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1,5-diphenylpenta-1,4 dien-3-ones: A novel class of free radical scavengers

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A series of 1,5-diphenylpenta-1,4-dien-3-ones were synthesized by conjugating benzalacetone with different substituted benzaldehydes. The general synthetic strategy employed for the synthesis of the compounds was based on mixed aldol condensation reaction in the presence of strong base. The structures of newly synthesized compounds were characterized by spectral and elemental analysis. Their radical scavenging activity was evaluated through the determination of their abilities to inhibit free radicals using $DPPH^{\cdot}$ as a stable radical. Ascorbic acid (**AA**) was used as reference antioxidant compound and also the comparative study with the synthesized compounds were done. The compounds that showed potential antioxidant activity based on IC_{50} value from the highest level were**: 2e, 2b** and **2a**.

Key words : Benzalacetone, aldehydes and radical scavenging activity

INTRODUCTION

Free radical is an atom or molecule that has one or more unpaired electrons. Theoretically, free radical will be formed if a covalent bond happens to break [1]. Recent evidence [2] suggests that free radicals, which are generated in many bioorganic redox processes, may induce oxidative damage in various components of the body (e.g., lipids, proteins and nucleic acids) and may also be involved in the process leading to the formation of mutations. Furthermore, radical reactions play a significant role in the development of life – limiting chronic diseases such as cancer, hypertension, cardiac infarction, arteriosclerosis, rheumatism, cataracts and others [3]. Oxidative stress seems to be caused by increased production of reactive oxygen species (ROS) and altered cellular redox states [4,5]. ROS are by–products of a variety of pathways of aerobic metabolism. They are unstable and react readily with a wide range of biological substrates such as lipids, DNA and protein resulting in cell damage [6–8].

A series of compounds that can scavenge radicals by trapping, initiating and/or propagating radicals are called as 'antioxidants' [9]. Since the pioneering work on antioxidants by Ingold in the 1980s [10–12] there has been a continuous search for structures with improved or modified antioxidant properties. In recent years, epidemiological studies show that consumption of food with high phenolic content correlates with decreasing cardiovascular diseases [13, 14]. Phenolic compounds may produce their beneficial effect by scavenging free radicals [15, 16]. Study of chemical compounds and their derivatives related with a specific pharmacological activity to find a quantitative structure – activity relationships is very useful for the rational planning of new drugs and have brought many benefits for developing new treatments.

Recently, synthesis of hydroxyl radical scavengers from benzalacetone and its derivatives have been reported [17]. Benzalacetone has a conjugated system and is expected to be easily oxidized [18, 19]. The more the double bond, the easier it will be oxidized. Therefore it is assumed that benzalacetone and its derivatives may show antioxidant activity. With this background, in an effort to develop novel antioxidants of this class, we synthesized a series of benzalacetone derivatives and evaluated their antioxidant activities. The coupling of different aldehydes having different active sites with benzalacetone was introduced to explore the structure-activity relationship.

CHEMISTRY

The general synthetic strategy employed for the preparation of the compounds under study was based on mixed aldol condensation reaction. The starting benzaldehyde (**1)** was treated with acetone in the presence of 10% NaOH at room temperature to get benzalacetone (**2)** (**Scheme 1**). Coupling of benzalacetone with various substituted aldehydes

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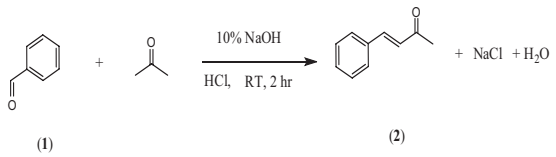
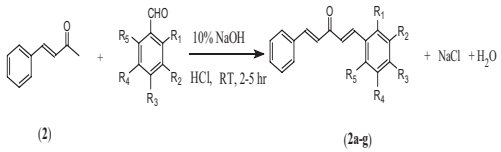
(hydroxy benzaldehyde, nitro benzaldehyde, trimethoxy benzaldehyde, methoxy benzaldehyde, chloro benzaldehyde, tolualdehyde) using NaOH as base gave respective 1,5-diphenylpenta-1,4-dien-3-ones (**2a–g**) as outlined in **Scheme 2**.



Scheme 1. Reaction protocol for the synthesis of benzalacetone (2)



Scheme 2. Reaction pathway for the synthesis of 1,5-diphenylpenta-1,4-dien-3-ones (2a–g).

where the radicals R_i are given in Table 1.

Table 1.

Compound	R_1	R_2	R_3	R_4	R_5
2a	H	H	H	H	H
2b	H	H	OCH_3	H	H
2c	H	OCH_3	OCH_3	OCH_3	H
2d	H	H	Cl	H	H
2e	H	H	OH	H	H
2f	H	H	NO_2	H	H
2g	H	H	CH_3	H	H

EXPERIMENTAL

Materials and Methods

Thin-layer chromatography (TLC) was used to assess the reactions and the purity of the products. The compounds (**2** and **2a–g**) were purified by column chromatography using 9:1 hexane : ethyl acetate. All the reported melting points were taken in open capillaries and are reported uncorrected. IR spectra were recorded in KBr (pellet form) on a Nicolet 5700 FT–IR spectrophotometer and only noteworthy absorption values (cm^{-1}) are listed. ^1H NMR spectra were recorded at 500 MHz on Bruker DRX–500MHz spectrometer using $CDCl_3$ as solvent. Mass spectra of the synthesized compound were obtained using a Q-TOF Waters Ultima instrument (No-Q-Tof GAA 082, Water Corporation, Manchester, UK) fitted with an Electron spray ionization (ESI) source. The data acquisition software used was Version 4.0. UV–visible spectrophotometer (Shimadzu 160A) was used for recording the absorbance of the test solutions. All the chemicals were purchased from the Sigma-Aldrich, Himedia, S.d.fine.chem, Rankem, Ranbaxy, E-Merck, India.

Procedure for the synthesis of benzalacetone (2)

To a well stirred solution of benzaldehyde (0.1 mole) and acetone (0.3mole), 2.5 ml of 10% NaOH solution was added drop wise until the solution turned pale yellow. The solution was stirred for 2 hr at room temperature (RT). The progress of the reaction was monitored by using TLC using hexane: ethyl acetate as mobile solvent. After the completion of the reaction, the reaction mixture was treated with dil. HCl until the mixture become slightly acidic. Further, the product was extracted by using diethyl ether. The aqueous layer was removed and the organic layer was washed thrice with triple distilled water and dried over anhydrous sodium sulphate. The solvent was removed by using a rotary evaporator at 35 ºC. The residual portion was distilled under reduced pressure and the fraction boiling at 150–155ºC / 25 mm Hg was collected. The distillate was cooled and the light yellow crystals of benzalacetone separated out.

Yellow solid, Yield (86.3%), M.p. 40-43ºC. IR (KBr) ν_{max} (cm^{-1}): 3053 – 2829.5 (Ar C–H), 1670.9 (C=O); ^1H NMR (250 mHz) ($CDCl_3$) δ (ppm): δ 7.3 – 7.6 (m, 5H, Ar-H), 6.6-7.6 (d, 2H, olefinic protons), 2.2 (s, 3H, methylene proton); Mass (m/z %): M^+ 146.19; Anal. Calcd. for $C_{10}H_{10}O$: C, 82.16; H, 6.89; O, 10.94%; Found: C,82.13; H,6.87; O, 10.96%

*General procedure for the synthesis of substituted 1,5-diphenylpenta-1,4dien-3-ones (**2a-g**)*

To a well stirred solution substituted benzaldehyde (0.01mole) and benzalacetone (0.012 mole), 2.5 ml of 10% NaOH solution was added drop wise. The solution was stirred for 2–5 hr at room temperature (RT). The progress of the reaction was monitored by using TLC using hexane: ethyl acetate as mobile solvent. After the completion of the reaction, the reaction mixture was treated with dil. HCl until the mixture become slightly acidic. Further, the product was extracted by using diethyl ether. The aqueous layer was removed and the organic layer was washed thrice with triple distilled water. The product was obtained by further desolventation in rotary evouporator at 35 ºC.

*1,5-diphenylpenta-1,4-dien-3-one, Compound (**2a**)*

Yellow solid, Yield (82.7%), M.p. 108 – 110.5ºC. IR (KBr) ν_{max} (cm^{-1}): 3065 – 2698.5 (Ar

C–H), 1677.9 (C=O); ^{1}H NMR (250 mHz) ($CDCl_3$) δ (ppm): δ 7.3 – 7.6 (m, 10H, Ar-H), 7.0–7.8 (d, 4H, olefinic proton); Mass (m/z %): M^+ 234.29; Anal. Calcd. For$C_{17}H_{14}O$: C,87.15; H,6.02; O, 6.83%; Found: C,87.13; H,6.05; O, 6.86%

*1-(4-methoxyphenyl)-5-phenylpenta-1,4-dien-3-one Compound (**2b**)*

Yellow semisolid, Yield (69.48%). IR (KBr) ν_{max} (cm^{-1}): 3045–2789.5 (Ar C–H), 1666.9 (C=O); ^{1}H NMR (250 mHz) ($CDCl_3$) δ (ppm): δ 6.9–7.6 (m, 9H, Ar-H), 7-7.8 (m, 4H, olefinic proton), 3.8 (s, 3H, OCH_3); Mass (m/z %): M^+ 264.10; Anal. Calcd. for $C_{18}H_{16}O_2$: C, 81.79; H, 6.10; O, 12.11%; Found: C, 81.77; H, 6.12; O, 12.12%

*1-phenyl-5-(3,4,5-trimethoxyphenyl)penta-1,4dien-3-one, Compound (**2c**)*

Yellow solid, Yield (94.71%), M.p. 64-67ºC. IR (KBr) ν_{max} (cm–^{1}): 3051–2811 (Ar C–H), 1670.9 (C=O); ^{1}H NMR (250 mHz) ($CDCl_3$) δ (ppm): δ 6.7-7.6 (m, 7H, Ar-H), 7.0-7.8 (m, 4H, olefinic proton), 3.81 (s, 9H, $3OCH_3$); Mass (m/z %): M^+ 324.14; Anal. Calcd. for $C_{20}H_{20}O_4$: C,74.06; H,6.21; O,19.73 %; Found: C,74.04; H,6.24; O, 19.71%

*1-(4-chlorophenyl)-5-phenylpenta-1,4-dien-3-one, Compound (**2d**)*

Yellow solid, Yield (92.42%), M.p. 70-75^{0}C. IR (KBr) ν_{max} (cm^{-1}): 3100–2729.5 (Ar C–H), 1650.2 (C=O); ^{1}H NMR (250 mHz) ($CDCl_3$) δ (ppm): δ 7.3–7.6 (m, 9H, Ar-H), 7.3–7.8 (m, 4H, Olefinic proton); Mass (m/z %): M^+ 268.74; Anal. Calcd. for $C_{17}H_{13}ClO$: C,75.98; H,4.88; Cl,13.19; O, 5.95%; Found: C,75.96; H,4.85; Cl,13.20; O, 5.91%

*1-(4-hydroxy)-5-phenylpenta-1,4-dien-3-one, Compound (**2e**)*

Yellow semisolid, Yield (70.38%). IR (KBr) ν_{max} (cm^{-1}): 3043–2787.5 (Ar C–H), 1670.1 (C=O), 3222.4–3500.8 (phenolic-OH); ^{1}H NMR (250 mHz) ($CDCl_3$) δ (ppm): δ 7.3–7.6 (m, 9H, Ar-H), 7.3-7.8 (m, 4H, Olefinic proton), 9.4 (s, 1H, phenolic OH); Mass (m/z %): M^+ 250.29; Anal. Calcd. for $C_{17}H_{14}O_2$: C,81.58; H,5.64; O, 12.78%; Found: C,81.56; H, 5.63; O, 12.75%

Brown solid, Yield (93.39%), M.p. 148–152ºC. IR (KBr) ν_{max} (cm^{-1}): 3003–2729.9 (Ar C–H), 1674.9 (C=O); ^{1}H NMR (250 mHz) ($CDCl_3$) δ (ppm): δ 7.3–7.6 (m, 9H, Ar-H), 7.3-7.8 (m, 4H, Olefinic proton); Mass (m/z %): M^+ 279.09; Anal. Calcd. for $C_{17}H_{13}NO_3$: C,73.11; H,4.69; N,5.02; O, 17.19%; Found: C,73.14; H,4.71; N, 5.04; O, 17.18%

*1-phenyl-5-p-tolylpenta-1,4-dien-3-one, Compound (**2g**)*

Yellow semisolid, Yield (73.71%). IR (KBr) ν_{max} (cm^{-1}): 3033 – 2723.2 (Ar C–H), 1661.9 (C=O); ^{1}H NMR (250 mHz) ($CDCl_3$) δ (ppm): δ 7.3 – 7.6 (m, 9H, Ar-H), 7.3-7.8 (m, 4H, Olefinic proton), 2.3 (s, 3H, methylene proton); Mass (m/z %): M^+ 248.32; Anal. Calcd. for $C_{18}H_{16}O$: C,87.06; H,6.49; O, 6.44%; Found: C,87.02; H,6.47; O, 6.41%

DPPH RADICAL SCAVENGING ASSAY

The radical scavenging activities of the synthesized compounds were evaluated using a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH). A number of methods are available for the determination of free radical scavenging activity but the assay employing stable DPPH radical has received most attention owing to its ease of use and its convenience [20]. The solutions of respective compounds were prepared in different concentrations (10, 25, 50, 100, 200 and 500 μM/mL) in methanol and mixed with constant volume of 0.1mM DPPH solution. The absorbance was measured at 517 nm against methanol as blank after the incubation for 20 min. The percentage (%) radical scavenging activity (RSA) was calculated by using the formula,

$$\% \text{ RSA} = [(A_o - A_1)/ A_o] \times 100$$

Where, A_o is the absorbance of the control (blank, without compound) and A_1 is the absorbance of the compound.

RESULTS AND DISCUSSION

In the course of the present research aimed at the development of benzalacetone analogues as a novel class of free radical scavengers, mixed aldol condensation reaction was employed as a method of choice to obtain the target compounds. The synthetic protocol employed for the preparation of the new analogues was based on mixed aldol condensation reaction. Benzaldehyde (**1**) was treated with acetone in the presence of 10% NaOH at room temperature to get benzalacetone (**2**) (**Scheme 1**). Further, coupling of benzalacetone

with various substituted aldehydes using NaOH as base gave respective 1,5-diphenylpenta-1,4-dien-3-ones (**2a**–**g**) as outlined in **Scheme 2**. ^{1}H NMR, IR, mass spectra and elemental analysis were used to confirm the structure in purity of the newly synthesized compounds.

The IR spectra of benzalacetone and analogues revealed the characteristic absorption of aromatic ketone. The strong bands in the region 1665 – 1648 cm^{-1} were assigned to C=O stretching of the aromatic ring system. In the all the compounds, the aromatic stretching was found at the region of 3100 – 2698 cm^{-1}. On the other hand, in ^{1}H NMR spectra of all 1,5-diphenylpenta-1,4-dien-3-ones (**2a-g**), the aromatic protons appeared at 6.6 – 7.6 ppm as multiplet and all the olefinic protons were appeared at 7.0–7.8 ppm as doublet. Addition to this in compound (**2b**) methoxy proton resonated as singlet at 3.8 ppm. Whereas, nine protons corresponding to three methoxy group in compound (**2c**)were observed as singlet at 3.4 – 3.8 ppm. In compound (**2e**) phenolic –OH proton appeared as singlet at 9.4 ppm and methyl proton was observed as singlet at 2.3 – 3.0 ppm in compound (**2g**). Mass spectra of all synthesized analogues showed M^+ peak in agreement with their molecular formula.

In order to evaluate antioxidant properties of the newly synthesized 1,5–diphenylpenta-1,4-dien-3-ones (**2a**–**g**) the effects on free radical were examined using DPPH assay according to the Blois method [21]. Their percentage DPPH activity at different concentration is depicted in Figure 1.

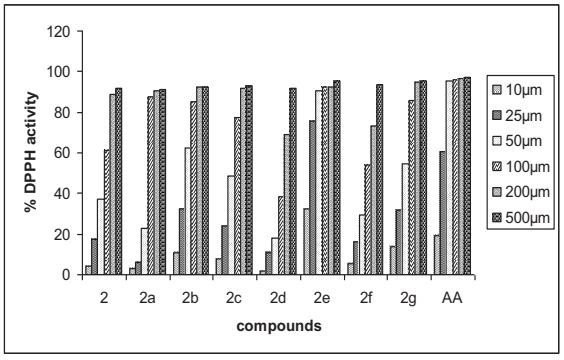


Fig.1. Percentage DPPH activity for the benzalacetone and 1,5-diphenylpenta-1,4-dien-3-ones (**2a**–**g**) with internal standard (**AA**).

All the synthesized compounds scavenged DPPH radical significantly in a concentration dependent manner. IC_{50} value was calculated by linear regression algorithm and the results were compared with that of internal standard ascorbic acid (**AA**). The respective IC_{50} values of the synthesized compounds and standard are listed in Table 2.

Table 2. 50% Inhibition of DPPH radical (IC_{50}) by benzalacetone and 1,5-diphenylpenta-1,4-dien-3-ones.

Compound	[a]IC_{50} (µM/mL)
2	62.5±0.65
2a	22.5±0.12
2b	17.5±1.02
2c	52.5±0.97
2d	125±0.43
2e	10±0.64
2f	87.5±0.86
2g	37.5±0.33
AA	22.5±0.21

[a]Each value represents means ± SD (n=3)

Initially, compound (**2a)** showed comparatively good activity. Further, incorporation of different substituents in to the phenolic moiety of compound (**2a)** was done and their influence on radical scavenging activity was studied. The introduction of electron withdrawing groups (NO_2 and Cl) in compounds (**2f**) and (**2d**) significantly reduced the DPPH activity, while that of electron donating groups (OCH_3, OH) in compound (**2e**) and (**2b**) enhanced the activity. On the other hand, compound (**2c)** bearing three methoxy group on the benzene ring exhibits less activity compared to compound (**2b)** having single methoxy group. This might be due to the steric factor and probability of the formation of the intramolecular hydrogen bonding. Incorporation of –OH group in to the benzene ring enhance the RSA exhibiting dominant activity among the analogues. The increasing orders of DPPH activity of newly synthesized compounds are as follows: **2e > 2b > 2a ≥ AA > 2g > 2c > 2 > 2f > 2d**

CONCLUSION

The compounds under study were synthesized by mixed aldol condensation. The radical scavenging activities of newly synthesized compounds were evaluated by using DPPH radical assay. Although most of the compounds synthesized showed considerable inhibition percentages, the compounds **(2e), (2b),** and **(2a)** were the most active. From the present study, conclusion could be drawn that the nature of the substituent on the aromatic ring and the presence of conjugated double bonds played a critical role in exerting the antioxidant activity. The information from the present study may warrant further in-depth biological evaluations.

ABBREVIATIONS

^oC = centigrade
min = minute
hr = hour
mL = milli Liter
μM = micro molar
% = percentage
IC_{50} = 50 percent Inhibition concentration
nm = nano meter
mM = milli molar
M= molar
RT = room temperature
AA = Ascorbic acid
DPPH = 2, 2-diphenyl-1-picrylhydrazyl
TLC = Thin layer chromatography
IR= Infrared
^{1}H NMR= proton nuclear magnetic resonance
MP= melting point
KBr= potassium bromide
RSA= radical scavenging activity

REFERENCES

1. S. Atun, R. Arianingrum, S. Handayani, Rudyansyah, M. Garson, *Indonesian J. Chem.,* **7**, 83 (2007)
2. G.C. Yen, H.Y Chen, *J. Agric. Food Chem.,* **43**, 27 (1995)
3. C. Soler–Rivas, J.C. Espin, H.J. Wichers, *Phytochem. Anal.,* **11**, 330 (2000).
4. H. Kaneto, Y. Kajimoto, J. Miyagawa, T. Matsuoka, Y. Fujitani, M. Hori, *Diabetes*., **48**, 2398 (1999).
5. A. Rudich, A. Tirosh, R. Potashnik, R. Hem, H. Kanety, N. Bashan, *Diabetes.*, **47,** 1562 (1998).
6. B. Halliwell, J.M.C. Gutteridge (1989) In Free Radicals in Biology and Medicine, 2nd ed.; Halliwell, B.; Gutteridge, J. M.C.; Eds.; Oxford University Press, Clarendon: Oxford, pp 1 – 20.
7. M. Roberfroid, P.P. Colderon In Free Radicals and Oxidation Phenomena in Biological Systems; M. Roberfroid, B. Colderon; Eds.; University Of Catholique de Lauvain Brussels: New York, pp **11** (1995).
8. J.M. Braughler, L.A. Duncan, R.L. Chase, *J. Biol. Chem*., **261**, 10282 (1986)
9. F. Shahidi, P.K.J.P.D. Wanasundara, *Crit. Rev. Food Sci. Nutr.,***32**, 67 (1992).
10. G.W. Burton, K.U. Ingold, *J Am Chem Soc*., **102**, 7791 (1980)
11. G.W. Burton, K.U. Ingold, *J.Am Chem Soc*., **103,** 6472 (1981)
12. L.R.C. Barclay, K.U. Ingold, *J Am Chem Soc*., **103**, 6478 (1981)
13. M.G.L. Hertog, P.M. Sweetnam, A.M. Fehily, P.C. Elwood, D. Kromhout, *Am. J. Clin. Nutr.,* **65**, 1489 (1997).
14. M.I. Gil, F.A. Thomas-Barberan, B. Hess-Pierce, B. M. Holcroft, A.A. Kader., *J. Agr. Food Chem*., **48**, 4581 (2000)
15. B.N. Ames, *Science*., **221**,1256 (1983)
16. R. Pulido, L. Bravo, F. Saura-Calixto *J. Agr. Food Chem.,* **48**, 3396 (2000).
17. S. Handayani, S. A. Indyah, *J. Physical Science.,* **19**, 61 (2008).
18. S.Handayani Synthesis of 4'-methoxyflavanon by chalcone isomerization,. Proceeding of the International Seminar on Organic Chemistry, UGM, Yogyakarta (2001).
19. S. Handayani, *Indonesian J. Chem.,* **5**, 22 (2005).
20. A. Abdelnaser, Elzaawely, D.X Tran, K. Haruo, T. Shinkichi, *Food Chem.*, **104**, 1648 (2007).
21. M.S. Blois, *Natur*e., **181**, 1199 (1958).

1,5-ДИФИНИЛПЕНТА-1,4 ДИЕН-3-ОНИ: НОВ КЛАС АНТИОКСИДАНТИ

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(Резюме)

Синтезирани са серия от 1,5 дифенилпента-1,4-диен-3-они чрез свързване на бензалацетон с различни заместени бензалалдехиди. Общата стратегия, приложена за синтеза на съединенията се основава на смесена алдол кондензационна реакция в присъствието на силна база. Структурите на новосинтезираните съединения се характеризират чрез спектрален и елементен анализ. Тяхната антиоксидантна активност е оценена чрез определяне способността им да инхибират свободните радикали, като използват DPPH$^{\cdot}$ като стабилен радикал. Аскорбинова киселина (АК) е била използвана като сравнителен антиоксидант, и е извършено сравнително изследване със синтезираните съединения. Съединенията, които показват най-висока потенциална антиоксидантна активност, въз основа на IC_{50} стойностите са: **2e**, **2b** и **2a**.