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Microwave-Assisted In Situ Cyclization of Curcumin Derivatives as Dominant Chemotherapeutic Agents for Leukemia and Colon Cancer

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Abstract—A rapid and efficient protocol has been proposed for the synthesis of 7-(4-substituted benzylidene)-3-(4-substituted phenyl)-3,3a,4,5,6,7-hexahydro-2*H*-indazoles under microwave irradiation. The synthesized compounds were screened for anticancer activity against 60 human cancer cell lines, and some of them exhibited significant anticancer activity against leukemia and colon cancer cell lines.

Keywords: curcumin type derivatives, in situ cyclization, leukemia, colon, microwave irradiation

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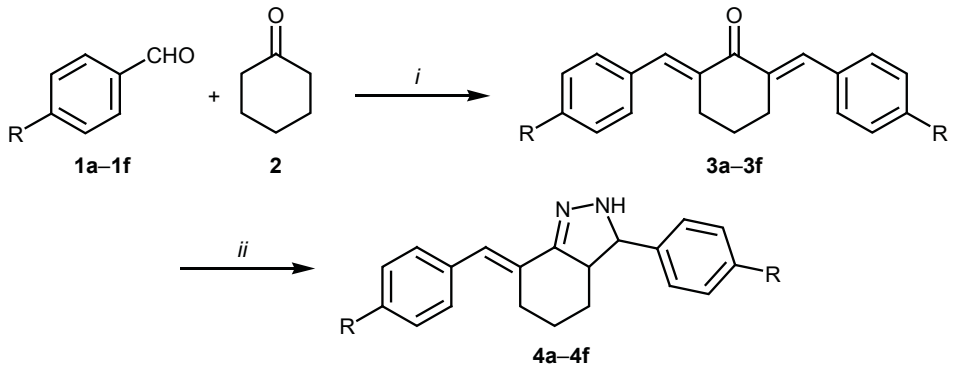
INTRODUCTION

Heterocyclic compounds attract chemists due to their pharmacological importance [1–5]. Heterocyclic compounds containing C=O and C=N bonds show diverse biological activities such as antidiabetic [6], antimicrobial [7], and anticancer [8]. Some naturally occurring compounds play an important role in the field of medicinal chemistry. Curcumin [(1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione] is a natural compound isolated from *Curcuma longa*. Its molecule possesses an active methylene group which is a potential site for further derivatization. Monocarbonyl curcumin analogs exhibit a more powerful inhibition in various cancer cell lines. Over a period of research, curcumin was reported to inhibit carcinogen-induced mutations and the formation of tumors in several experimental systems [9–11]. It also exhibits antiproliferation capability as a potent tool in cancer therapy [12, 13]. Therefore, in the present work we wish to report some curcumin analogs bearing a carbonyl group and their further conversion into 2*H*-indazole derivatives as potent anticancer agents.

RESULTS AND DISCUSSION

The condensation of various substituted aromatic aldehydes and cyclohexanone in the presence of boric acid and HCl catalyst gave 2,6-dibenzylidenecyclohexan-1-ones **3a**–**3f** which were then reacted with hy-

Scheme 1.



i: H_3BO_3, HCl, MeOH, MW (180 W), 40 s; *ii*: $NH_2NH_2 \cdot H_2O$, MeOH, MW (180 W), 1 min.
R = Br (**a**), Me (**b**), NO_2 (**c**), Cl (**d**), OH (**e**), OMe (**f**).

Table 1. Anticancer screening of compounds **4a**, **4c**, **4d**, and **4e**

Compound no.	Cell line/cancer type[a]	Growth percent	% Growth inhibition
4a	**CCRF-CEM**/L	**11.62**	**88.38**
	HL-60(TB)/L	16.96	83.04
	MOLT-4/L	26.07	73.93
	RPMI-8226/L	39.67	60.33
	NCI-H522/NSCL	57.48	42.52
	SNB-75/CNS	57.69	42.31
	UO-31/RC	60.62	39.38
4c	RPMI-8226/L	15.19	84.81
	SR/L	75.28	24.72
	HCT-116/CC	**16.26**	**83.74**
	HT29/CC	73.42	26.58
	KM12/CC	81.68	18.32
4d	**MCF7/B**C	**31.72**	**68.28**
	T-47D/BC	72.97	27.03
	CCRF-CEM/L	93.15	6.85
	HCT-116/CC	90.54	9.46
	SNB-75/CNS	69.72	30.28
	A498/RC	76.67	23.33
4e	SNB-75/CNS	55.77	44.23
	A498/RC	62.90	37.10

[a] "L" stands for leukemia, "NSCL" stands for non-small-cell lung cancer, "CC" stands for colon cancer, "CNS" stands for central nervous system cancer, "RC" stands for renal cancer, and "BC" stands for breast cancer.

drazine hydrate under microwave irradiation (180 W) for 1 min in 10-s intervals to afford final 7-(4-substituted benzylidene)-3-(4-substituted phenyl)-3,3a,4,5,6,7-hexahydro-2*H*-indazole derivatives **4a**–**4f** (Scheme 1). The reaction conditions were optimized using conventional heating and microwave irradiation. The reaction carried out under reflux conditions required ~3 h for completion, whereas under microwave irradiation it was complete in 40 s. Furthermore, the microwave-assisted reaction provided better yields.

All compounds **4a**–**4f** were submitted to the National Cancer Institute (USA) for biological screening, and compounds **4a**, **4c**, **4d**, and **4e** were selected for a single-dose (10^{-5} M) study. Table 1 shows the percentage growth inhibition (GI, %) of cancer cells treated with compounds **4a**, **4c**, **4d**, and **4e** at a concentration of 10^{-5} M. The tested compounds exhibited good to moderate activity. Compound **4a** showed a good activity against CCRF-CEM, HL-60(TB), and MOLT-4 cell lines, and compound **4c** exhibited a good potency against RPMI-8226, HCT-116, and MCF7 cell lines. Compounds **4d** and **4e** showed moderate to low inhibition.

EXPERIMENTAL

All melting points are uncorrected. Commercial chemicals, reagents, and solvents were used without further purification. The purity of the reaction products was monitored by TLC on Merck silica gel G60 F254 plates with spot visualization with UV light (λ 254 and 365 nm), iodine vapor, and aqueous $KMnO_4$. The ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer at 400 (^1H) and 101 MHz (^{13}C) in $CDCl_3$; the chemical shifts were measured relative to internal tetramethylsilane. The IR spectra (diffuse reflectance) were recorded on a Shimadzu

FTIR-8400 instrument from samples prepared as KBr discs. Elemental analysis was carried out on a Euro EA 3000 elemental analyzer. The reaction temperature was controlled by a ruby thermometer. The mass spectra (electron impact, 70 eV) were recorded on a Shimadzu GCMS QP-2010 mass spectrometer using a direct inlet probe. Microwave-assisted reactions were carried out in a Samsung MS23J5133AG domestic microwave oven.

7-(4-Substituted benzylidene)-3-(4-substituted phenyl)-3,3a,4,5,6,7-hexahydro-2*H*-indazoles 4a–4f (*general procedure*). A solution of 5 mmol of cyclohexanone (**2**) and 10 mmol of substituted aromatic aldehyde **1a**–**1f** in 20 mL of methanol. Boric acid (1 mmol) and concentrated aqueous HCl (5 mL) were then added, and the mixture was subjected to microwave irradiation (180 W) for 40 s in 10-s intervals. After completion of the reaction, hydrazine hydrate (1 mL, 2 mmol) was added, and the mixture was subjected to microwave irradiation for 1 min in 10-s intervals. After completion of the reaction (TLC), the mixture was poured into cold water, and the product was filtered off and dried.

7-(4-Bromobenzylidene)-3-(4-bromophenyl)-3,3a,4,5,6,7-hexahydro-2*H*-indazole (4a). Yield 78%, mp 198–200°C. IR spectrum, ν, cm^{-1}: 3338 (N–H), 1593 (C=N), 1497 (C=C), 1221(C–N). ^1H NMR spectrum, δ, ppm: 7.26–7.72 m (8H, H_{arom}), 6.42 s (1H, =CH), 5.32 d.d (1H, NCH), 5.00 s (1H, NH), 1.55–3.42 m (7H, CH_2, CH). ^{13}C NMR spectrum, δ_C, ppm: 152.45, 146.07, 139.51, 134.87, 130.91, 129.75, 127.96, 126.20, 123.20, 120.10, 114.40, 69.04, 57.43, 30.24, 29.11, 24.63. Mass spectrum: *m*/*z* 446/448. Found, %: C 53.80; H 4.02; N 6.24. $C_{20}H_{18}N_2Br_2$. Calculated, %: C 53.84; H 4.07; N 6.28.

7-(4-Methylbenzylidene)-3-(4-methylphenyl)-3,3a,4,5,6,7-hexahydro-2*H*-indazole (4b). Yield 71%, mp 168–170°C. IR spectrum, ν, cm^{-1}: 3328 (N–H), 1585 (C=N), 1490 (C=C), 1224 (C–N). ^1H NMR spectrum, δ, ppm: 7.35–7.74 m (8H, H_{arom}), 6.48 s (1H, =CH), 5.23 d.d (1H, NCH), 4.98 s (1H, NH), 2.35 s (3H, CH_3), 2.10 s (3H, CH_3), 1.60–3.38 m (7H, CH_2, CH). ^{13}C NMR spectrum, δ_C, ppm: 150.85, 145.24, 138.51, 134.62, 130.27, 129.44, 127.86, 126.28, 123.72, 120.37, 114.19, 69.46, 57.34, 30.24, 29.81, 28.34, 27.95, 24.28. Mass spectrum, *m*/*z*: 316 $[M]^+$, 317. Found, %: C 83.48; H 7.59; N 8.81. $C_{22}H_{24}N_2$. Calculated, %: C 83.50; H 7.64; N 8.85.

7-(4-Nitrobenzylidene)-3-(4-nitrophenyl)-3,3a,4,5,6,7-hexahydro-2*H*-indazole (4c). Yield 82%, mp 170–171°C. IR spectrum, ν, cm^{-1}: 3330 (N–H), 1590 (C=N), 1450 (NO_2), 1497 (C=C), 1221 (C–N). ^1H NMR spectrum, δ, ppm: 7.41–7.83 m (8H, H_{arom}), 6.50 s (1H, =CH), 5.26 d.d (1H, NCH), 5.02 s (1H, NH), 1.60–3.38 m (7H, CH_2, CH). ^{13}C NMR spectrum, δ_C, ppm: 152.45, 145.07, 139.10, 134.43, 130.73, 129.27, 127.16, 126.89, 122.20, 120.90, 114.85, 69.02, 58.12, 31.02, 30.01, 24.51. Mass spectrum, *m*/*z* 378 $[M]^+$, 379. Found, %: C 63.46; H 4.77; N 14.76. $C_{20}H_{18}N_4O_4$. Calculated, %: C 63.49; H 4.80; N 14.81.

7-(4-Chlorobenzylidene)-3-(4-chlorophenyl)-3,3a,4,5,6,7-hexahydro-2*H*-indazole (4d). Yield 69%, mp 186–188°C. IR spectrum, ν, cm^{-1}: 3335 (N–H), 1595 (C=N), 1491 (C=C), 1223 (C–N), 853 (C–Cl), 810 (δC–H_{arom}). ^1H NMR spectrum, δ, ppm: 7.26–7.72 m (8H, H_{arom}), 6.42 s (1H, =CH), 5.30 d.d (1H, NCH), 5.03 s (1H, NH), 1.52–3.44 m (7H, CH_2, CH). ^{13}C NMR spectrum, δ_C, ppm: 150.25, 145.17, 138.53, 134.43, 130.21, 129.25, 127.87, 126.24, 123.68, 119.18, 114.21, 69.71, 57.67, 30.12, 28.54, 26.61. Mass spectrum: *m*/*z* 357/359. Found, %: C 67.20; H 5.05; N 7.82. $C_{20}H_{18}N_2Cl_2$. Calculated, %: C 67.24; H 5.08; N 7.84.

4-[7-(4-Hydroxybenzylidene)-3,3a,4,5,6,7-hexahydro-2*H*-indazol-3-yl]phenol (4e). Yield 65%, mp 166–168°C. IR spectrum, ν, cm^{-1}: 3410 (O–H), 3335 (N–H), 1589 (C=N), 1491 (C=C), 1221(C–N), 810 (δC–H_{arom}). ^1H NMR spectrum, δ, ppm: 9.45 s (2H, OH), 7.41–7.83 m (8H, H_{arom}), 6.50 s (1H, =CH), 5.26 d.d (1H, NCH), 5.02 s (1H, NH), 1.60–3.38 m (7H, CH_2, CH). ^{13}C NMR spectrum, δ_C, ppm: 151.95, 146.07, 139.59, 134.24, 131.45, 129.99, 127.74, 126.86, 123.82, 120.63, 114.28, 69.92, 57.19, 30.74, 29.36, 25.55. Mass spectrum, *m*/*z* 320 $[M]^+$, 321. Found, %: C 74.95; H 6.26; N 8.71. $C_{20}H_{20}N_2O_2$. Calculated, %: C 74.98; H 6.29; N 8.74.

7-(4-Methoxybenzylidene)-3-(4-methoxyphenyl)-3,3a,4,5,6,7-hexahydro-2*H*-indazole (4f). Yield 71%, mp 170–172°C. IR spectrum, ν, cm^{-1}: 3335 (N–H), 1589 (C=N), 1491 (C=C), 1221 (C–N), 812 (δC–H_{arom}). ^1H NMR spectrum, δ, ppm: 7.37–7.80 m (8H, H_{arom}), 6.39 s (1H, =CH), 5.25 d.d (1H, NCH), 5.05 s (1H, NH), 3.58 s (6H, OCH_3), 1.59–3.40 m (7H, CH_2, CH). ^{13}C NMR spectrum, δ_C, ppm: 152.45, 146.07, 139.51, 134.87, 130.46, 128.75, 127.09, 125.14, 123.20, 120.10, 114.42, 69.08, 60.75, 57.43, 53.69, 30.24, 29.11, 24.63. Mass spectrum, *m*/*z* 348 $[M]^+$, 349. Found, %: C 75.80; H 6.91; N 8.02. $C_{22}H_{24}N_2O_2$. Calculated, %: C 75.83; H 6.94; N 8.04.

Anticancer activity. The in vitro anticancer activity of the synthesized compounds was evaluated according

to the Developmental Therapeutics Program of the National Cancer Institute (Bethesda, USA) [14–16]. Compounds **4a**, **4c**, **4d**, and **4e** were selected and initially screened at a single dose of 10^{-5} M concentration. The entire 60 human cancer cell lines were organized into nine subpanels derived from nine different human cancer types: leukemia, non-small-cell lung cancer, colon, central nervous system, melanoma, ovarian, renal, prostate, and breast cancer cell lines (see Supplementary Information). Output from the single-dose screen is reported as a graph of mean growth percent of the treated cells.

CONCLUSIONS

7-(4-Substituted benzylidene)-3-(4-substituted)-3,3a,4,5,6,7-hexahydro-2*H*-indazole derivatives have been synthesized by condensation of aromatic aldehydes, cyclohexanone, and hydrazine hydrate under microwave irradiation. From the synthesized compounds, **4a** shows excellent inhibition against CCRF-CEM cell line for leukemia, while **4c** exhibits good inhibition against HCT-116 cell line for colon cancer.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at https://doi.org/10.1134/S1070428022030150.

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