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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF 6-CARBETHOXY-5-(3'-BROMOPHENYL)-3-ARYL-2-CYCLOHEXENONES AND 6-ARYL-4-(3'-BROMOPHENYL)-3-OXO-2,3A,4,5-TETRAHYDRO-2H-INDAZOLES

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Abstract

6-Carbethoxy-5-(3'-bromophenyl)-3-aryl-2-cyclohexenones **2a-j** were obtained from the1-Aryl-3-(3'-bromophenyl)-2-propene-1-ones **1a-j** by Micheal addition of ethyl acetoacetate, followed by internal Claisen condensation. Reaction of **2a-j** with hydrazine hydrate afforded the corresponding 6-Aryl-4-(3'-bromophenyl)-3-oxo-2,3a,4,5-tetrahydro-2H-indazoles **3a-j**. The structures of newly synthesized compounds were established on the basis of elemental analyses, IR, NMR and Mass spectral data. The pharmacological evaluations were performed for their anticancer, antitubercular and antimicrobial activities.

Keywords: Cyclohexenones; Indazoles; Anticancer activity; Antitubercular activity; Antimicrobial activity

Introduction

Much attention has been paid to the synthesis of heterocyclic compounds bearing a 1,2-diazole ring system like indazoles, mainly because of the interest concerning their broad spectrum of pharmacological activities. Indazole derivatives exhibit variety of pharmacological properties such as anti-inflammatory [1,2], antidepressant [3], antitumor [4], antihypertensive [5] and antiviral [6] activities. Keeping in view of these findings, we are reporting herein the synthesis of some novel cyclohexenone **(2a-j)** and indazole derivatives **(3a-j)**.

Condensation of 3-bromo benzaldehyde with different aryl methyl ketones by the known method [7] gave the required compounds 1-Aryl-3-(3′-bromophenyl)-2-propene-1-ones **(1a-j)**. Micheal addition of **(1a-j)** with ethyl acetoacetate in presence of K_2CO_3 followed by internal Claisen condensation afforded 6-Carbethoxy-5-(3′-bromophenyl)-3-aryl-2-cyclohexenones **(2a-j)**. Reaction of **(2a-j)** with hydrazine hydrate gave corresponding 6-Aryl-4-(3′-bromophenyl)-3-oxo-2,3a-4,5-tetrahydro-2H-indazoles **(3a-j)**.

The structures of the synthesized compounds were assigned on the basis of elemental analyses, ^{1}H NMR

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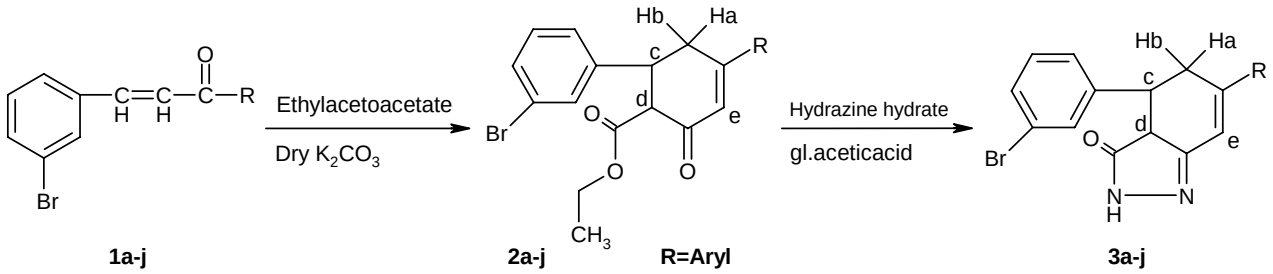

1a-j **2a-j** **R=Aryl** **3a-j**

Reaction Scheme

and IR spectral data. The compounds were screened for their anticancer, antitubercular and antimicrobial activities.

Experimental

Thin layer chromatography was used for follow up of the reaction and purity of the compounds. The melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded in Shimadzu FTIR-8400 instrument in KBr disc and only noteworthy absorption peaks (cm^{-1}) are listed. ^{1}H-NMR spectra were recorded on a Bruker AC-300 MHz FT NMR using TMS as an internal standard, chemical shift in δ ppm. Mass spectra were recorded on Jeol D-300 spectrometer. All the compounds gave satisfactory elemental analysis.

Preparation of 6-Carbethoxy-5-(3'-bromophenyl)-3-aryl-2-cyclohexenones 2a-j

To a solution of 1-Aryl-3-(3′-bromophenyl)-2-propene-1-one (0.01 mol) in dry acetone (50 ml), dry K_2CO_3 (0.04 mol) and ethyl acetoacetate (0.02 mol) were added and the reaction mixture stirred at room temperature for 5 hours. It was left at room temperature overnight and was filtered. The filtrate was evaporated. The residue which was recrystallised from ethanol. **2h:** Yield-64%, m.p. 299-301°C. Calculated for $C_{22}H_{21}O_4Br$: C-61.53, H-4.89 %; found: C- 61.50, H-4.85%. IR KBr (cm^{-1}): 1716 (C=O, ester), 1670 (C=O, ketone). ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ ppm: 1.08 [t(J_{AB}=7.24, J_{BC}=6.76 Hz), 3H, -CH_2-CH_3], 2.62 [dd, (J=2.41 Hz), 1H, methylene -CH_a],2.96 [dd, (J=4.14 Hz), 1H, methylene -CH_b)], 3.73 (m, 1H, -CH_c), 3.75 (s,1H,-CH_e), 3.89 (s, 3H ,-OCH_3), 4.09 [q(J_{AB} =6.93, J_{BC}=7.32, J_{CD}=7.20 Hz), 2H,-CH_2-CH_3], 7.06-7.47 [d(J=2.17 Hz), 1H, -CH_d], 6.92-7.52 (m, 8H, Ar-H). MS = m/z(430,M^+).

Other compounds were prepared similarly. Physical and analytical data of the compounds are recorded in Table 1.

Preparation of 6-Aryl-4-(3'-bromophenyl)-3-oxo-2,3a,4,5-tetrahydro-2H-indazoles 3a-j

A mixture of 6-Carbethoxy-5-(3′-bromophenyl)-3-aryl-2-cyclohexenone (0.01 mol) and hydrazine hydrate (0.02 mol) was refluxed in ethanol (50 ml) containing few drops of glacial acetic acid, on a water bath for 6 hours. The residue obtained after cooling was filtered and crystallized from methanol. **3h:** Yield 65%, m.p. 229-231°C. Calculated for $C_{20}H_{17}N_2O_2Br$: C- 60.45, H-4.28, N- 7.05 %, found: C- 60.40, H- 4.25, N- 7.01 %. IR KBr (cm^{-1}): 3241 (N-H, sec. amine), 1658 (C=O, sec. amide). ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ ppm: 2.84 [dd (J=2.43 Hz), 1H, methylene -CH_a], 3.05 [dd(J=4.15 Hz), 1H, methylene –CH_b], 3.73 (m, 1H, –CH_c), 3.80(s,1H,-CH_e), 3.85 (s, 3H, -OCH_3), 6.52 [d(J=2.15 Hz), 1H, -CH_d], 6.97-7.94 (m, 8H, Ar-H), 8.01 (s, 1H, N-H). MS = m/z (397 M^+).

Other compounds were prepared similarly. Physical and analytical data of the compounds are recorded in Table 1.

Results and Discussion

Anticancer Activity

The anticancer screening of some selected compounds was carried out at National Cancer Institute, Department of health & human service, Bethesda, U.S.A. The study is related with *in vitro* anticancer screen aimed at identifying agents having cell type specificity using batteries of cell slines derived from human solid tumors. At its primary anticancer assay, a 3-cell panel consisting of NCI-H 460 (Lung), MCF-7 (Breast) and SF-268 (CNS) has been used. A 48 hours continuous drug exposure protocol is used, and a sulforhodamine B (SRB) protein assay is used for estimating cell viability or growth [8].

Looking to the structure activity relationship, three compounds **(2g, 3c, 3g)** have been selected for the primary anticancer screening (Table 2).

Table 1. Physical and analytical data of compounds **2a-j** and **3a-j**

Compound	R	Molecular Formula	m.p. °C	Yield %	% of N calcd.	found
2a	$-C_6H_5$	$C_{21}H_{19}O_3Br$	144	63	-	-
2b	4-Br-C_6H_4	$C_{21}H_{18}O_3Br_2$	204	67	-	-
2c	4-Cl-C_6H_4	$C_{21}H_{18}O_3BrCl$	76	58	-	-
2d	2,4-$(Cl)_2$-C_6H_3	$C_{21}H_{17}O_3BrCl_2$	110	55	-	-
2e	2-OH-C_6H_4	$C_{21}H_{19}O_4Br$	160	61	-	-
2f	4-OH-C_6H_4	$C_{21}H_{19}O_4Br$	202	52	-	-
2g	2-OH-5-CH_3-C_6H_3	$C_{22}H_{21}O_4Br$	110	68	-	-
2h	4-OCH_3-C_6H_4	$C_{22}H_{21}O_4Br$	300	64	-	-
2i	2-CH_3-C_6H_4	$C_{22}H_{21}O_3Br$	220	61	-	-
2j	4-NO_2-C_6H_4	$C_{21}H_{18}NO_5Br$	180	62	3.15	3.10
3a	$-C_6H_5$	$C_{19}H_{15}N_2OBr$	226	55	7.62	7.60
3b	4-Br-C_6H_4	$C_{19}H_{14}N_2OBr_2$	165	53	6.27	6.24
3c	4-Cl-C_6H_4	$C_{19}H_{14}N_2OBrCl$	195	60	6.97	6.94
3d	2,4-$(Cl)_2$-C_6H_3	$C_{19}H_{13}N_2OBrCl_2$	180	62	6.42	6.40
3e	2-OH-C_6H_4	$C_{19}H_{15}N_2O_2Br$	208	57	7.31	7.28
3f	4-OH-C_6H_4	$C_{19}H_{15}N_2O_2Br$	165	63	7.31	7.27
3g	2-OH-5-CH_3-C_6H_3	$C_{20}H_{17}N_2O_2Br$	230	71	7.05	7.00
3h	4-OCH_3-C_6H_4	$C_{20}H_{17}N_2O_2Br$	230	65	7.05	7.01
3i	2-CH_3-C_6H_4	$C_{20}H_{17}N_2OBr$	180	58	7.34	7.30
3j	4-NO_2-C_6H_4	$C_{19}H_{14}N_3O_3Br$	200	59	10.16	10.16

Table 2. Anticancer screening result of compound which shows percent of growth

Compound	Concentration	Anticancer activity (% growth)		
		(Lung) NCI-H460	(Breast) NCF-7	(CNS) SF-268
2g	1.00E-04Molar	-36	-32	-48
3c	1.00E-04Molar	35	11	38
3g	1.00E-04Molar	-8	-40	8

Antitubercular Activity

The antitubercular evaluation of the compounds was carried out at Tuberculosis Antimicrobial Acquisition Co-ordinating Facility (TAACF) U.S.A. Primary screening of the compounds for antitubercular activity has been conducted at 6.25 μg/ml concentration against *Mycobacterium tuberculosis* $H_{37}Rv$ in BACTEC 12B medium using the ALAMAR radiometric system.

The antimycobacterial activity data were compared with standard drug Rifampin at 0.25 μg/ml concentration which showed 98% inhibition (Table 3).

Antimicrobial Activity

The antimicrobial activity was assayed by using the cup-plate agar diffusion method [9] by measuring the zone of inhibition in mm. All the compounds were screened *in vitro* for their antimicrobial activity against varieties of bacterial strains such as *E. coli*, *P. vulgaris*, *B. mega*, *S. aureus* and fungus *A. niger* at 40 μg/ml concentration. Standard drugs like Amoxycillin, Ampicillin, Ciprofloxacin, Erythromycin and Griseofulvin were used for the comparison purpose (Table 4).

Table 3. Antitubercular screening results of compounds which shows high percent of inhibition

Compound	R	Assay	MTb Strain	% Inhibition
2a	-C_6H_5-	Alamar	$H_{37}Rv$	20
2c	4-Cl-C_6H_4-	Alamar	$H_{37}Rv$	**52**
2d	2,4-$(Cl)_2$-C_6H_3-	Alamar	$H_{37}Rv$	20
2g	2-OH-5-CH_3-C_6H_3-	Alamar	$H_{37}Rv$	21
2i	4-CH_3-C_6H_4-	Alamar	$H_{37}Rv$	23
3a	-C_6H_5-	Alamar	$H_{37}Rv$	25
3b	4-Br-C_6H_4-	Alamar	$H_{37}Rv$	14
3c	4-Cl-C_6H_4-	Alamar	$H_{37}Rv$	**50**
3d	2,4-$(Cl)_2$-C_6H_3-	Alamar	$H_{37}Rv$	26
3g	2-OH-5-CH_3-C_6H_3-	Alamar	$H_{37}Rv$	18

Table 4. Antimicrobial data of the compounds which exhibited highest activity

Standard drugs	*E. coli*	*P. vulgaris*	*B. megaterium*	*S. aureus*	*A. niger*
Amoxycillin	**2h**(16)	**2e**(20)	**2b**(19)	**2c**(18)	**2e**(22)
(22-26 mm)	**2i**(26)	**2f**(20)	**2i**(19)	**2h**(19)	**2f**(20)
Ampicillin	**2j**(17)	**2g**(17)	**2j**(17)	**2j**(20)	**3b**(25)
(18-24 mm)	**3d**(18)	**2h**(17)	**3c**(19)	**3d**(19)	**3c**(21)
Erythromycin	**3f**(20)	**2i**(19)	**3d**(21)	**3e**(18)	
(22-26 mm)	**3g**(18)	**2j**(22)	**3i**(18)	**3f**(19)	
Ciprofloxacin	**3i**(19)	**3a**(19)	**3j**(17)	**3g**(18)	
(18-26 mm)	**3j**(20)	**3h**(21)			
Griseofulvin		**3i**(21)			
(21 mm)					

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