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ECOFRIENDLY SYNTHESIS OF SOME BIOACTIVE PYRAZOLINE AND ISOXAZOLINE DERIVATIVES FROM α, β-UNSATURATED CYCLOHEXANONE

A. WASI, BHUPENDRA KUMAR SHARMA[a], ASHISH KUMAR GUPTA[b] and KUMUD INTODIA[*]

Department of Chemistry, S. M. B. Government College, NATHDWARA, Dist. Rajsamand (Raj.) INDIA
[a]Department of Chemistry, S. G. G. Government College, BANSWARA (Raj.) INDIA
[b]Amity Institute of Biotechnology, Amity University, NOIDA (U.P.) INDIA

ABSTRACT

Condensation of cyclohexanone with substituted benzaldehyde in presence of neutral alumina under microwave irradiation affords substituted α, β-unsaturated cyclohexanone in quantitative yields, which undergoes 1.3-dipolar cycloaddition with hydrazines (Phenylhydrazine, 2, 4-dinitrophenyl hydrazine, semicarbazide and thiosemicarbazide) to afford a series of novel 7-(substituted benzylidene)-3-(substituted phenyl)-2-(substituted)-3, 3a, 4, 5, 6, 7-hexahydro-2H-indazole derivatives. Some new 7-(substituted benzylidene)-3-(substituted phenyl)-3, 3a, 4, 5, 6, 7-hexahydrobenzo[c] isoxazole derivatives were also obtained in quantitative yields by cyclococondensation of substituted α, β-unsaturated cyclohexanone with hydroxylamine hydrochloride under microwave irradiation. The structures of all the compounds were confirmed by their analytical and spectral data. All the synthesized compounds were screened for antibacterial and antifungal activity. Almost all compounds have shown excellent activity against some Multi Drug Resistant bacteria.

Key words: Microwave irradiation, Cyclohexanone, Hexahydroindazole, Hexahydrobenzo[c] isoxazole, Heterocyclic synthesis, Multi drug resistant bacteria.

INTRODUCTION

The α, β-unsaturated ketones have attracted much attention, particularly the α, β-unsaturated derivatives of cyclohexanone, not only due to their intriguing biological activities such as antiangiogenic[1,2], cytotoxicity[3,4], cholesterol-lowering activity[5], use in agrochemicals, pharmaceuticals and perfumes[6], and as liquid crystalline polymer units[7], but

[*]Author for correspondence; E-mail: info@agtechnology.in, bhoopendrasharma@ymail.com

also as important precursors for the synthesis of heterocyclic compounds such as pyrazolines and isoxazolines. Generally, these compounds are prepared by Claisen-Schmidt condensation from aromatic aldehydes and ketones[8-16].

1, 3-Dipolar cycloaddition is a general methodology that has been applied for the synthesis of five-membered heterocyclic compounds such as 2-pyrazolines and isoxazolines using α, β-unsaturated ketones as starting materials[17].

Several pyrazoline derivatives possess important biological activities such as anti-inflammatory[18-20], antidepressant[21,22], antipyretic[23], antibacterial[24-29], antifungal[30] and antitumoral[31]. Over the years, the structure of pyrazoline has received considerable attention and of particular interest is the use of pyrazolines as synthetic intermediates for preparing cyclopropane[32-34] and pyrazole[35-38] derivatives. Moreover, pyrazolines have played a crucial role in the development of heterocyclic chemistry and also are extensively useful synthons in organic chemistry. Isoxazoline derivatives have also been reported to possess antidiabetic[39], diuretic[40], analgesic[41], anthelimintic[42] and hypolipaemic activity[43]. In the light of interesting biological activities exhibited by these compounds, the synthesis of pyrazoline and isoxazoline derivatives from α, β-unsaturated cyclohexanone was carried out under microwave irradiation.

EXPERIMENTAL

Instrumentation

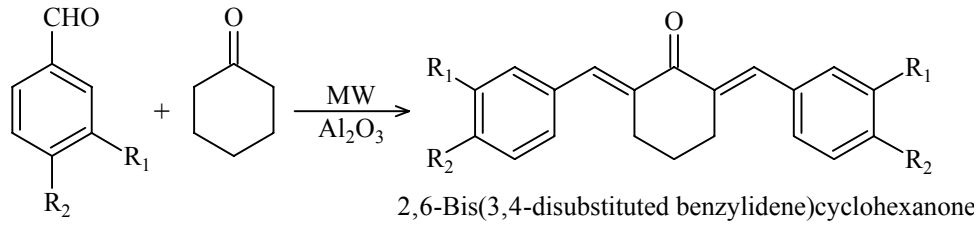
The melting points of all the products was determined in open glass capillaries and are uncorrected. The IR spectra (cm^{-1}) were recorded using KBr on Shimadzu FT-IR 8201 PC spectrometer. PMR spectra ($CDCl_3$/DMSO-d_6) were recorded on Brucker-FT-WM-400 (400 MHz) using TMS as internal standard (chemical shift in δ ppm). Mass spectra were recorded on a Jeol-D-300 spectrometer. Elemental analysis was within + or - 0.5% from their calculated values.

Synthesis

Synthesis of α, β-unsaturated derivatives of cyclohexanone (1, 2, 3)

A mixture of the substituted aromatic aldehyde (2 eq.) and cyclohexanone (1 eq.) was exposed to microwave irradiation in an open flask in the presence of neutral aluminium oxide for about five minutes. The progress of the reaction was monitored with TLC. The resulting crude yellow solid was extracted with ethanol and filtered to remove aluminium

oxide. The product, so-obtained was washed with cold water, dried and crystallized with ethanol to give the desired compounds in 80-95% yield **(Scheme 1)**.



2,6-Bis(3,4-disubstituted benzylidene)cyclohexanone

1: R_1 = H R_2 = H

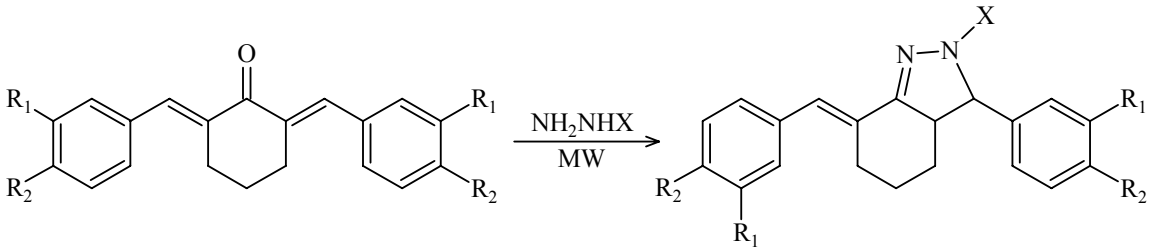
2: R_1 = OH R_2 = OCH_3

3: R_1 = H R_2 = OH

Scheme 1

General synthesis of 7-(substituted benzylidene)-3-(substituted phenyl)-2-(substituted)-3, 3a, 4, 5, 6, 7-hexahydro-2H-indazole derivatives

A slurry of compound **(1, 2 or 3)** (0.05 mol) and hydrazines ($RNHNH_2$) (0.05 mol) in ethanol (5 mL) was exposed to microwave radiations in an open flask for about 3-6 minutes. After completion of the reaction as indicated by TLC, the reaction mixture was cooled to room temperature. The product formed was extracted with ethanol. The products are recrystallized from ethanol to give good yield (75%-87%) of pure crystals of (**1a-1d)**, (**2a-2d)** and (**3a-3d**) **(Scheme 2)**.



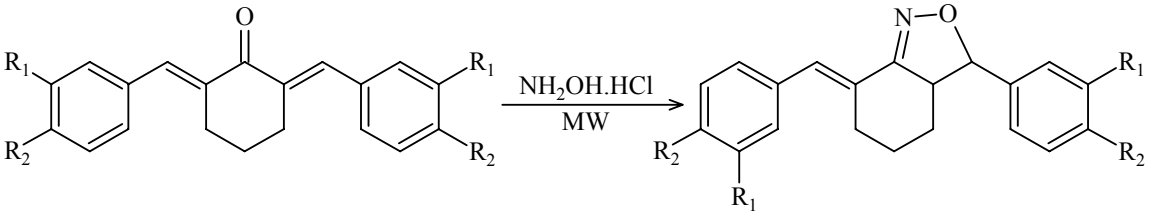
(1, 2, 3) **(1a-d), (2a-d), (3a-d)**

X = a; b : NO_2 NO_2 c : $CONH_2$ d : $CSNH_2$

Scheme 2

General Synthesis of 7-(substituted benzylidene)-3-(substituted phenyl)-3, 3a, 4, 5, 6, 7-hexahydrobenzo[c] isoxazole derivatives

Slurry of α, β-unsaturated derivatives of cyclohexanone (0.05 mol) and hydroxylamine hydrochloride (0.05 mol) in 5.0 mL of ethanol was exposed to microwave radiations in an open flask for about 4-6 minutes. After completion of the reaction as indicated by TLC, the reaction mixture was cooled to room temperature. The product was extracted and recrystallized from ethanol to give good yield (82%-85%) of pure crystals of 7-(substituted benzylidene)-3-(substituted phenyl)-3, 3a, 4, 5, 6, 7-hexahydrobenzo[c] isoxazole derivatives **(1e, 2e and 3e)**. **(Scheme 3)**.



(1, 2, 3) **(1e, 2e, 3e)**

Scheme 3

Table 1: Physical and analytical data

Comp.	Mol. formula	Mol. wt.	M.P. (°C)	% Yield	Found (Calculated)		
					% C	% H	% N
1	$C_{20}H_{18}O$	274	194	80	87.59 (87.56)	6.68 (6.61)	-
2	$C_{22}H_{22}O_5$	366	285	84	72.18 (72.12)	6.07 (6.05)	-
3	$C_{20}H_{18}O_3$	306	292	83	78.47 (78.41)	5.93 (5.92)	-
1a	$C_{26}H_{24}N_2$	364	303	80	85.69 (85.68)	6.69 (6.64)	7.68 (7.69)
1b	$C_{26}H_{22}N_4O_4$	454	211	75	68.76 (68.71)	4.92 (4.88)	12.36 (12.33)
1c	$C_{21}H_{21}N_3O$	331	304	83	76.16 (76.11)	6.38 (6.39)	12.70 (12.68)

Cont…

Comp.	Mol. formula	Mol. wt.	M.P. (^oC)	% Yield	Found (Calculated)		
					% C	% H	% N
1d	$C_{21}H_{21}N_3S$	347	272	81	72.63 (72.59)	6.13 (6.09)	12.13 (12.09)
1e	$C_{20}H_{19}NO$	289	238	83	83.06 (83.01)	6.69 (6.62)	4.88 (4.84)
2a	$C_{28}H_{28}N_2O_4$	456	298	86	73.69 (73.66)	6.23 (6.18)	6.18 (6.14)
2b	$C_{28}H_{26}N_4O_8$	546	198	73	61.58 (61.53)	4.86 (4.80)	10.28 (10.25)
2c	$C_{23}H_{25}N_3O_5$	423	289	82	65.28 (65.24)	5.99 (5.95)	9.94 (9.92)
2d	$C_{23}H_{25}N_3O_4S$	439	279	84	62.88 (62.85)	5.78 (5.73)	9.59 (9.56)
2e	$C_{22}H_{23}NO_5$	381	305	82	69.31 (69.28)	6.11 (6.08)	3.69 (3.67)
3a	$C_{26}H_{24}N_2O_2$	396	297	83	78.79 (78.76)	6.15 (6.10)	7.10 (7.07)
3b	$C_{26}H_{22}N_4O_6$	486	199	76	64.21 (64.19)	4.59 (4.56)	11.54 (11.52)
3c	$C_{21}H_{21}N_3O_3$	363	298	81	69.46 (69.41)	5.87 (5.82)	11.58 (11.56)
3d	$C_{21}H_{21}N_3O_2S$	379	281	83	66.49 (66.47)	5.59 (5.58)	11.12 (11.07)
3e	$C_{20}H_{19}NO_3$	321	301	84	74.78 (74.75)	5.99 (5.96)	4.39 (4.36)

Spectral data

(1). 2,6-Dibenzylidenecyclohexanone

IR (KBr) cm^{-1}: 1670 (C=O conjug.), 3055 (=C-H), 1630 (exocyclic), 3022 (C-H Ar), 1601, 1580, 1500, 1452 (C=C Ar), 705, 750 (monosub.), 2950, 2800 (CH_2 str.), 1440, 725 (CH_2 bend.). **NMR** ($CDCl_3$) δ: 1.62 (p, 2H, C_4), 2.81 (t, 4H, C_3 & C_5), 7.35 (s, 2H, =CH), 7.36 -7.63 (m, 10 H Ar). **Mass** m/z: 274 (M^+), 273, 257, 247, 246, 218, 217, 203, 202, 170, 131, 91, 77.

(2). 2,6-Bis(3-hydroxy-4-methoxybenzylidene)cyclohexanone

IR (KBr) cm^{-1}: 3370 (O-H), 1360 (O-H bend.), 1665 (C=O conjug.), 3060 (=C-H), 1620 (O-CH_3), 1040 (C-O-C), 3010 (C-H Ar), 1603, 1580, 1450 (C=C Ar), 810, 907 (1, 2, 4-trisub.), 2951, 2835, 2815 (CH_2 str.), 1320 (CH_2 bend.). **NMR** ($CDCl_3$) δ: 1.62 (p, 2H, C_4), 2.81 (t, 4H, C_3 & C_5), 3.87 (s, 6H, -OCH_3), 5.38 (s, 2H, OH), 7.39 (s, 2H, =CH), 6.68 (m, 2H Ar.), 7.18 -7.25 (m, 4H Ar). **Mass** m/z: 366 (M^+), 365 (100%), 351, 338, 337, 323, 310, 309, 295, 294, 243, 230, 177, 149, 137, 123.

(3). 2,6-Bis(4-hydroxybenzylidene)cyclohexanone

IR (KBr) cm^{-1}: 3357 (O-H), 1362 (O-H bend.), 1668 (C=O conjug.), 3068 (=C-H), 1640 (exocyclic), 3033 (C-H Ar), 1600, 1582, 1507, 1450 (C=C Ar), 815 (p-disub.), 2950, 2849, 2810 (CH_2 str.), 1321 (CH_2 bend.). **NMR** ($CDCl_3$) δ: 1.62 (p, 2H, C_4), 2.84 (t, 4H, C_3 & C_5), 5.39 (s, 2H, OH), 7.29 (s, 2H, =CH), 6.68-6.91 (m, 4H Ar.), 7.58-7.62 (m, 4H Ar). **Mass** m/z: 306 (M^+), 305 (100%), 278, 277, 250, 249, 235, 234, 213, 200, 147, 119, 107, 93.

(1a). 7-Benzylidene-2,3-diphenyl-3,3a,4,5,6,7-hexahydro-2H-indazole

IR (KBr) cm^{-1}: 1585 (C=N), 1294 (C-N), 3029 (=C-H str.), 1640 (exocyclic), 3015 (C-H Ar.), 1602, 1501, 1450, (C=C Ar.), 706, 740 (monosub.), 2945, 2801 (CH_2 str.), 1435, 728 (CH_2 bend.). **NMR** ($CDCl_3$) δ: 1.39-1.59 (m, 4H, C_4 & C_5), 1.94-2.04 (m, 2H, C_6), 2.31 (q, 1H, C_{3a}), 4.81 (d, 1H, C_3), 6.36 (s, 1H, =CH), 6.78 -6.84 (m, 3H Ar.), 7.22-7.41 (m, 10H Ar.), 7.59-7.60 (m, 2H Ar.). **Mass** m/z: 364 (M^+), 363, 362, 287, 286 (100%), 274, 195, 194, 180, 129, 103, 90, 78, 77.

(1b). 7-Benzylidene-2-(2,4-dinitrophenyl)-3-phenyl-3, 3a, 4, 5, 6, 7-hexahydro-2H-indazole

IR (KBr) cm^{-1}: 1528 (NO_2 Assym.), 1342 (NO_2 Symm.), 1584 (C=N), 1295 (C-N), 3048 (=C-H str.), 1658 (exocyclic), 3015 (C-H Ar.), 1608, 1498, 1450 (C=C Ar.), 708, 750 (monosub.), 811, 910 (1, 2, 4-trisub.), 2925, 2858, 2812 (CH_2 str.), 1163 (CH_2 bend.). **NMR** ($CDCl_3$) δ: 1.38-1.58 (m, 4H, C_4 & C_5), 1.95-2.03 (m, 2H, C_6), 2.31 (q, 1H, C_{3a}), 4.81 (d, 1H, C_3), 6.37 (s, 1H, =CH), 7.11-7.13 (m, 1H Ar.), 7.25-7.40 (m, 8H Ar.), 7.58-7.61 (m, 2H Ar.), 8.41-8.44 (m, 1H Ar.), 8.87-8.88 (m, 1H Ar.). **Mass** m/z: 454 (M^+), 453, 452, 424, 408, 396, 382, 377, 287 (100%), 285, 284, 270, 168, 167, 129, 103, 91, 77, 30.

(1c). 7-Benzylidene-3-phenyl-3, 3a, 4, 5, 6, 7-hexahydro-2H-indazole-2-carboxamide

IR (KBr) cm^{-1}: 3350, 3168 (NH_2 amide), 1668 (C=O), 659 (N-H bend.), 1589 (C=N), 1293 (C-N), 3045 (=C-H str.), 1630 (exocyclic), 3018 (C-H Ar.), 1600, 1499, 1451 (C=C Ar.),

710, 745 (monosub.), 2930, 2865 (CH_2 str.), 1418, 785 (CH_2 bend.). **NMR** ($CDCl_3$) δ: 1.37-1.57 (m, 4H, C_4 & C_5), 1.95-2.03 (m, 2H, C_6), 2.69 (q, 1H, C_{3a}), 5.18 (d, 1H, C_3), 6.35 (s, 1H, =CH), 9.55 (s, 2H, NH_2), 7.26-7.40 (m, 8H Ar.), 7.59-7.60 (m, 2H Ar.). **Mass** m/z: 331 (M^+), 330, 329, 287, 286 (100%), 254, 253, 240, 228, 162, 161, 147, 129, 103, 91, 78, 77, 45, 40.

(1d). 7-Benzylidene-3-phenyl-3, 3a, 4, 5, 6, 7-hexahydro-2H-indazole-2-carbothioamide

IR (KBr) cm^{-1}: 1230 (C=S), 3360, 3165 (NH_2), 1395 (NH bend.), 1591 (C=N), 1285 (C-N), 3038 (=C-H str.), 1628 (exocyclic), 3018 (C-H Ar.), 1600, 1500, 1450 (C=C Ar.), 706, 748 (monosub.), 2948, 2850 (CH_2 str.), 1420, 728 (CH_2 bend.). **NMR** ($CDCl_3$) δ: 1.38-1.58 (m, 4H, C_4 & C_5), 1.95-2.02 (m, 2H, C_6), 2.30 (q, 1H, C_{3a}), 4.81 (d, 1H, C_3), 6.34 (s, 1H, =CH), 8.54 (s, 2H, NH_2), 7.26 -7.41 (m, 8H Ar.), 7.60 – 7.61 (m, 2H Ar.). **Mass** m/z: 347 (M^+), 348, 346, 345, 287, 286, 270, 269, 256, 178, 177, 163, 129, 103, 91, 78, 77, 60.

(1e). 7-Benzylidene-3-phenyl-3, 3a, 4, 5, 6, 7- hexahydrobenzo [c] isoxazole

IR (KBr) cm^{-1}: 1567(C=N), 866 (N-O), 3062 (=C-H str.), 1622 (exocyclic), 3019 (C-H Ar.), 1600, 1581, 1500, 1450 (C=C Ar.), 710, 738 (monosub.), 2951, 2848 (CH_2 str.), 1414, 806 (CH_2 bend.). **NMR** ($CDCl_3$) δ: 1.23-1.64 (m, 4H, C_4 & C_5), 1.92-1.97 (m, 2H, C_6), 2.14 (q, 1H, C_{3a}), 5.92 (d, 1H, C_3), 6.35 (s, 1H, =CH), 7.32-7.41 (m, 8H Ar.), 7.59-7.60 (m, 2H Ar.). **Mass** m/z: 289 (M^+), 287 (100%), 288, 271, 212, 211, 198, 169, 145, 143, 142, 129, 120, 103, 102, 91, 78, 77, 41, 40.

(2a). 7-(3-Hydroxy-4-methoxybenzylidene)-3-(3-hydroxy-4-methylphenyl)-2-phenyl-3, 3a, 4, 5, 6, 7-hexahydro-2H-indazole

IR (KBr) cm^{-1}: 3377 (O-H), 1355 (O-H bend.), 1227 (C-O), 1041 (C-O-C), 1588 (C=N), 1289 (C-N), 3059 (=C-H), 1631 (exocyclic), 3018 (C-H Ar.), 1600, 1500, 1450 (C=C Ar.), 812, 902 (1, 2, 4- trisub.), 706, 740 (monosub.) 2948, 2849 (CH_2 str.), 1415, 829 (CH_2 bend.), 1372 (CH_3 bend.). **NMR** ($CDCl_3$) δ: 1.29-1.52 (m, 4H, C_4 & C_5), 1.95-2.03 (m, 2H, C_6), 2.31 (q, 1H, C_{3a}), 3.84 (s, 6H, OCH_3), 4.82 (d, 1H, C_3), 5.36 (s, 2H, OH), 6.35 (s, 1H, =CH), 6.74-6.89 (m, 7H Ar.), 7.17-7.24 (m, 4H Ar.). **Mass** m/z: 456 (M^+), 455, 454, 431, 428, 413, 379, 378, 333, 319, 241, 240, 229, 226, 175, 149, 137, 123, 78, 77.

(2b). 2-(2,4-Dinitrophenyl)-7-(3-hydroxy-4-methoxybenzylidene)-3-(3-hydroxy-4-methoxylphenyl)-3, 3a, 4, 5, 6, 7-hexahydro-2H-indazole

IR (KBr) cm^{-1}: 3370 (O-H), 1350 (O-H bend.), 1223 (C-O), 1041 (C-O-C), 1528 (NO_2 assym.), 1340 (NO_2 symm.), 1582 (C=N), 1290 (C-N), 3061 (=C-H), 1632 (exocyclic), 3020 (C-H Ar.), 1600, 1500, 1451 (C=C Ar.), 814, 909 (1, 2, 4-trisub.), 2951, 2856 (CH_2 str.), 1410, 816 (CH_2 bend.), 1370 (CH_3 bend.). **NMR** ($CDCl_3$) δ: 1.28-1.51 (m, 4H, C_4 &

C_5), 1.96-2.01 (m, 2H, C_6), 2.31 (q, 1H, C_{3a}), 3.83 (s, 6H, OCH_3), 4.82 (d, 1H, C_3), 5.35 (s, 2H, OH), 6.34 (s, 1H, =CH), 6.73-6.78 (m, 2H Ar.), 6.87-6.93 (m, 2H Ar.), 7.12-7.24 (m, 3H Ar.), 8.42-8.43 (m, 1H Ar.), 8.77-8.88 (m, 1H Ar.). **Mass** m/z : 546 (M^+), 545, 531, 518, 503, 500, 423, 409, 379, 378 (100%), 331, 330, 316, 168, 167, 165, 149, 137, 124, 123, 30.

(2c). 7-(3-Hydroxy-4-methoxybenzylidene)-3-(3-hydroxy-4-methoxyphenyl)-3, 3a, 4, 5, 6, 7-hexahydro-2H-indazole-2-carboxamide

IR (KBr) cm^{-1}: 1670 (C=O), 3369, 3170 (NH_2), 1410 (NH bend.), 3400 (O-H), 1358 (O-H bend.), 1224 (C-O), 1040 (C-O-C), 1592 (C=N), 1290 (C-N), 3038 (=C-H), 1638 (exocyclic), 3010 (C-H Ar.), 1580, 1500, 1450 (C=C Ar.), 808, 908 (1, 2, 4-trisub.), 2950, 2851 (CH_2 str.), 1413, 828 (CH_2 bend.), 1375 (CH_3 bend.). **NMR** ($CDCl_3$) δ: 1.29-1.51 (m, 4H, C_4 & C_5), 1.94-2.02 (m, 2H, C_6), 2.69 (q, 1H, C_{3a}), 3.84 (s, 6H, OCH_3), 5.18 (d, 1H, C_3), 5.36 (s, 2H, OH), 6.34 (s, 1H, =CH), 9.71 (s, 2H, NH_2), 6.73-6.77 (m, 2H Ar.), 6.89-6.91 (m, 2H Ar.), 7.19-7.22 (m, 2H Ar.). **Mass m/z:** 423 (M^+), 422, 421, 424, 408, 395, 380, 379, 378 (100%), 300, 286, 208, 207, 175, 193, 149, 137, 124, 123, 45, 44.

(2d). 7- (3-Hydroxy-4-methoxybenzylidene) -3-(3-hydroxy-4-methoxyphenyl)-3, 3a, 4, 5, 6, 7-hexahydro-2H-indazole-2-carbothioamide

IR (KBr) cm^{-1}: 1240 (C=S), 3365, 3168 (NH_2), 1402 (NH bend.), 3405 (O-H), 1360 (O-H bend.), 1221 (C-O), 1041 (C-O-C), 1589 (C=N), 1288 (C-N), 3049 (=C-H), 1641 (exocyclic), 3015 (C-H Ar.), 1600, 1501, 1452 (C=C Ar.), 805, 911 (1, 2, 4- trisub.), 2948, 2849 (CH_2 str.), 1415, 820 (CH_2 bend.), 1371 (CH_3 bend.). **NMR** ($CDCl_3$) δ: 1.30-1.58 (m, 4H, C_4 & C_5), 1.94-2.0 (m, 2H, C_6), 2.51 (q, 1H, C_{3a}), 3.84 (s, 6H, OCH_3), 4.82 (d, 1H, C_3), 5.36 (s, 2H, OH), 6.31 (s, 1H, =CH), 8.57 (s, 2H, NH_2), 6.74-6.78 (m, 2H Ar.), 6.88-6.90 (m, 2H Ar.), 7.19-7.22 (m, 2H Ar.). **Mass m/z:** 439 (M^+), 438, 437, 424, 411, 396, 379, 378 (100%), 316, 315, 302, 224, 223, 209, 175, 149, 137, 124, 123, 61, 60.

(2e). 7-(3-Hydroxy-4-methoxybenzylidene)-3-(3-hydroxy-4-methoxyphenyl)-3, 3a, 4, 5, 6, 7- hexahydrobenzo[c]isoxazole

IR (KBr) cm^{-1} 1568 (C=N), 865 (N-O), 3382 (O-H), 1360 (O-H bend.), 1224 (C-O), 1043 (C-O-C), 3062 (=C-H), 1651 (exocyclic), 3008 (C-H Ar.), 1608, 1581, 1506, 1456 (C=C Ar.), 800, 910 (1, 2, 4- trisub.), 2956, 2842, 2781 (CH_2 str.), 1325, 1261 (CH_2 bend.). **NMR** ($CDCl_3$) δ: 1.22-1.64 (m, 4H, C_4 & C_5), 1.93-1.97 (m, 2H, C_6), 2.13 (q, 1H, C_{3a}), 3.81 (s, 6H, OCH_3), 5.93 (d, 1H, C_3), 5.33 (s, 2H, OH), 6.38 (s, 1H, =CH), 6.73-6.81 (m, 2H Ar.), 6.86-6.98 (m, 2H Ar.), 7.15-7.18 (m, 1H Ar.), 7.21-7.22 (m, 1H Ar.). **Mass** m/z: 381 (M^+), 380, 379, 366, 363, 353, 338, 258, 244, 215, 192, 189, 188, 175, 149, 148, 137, 123, 120, 41, 40.

(3a). 7-(4-Hydroxybenzylidene)-3-(4-hydroxyphenyl)-2-phenyl-3, 3a, 4, 5, 6, 7-hexahydro-2H-indazole

IR (KBr) cm^{-1}: 3390 (O-H), 1362 (O-H bend.), 1220 (C-O), 1586 (C=N), 1290 (C-N), 3042 (=C-H), 1650 (exocyclic), 3010 (C-H Ar.), 1598, 1506, 1451 (C=C Ar.), 816 (p-disub.), 710, 735 (monosub.), 2947, 2845, 2810 (CH_2 str.), 1442 (CH_2 bend.). **NMR** ($CDCl_3$) δ: 1.26-1.49 (m, 4H, C_4 & C_5), 1.92-2.00 (m, 2H, C_6), 2.28 (q, 1H, C_{3a}), 4.81 (d, 1H, C_3), 5.38 (s, 2H, OH), 6.38 (s, 1H, =CH), 6.63-6.84 (m, 7H Ar.), 7.10-7.12 (m, 2H Ar.), 7.19-7.24 (m, 2H Ar.), 7.54-7.56 (m, 2H Ar.). **Mass** m/z: 396 (M^+), 397, 395, 394, 368, 319, 318 (100%), 303, 289, 211, 210, 196, 145, 119, 107, 93, 78, 77.

(3b). 2-(2, 4-Dinitrophenyl)-7-(4-hydroxybenzylidene)-3-(4-hydroxyphenyl)-3a, 4, 5, 6-tetrahydro-2H-indazole

IR (KBr) cm^{-1}: 3410 (O-H), 1365 (O-H bend.), 1224 (C-O), 1527 (NO_2 assym.), 1339 (NO_2 symm.), 1587 (C=N), 1288 (C-N), 3048 (=C-H), 1635 (exocyclic), 3010 (C-H Ar.), 1600, 1500, 1450 (C=C Ar.), 815, 910 (1, 2, 4-trisub.), 802 (p-disub.), 2950, 2851 (CH_2 str.), 1416 (CH_2 bend.). **NMR** ($CDCl_3$) δ: 1.27-1.51 (m, 4H, C_4 & C_5), 1.92-2.06 (m, 2H, C_6), 2.68 (q, 1H, C_{3a}), 4.81 (d, 1H, C_3), 5.36 (s, 2H, OH), 6.34 (s, 1H, =CH), 6.64-6.71 (m, 4H Ar.), 7.11-7.13 (m, 3H Ar.),7.55-7.56 (m, 2H Ar.), 8.42-8.43 (m, 1H Ar.), 8.86-8.87 (m, 1H Ar.). **Mass m/z:** 486 (M^+), 487, 485, 484, 458, 457, 456, 440, 393, 392, 379, 319, 318 (100%), 301, 300, 286, 168, 167, 145, 119, 107, 94, 93, 30.

(3c). 7-(4-Hydroxybenzylidene)-3-(4-hydroxyphenyl)-3, 3a, 4, 5, 6, 7-hexahydro-2H-indazole-2-carboxamide

IR (KBr) cm^{-1}: 3440 (O-H), 1368 (O-H bend.), 1226 (C-O), 3369, 3172 (NH_2), 1409 (NH bend.), 1668 (C=O), 1587 (C=N), 1290 (C-N), 3049 (=C-H), 1630 (exocyclic), 3024 (C-H Ar.), 1600, 1501, 1450 (C=C Ar.), 818 (p- disub.), 2950, 2851 (CH_2 str.), 1416, 819 (CH_2 bend.). **NMR** ($CDCl_3$) δ: 1.28-1.54 (m, 4H, C_4 & C_5), 1.93-1.98 (m, 2H, C_6), 2.69 (q, 1H, C_{3a}), 5.13 (d, 1H, C_3), 5.36 (s, 2H, OH), 6.35 (s, 1H, =CH), 9.61 (s, 2H, NH_2), 6.66-6.71 (m, 4H Ar.), 7.00-7.12 (m, 2H Ar.), 7.55-7.56 (m, 2H Ar.). **Mass m/z:** 363 (M^+), 364, 362, 361, 319, 318 (100%), 270, 269, 256, 235, 234, 178, 177, 145, 163, 119, 107, 94, 93.

(3d). 7-(4-Hydroxybenzylidene)-3-(4-hydroxyphenyl)-3, 3a, 4, 5, 6, 7-hexahydro-2H-indazole-2-carbothioamide

IR (KBr) cm^{-1}: 1238 (C=S), 3368, 3169 (NH_2), 1412 (NH bend.), 3430 (O-H), 1367 (O-H bend.), 1225 (C-O), 1588 (C=N), 1291 (C-N), 3049 (=C-H), 1629 (exocyclic), 3019

(C-H Ar.), 1600, 1501, 1450 (C=C Ar.), 821 (p- disub.), 2950, 2851 (CH_2 str.), 1414, 805 (CH_2 bend.). **NMR** ($CDCl_3$) δ: 1.27-1.51 (m, 4H, C_4 & C_5), 1.93-1.99 (m, 2H, C_6), 2.28 (q, 1H, C_{3a}), 4.81 (d, 1H, C_3), 5.36 (s, 2H, OH), 6.31 (s, 1H, =CH), 8.57 (s, 2H, NH_2), 6.66-6.71 (m, 4H Ar.), 7.11-7.12 (m, 2H Ar.), 7.54-7.55 (m, 2H Ar.). **Mass m/z:** 379 (M^+), 380, 378, 377, 351, 350, 319, 318 (100%), 286, 285, 272, 194, 193, 179, 145, 119, 107, 94, 93, 60.

(3e). 7-(4-Hydroxybenzylidene)-3-(4-hydroxyphenyl)-3, 3a, 4, 5, 6, 7-hexahydro-benzo[c] isoxazole

IR (KBr) cm^{-1}: 1569 (C=N), 868 (N-O), 3390 (O-H),1362 (O-H bend.), 1222 (C-O), 3049 (=C-H), 1622 (exocyclic), 3021 (C-H Ar.), 1600, 1500, 1450 (C=C Ar.), 828 (p-disub.), 2957, 2846 (CH_2 str.), 1399, 807 (CH_2 bend.). **NMR** ($CDCl_3$) δ: 1.24-1.65 (m, 4H, C_4 & C_5), 1.93-1.98 (m, 2H, C_6), 2.14 (q, 1H, C_{3a}), 5.91 (d, 1H, C_3), 5.36 (s, 2H, OH), 6.34 (s, 1H, =CH), 6.65-6.68 (m, 4H Ar.), 7.18-7.19 (m, 2H Ar.), 7.56-7.57 (m, 2H Ar.). **Mass** m/z: 321 (M^+), 322, 320, 319 (100%), 303, 293, 292, 228, 227, 214, 185, 161,159, 158, 145, 136, 119, 117, 107, 94, 93, 41, 40.

Microbial activity

The *in vitro* antibacterial and antifungal activities of all the synthesized compounds were evaluated by Kirby-Bayer method, using standard literature protocol. The MDR (Multi Drug Resistant) bacterial pathogens viz. *Pseudomonas aeruginosa (PA), Staphylococcus aureus (SA)* and *Acinetobacter spp (AB)* were obtained from Apollo Hospital, New Delhi. The fungal pathogens *viz. A. flavus* and *Penicillum spp.* were obtained from clinical microbiology lab, Amity Universiy, Noida, UP. In evaluation method, the microorganisms of interests is swabbed uniformly across an autoclaved culture dish having nutrient medium in agar base under sterile condition. This culture dish was sealed and incubated. When a layer of microbial growth takes place, a filter paper disk, impregnated of the compound to be tested, was placed on the surface of the agar and incubated at 40°C for 18-20 hrs. The compound diffuses out from the filter paper into the agar. If the compound is effective against bacteria/fungi at a certain concentration, no colonies will grow, wherever the concentration in agar was greater or equal to that effective concentration. This region is called zone of inhibition that is a measure of the compound's effectiveness. DMSO was used as a solvent to prepare solution of the test compounds. Standard drugs Gentamycin and Ampicillin were used for antibacterial activity. Standard drug Ampicillin showed no activity against *Staphylococcus aureus (SA), Pseudomonas aeruginosa (PA)* and *Acinetobacter (AB)* whereas Gentamycin showed no activity against *Pseudomonas aeruginosa (PA)*. Nystatin (10 mcg), and Amphotericin B (10 mcg) were used for antifungal activity as standard drugs.

Table 2: Biological evaluation

Comp.	Antibacterial activity			Antifungal activity	
	S. aureus	***P. aeruginosa***	***Acinetobacter***	***A. flavus***	***Penicillium spp***
1	18 (1.8)	16 (16)	16 (2.0)	24 (1.84) (2.40)	30 (2.5) (3.00)
2	16 (1.6)	14 (14)	18 (2.25)	35 (2.91) (3.50)	25 (1.92) (2.50)
3	24 (2.4)	24 (24)	25 (3.12)	37 (9.06) (3.70)	31 (2.38) (3.10)
1a	18 (1.8)	19 (19)	19 (2.37)	9 (0.60) (0.60)	00 (00)
1b	21 (2.1)	20 (20)	21 (2.62)	13 (1.00) (1.30)	6 (0.50) (0.60)
1c	19 (1.9)	19 (19)	20 (2.50)	20 (1.53) (2.00)	32 (2.66) (3.20)
1d	22 (2.2)	20 (20)	23 (2.87)	16 (1.23) (1.60)	28 (2.33) (2.80)
1e	28 (2.8)	27 (27)	27 (3.37)	30 (2.30) (3.00)	35 (2.90) (3.50)
2a	24 (2.4)	24 (24)	23 (2.80)	22 (1.69) (2.20)	00 (00)
2b	17 (1.7)	17 (17)	18 (2.25)	14 (1.07) (1.40)	6 (0.50) (0.60)
2c	25 (2.5)	24 (24)	24 (3.00)	24 (1.84) (2.40)	29 (2.41) (2.90)
2d	20 (2.00)	18 (18)	19 (2.37)	22 (1.69) (2.20)	26 (2.16) (2.60)
2e	31 (3.10)	30 (30)	31 (3.87)	33 (2.53) (3.30)	45 (3.75) (4.50)
3a	22 (2.20)	20 (20)	00 (00)	25 (1.92) (2.5)	00 (00)
3b	21 (2.10)	19 (19)	19 (2.37)	18 (1.38) (1.80)	8 (0.66) (0.80)
3c	19 (1.90)	18 (18)	20 (2.50)	21 (1.61) (2.10)	31 (2.58) (3.10)
3d	18 (1.80)	19 (19)	20 (2.50)	15 (1.15) (1.50)	24 (2.00) (2.40)
3e	26 (2.60)	28 (28)	25 (3.12)	30 (2.30) (3.00)	40 (3.30) (4.00)
Std. 1	**10**	**00**	**08**	**13**	**12**
Std. 2	**00**	**00**	**00**	**10**	**10**

Zone of Inhibition (mm) (Activity Index)

Standard: **Antibacterial:** **1.** Gentamycin **2.** Ampicillin
Antifungal: **1.** Nystatin **2.** Amphotericin B

RESULTS AND DISCUSSION

The α, β-unsaturated cyclohexanone derivatives (**1, 2, 3)**, (**Scheme 1**) were obtained by condensation of cyclohexanone with substituted benzaldehydes, under microwave

irradiation in presence of neutral aluminum oxide in excellent (80-87%) yields. Compounds **(1, 2, 3)** were identified by their physical, analytical and IR, Mass and ^{1}H NMR spectral data. In the IR spectrum, a strong band around 1668 cm^{-1} indicates the presence of conjugated carbonyl group. In the ^{1}H NMR spectra, the olefinic protons gave a singlet signal at 7.35 ppm.

Pyrazoline derivatives **(1a-d)**, were synthesized by reaction of compound **(1)** with phenylhydrazine, 2, 4-dinitrophenylhydrazine, semicarbazide and thiosemicarbazide, respectively in an open flask under microwave irradiation (**Scheme 2**). Pyrazolines **(2a-d)** and **(3a-d)** were obtained by the same procedure given for compounds **(1a-d)**.

Isoxazoline derivatives **(1e), (2e)** and **(3e)**, were synthesized by reaction of compound **(1)**, **(2)** and **(3)**, respectively with hydroxylamine hydrochloride in an open flask under microwave irradiation (**Scheme 3**).

All products were characterized on the basis of their IR, ^{1}H-NMR, Mass spectral and elemental analysis. In pyrazolines derivatives the IR spectra showed an absorption band for the (C=N) group at 1580-1595 cm^{-1} and a band at 1290 cm^{-1} for (C-N) group. In the ^{1}H NMR spectra, the olefinic protons gave a singlet signal at 6.31-6.38 ppm.

The IR spectra of isoxazoline derivatives showed an absorption band for the (C=N) group at 1568 cm^{-1} and a band at 866 cm^{-1} for (N-O) group. In the ^{1}H NMR spectra, the olefinic protons gave a singlet signal at 6.38 ppm.

All 18 compounds were screened for biological activity against different MDR bacteria and fungi. All the synthesized compounds have shown excellent activity against all Multi Drug Resistant (MDR) bacteria in comparison to standard drug Ampicillin and Gentamycin. Only compound **(3a)** showed no activity against *Acinetobacter.*

All the synthesized compounds except **(1a), (2a)** and **(3a)** have shown excellent activity against all fungi in comparison to standard drugs Nystatin and Amphotericin B. Compound **(1a)**, **(2a)** and **(3a)** showed no activity against *Penicillium spp.*

CONCLUSION

This work demonstrates a rapid, efficient and environment friendly method for the synthesis of excellent potentially bioactive α, β-unsaturated cyclohexanone and their 2-pyrazolines and isoxazolines derivatives in excellent (75%-86%) yield under microwave irradiation. This microwave assisted synthesis is a dry media reaction condition that leads to considerable saving in reaction time and energetically profitable over the conventional

method. All the synthesized compounds have shown excellent activity in some MDR bacterial and fungal studies. The biological activity of these compounds will trigger more interest in the synthesis of such compounds from the easily available starting materials in an eco-friendly manner.

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