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Synthesis and anticancer (MCF-7, PC-3) activities of new 2-hydroxy-2,2-bis(4-substitutedphenyl)-*N*'-[(1*E*)-(3/4-substitutedphenyl)methylene]-acetohydrazides

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Abstract: A series of five $–CH_3$, $-NO_2$, $-OCH_3$ and -Cl substituted 2-hydroxy-2,2-bis(4-phenyl)-*N*'-[(1*E*)-(3/4-phenyl)methylene]acetohydrazide (**1a-1e**) was synthesized by the reaction of 2-hydroxy-2,2-diphenylacetohydrazide with substituted aromatic aldehydes to give intermediate Schiff bases. Structures of the synthesized compounds were characterized using NMR (1D; ^1H, ^{13}C/APT and 2D ^1H-^1H COSY, and NOESY), FT-IR, UV, LC-MS/MS spectral data and elemental analysis. The geometry of compounds **1a-1e** were determined to be "*E*" by NOESY. All the tested compounds showed cytotoxic activity on MCF-7 and PC-3 cell line at highest experimental concentration (100 µM). Compounds **1b** (18.24 ± 7.62 µM) and **1e** (7.62 ± 1.85 µM) have strong anti-proliferative activity on MCF-7 cell line, while compound **1b** (45.81 ± 1.10 µM) has the strongest activity on PC-3 cell line.

Keywords: 2-Hydroxy-2,2-diphenylacetic acid; acetohydrazide derivatives; MCF-7; PC-3.

1. Introduction

Carbon-carbon bond formation is the most important reaction for the synthesis of target molecules. Many of the carbon bond formation have been mentioned in the literature.[1-8] One of the most useful carbon-carbon formation reactions is the benzoin reaction that is a promising method for the preparation of α-hydroxyl diphenyl ketone.[9,10] Diarylethanones and arylethanoic acids are important subunits and they have been found in a variety of natural products, which are important for the synthesis of pharmaceutical and agrochemical compounds. Benzil undergos a rearrangement to yield α-hydroxy acetic acid in the presence of a strong base. It could be converted to acetohydrazide and its derivatives to display biological activity due to the presence of potent pharmacophores.[11-14] Moderate antibacterial activity of arylhydrazones of benzylic acid reacted with various aryl aldehydes has been mentioned.[15]

Recently, there has been considerable interest for the synthesis of substituted acetohydrazide and their thiazolidin-4-one derivatives. These compounds showed a broad biological activities such as anticancer[12,16], antiviral[17-21], anticonvulsant[22], antimycobacterial[23-25], cytotoxic[26] and antifungal.[27] Derivatives of acetohydrazide have already been reported to have anticancer or antitumor properties as kinase inhibitors.[12, 16] Therefore, there is still need to synthesize and explore the pharmaceutical relevance of substituted acetohydrazides. Hence, herein, we disclose the synthesis of five new analogs of acetohydrazides and screen for their MCF-7 and P-3 human breast and prostate cancer activities, which displayed promising results.

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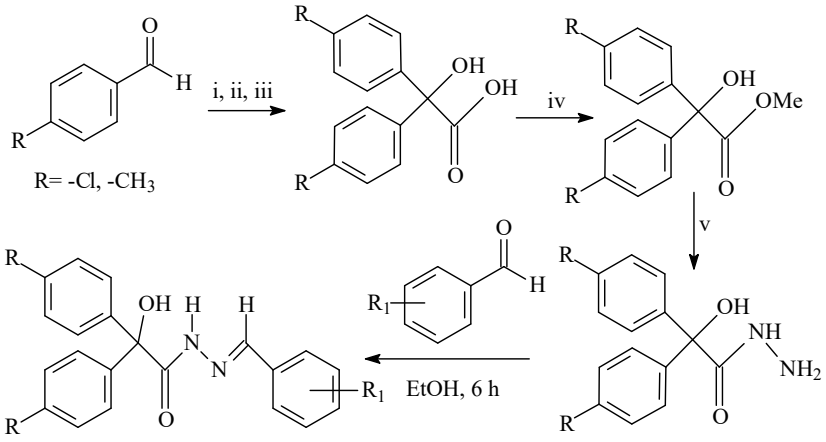
2. Experimental

2.1. Materials and apparatus

All of the chemical reagents used in the synthesis were high grade of commercial products purchased from Sigma and used without further purification. The solvents (n-hexane, ethyl acetate, chloroform, diethyl ether, ethanol, methanol) were either analytical grade or bulk solvents distilled before use. Thin-layer chromatographies (TLC) were carried out on Merck pre-coated 60 Kieselgel F_{254} analytical aluminum acidic plates. Melting points were determined using Thermo-var apparatus fitted with a microscope and are uncorrected. ^{1}H, ^{13}C/APT and NOESY NMR spectra were recorded on a Bruker 400/100 MHz NMR with tetramethylsilane (TMS) as an internal standard, respectively. Infrared spectra were obtained with a Perkin-Elmer 1600 FT-IR (4000-400 cm^{-1}) spectrometer. The mass spectral analyses were carried out on a Micromass Quattro LC-MS/MS spectrophotometer. The elemental analyses were performed on a Costech ESC 4010 instrument.

2.2. Methods

The known sequential benzoin,[9] benzil,[10] 2-hydroxy-2,2-diphenylacetic acid,[11] ethyl-2-hydroxy-2,2-diphenylacetate[12,13,15] and 2-hydroxy-2,2-diphenylacetohydrazide[15] syntheses (Scheme) were conducted according to the literature procedures.



1a-1e

Compounds	-R	-R_1
1a	-Cl	*p*-Cl
1b	-Cl	*m*-CH_3
1c	-Cl	*m*-NO_2
1d	-Cl	*p*-OCH_3
1e	-CH_3	*m*-CH_3

Scheme. Chemical synthesis of **1a-e**.
(i) KCN, EtOH, reflux, 3 h; (ii) HNO_3, reflux, 4 h; (iii) KOH, EtOH, reflux, 3 h;
(iv) MeOH, H_2SO_4, reflux, 5 h; (v) $H_2NNH_2.H_2O$, EtOH, reflux, 4 h.

*2.2.1. General procedure for the synthesis of 2-hydroxy-2,2-bis(4-substitutedphenyl)-N'-[(1E)-(3/4-substitutedphenyl)methylene]acetohydrazides (**1a-1e**)*

A mixture of substituted 2-hydroxy-2,2-diphenylacetohydrazide (6 mmol) and appropriately substituted benzaldehyde (6.6 mmol) was refluxed in absolute ethanol (30 mL) for 4 h. The reaction mixture was checked by TLC on silica gel plate. After the completion, the reaction mixture was cooled and excess

ethanol was evaporated under reduced pressure. The resulting residue was allowed to stand overnight or, in some cases, refrigerated until it becomes solid which was then washed with water, dried and recrystallized from ethanol to give compounds **1a-1e**.

*2-Hydroxy-2,2-bis(4-chlorophenyl)-N'-[(1E)-(4-chlorophenyl)methylene]acetohydrazide (**1a**)*: Yield: 58%, white solid, R_f=0.87 (hexane-diethyl ether, 1:3), m.p. (°C): 219-220, IR (KBr, cm^{-1}): 3340 (-OH), 3268 (-NH), 3050 (=CH), 2950 (-CH), 1664 (-NHC=O), 1593 (-C=N), 1570, 1448 (-C=C- aromatic), 802 (-CCl), Anal. calcd for $C_{21}H_{15}N_2O_2Cl_3$ (m.w.: 433.72): C, 58.15; H, 3.49; N, 6.46. Found: C, 58.33; H, 3.83; N, 6.46. LC-MS/MS: (*m/z*) (%) 434 (80), $[M+H]^+$: 436 (65), $[M+2H]^+$: 438 (15), $[M+4H]^+$: 283(85) $[M-NHN=CHC_6H_4Cl]^+$.

*2-Hydroxy-2,2-bis(4-chlorophenyl)-N'-[(1E)-(3-methylphenyl)methylene]acetohydrazide (**1b**)*: Yield: 52%, white solid, R_f=0.86 (ethyl acetate-hexane, 6:4), m.p. (°C): 273-274, IR (KBr, cm^{-1}): 3338 (-OH), 3250 (-NH), 3050 (=CH), 2950 (-CH), 1664 (-NHC=O), 1593 (-C=N), 1570, 1448 (-C=C- aromatic), 804 (-CCl), Anal. calcd for $C_{22}H_{18}N_2O_2Cl_2$ (m.w.: 413.30): C, 63.93; H, 4.39; N, 6.78. Found: C, 64.79; H, 4.47; N, 6.82. LC-MS/MS: (*m/z*) (%) 414 (25), $[M+H]^+$: 416 (45), $[M+2H]^+$: 418 (17), $[M+4H]^+$: 283(80) $[M-NHN=CHC_6H_4Cl]^+$.

*2-Hydroxy-2,2-bis(4-chlorophenyl)-N'-[(1E)-(3-nitrophenyl)methylene]acetohydrazide (**1c**)*: Yield: 72%, light white solid, R_f=0.64 (ethyl acetate-hexane, 6:4), m.p. (°C): 189-191, IR (KBr, cm^{-1}): 3345 (-OH), 3248 (-NH), 3050 (=CH), 2950 (-CH), 1664 (-NHC=O), 1593 (-C=N), 1570, 1448 (-C=C- aromatic, $-NO_2$), 806 (-CCl), Anal. calcd for $C_{21}H_{15}N_3O_4Cl_2$ (m.w.: 444.27): C, 56.77; H, 3.40; N, 9.46. Found: C, 56.59; H, 3.50; N, 9.38. LC-MS/MS: (*m/z*) (%) 443 (10), $[M-H]^+$: 305(100) $[M-NHN=CHC_6H_4Cl+Na-2H]^+$: 283(18) $[M-NHN=CHC_6H_4Cl]^+$.

*2-Hydroxy-2,2-bis(4-chlorophenyl)-N'-[(1E)-(4-methoxyphenyl)methylene]acetohydrazide (**1d**)*: Yield: 77%, light brown solid, R_f=0.63 (ethyl acetate-hexane, 6:4), m.p. (°C): 187-190, IR (KBr, cm^{-1}): 3346 (-OH), 3265 (-NH), 3050 (=CH), 2950 (-CH), 1664 (-NHC=O), 1593 (-C=N), 1570, 1448 (-C=C- aromatic), 806 (-CCl), Anal. calcd for $C_{22}H_{18}N_2O_3Cl_2$ (m.w.: 429.30): C, 61.55; H, 4.23; N, 6.53. Found: C, 61.49; H, 4.35; N, 6.66. LC-MS/MS: (*m/z*) (%) 428 (19), $[M-H]^+$: 430 (15), $[M+H]^+$: 438 (15), $[M+4H]^+$: 288(100) $[M-C_6H_4Cl-OCH_3+H]^+$.

*2-Hydroxy-2,2-bis(4-methylphenyl)-N'-[(1E)-(3-methylphenyl)methylene]acetohydrazide (**1e**)*: Yield: 40%, brown solid, R_f=0.74 (ethyl acetate-hexane, 6:4), m.p. (°C): 180-182, IR (KBr, cm^{-1}): 3319 (-OH), 3250 (-NH), 3050 (=CH), 2950 (-CH), 1645 (-NHC=O), 1580 (-C=N), 1570, 1450 (-C=C- aromatic), 825 (*p*-disubs.), 734, 684 (*p*-disubs.), Anal. calcd for $C_{24}H_{24}N_2O_2$ (m.w.: 372.47): C, 77.39; H, 6.49; N, 7.52. Found: C, 77.13; H, 6.61; N, 7.45. LC-MS/MS: (*m/z*) (%) 373 (05), $[M+H]^+$: 283(100) $[M-C_6H_4CH_3+2H]^+$.

Table 1. ^{1}H NMR data for the compounds 1a-1e*, DMSO-d_6, 400 MHz (*J*= Hz)

H	**1a**	**1b**	**1c**	**1d**	**1e**
2',6'	7.41, bs	7.41, bs	7.45, bs	7.61, d, *J* =7.6	7.64, d, *J*=7.6
3',5'	7.40, bs	7.45, bs	7.45, bs	7.69, d, *J* =7.6	7.25, d, *J* =7.6
2"	7.64, d, *J* =8.0	7.45, s	8.05, bs, J=3.0	7.05, d, *J* =8.0	7.5, bs, *J* =3.0
3"	7.48, d, *J* =8.0	-	-	7.03, d, *J* =8.0	-
4"	-	7.21, d, *J* =7.2	8.26, d, *J* =7.4	-	7.39, m
5"	7.48, d, *J* =8.0	7.30, d, *J* =7.2	7.70, t, *J* = 7.4	7.03, d, *J* =8.0	7.39, m
6"	7.64, d, *J* =8.0	7.21, d, *J* =7.2	7.42, d, *J* =7.5	7.05, d, *J* =8.0	7.63, m
CH=N	8.51, bs	8.48, bs	8.48, s	8.41, s	8.51, s
NH	11.59, bs	11.59, bs	11.63, bs	11.81, bs	11.72, bs
$-CH_3$	-	2.31, s, 3H	-	-	2.35, s, 6H
$-OCH_3$	-	-	-	3.81, s, 3H	-
$-CH_3$	-	-	-	-	2.33, s, 3H

*^{1}H NMR data is assigned by the help of 2D-COSY and ACD NMR program.

Table 2. ^{13}C NMR data for the compounds **1**a-**1**e*, DMSO-d_6, 100 MHz.

C	1a	1b	1c	1d	1e
1	169.35	169.24	169.78	169.02	169.13
2	80.13	80.21	80.24	80.11	80.12
1'	142.75	142.82	142.66	142.89	142.88
2',6''	128.26	128.25	128.29	129.00	128.24
3',5'	129.75	129.75	129.75	129.96	129.77
4'	135.04	134.64	132.94	132.73	132.81
1''	132.81	132.79	136.54	127.24	140.47
2''	129.16	129.71	124.75	129.24	127.56
3''	129.40	138.50	147.26	114.81	137.02
4''	133.62	131.31	121.36	161.39	131.10
5''	129.40	127.82	131.10	114.81	129.93
6''	129.16	124.95	133.83	129.24	128.90
CH=N	148.30	149.66	148.63	148.55	148.70
$-CH_3$	-	21.31	-	-	21.48
$-OCH_3$	-	-	-	55.75	-

*^{13}C NMR data is assigned by the help of ACD NMR program.

2.3. MCF-7 and PC-3 Assay (Cell viability)

Cell viability was determined by 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. MCF-7 human breast[28] and PC-3 human prostate cancer cell lines[29] (ATCC, USA) were seeded in 48-well plate and incubated for 24 h to form a semi-confluent layer. After 24 h, cells were exposed to four different concentrations of compounds (2, 10, 50 and 100 µM) dissolved in dimethyl sulfoxide (DMSO). After 24 h incubation, MTT was added to all wells at 0.5 mg/mL of concentration and incubated an additional 2 h at 37 °C. After discarding the medium from plates, 100 µl of isopropanol was added to the wells. Absorbance of the MTT formazan was determined at 570 nm by a UV-spectrophotometric plate reader (BioTek ELx808™, Turkey). Viability was defined as the ratio (expressed as a percentage) of absorbance of the cells exposed to compounds to the cells treated with 0.5% DMSO (as control). As a reference, doxorubicin HCl (Sigma-Aldrich, EP) was used. All the measurements were conducted in triplicates.

2.3.1. Statistical analysis

GraphPad Prism 6 was used for all the statistical analyses. Data related to cell viability was analyzed by using one-way ANOVA following the post-hoc tests. Differences were considered as significant at $p < 0.05$.

3. Result and Discussion

3.1. Synthesis

In this work, due to the biological evaluation, novel 2-hydroxy-2,2-bis(4-substitutedphenyl)-*N*'-[(1*E*)-(3/4-substitutedphenyl)methylene]-acetohydrazides (**1a-1e**) were synthesized (Scheme). Substituted benzaldehyde was converted to benzoin by refluxing in ethanol in the presence of KCN. Benzoin was oxidized to benzil with HNO_3. Alkali treatment of benzil yielded 2-hydroxy-2,2-diphenylacetic acid, which was then esterified and reacted with hydrazine hydrate in ethanol to give 2-hydroxy-2,2-diphenylacetohydrazide. Finally, acetohydrazide compounds reacted with substituted aromatic aldehydes to give intermediate Schiff bases (**1a-1e**) in the range of 40-77% yields. All of the synthesized compounds (**1a-1e**) were identified by spectroscopic methods such as 1D-NMR (^1H, ^{13}C/APT) and 2D-NMR (^1H-^1H COSY and NOESY), FT-IR, LC-MS/MS, elemental analyses and by the help of ACD NMR program.

In the ^1H NMR spectra (Table 1), the low-field signals of the amide group were observed at 11.59-11.81 ppm, which resonate together to give a singlet (**1a, 1b,** and **1d**). The aromatic protons of the *p*-

substituted phenyl group of compounds **1a-1e** form the characteristic AB-system (doublets H-2,6 and H-3,5 or bs). In the ^{1}H NMR spectra of compounds **1a-1e**, a characteristic singlet of azomethine proton at 8.48–8.51 ppm was observed. The location of the azomethine (N=CH) proton signals at 8.48–8.51 ppm provided an opportunity to confirm the *E/Z* arrangement of the substituents around the double bond. The 2D NOESY spectra (Figure S1) showed correlation between amide –NH to azomethine (N=CH) protons, leading to an *E* geometrical isomer of all the synthetic compounds (**1a-1e**). The signals of the carbon at 148.30-149.66 ppm in the ^{13}C NMR spectra (Table 2) were characteristic for azomethine (N=CH) carbon, which are an indication of hydrazine group of acetohydrazide (**1a-1e**).

3.2. Anticancer activity (MCF-7 and PC-3 Cell viability)

Synthesized 2-hydroxy-2,2-bis(4-substitutedphenyl)-*N*'-[(1*E*)-(3/4-substituted-phenyl)-methylene]-acetohydrazides (**1a-1e**) were evaluated for anticancer activity against MCF-7 and PC-3 in a concentration of 100 μM. IC_{50} values of the tested compounds on MCF-7 and PC-3 cell lines are given in Table 3. All the tested compounds showed cytotoxic activity on MCF-7 and PC-3 cell lines at the highest experimental concentration (100 μM). As seen in Figure S2, all the compounds showed statistically significant cytotoxicity compared to control group. Notably, compounds **1b** and **1e** have strong anti-proliferative activity on MCF-7 cell line, while compound **2** has the strongest activity on PC-3 cell line. In contrast to activity observed on MCF-7 cell line, compound **1e** has the weakest inhibitory activity on PC-3 cell line compared to other derivatives. Figure 2 represents anti-proliferative activities of the compounds at the highest experimental dose (100 μM) on MCF-7 and PC-3 cell lines. [28-29]

In the literature, various substituted carboxylic acid hydrazides compounds were reported to show selective cytotoxic activities toward cell lines of the NSC lung and breast cancers. Moreover, hydrazones inhibited the growth of ovarian cancer cell lines by 35.2–44.0%. But, it is mentioned that hydrazides of dicarboxylic acids were practically inactive compounds which had an antiproliferative effect against the cell line HS 578T of breast cancer. In our case, screening of the –Cl, $–CH_3$, $-NO_2$, and $-OCH_3$ substituted 2-hydroxy-2,2-bis(4-phenyl)-*N*'-[(1*E*)-(3/4-phenyl)methylene]acetohydrazide (**1a-1e**) against MCF-7 and PC-3 cell lines revealed that –Cl and $–CH_3$ substituted hydrazones **1b** and **1e** were found to be the best inhibitors against breast and prostate cancers.

Table 3. IC_{50} values on MCF-7 and PC-3 cell line for compounds **1a-1e**.

Compounds	MCF-7	PC-3
	IC_{50} (μM) ± SD	
1a	100 ≤	100 ≤
1b	18.24 ± 7.62	45.81 ± 1.10
1c	27.56 ± 5.66	53.12 ± 5.41
1d	43.13 ± 3.13	90.17 ± 6.83
1e	07.62 ± 1.85	100 ≤
Doxorubicin[a]	0.065 ± 0.016	2.96 ± 0.08

[a]Reference compound.

4. Conclusion

In the present paper, synthetic procedure for the new 2-hydroxy-2,2-bis(4-substitutedphenyl)-*N*'-[(1*E*)-(3/4-substitutedphenyl)-methylene]-acetohydrazide (**1a-1e**) was described and they were found to be very useful reactive intermediates of various N-heterocycles. Five synthesized compounds were tested for anticancer activity against breast and prostate cancer cell lines. This investigation showed that the most active compounds were 2-hydroxy-2,2-bis(4-methylphenyl)-N'-[(1E)-(3-methylphenyl)-methylene]acetohydrazide (**1e**) and 2-hydroxy-2,2-bis(4-chlorophenyl)-N'-[(1E)-(3-methylphenyl)-methylene]acetohydrazide (**1b**) on MCF-7 cell line, and 2-hydroxy-2,2-bis(4-chlorophenyl)-N'-[(1E)-(3-methylphenyl)methylene]aceto-hydrazide (**1b**) has the strongest activity on PC-3 cell line. Thus, compounds **1b** and **1e** would be useful for the development of new anticancer drugs against breast and prostate cancers.

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Supporting Information

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