMR: δ 20.0 (1C, s), 40.7-40.8 (2C, 40.7 (s), 40.7 (s)), 45.9 (1C, s), 56.6 (1C, s), 86.0-86.1 (2C, 86.1 (s), 86.1 (s)), 112.8 (1C, s), 115.8 (1C, s), 117.6 (1C, s), 124.1 (1C, s), 143.2 (1C, s), 154.4 (1C, s), 155.9 (1C, s).

Mass (m/z):284

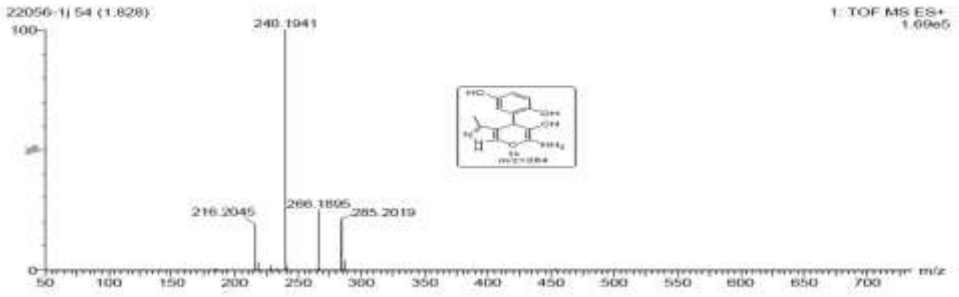


Fig 27: mass spectra of compound 1 k

IR(Cm^{-1}): 1251-1023(-CN- stretching), 1211-1160(-CO- stretching), 3501 – 3302(-NH- stretching), 1701 – 1510 (-C=C- stretching)

Compound 1l

^{13}C NMR: δ 20.0 (1C, s), 40.7-40.8 (2C, 40.7 (s), 40.7 (s)), 45.9 (1C, s), 56.6 (1C, s), 86.0-86.1 (2C, 86.1 (s), 86.1 (s)), 115.1 (1C, s), 115.6 (1C, s), 116.5 (1C, s), 136.1 (1C, s), 143.2 (1C, s), 143.4 (1C, s), 161.2 (1C, s).

^{1}H NMR: δ 1.30 (3H, d, J = 6.8 Hz), 2.82-3.07 (2H, 2.90 (d, J = 6.4, 6.1, 5.8 Hz), 3.00 (d, J = 5.5, 4.4, 2.5 Hz)), 3.45 (1H, d, J = 6.4, 5.5 Hz), 4.45 (1H, d, J = 6.8, 6.1 Hz), 5.04 (1H, d, J = 2.5 Hz), 5.67 (1H, d, J = 5.8 Hz), 6.45 (1H, d, J = 8.3, 0.5 Hz), 6.84 (1H, d, J = 8.3, 2.2 Hz), 7.09 (1H, d, J = 2.2, 0.5 Hz), 8.00 (1H, d, J = 4.4 Hz).

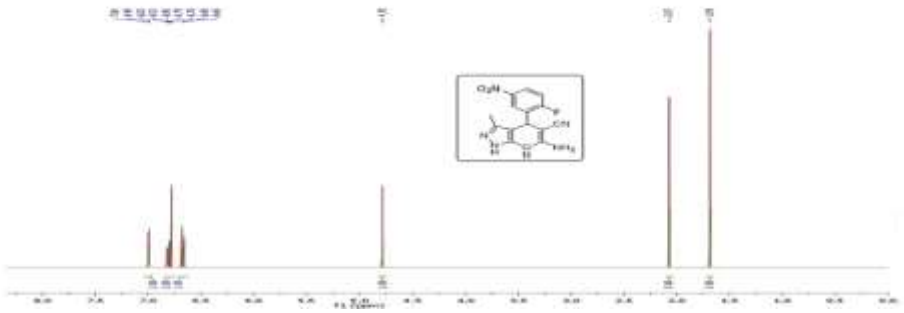


Fig 28 : ^{1}H NMR spectra of 1l Mass(m/z): 315

Fig: mass spectra of 1l

IR(Cm^{-1}): 1251-1023(-CN- stretching), 1211-1160(-CO- stretching), 3501 – 3302(-NH- stretching), 1701 – 1510 (-C=C- stretching)

Antibacterial Assay.

All the compounds synthesized in the present work were screened for antibacterial activity against Staphylococcus aureus, and E. coli by well diffusion method. The bacteria cultures used were from ATCC number 23235 for Staphylococcus aureus and E. coli ATTC number was 25922, it was then sub-cultured on nutrient agar slants. They were pre-cultured on nutrient broth overnight and incubated at 37ºC. The culture broths were centrifuged at 1000 rpm for 5minutes; bacterial pellet was suspended in double-distilled sterile water. In this assay, a concentration of 1mg/ml of synthesized compounds was placed into respective wells cut in nutrient agar plates inoculated with test bacteria. Similarly, reference antibiotics amoxicillin and ciprofloxacin were also placed into their respective wells. All the plates were incubated at 37o C for 24 hours. After incubation, the plates were observed for the inhibition zone surrounding the well, and its diameter was measured.

Table 3: Antimicrobial activity of compounds against E coli and Staphylococcus aureus

Compounds	Staphylococcus aureus	Escherichia coli
1a	24.3 ± 0.84	23.4 ± 1.37
1b	23.4 ± 1.23	26.1 ± 1.04
1c	22.3 ± 1.24	25.3 ± 0.95
1d	26.3 ± 1.04	25.8 ± 0.92
1e	25.7 ± 0.87	24.3 ± 1.23
1f	23.4 ± 0.97	26.7 ±1.07
1g	22.3 ± 0.92	23.4 ± 0.91
1h	26.4 ± 0.98	22.3 ± 1.28
1i	27.2 ± 1.12	24.9 ± 1.34
1j	27.3 ± 1.36	25.7 ± 1.45
1k	24.6 ± 1.85	25.1 ± 1.72
1l	27.6 ± 1.27	26.4 ± 1.24
Amoxicillin	28.4 ± 1.06	---
Ciprofloxacin	---	26.5 ± 1.27

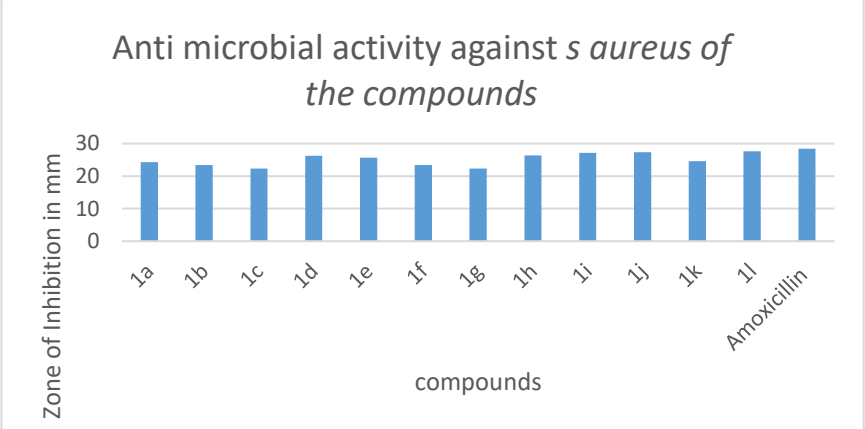


Fig 29: Antimicrobial activity of compounds against S aureus

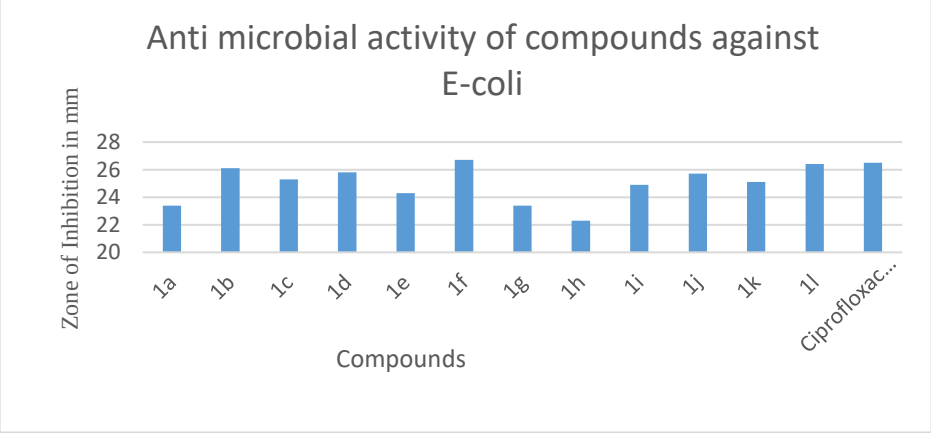


Fig 30 : Antimicrobial activity of compounds against E coli

RESULTS AND DISCUSSION

We began our investigation by using baker's yeast as a catalyst in the reaction of benzaldehyde, malononitrile, ethyl acetoacetate, and hydrazine hydrate in ethanol (Scheme 1). The first and most important step in a multicomponent reaction, particularly a bio catalytic reaction, is the selection of a solvent. So, first, we looked at how solvent affected our model reaction. In the presence of baker's yeast, we performed our reaction in some protic and aprotic solvents. To our

delight, the first glimpse of success was obtained by running a model reaction in ethanol as a solvent at room temperature and pH with stirring and delivering a target product 1a with an 84% yield after 34 hours (Table 1, entry 1). We continuously monitored the model reaction and discovered that the intermediates (Intermediate I and II) formed within a few hours and that the complete conversion of the intermediates to product 5a requires 34 hours of constant stirring. Invigorated by the above-mentioned intriguing result, other solvents such as methanol (MeOH), acetonitrile (ACN), dichloromethane (DCM), dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), and tetrahydrofuran (THF) were also used as media for the model reaction, with only methanol, acetonitrile, and DCM yielding the desired product in 82 (Table 1, entries 2–4). Other solvents, unfortunately, did not work well for the model reaction (Table 1, entries 5–8). The model reaction proceeds more smoothly in protic solvents than in aprotic solvents, where no reaction or only a trace amount of product 5a is formed after 50 hours. As a result, we focused on using an abundant protic solvent that is widely accepted for bio catalysis namely water, but unfortunately, a trace amount of product was formed (Table 1, entry 9).This could be due to the insolubility of organic substrates; thus, we tried the model reaction with water/ethanol and water/methanol. This attempt yields 75 and 70% of the target product, respectively (Table 1, entries 10–11). It shows that adding water to the reaction lowers the yield of 1a from 84 to 75% and from 82 to 70%. We also ran the model reaction in a solvent-free environment with baker's yeast and in the absence of baker's yeast at room temperature, but the expected products were not observed even after 50 hours (Table 1, entries 12, 13). Following the screening of the solvents, ethanol emerges as the clear winner over all other solvents as a result, ethanol was chosen as the best solvent for future research. The control experiment demonstrated that even after 50 hours of stirring, the reaction did not produce the desired product in the absence of baker's yeast (Table 1, entry 14). Because the efficiency of multicomponent reactions is affected by the catalyst dose and reaction time in addition to the solvent, we performed the condensation of benzaldehyde, malononitrile, ethyl acetoacetate, and hydrazine hydrate using a variable amount of baker's yeast ranging from 0.6 to 3.1 g. The reaction initially failed to work when we used 0.6 and 1.1 g of the catalyst, and no product yield was obtained. When the amount of catalyst is increased to 1.6 g, the product formation begins at 30%. It was discovered that increasing the amount of catalyst resulted in a higher product yield. As a result, the amount was increased to 2.6and 3.1 g, yielding 73 and 65%, respectively (Table 1, entries 15–19). As shown in Table 1, entry 1, the maximum yield obtained for product 5a was 84% when the reaction was carried out with 2 g of the catalyst. It means that increasing the catalyst loading lowers the yield, which could be due to an increase in reaction mass, which slows down the mixing of reactants and thus affects product formation. As a result, it has been concluded that using 2 g of baker's yeast as a catalyst and ethanol as a medium, this multicomponent cyclo condensation occurs well. We investigated the scope of the aldehydes for this methodology after establishing an efficient condition for the model reaction (Scheme 2). Table 2 summarises the results of a wide range of substituted aromatic aldehydes successfully treated with hydrazine hydrate, malononitrile, and ethyl acetoacetate. The attached functional group on the aromatic ring of the aldehyde influences the product yield slightly. Under these conditions, aldehydes with electron-withdrawing and electron-donating groups could be tolerated. The substrate scope is also tested with a heterocyclic aldehyde, 2-chloro-3-formyl quinoline, which yielded a surprising amount of the target product (1i). A series of control experiments were carried out in order to obtain some mechanistic details about this transformation. After 3 h and 1 h of constant stirring with baker's yeast, we obtained 2-benzylidenemalononitrile and 3-methyl-1H-pyrazol-5(4H)-one, respectively, from separate reactions of di substituted benzaldehyde and malononitrile in ethanol and ethyl acetoacetate with hydrazine. In the presence of baker's yeast, the first two intermediates are formed and then condensed to form pyranopyrazoles. Based on the obtained results and the literature, a plausible mechanism for the synthesis of pyranopyrazole 1a using benzaldehyde, hydrazine hydrate, malononitrile, and ethyl acetoacetate can be considered (Fig. 1). The imidazole residue [56] found in baker's yeast can act as a catalyst for this cyclocondensation. Imidazole removes the proton from malononitrile while also activating the aldehyde, resulting in malononitrile attacking aldehydes to produce 2-benzylidenemalononitrile (I) [40]. Concurrently, cyclocondensation occurs between ethyl acetoacetate and hydrazine hydrate, yielding a five-membered ring, 3-methyl-1H-pyrazol-5(4H)-one II, which is then converted to its corresponding enol form III in the presence of imidazole residue. Following that, Michael-type addition of intermediate III to intermediate I yielded intermediate IV, which undergoes intramolecular cyclization via oxygen nucleophilic attack on the nitrile group, yielding intermediate V. Finally, the tautomerization of intermediate V resulted in dihydropyrano[2,3-c]pyrazole 1a. A comparison of reaction conditions and yield with some of the reported catalysts in the literature was performed to evaluate the advantages of our protocol for the synthesis of pyranopyrazole derivatives. The majority of the reported protocols for the synthesis of pyranopyrazole derivatives involve condensations of aromatic aldehyde, ethyl acetoacetate, malononitrile, and hydrazine hydrate. Table 3 shows the results of comparing baker's yeast to other catalysts, demonstrating clearly the superiority of the current catalysts over other catalysts. It allows for a truly green process with higher product yields.

After incubation, inhibition zones formed around the wells were measured in millimetres. This study was performed in triplicates. The results showed (in Fig. 29 and 30 and table 3) antibacterial activity of all the synthesized compounds tested at 1mg/ml concentration showed low to high activity against Staphylococcus aureus, and E. coli. Revealed the study of antibacterial activity of compounds. Zone inhibition most synthesized compounds were found to good activity. We referred standard. Antibacterial activity of compound 1d 1h 1i 1j 1l were very good near to the standard amoxicillin as shown in table and fig against grams positive organism

On the other hand Antibacterial activity of compound 1b, if, 1l, 1j, 1k. 11c and 1dwere very good near to the standard ciprofloxacin as shown in table and fig against grams negative organism.

This showed that the compounds synthesized in our work are effective antibacterial activity.

CONCLUSION

Here, employing baker's yeast as a biocatalyst, we have effectively established a green method for the one-pot, four-component synthesis of pyranopyrazole in a non-aqueous media. Baker's yeast has excellent functional group tolerance. Without any specific workup setup, the products are produced with good to moderate yields at ambient temperature and neutral pH. The given protocol's encouraging features include the catalyst's total biodegradability, low cost relative to alternative chemical catalysts, ease of chemical isolation, and better reaction profile. Antibacterial activity against both gram positive and grams negative organisms showed good results against standard.

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