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ORIGINAL ARTICLE

THE ANTIOXIDANT POTENTIAL EVALUATION OF DICLOFENAC DERIVATIVES WITH HYDRAZONES STRUCTURE

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Abstract

A series of diclofenac hydrazones was synthesized and the structure of the compounds was proved using different spectral methods such as infrared (IR), nuclear magnetic resonance (^{1}H-NMR) and high resolution mass spectroscopy (HR-MS). The biological evaluation, focused on *in vitro* antioxidant effects using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)) free radicals scavenging assays was also performed. An improved antioxidant effect of the synthesized compounds compared to diclofenac was observed.

Rezumat

O serie de derivați ai diclofenacului cu structură de hidrazonă a fost sintetizată, iar structura compușilor a fost confirmată utilizându-se diferite metode spectrale: spectroscopie în infraroșu (IR), rezonanță magnetică nucleară (^{1}H-RMN) și spectroscopie de masă de înaltă rezoluție (HR-MS). Evaluarea biologică a urmărit determinarea *in vitro* a efectelor antioxidante față de radicalii DPPH (1,1-difenil-2-picrilhidrazil) și ABTS (acid 2,2'-azinobis(3-etilbenzotiazolin-6-sulfonic)). Pentru compușii sintetizați s-a observat o acțiune antioxidantă îmbunătățită comparativ cu cea a diclofenacului.

Keywords: diclofenac, hydrazone, antioxidant effects

Introduction

Classical NSAIDs (nonsteroidal anti-inflammatory drugs) are one of the most used class of medication worldwide, primarily based on their effectiveness as anti-inflammatory, analgesic and antipyretic agents [9, 12, 15]. Since diclofenac therapy is associated with several side effects, especially at the gastrointestinal and renal level, the researchers have focused their research work on the derivatization at the free carboxyl group in order to obtain new compounds with improved toxicological profile. Recent studies have also highlighted that reactive oxygen species are involved in triggering of various diseases including inflammatory conditions and the diseases associated [2, 5].

Advances in molecular biology and rational drug design approaches have led to the successful identification of novel anti-inflammatory targets [10, 11]. In order to improve the pharmacological profile and to reduce the side effects of the diclofenac, a series of hydrazones derivatives of diclofenac has been synthetized and biologically evaluated in terms of antioxidant effects.

Materials and Methods

Reagents: diclofenac sodium, hydrazine hydrate 64%, aromatic aldehydes (2-nitro/4-cyan/3-nitro/3-ethoxy-4-hydroxy/4-bromo-2-nitro/2-chloro-5-trifluoro-methyl/4-methoxy/4-methyl/3-trifluormethyl/3,4-difluoro/3-bromo-4-hydroxy/2,5-dibromo/2-bromo-4-fluoro/4-bromo-2-fluoro/3-fluoro-4-methyl/4-fluoro/3-methoxy-4-methyl/2-bromo-3-hydroxy-4-methoxy - benzaldehyde), solvents proanalysis (p.a.) (ethanol, dioxane, diethyl ether, ethyl acetate, petroleum ether, n-pentane), reagents used as catalysts (acetic acid, sulfuric acid, hydrochloric acid), dimethylsulph-oxide-d_6 (DMSO-d_6), standard reagents for radical scavenging assays were purchased from Sigma Aldrich. All reagents and solvents were used without prior purification.

Synthesis. The synthesis of hydrazone derivatives of diclofenac was performed according to the procedure described in Figure 1 [3, 6].

The reactions were monitored using TLC (Thin Layer Chromatography) Silica gel 60 F254 plates produced by Merck Company and the spots were visualized using UV light.

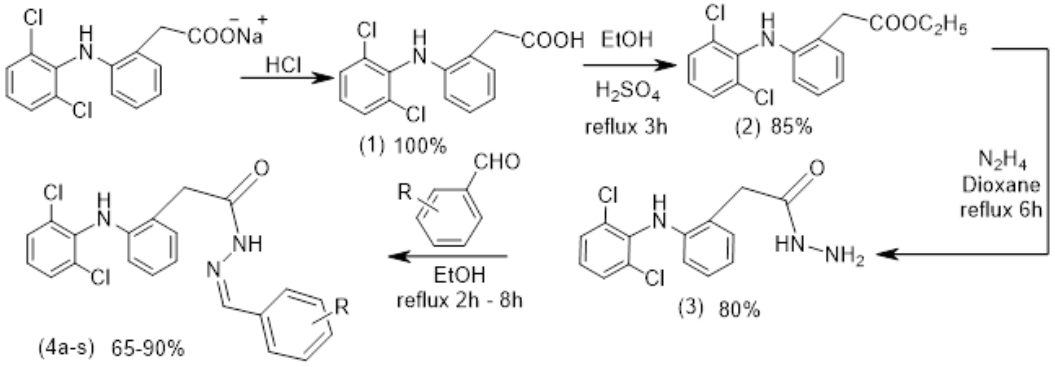


Figure 1.
The synthesis of hydrazone derivatives of diclofenac (**4a-s**)

Physico-chemical and spectral characterization. The melting points were measured using a Buchi Melting Point B-540 apparatus and they are uncorrected. The infrared (IR) spectra were recorded on a Thermo-Nicolet AVATAR 320 AEK0200713 FT-IR Spectrometer, at a resolution of 4 cm^{-1} after 6 scans in the 4000 - 500 cm^{-1} range. The spectra were processed using the Omnic Spectra Software. The ^{1}H-NMR spectra were recorded with a Bruker Avance Spectrometer 400 MHz, using tetramethylsilane (TMS) as internal standard and DMSO-d_6 as solvent. The chemical shifts were shown in δ values (ppm). The mass spectra were recorded using a Bruker MaXis Ultra-High Resolution Quadrupole Time-of-Flight Mass Spectrometer.

Biological evaluation. The antioxidant effects were evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)) free radicals scavenging assays.

The DPPH Radical Scavenging Assay. The tested compounds were dissolved in DMSO to obtain a stock solution of 10 mg/mL. From the stock solution there were taken different samples (50 µL, 100 µL, 200 µL, 300 µL, 400 µL, 500 µL) which were diluted with methanol in order to obtain 1300 µL. Over the resulting samples 1200 µL of methanol solution of DPPH (0.1 mM, A_{517nm} = 1.0 ± 0.05) were added. The concentration of the compounds tested in the obtained samples was 0.2 mg/mL, 0.4 mg/mL, 0.8 mg/mL, 1.2 mg/mL, 1.6 mg/mL and 2.0 mg/mL respectively. The mixture was stirred and left for 1 h in the dark, after which the absorbance at 517 nm was measured using methanol as blank sample [4, 8, 13]. The DPPH radical scavenging ability of the tested compounds was calculated as the inhibition percentage (I%) using the formula:

$$I\% = ((A_0 - A_t)/A_0) \times 100,$$

wherein A_0 = the absorbance value of the DPPH methanolic solution of 0.1 mM; A_t = the absorbance value of the tested compounds. For each compound it was calculated the effective concentration 50 (EC_{50}) by linear regression and ascorbic acid (1 mg/mL) was used as a positive control. All determinations were performed in triplicate and the results were expressed as arithmetic average ± standard deviation (SD).

The ABTS$^{\bullet+}$ Radical Scavenging Assay. The ABTS$^{\bullet+}$ free radical was generated by treating of the aqueous solution of 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (7 mM) with ammonium persulfate (2.45 mM), after that the resulting mixture was kept in the dark for 12 to 16 h to promote the formation of ABTS$^{\bullet+}$ free radical. The tested compounds were dissolved in DMSO to obtain a stock solution of 10 mg/mL. From the stock solution were taken different samples (12.5 µL, 25 µL, 50 µL, 100 µL, 150 µL, 200 µL) which were diluted with DMSO up to the volume of 200 µL and then 1800 µL of ABTS$^{\bullet+}$ solution was added. The concentration of the compounds tested in the obtained samples was 0.061 mg/mL, 0.125 mg/mL, 0.25 mg/mL, 0.50 mg/mL, 0.75 mg/mL and 1.0 mg/mL respectively. The mixture was left to stand for 6 min, after which the absorbance at 734 nm was measured using ethanol as blank sample [7, 14, 16, 17].

The ABTS radical scavenging ability of the tested compounds was calculated as the inhibition percentage (I%) using the formula:

$$I\% = ((A_0 - A_t)/A_0) \times 100,$$

wherein A_0 = the absorbance value of the ethanolic solution of ABTS$^{\bullet+}$, A_t = the absorbance value of the tested compounds. For each compound it was calculated the effective concentration 50 (EC_{50}) by linear regression and ascorbic acid (1 mg/mL) was used as a positive control. All determinations were performed in triplicate and the results were expressed as arithmetic average ± standard deviation (SD).

Results and Discussion

Synthesis. The hydrazones derivatives of diclofenac (**4a-s**) were synthesized according to Figure 1. Sodium diclofenac, in the presence of HCl, gave diclofenac, acid form (**1**) which was transformed into the corresponding ethyl ester (**2**) by reaction with ethyl alcohol. The reaction of the compound (**2**) with an excess of hydrazine hydrate 64% resulted diclofenac hydrazide (**3**). Then, the condensation of the compound

(**3**) with different aromatic aldehydes led to the formation of the corresponding hydrazones (**4a–s**) in good yields.

Physico-chemical and spectral characterization. The hydrazone derivatives of diclofenac (**4a-s**) are crystalline powders, which have a coloration that varies from white to yellow, are soluble in DMSO, sparingly soluble in acetone, insoluble in methanol, chloroform, benzen, dioxane, diethyl ether. The molecular weight calculated (m/z) and found ($[M+H]^+$) are presented in Table I together with physico-chemical features (yield, melting point, Rf).

Table I

The physico-chemical and mass spectra data of hydrazone derivatives (**4a-s**)

Comp.	*R*	*η (%)*	*m.p. (^{0}C)*	*Rf**	*m/z calculated*	*$[M+H]^+$ found*
4a	2-NO_2	91	223	0.39	442.108359	442.108114
4b	4-CN	70	249	0.41	423.077416	423.077393
4c	3-NO_2	82	234	0.40	442.108359	442.108217
4d	3-OCH_2CH_3-4-OH	65	243	0.37	458.103273	458.103331
4e	4-Br-2-NO_2	90	238	0.42	522.977734	522.977581
4f	2-Cl-5-CF_3	80	235	0.41	500.030556	500.030460
4g	4-OCH_3	75	256	0.40	428.092709	428.092864
4h	-H	72	262	0.40	398.082144	398.082158
4i	4-CH_3	92	272	0.38	412.146230	412.146128
4j	3-CF_3	82	261	0.39	466.069529	466.069573
4k	3,4-diF	78	239	0.43	434.063300	434.063406
4l	3-Br-4-OH	84	233	0.42	493.156794	493.156548
4m	2,5-diBr	71	287	0.41	556.156794	556.156508
4n	2-Br-4-F	65	229	0.43	495.151315	495.151159
4o	4-Br-2-F	69	230	0.43	495.151315	495.151644
4p	3-F-4-CH_3	85	268	0.39	430.088372	430.088427
4q	4-F	88	225	0.39	416.072722	416.072626
4r	3-OCH_3-4-CH_3	83	274	0.33	442.156794	442.156693
4s	2-Br-3-OH-4-OCH_3	67	262	0.41	523.156794	523.156693

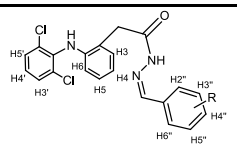
*petroleum ether:ethyl acetate = 8.0:2.0 v/v;

In the FT-IR spectra the appearance of the stretching band of the imine group (-N=CH-) at 1628 - 1474 cm^{-1} [6] confirms the successfully formation of the hydrazones derivatives of diclofenac. The characteristic bands of the aromatic ring appeared in the range of 3024 - 2839 cm^{-1}, and between 1580 - 1287 cm^{-1}, and the phenyl ring substituents were observed at: 3194 cm^{-1} (OH), 2222 cm^{-1} (CN), 1266 - 1180 cm^{-1} (OCH_3), 1342 - 1304 cm^{-1} (NO_2), 1297 - 1080 cm^{-1} (C-F), 1080 - 941 cm^{-1} (C-Br), 887 - 663 cm^{-1} (C-Cl).

The structure of the hydrazone derivatives is strongly supported also by the ^{1}H-NMR spectra where the proton of -CH=N- resonates as singlet, between 8.37 - 7.85 ppm [1]. FT-IR and ^{1}H-RMN data are listed in Table II.

Table II

The FT-IR and ^{1}H-RMN data of hydrazone derivatives (**4a-s**)



No	*^{1}H-NMR signals δ (ppm)*	*FT-IR characteristic band (cm^{-1})*
4a	3.73 - 4.13 (2H, 2s, CH_2CO), 6.31 (1H, m, H3), 6.87 (1H, dt, H5), 7.17 (3H, m, H4, H4″, H5″), 7.64 (4H, m, H6, H3′, H4′, H5′), 8.07 (2H, m, H3″, H6″), 8.37 (1H, d, CH=N), 8.65 (1H, s, NH), 12.01 (1H, d, CONH)	3734, 3279, 3032 (-NH-), 2361, 1580, 1342 (CH_{Ar}), 1651 (-CO-NH), 1628 (-CH=N), 1566, 1304 (C-NO_2), 849, 787, 748, 702 (-C-Cl)
4b	3.74 - 4.17 (2H, 2s, CH_2CO), 6.31 (1H, m, H3), 6.87 (1H, dt, H5), 7.16 (3H, m, H4, H6, H4′), 7.54 (3H, m, H3′, H5′, H5″), 7.92 (3H, m, H2″, H3″, H6″), 7.96 (1H, s, CH=N), 8.20 (1H, d, NH), 11.92 (1H, s, CONH)	3742, 3263, 3040 (-NH-), 3001, 1504, 1450 (CH_{Ar}), 2222 (CN), 1666 (-CO-NH), 1589 (-CH=N), 841, 764, 702 (-C-Cl)
4c	3.71 - 4.11 (2H, 2s, CH_2CO), 6.32 (1H, m, H3), 6.88 (1H, dt, H5), 7.19 (3H, m, H4, H4″, H5″), 7.64 (4H, m, H6, H3′, H4′, H5′), 8.07 (2H, m, H2″, H6″), 8.37 (1H, d, CH=N), 8.65 (1H, s, NH), 12.09 (1H, d, CONH)	3734, 3271, 3024 (-NH-), 2361, 1583, 1515 (CH_{Ar}), 1651 (-CO-NH), 1528 (-CH=N), 1566, 1304 (C-NO_2), 872, 810, 741, 679 (-C-Cl)

No	*^{1}H-NMR signals δ (ppm)*	*FT-IR characteristic band (cm^{-1})*
4d	1.35 (3H, m, CH_3), 3.68 - 4.11 (2H, 2s, CH_2CO), 4.03 (2H, dq, OCH_2), 6.30 (1H, dd, H3), 6.85 (2H, m, H5, H5″), 7.17 (5H, m, H6, H4, H4′, H2″, H6″), 7.53 (2H, m, H2′, H5′), 7.83 (1H, d, CH=N), 8.12 (1H, d, NH), 9.45 (1H, d, OH), 11.54 (1H, d, CONH)	3734, 3518, 3286 (-NH-), 3194 (-OH), 2361, 1512, 1443 (CH_{Ar}), 1643 (-CO-NH-), 1512 (-CH=N), 1180 (O-C), 864, 833, 748, 717 (-C-Cl)
4e	3.69 - 4.08 (2H, 2s, CH_2CO), 6.27 (1H, m, H3), 6.83 (1H, m, H5), 7.14 (3H, m, H4, H6, H4′), 7.49 (3H, m, H2′, H5′, H6″), 7.96 (2H, m, H3″, H5″), 8.25 (1H, m, CH=N), 8.43 (1H, d, NH), 12.01 (1H, d, CONH)	3734, 3271, 3186 (-NH-), 2993, 1529, 1257 (CH_{Ar}), 1649 (-CO-NH), 1574 (-CH=N), 1450, 1342 (C-NO_2), 995 (-C-Br), 879, 748, 687 (-C-Cl)
4f	3.70 - 4.12 (2H, 2s, CH_2CO), 6.26 (1H, dd, H3), 6.82 (1H, m, H5), 7.12 (3H, t, H4, H3″, H4″), 7.45 (2H, m, H6, H4′), 7.77 (3H, m, H3′, H5′, H6″), 8.12 (1H, d, CH=N), 8.51 (1H, 2s, NH), 12.03 (1H, 2s, CONH)	3302, 3178 (-NH-), 3024, 1566, 1504 (CH_{Ar}), 1659 (-CO-NH-), 1504 (-CH=N), 1257, 1149, 1080 (C-F), 825, 748, 717, 663 (-C-Cl)
4g	2.04 (3H, s, CH_3), 3.65 - 4.07 (2H, 2s, CH_2CO), 6.26 (1H, m, H3), 6.82 (1H, m, H5), 7.08 (5H, m, H4, H6, H4′, H2″, H6″), 7.55 (4H, m, H3′, H5′, H3″, H5″), 7.85 (1H, d, CH=N), 8.12 (1H, d, NH), 11.56 (1H, d, CONH)	3256, 3009 (-NH-), 2901, 1512, 1450 (CH_{Ar}), 1643 (-CO-NH-), 1597 (-CH=N), 1257 (O-C), 833, 756, 717, 679 (-C-Cl)
4h	3.71 - 4.14 (2H, 2s, CH_2CO), 6.31 (1H, dd, H3), 6.87 (1H, dt, H5), 7.19 (3H, m, H4, H6, H4′), 7.50 (5H, m, H3′, H5′, H3″, H4″, H5″), 7.74 (2H, dd, H2″,H6″), 8.07 (1H, d, CH=N), 8.24 (1H, s, NH), 11.73 (1H, d, CONH)	3286, 3016 (-NH-), 1504, 1450 (CH_{Ar}), 1643 (-CO-NH), 1574 (-CH=N), 748, 694, 687 (-C-Cl)
4i	2.41 (3H, s, CH_3), 3.70 - 4.13 (2H, 2s, CH_2CO), 6.31 (1H, dd, H3), 6.86 (1H, dt, H5), 7.19 (5H, m, H4, H6, H4′, H6″, H2″), 7.58 (4H, m, H3′, H5′, H3″, H5″), 8.07 (1H, d, CH=N), 8.37 (1H, d, NH), 11.66 (1H, d, CONH)	3256, 3178 (-NH-), 2901, 1504, 1450 (CH_{Ar}), 1643 (-CO-NH-), 1566 (-CH=N), 818, 748, 710, 679 (-C-Cl)
4j	3.74 - 4.16 (2H, 2s, CH_2CO), 6.31 (1H, dd, H3), 6.87 (1H, dt, H5), 7.18 (3H, m, H4, H6, H4′), 7.62 (5H, m, H3′, H5′, H4″, H5″, H6″), 8.02 (1H, dd, H2″), 8.11 (1H, d, CH=N), 8.33 (1H, s, NH), 11.90 (1H, d, CONH)	3279, 3194 (-NH-), 3016, 1566, 1450 (CH_{Ar}), 1651 (-CO-NH-), 1504 (-CH=N), 1297, 1273, 1157 (C-F), 771, 748, 694, 671 (-C-Cl)
4k	3.72 - 4.15 (2H, 2s, CH_2CO), 6.31 (1H, dd, H3), 6.87 (1H, dt, H5), 7.19 (3H, m, H4, H6, H4′), 7.89 (5H, m, H2′, H5′, H2″, H5″, H6″), 7.94 (1H, d, CH=N), 8.13 (1H, d, NH), 11.83 (1H, d, CONH)	3317, 3070 (-NH-), 2962, 1582, 1450 (CH_{Ar}), 1659 (-CO-NH-), 1512 (-CH=N), 1281, 1203, 1165 (C-F), 856, 810, 764, 656 (-C-Cl)
4l	3.69 - 4.10 (2H, 2s, CH_2CO), 6.30 (1H, dd, H3), 6.86 (1H, dt, H5), 7.16 (4H, m, H4, H6, H4′, H2″), 7.62 (4H, m, H3′, H5′, H5″, H6″), 7.89 (1H, d, CH=N), 8.11 (1H, d, NH), 10.76 (1H, d, OH), 11.63 (1H, d, CONH)	3495, 3279 (-NH-), 3186 (-OH), 3001, 1497, 1443 (CH_{Ar}), 1643 (-CO-NH-), 1589 (-CH=N), 1080 (-C-Br), 818, 748, 710, 671 (-C-Cl)
4m	3.70 - 4.12 (2H, 2s, CH_2CO), 6.26 (1H, dd, H3), 6.82 (1H, m, H5), 7.12 (3H, t, H4, H3″, H4″), 7.45 (2H, m, H6, H4′), 7.77 (3H, m, H3′, H5′, H6″), 8.12 (1H, d, CH=N), 8.51 (1H, 2s, NH), 12.03 (1H, 2s, CONH)	3904, 3734 (-NH-), 3078, 1497, 1443 (CH_{Ar}), 1612 (-CO-NH-), 1589 (-CH=N), 1080, 1026 (-C-Br), 802, 725, 694 (-C-Cl)
4n	3.69 - 4.08 (2H, 2s, CH_2CO), 6.27 (1H, m, H3), 6.83 (1H, m, H5), 7.14 (3H, m, H4, H6, H4′), 7.49 (3H, m, H2′, H5′, H6″), 7.96 (2H, m, H3″, H5″), 8.25 (1H, m, CH=N), 8.43 (1H, d, NH), 12.01 (1H, d, CONH)	3205, 3101 (-NH-), 2361, 1389 (CH_{Ar}), 1589 (-CO-NH), 1474 (-CH=N), 1281, 1203, 1180 (C-F), 1034, 941 (-C-Br), 887, 856, 818, 663 (-C-Cl)
4o	3.70 - 4.08 (2H, 2s, CH_2CO), 6.28 (1H, m, H3), 6.83 (1H, m, H5), 7.14 (3H, m, H4, H6, H4′), 7.50 (3H, m, H2′, H5′, H6″), 7.96 (2H, m, H3″, H5″), 8.25 (1H, m, CH=N), 8.43 (1H, d, NH), 12.02 (1H, d, CONH)	3308, 3070 (-NH-), 1404, 1389 (CH_{Ar}), 1620 (-CO-NH), 1597 (-CH=N), 1265, 1211 (C-F), 1057, 957 (-C-Br), 879, 839, 671 (-C-Cl)
4p	2.27 (3H, s, CH_3), 3.71 - 4.14 (2H, 2s, CH_2CO), 6.31 (1H, dd, H3), 6.87 (1H, m, H5), 7.22 (4H, m, H4, H2″, H5″, H6″), 7.53 (4H, m, H6, H3′, H4′, H5′), 8.03 (1H, d, CH=N), 8.20 (1H, 2s, NH), 11.76 (1H, d, CONH)	3263, 3171, 3001 (-NH-), 2901, 1504, 1450 (CH_{Ar}), 1643 (-CO-NH), 1566 (CH=N), 1265, 1188, 1157 (C-F), 872, 833, 771, 656 (-C-Cl)
4q	3.70 - 4.13 (2H, 2s, CH_2CO), 6.31 (1H, dd, H3), 6.86 (1H, dt, H5), 7.06 (1H, q, H4), 7.25 (4H, m, H6, H4′, H3″,H5″), 7.53 (2H, m, H3′, H5′), 7.79 (2H, m, H2″,H6″), 8.06 (1H, d, CH=N), 8.24 (1H, s, NH), 11.73 (1H, d, CONH)	3340, 3070 (-NH-), 2962, 1582 (CH_{Ar}), 1660 (-CO-NH-), 1504 (-CH=N), 1296, 1203, 1165 (C-F), 856, 810, 764, 656 (-C-Cl)

No	*^{1}H-NMR signals δ (ppm)*	*FT-IR characteristic band (cm^{-1})*
4r	2.18 (3H, s, CH_3), 3.71 - 4.14 (2H, 2s, CH_2CO), 3.83 (3H, d, OCH_3), 6.31 (1H, dd, H3), 6.87 (1H, dt, H5), 7.21 (6H, m, H4, H6, H4′, H2″, H5″, H6″), 7.56 (2H, m, H3′, H5′), 8.04 (1H, d, CH=N), 8.20 (1H, 2s, NH), 11.69 (1H, d, CONH)	3286, 3001 (-NH-), 2908, 1574, 1450 (CH_{Ar}), 1643 (-CO-NH-), 1504 (-CH=N), 1266 (O-C), 810, 748, 710, 663 (-C-Cl)
4s	3.73 - 4.14 (2H, 2s, CH_2CO), 3.85 (3H, d, OCH_3), 6.34 (1H, dd, H3), 6.89 (1H, dt, H5), 7.23 (5H, m, H4, H6, H4′, H5″, H6″), 7.59 (2H, m, H3′, H5′), 8.06 (1H, d, CH=N), 8.22 (1H, 2s, NH), 10.78 (1H, d, OH), 11.72 (1H, d, CONH)	3263, 3032 (-NH-), 3117 (-OH), 2839, 2361, 1279 (CH_{Ar}), 1597 (-CO-NH-), 1489 (-CH=N), 1211 (O-C), 1026 (-C-Br), 825, 663 (-C-Cl)

The antioxidant effects evaluation

The DPPH Radical Scavenging Ability. From the obtained results (Table III) it could be observed that all tested compounds showed improved DPPH radical scavenging ability in reference with diclofenac, for which the EC_{50} was 1.71 ± 0.023. The most active compounds are **4d** (EC_{50} = 0.06 ± 0.004) and **4s** (EC_{50} = 0.07 ± 0.005) which were obtained by condensation of diclofenac hydrazide (**3**) with 3-ethoxy-4-hydroxybenzaldehyde (**4d**) and 2-bromo-3-hydroxy-4-methoxybenzaldehyde (**4s**) respectively. Appreciable anti-radicalic activity showed also **4c** (EC_{50} = 0.23 ± 0.012), **4n** (EC_{50} = 0.28 ± 0.002), **4h** (EC_{50} = 0.35 ± 0.015), **4r** (EC_{50} = 0.36 ± 0.008), **4l** (EC_{50} = 0.37 ± 0.013) and **4j** (EC_{50} = 0.40 ± 0.014). Compared to ascorbic acid, used as a positive control, all tested compounds were less active in similar conditions.

Table III

The DPPH radical scavenging ability (EC_{50}, mg/mL) of hydrazone derivatives (**4a-s**)

No	*EC_{50} mg/mL*	*No*	*EC_{50} mg/mL*	*No*	*EC_{50} mg/mL*
Diclofenac	1.71 ± 0.023	**4g**	1.42 ± 0.033	**4n**	0.28 ± 0.002
4a	2.33 ± 0.018	**4h**	0.35 ± 0.015	**4o**	1.56 ± 0.038
4b	2.02 ± 0.045	**4i**	0.96 ± 0.041	**4p**	1.31 ± 0.031
4c	0.23 ± 0.012	**4j**	0.40 ± 0.014	**4q**	2.14 ± 0.026
4d	0.06 ± 0.004	**4k**	2.23 ± 0.022	**4r**	0.36 ± 0.008
4e	1.65 ± 0.012	**4l**	0.37 ± 0.013	**4s**	0.07 ± 0.005
4f	2.65 ± 0.042	**4m**	2.33 ± 0.026	**AA**	0.006± 0,004

AA – ascorbic acid; Data are mean ± SD (n = 3, $p < 0.05$)

The $ABTS^{\bullet+}$ Radical Scavenging Ability. From the results obtained (Table IV) it could be observed that the most part of tested compounds showed improved $ABTS^{\bullet+}$ radical scavenging ability in reference with diclofenac, for which the EC_{50} was 0.62 ± 0.027. The most active compounds are **4d** (EC_{50} = 0.07 ± 0.004) and **4s** (EC_{50} = 0.08 ± 0.003) which were obtained by condensation of diclofenac hydrazide (**3**) with 3-ethoxy-4-hydroxybenzaldehyde (**4d**) and 2-bromo-3-hydroxy-4-methoxybenzaldehyde (**4s**) respectively. Appreciable anti-radicalic activity showed also **4p** (EC_{50} = 0.12 ± 0.001), **4g** (EC_{50} = 0.12 ± 0.007), **4l** (EC_{50} = 0.13 ± 0.008), **4r** (EC_{50} = 0.19 ± 0.008), **4h** (EC_{50} = 0.21 ± 0.010) and **4c** (EC_{50} = 0.31 ± 0.015). Compared to ascorbic acid, used as a positive control, all tested compounds were less active in similar conditions.

Table IV

The ABTS radical scavenging ability (EC_{50}, mg/mL) of hydrazone derivatives (**4a-s**)

No	*EC_{50} mg/mL*	*No*	*EC_{50} mg/mL*	*No*	*EC_{50} mg/mL*
Diclofenac	0.62 ± 0.027	**4g**	0.12 ± 0.007	**4n**	1.03 ± 0.022
4a	0.67 ± 0.020	**4h**	0.21 ± 0.010	**4o**	1.06 ± 0.038
4b	0.48 ± 0.017	**4i**	0.38 ± 0.014	**4p**	0.12 ± 0.001
4c	0.31 ± 0.015	**4j**	0.30 ± 0.020	**4q**	1.08 ± 0.026
4d	0.07 ± 0.004	**4k**	0.39 ± 0.009	**4r**	0.19 ± 0.008
4e	1.72 ± 0.048	**4l**	0.13 ± 0.008	**4s**	0.08 ± 0.003
4f	0.74 ± 0.018	**4m**	0.98 ± 0.029	**AA**	0.005 ± 0,004

AA – ascorbic acid; Data are mean ± SD (n = 3, $p < 0.05$)

Conclusions

A series of 19 hydrazones of diclofenac (**4a-s**) has been synthesized and optimal conditions of reaction were established. All the synthetized compounds were characterized in terms of solubility in different organic solvents, melting point, yield, molecular formula, and the chemical structure was proved using FT-IR, ^{1}H-NMR and HR-MS spectroscopy. The evaluation of the antioxidant potential of hydrazone derivatives was performed using two *in vitro* methods: DPPH and ABTS radical scavenging assay. The results obtained support that the antioxidant effect of tested compounds

increases with the concentration and is influenced by the nature of the substituent on the aromatic ring. The most active compounds were **4d** and **4s**, which were obtained by condensation of diclofenac hydrazide with 3-ethoxy-4-hydroxy-benzaldehyde and 2-bromo-3-hydroxy-4-methoxybenzaldehyde respectively, which showed the lowest values of EC_{50}, for both free radical assays. The results showed that the chemical modulation of diclofenac by introducing a imine group has a favourable influence on antioxidant potential of the synthetized compounds.

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Conflict of interest

The authors declare no conflict of interest.

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