https://doi.org/10.31925/farmacia.2022.4.3 **ORIGINAL ARTICLE**

RECENT ADVANCES IN THE STUDY OF DERIVATIVES OF (*EZ*)-N'-BENZYLIDENE-(2RS)-2-(6-CHLORO-9*H*-CARBAZOL-2-YL) PROPANOHYDRAZIDE

ALEXANDRA TEODORA (TELEHOIU) BORDEI [1], CARMEN LIMBAN [1]*, DIANA CAMELIA NUȚĂ [1], IRINA ZARAFU [2], ELEONORA DENES [3], LUMINIȚA MĂRUȚESCU [4,5], MARIANA CARMEN CHIFIRIUC [4,5,6], MARCELA POPA [4,5], CORINA ARAMĂ [1]

[1]*Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy, 6 Traian Vuia, 020956, Bucharest, Romania*
[2]*Faculty of Chemistry, University of Bucharest, 4-12 Regina Elisabeta, 030018 Bucharest, Romania*
[3]*Supramolecular Organic and Organometallic Chemistry Centre, Department of Chemistry, Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University, 11 Arany János, 400028 Cluj-Napoca, Romania*
[4]*Department of Microbiology and Immunology, Faculty of Biology, University of Bucharest, 91-96 Splaiul Independenței, Bucharest, Romania*
[5]*Research Institute of the University of Bucharest, University of Bucharest, 90 Panduri Road, Bucharest, Romania*
[6]*Academy of Romanian Scientist, 54 Splaiul Independetei, Bucharest, Romania*

**corresponding author: carmen.limban@umfcd.ro*

Manuscript received: February 2022

Abstract

This study aims to complete the molecular characterization of some compounds with the original structure, derivatives of (*EZ*) -N'-benzylidene-(2RS) -2- (6-chloro-*9H*-carbazol-2-yl) propanohydrazide, by HR-MS and thermal analysis. The results of the antimicrobial action tests are also presented. The tests were performed on two Gram-negative bacterial strains (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853), two Gram-positive bacterial strains (*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212) and one fungal strain (*Candida albicans* ATCC 10231) and including qualitative and quantitative tests, being also determined the anti-biofilm activity. Compounds **1a**, **1d** and **1b** exhibited good *in vitro* antimicrobial activity, with a MIC (minimum inhibitory concentration) value of 0.31 mg/mL against Gram-positive bacterial and fungal strain. The results also indicated that the compounds are more potent against microbial biofilms.

Rezumat

Acest studiu își propune să completeze caracterizarea moleculară a unor compuși cu structură originală, derivați ai (*EZ*)-N'-benziliden-(2RS)-2-(6-cloro-*9H*-carbazol-2-il) propanohidrazidei prin analiză spectrală HR-MS și analiză termică. Sunt prezentate și rezultatele testării acțiunii antimicrobiane. Testele au fost efectuate pe două tulpini de bacterii Gram negative (*Escherichia coli* ATCC 25922 și *Pseudomonas aeruginosa* ATCC 27853), două tulpini de bacterii Gram pozitive (*Staphylococcus aureus* ATCC 25923 și *Enterococcus faecalis* ATCC 29212) și o tulpină fungică (*Candida albicans* ATCC 10231), utilizând metode de testare calitative și cantitative, fiind determinată inclusiv activitatea anti-biofilm. Compușii **1a**, **1d** și **1b** au prezentat o activitate antimicrobiană bună *in vitro*, cu o valoare CMI (concentrație minimă inhibitorie) de 0,31 mg/mL împotriva bacteriilor Gram pozitive și a tulpinii fungice. Rezultatele au indicat, de asemenea, că acești compuși sunt mai activi împotriva biofilmelor microbiene.

Keywords: carbazole, carprofen, Schiff bases, thermal analysis, HR-MS analysis, antimicrobial activity

Introduction

The carbazole nucleus can be found in the structure of some chemical substances having different biological activities such as olivacin – antimalarial, rimcazole – antipsychotic and anticonvulsant, carvedilol – anti-hypertensive, ondansetron – antiemetic, 1,8-dimethoxy-3-formylcarbazole – antibacterial and antifungal agent [4], or some active natural compounds (ellipticine – antimalarial [14], koenidine – antidiabetic [18] staurosporine with antibacterial, antifungal and antitumor activity [23], carbazomycins with antibacterial and antifungal activity [10], murrayafolin A – antifungal on *Cladosporium cucumerinum* [6]).

A number of isolated natural compounds of the species *Murraya koenigii*, namely mahanine, murayanin, mahanimbine, girinimbine and murayanol, have anti-fungal activity against human pathogenic fungi or anti-bacterial activity and also inhibit topoisomerases I and II [28].

A study from 2014 about new derivatives of 5-[(*9H*-carbazol-9-yl)methyl]-2-[(R-phenyl)(piperazine-1-yl)aminomethyl]-1,3,4-oxadiazole, designed by the introduction of a pharmacophore with an oxadiazole nucleus, at position 9 of the carbazole ring showed

that they have good antimicrobial activity, especially on *Staphylococcus aureus* [26].

A series of diamide macrocyclic systems containing the carbazole ring, namely carbazolophane amides, have been synthesized and they have demonstrated antimicrobial activity. The antibacterial effect of these compounds was tested on different pathogens such as *Proteus mirabilis, Proteus vulgaris, Staphylococcus aureus* and *Salmonella typhi*, and the antifungal activity was tested on plant pathogenic species: *Rhizoctonia solani, Macrophomina phaseolina, Curvularia lunata*. One of the compounds showed even better activity compared to tetracycline and carbendazim, used as standards [22].

Zhang *et al.* studied the influence of the pharmacophore nuclei imidazole or triazole included in the structure of a carbazole derivative, on the antibacterial and antifungal action. *In vitro* studies were performed on *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus proteus*, *Candida albicans* and *Aspergillus fumigatus* strains and showed that the molecule containing imidazole has good antibacterial activity and compounds with a triazole nucleus have lower antibacterial activity, while antifungal activity on *Candida albicans* is increased [32].

Carprofen (2-(6-chloro-*9H*-carbazol-2-yl)propanoic acid) is a non-steroidal anti-inflammatory drug with a carbazole nucleus, a derivative of propionic acid, with anti-inflammatory, analgesic and antipyretic action, currently used exclusively in veterinary medicine, as a symptomatic treatment of osteoarthritis and post-operative pain in dogs [27]. It inhibits the activity of cyclooxygenases 1 and 2, resulting in decreased formation of prostaglandin and thromboxane precursors. It is also known that Schiff bases have demonstrated various pharmacodynamic actions such as antimicrobial [17, 29], tuberculostatic [12], anthelmintic [13], anti-inflammatory and analgesic [15], or antitumor [8]. In addition, they can be used in the synthesis of new heterocyclic compounds, in the identification, detection, analysis and purification of carbonyl compounds, or in the protection of carbonyl functional groups, in complex syntheses [1].

N-acyl hydrazones are more stable than ordinary Schiff bases and are obtained by condensing a hydrazide with a carbonyl compound. They may also have a more pronounced biological activity than ordinary Schiff bases, because they may form hydrogen bonds with biochemical systems through oxygen and nitrogen atoms [3].

New 6-phenyl substituted derivatives of N'-(*E*)-(methylidene)-2-methylpyridine-3-carbohydrazide were synthesized and tested for antibacterial activity on *Staphylococcus aureus, Enterococcus faecalis, Escherichia. coli* and *Pseudomonas aeruginosa* strains, using ciprofloxacin as standard, and the results showed a good antibacterial effect [16].

Starting from the structure of naproxen, an NSAID used in therapy, a new compound, N'-[(*E*)-(5-bromo-2-hydroxyphenyl)methylidene]-2-(6-methoxy-naphthalen-2-yl)propanehydrazide, was obtained, and it that was tested for its antimicrobial effect, by disc-diffusion method, on *Escherichia coli, Staphylococcus aureus, Salmonella typhi* and *Bacillus subtilis*, the effect being better than that of ciprofloxacin used as reference substance [25].

Another study focused on the synthesis of new 5-aryl-substituted N-(furan-2-yl)-methylidene)-hydrazides, which were evaluated for their *in vitro* antimicrobial effect on Gram-positive strains (*Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis* and *Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli, Salmonella typhi, Shigella dysenteriae* and *Klebsiella pneumoniae*), by the disc-diffusion method, using ampicillin as reference substance. All compounds showed good activity on the tested species [11].

Considering the previously presented data, we combined the pharmacophore fragments represented by the carbazole nucleus and the propionic acid fragment from the chemical structure of carprofen, as well as the azo-methine functional group of Schiff bases, in a single N-acyl hydrazone molecule. The new N-acyl hydrazones were analysed using HR-MS and tested for their anti-microbial action.

Materials and Methods

Chemistry

Starting from the chemical structure of caprofen, following structural modulations, derivatives of (*EZ*)-N'-benzylidene-(2RS)-2-(6-chloro-9*H*-carbazol-2-yl) propanohydrazide (**1a-f**) were obtained; we presented in our published articles [2, 9] the synthesis and confirmation of the structure of these original compounds, realized by IR and NMR spectral experiments

The obtaining of new derivatives was based on the principles of "green chemistry", a concept that represents the design, development and implementation of chemical products and processes, in order to reduce or eliminate the use and formation of substances dangerous to human health and the environment.

High-resolution mass spectrometry (HR-MS)

APCI+ high resolution mass spectra of compounds **1a-1f** were recorded on a Thermo Scientific LTQ-Orbitrap XL spectrometer equipped with a standard ESI/APCI source. The mass spectra were processed with Thermo Xcalibur software [30].

Thermal analysis

Thermal measurements (thermogravimetric analysis - TG/DTG and differential scanning calorimetry - DSC) were performed with Mettler Toledo equipment, TGA2 and DSC3 modules (Mettler Toledo International GmbH - Switzerland).

Measurements were made in a controlled atmosphere (nitrogen), within a temperature range 25 - 600°C

(DSC) and 25 - 900°C (TG). We used uncovered Al_2O_3 90 µL pans for TG and 60 µL Al pans. Samples were accurately weighed using a Mettler Toledo balance, XSR105DU model. The samples were analysed in duplicate.

Determination of antimicrobial activity

The antimicrobial activity of the new compounds was determined against two Gram-negative bacterial strains (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853), two Gram-positive bacterial strains (*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212) and one fungal strain (*Candida albicans* ATCC 10231).

Qualitative screening of the antimicrobial activity

The antimicrobial activity was evaluated using an adapted agar diffusion method, a standardized method for antibiotic susceptibility testing, established by Clinical & Laboratory Standards Institute (CLSI M100-Ed30, 2020 [5, 31]. Briefly, microbial suspensions were prepared from 18 - 24 h cultures developed on a solid medium (Plate Count Agar for the bacterial strains and Sabouraud agar for microfungi) and their density was adjusted to 1.5×10^8 CFU/mL according to the 0.5 McFarland nephelometric standard. Then the inoculum was evenly distributed over the Muller-Hinton agar with a cotton swab. Filter paper discs (6 mm diameter; Oxoid) were placed on the inoculated agar surfaces and impregnated with 5 µL compound stock solutions prepared in DMSO (10 mg/mL). The Petri dishes were incubated at room temperature for 20 - 30 minutes to allow diffusion of the compound solutions into the medium and then incubated to 37°C for 24 hours. Inhibition zones were read manually using a ruler. Only tests with confluent growth and clearly delineated inhibition zones were measured.

Quantitative testing of antimicrobial activity

Broth microdilution testing was performed as outlined in document M100, 30th [5, 31], in 96-well microtiter plates. Microbial suspensions were prepared for each strain in sterile 0.9% saline solution. Turbidities were measured using 0.5 McFarland standard. Suspensions targeted an inoculum density equivalent to approximately 1.0×10^8 colony-forming units (CFU/mL). The suspensions were diluted 1:200 in Muller-Hinton Broth to target an inoculum concentration of approximately 5.0×10^5 CFU/mL. The tested concentrations of the solutions of the different compounds in DMSO were achieved through double serial dilutions, in columns 1 - 10, and were between 500 - 0.97 µg/mL. Then, the wells were inoculated with 10 µL of microbial suspensions. Column 11 contained 10 µL of standardized inoculum and 90 µL of Mueller Hinton Broth, and column 12 contained 100 µL of Mueller Hinton Broth (as a control to monitor sterility). Ciprofloxacin (Sigma-Aldrich, St. Louis, MO, USA) served as positive control. The 96-well plates were incubated without agitation for 24 h at 37°C. In order to confirm the minimum inhibitory concentrations (MIC), the assays were performed in triplicate. The MIC was determined as the lowest concentration of tested compound that inhibited the growth of the microorganism as detected spectrophotometrically at 620 nm with an Apollo LB 911 ELISA Reader (Berthold Technologies GmbH & Co. KG, Waltham, MA, USA) [19].

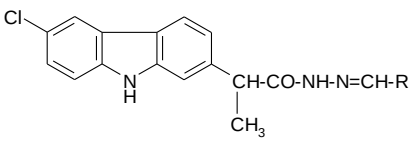
Evaluation of anti-biofilm activity

Microtiter biofilm inhibition assay was used for the evaluation of the anti-biofilm activity of tested compounds. Microbial suspensions were prepared from 18 - 24 hours cultures in sterile 0.9% saline, final OD600 = 0.01. A volume of 100 µL of Mueller Hinton Broth was added in the wells of a 96-well polystyrene microtiter plate. Stock solutions of the tested compounds (10 mg/mL in DMSO) were then pipetted in column 1. Serial two-fold dilutions were performed in columns 1 - 10. A volume of 10 µL microbial suspension was added over the different concentrations of the compounds. Column 11 contained 10 µL of standardized inoculum and 90 µL of Mueller Hinton Broth, and column 12 contained 100 µL of Mueller Hinton Broth (as a control to monitor sterility). After incubation for 24 h at 37°C under static conditions, the wells of the microplate were emptied and washed twice with phosphate-buffered saline. The microbial biofilms formed on the plastic surface of the wells were fixed with methanol for 5 min and coloured with 1% crystal violet solution for 15 min. The dye was removed with distilled water to remove the unbound dye. The fixed dye was put in suspension with 33% acetic acid and the absorbance at 492 nm was recorded with an Apollo LB 911 ELISA Reader. The biofilm inhibition was evaluated against the biofilm developed in the absence of the tested compound and the media sterility control. The minimal biofilm inhibition concentration (MBIC) was established as the lowest concentration of the tested compounds at which the decrease in absorbance value, measured at 492 nm, was observed in comparison to the positive control [21].

Results and Discussion

Chemistry

The synthesized compounds have the general formula shown in Figure 1.



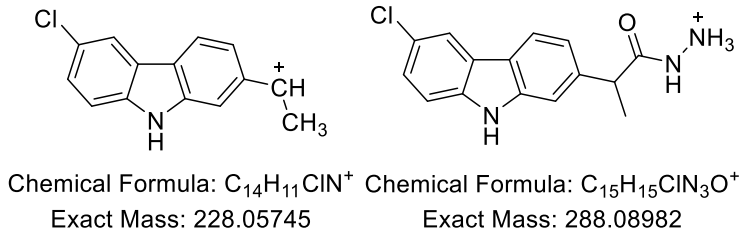
R = C_6H_4-*o*(OH) (**1a**), C_6H_4-*p*Cl (**1b**), C_6H_3-2(OH)-3(OCH_3) (**1c**), C_6H_3-2(OH)-5(OCH_3) (**1d**), C_6H_3-2,6$(Cl)_2$ (**1e**), C_6H_3-3,5$(Cl)_2$ (**1f**)

Figure 1.

Structural formula of (*EZ*)-N'-(mono/disubstituted-benzylidene)-(2*RS*)-2-(6-chloro-9*H*-carbazol-2-yl) propanehydrazide

High-resolution mass spectrometry

For all compounds, the APCI+ high resolution mass spectra were recorded in a mixture of DMSO and MeOH. The molecular peaks $[M+H]^+$ were observed as base peaks in all spectra, thus confirming the identity of the investigated species. A peak at *m/z* 228.057, corresponding to the $[C_{14}H_{11}ClN]^+$ fragment (Figure 2), was observed in all spectra, with intensities ranging from 1% to 37%, depending on the investigated compound. For compound **1f** an additional peak is detected at *m/z* 288.089, with an intensity of 38% and it was assigned to the $[C_{15}H_{15}ClN_3O]^+$ ion (Figure 2).



Chemical Formula: $C_{14}H_{11}ClN^+$ Chemical Formula: $C_{15}H_{15}ClN_3O^+$

Exact Mass: 228.05745 Exact Mass: 288.08982

Figure 2.

The structure of $[C_{14}H_{11}ClN]^+$ and $[C_{15}H_{15}ClN_3O]^+$ fragments identified in the APCI+ MS spectra

Thermal analysis

DSC curves recorded for the six compounds exhibit sharp endothermic peaks, with the melting temperatures in the 200 - 260°C range (Figure 3); melting enthalpies estimated from the peak area are presented in Table I. For compound 1a the DSC curve indicates very likely the presence of two polymorphic forms.

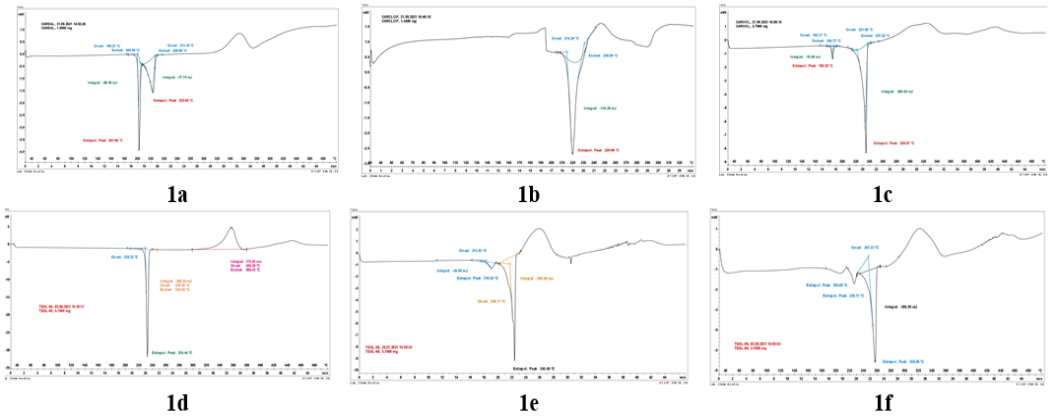


Figure 3.

DSC curves of the derivatives of (*EZ*)-N'-benzylidene-(2*RS*) -2-(6-chloro-9*H*-carbazol-2-yl)propanehydrazide (**1a-f**)

Table I

Melting temperatures and melting enthalpy of the derivatives of (*EZ*)-N'-benzylidene-(2RS) -2-(6-chloro-9H-carbazol-2-yl)propanehydrazide (**1a-f**)

Compound	Melting temperature T_m	t_{onset}	t_{endset}	$\Delta H_{melting}$		
	(°C)	(°C)	(°C)	(kJ/mol)	(J/g)	
1a	201.66	199.27	204.45	17.3458	44.4194	With decomposition
1b	229.9	214.24	230.88	33.5976	82.1458	With decomposition
1c	234.07	221.6	237.22	48.5449	96.9928	
1d	230.64	227.59	235.17	42.203	103.974	-
1e	254.99	238.21	255.12	39.089	87.9393	With decomposition
1f	256.55	239.37	259.43	28.8268	64.8522	With decomposition

During heating in the nitrogen atmosphere in the temperature range 25 - 900°C, all six compounds decompose completely without residue. The TG and DTG curves obtained for the six derivatives of (*EZ*)-N'-benzylidene-(2*RS*)-2-(6-chloro-9*H*-carbazol-2-yl)propanehydrazide (**1a-f**) are similar, with two main descending segments, first from 150 - 200°C to around 400°C, (weight loss around 60%) and the second from 400 - 500°C to 650 - 700°C (weight loss around 40%) (Figure 4, Table II).

It is noteworthy that for compounds **1a**, **1b**, **1e** and **1f** the endothermic peak corresponding to substance

melting is partially or completely superposed on the first descending segment in the TG curve, meaning that they all melt with decomposition, while thermal decomposition of **1c** and **1d** starts after melting.

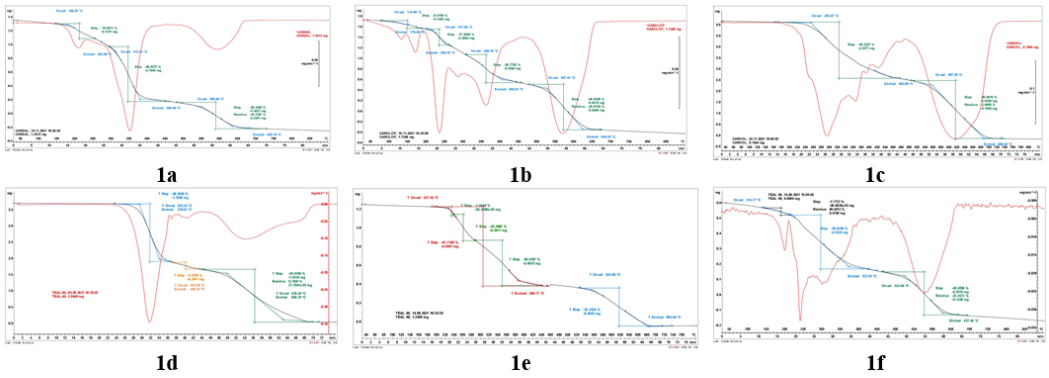


Figure 4.
TG and DTG curves of the derivatives of (*EZ*)-N'-benzylidene-(2*RS*) -2-(6-chloro-9*H*-carbazol-2-yl)propanehydrazide (**1a-f**)

Table II
Thermal decomposition stages of derivatives of (*EZ*)-N'-benzylidene-(2*RS*) -2-(6-chloro-9*H*-carbazol-2-yl)propanehydrazide (**1a-f**)

Compound	Temperature range	Weight loss
	°C	%
1a	180.57 - 373.48	80.87
	530.78 - 648.74	27.90
1b	217.04 - 405.81	48.17
	527.41 - 624.03	49.12
1c	253.47 - 452.6	49.22
	557.39 - 686.94	50.94
1d	323.52 - 440.25	53.82
	528.59 - 660.2	43.92
1e	227.45 - 386.77	67.71
	554.8 - 683.64	32.43
1f	225 - 372.91	58.63
	524.86 - 627.48	46.23

Determination of antimicrobial activity

Qualitative screening of antimicrobial activity

Qualitative screening of the antimicrobial activity revealed that the tested compounds showed a low ability to diffuse in the solid culture medium as demonstrated by the absence of clear areas of microbial growth inhibition around the discs impregnated with the tested substances. A small growth inhibition zone was obtained for *C. albicans* ATCC 10231 in case of compounds **1f.** The absence of a growth inhibition zone could be also explained by the marked hydrophobicity of the tested compounds, thus further additional studies are required to verify the antimicrobial potential, with optimized working methods.

Quantitative testing of antimicrobial activity to determine MIC

The discovery of new chemical entities with biological activity is essential for the development of novel therapeutic agents active against drug resistant pathogens [20], and in this context, we designed and synthesized a new compound having carbazole nucleus.

The quantitative testing of the antimicrobial activity showed that the majority of the tested compounds exhibited antimicrobial effects against Gram positive and Gram negative bacterial and fungal strains, with MIC values from 5 mg/mL to 0.15 mg/mL. Among the tested compounds, **1b** was the most active, with MIC value of 0.15 mg/mL against Gram positive bacteria *E. faecalis* ATCC 29212. Compounds **1a**, **1d** and **1b** exhibited good *in vitro* antimicrobial activity, with MIC value of 0.31 mg/mL against Gram positive bacteria: *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 and fungal strain *C. albicans* ATCC 10231. The microbial growth of Gram-negative bacteria was less affected by the tested compounds.

Evaluation of anti-biofilm activity by determining MBIC

Because the planktonic forms of microorganisms are less resistant than their forms in biofilms, the MIC value does not provide sufficient information concerning the efficacy of antimicrobial agents against infections involving biofilms [7]. Adhesion is the first and a key step in biofilm development and several target specific strategies are being investigated to control/inhibit bacterial biofilm development [24]. In the present study using the biofilm inhibition assay, we investigated if the compounds synthesized exhibit anti-biofilm activity. The formation of microbial biofilms on the inert surfaces was inhibited by the tested compounds at different MBIC values (5 - 0.009 mg/ mL). The results showed that the biofilms of Gram-positive bacteria and of the fungal strain *C. albicans* were more susceptible to the compounds tested in comparison with the Gram-negative ones. Compound **1f** did not influence microbial adherence of the tested microorganisms. A very good anti-biofilm activity was noticed for compound **1d** against *C. albicans* ATCC 10231, the MBIC value of 0.009 mg/mL. Also, compound **1b** was very active,

with MBIC of 0.078 mg/mL, in case of biofilms developed by the Gram-positive bacterial strains, E. *faecalis* ATCC 29212 and *S. aureus* ATCC 25923.
Comparing the two effects, it is obvious that the antibiofilm action of the tested compounds is much more intense then the antimicrobial activity. At the same time, the lack of inhibitory effect on Gram-negative strains should be noted, this being probably caused by the substitution on the benzene nucleus.
Compound **1f**, 3,5-dichloro-substituted, was totally devoid of antibacterial and antifungal effect in the performed experiments. The substitution of chloro at the 4-position of the benzene nucleus increased the antibacterial activity against *E. faecalis* ATCC 29212 and *C. albicans* ATCC 10231 (compound **1b**), and when the compound is 2-hydroxy-substituted (compound **1a**) enhanced the antimicrobial activity against *S. aureus* ATCC 25923.

Conclusions

In this study, we continued the investigation of some compounds with the original structure, derivatives of (*EZ*)-N'-(mono/disubstituted-benzylidene)-(2*RS*)-2-(6-chloro-9*H*-carbazol-2-yl)propanehydrazide.
They were characterized using HR-MS and thermal analysis. The novel compounds were evaluated against planktonic cells and biofilms of Gram-positive *E. faecalis* ATCC 29212 and *S. aureus* ATCC 25923, Gram-negative *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 and fungi *C. albicans* ATCC 10231. The results indicated that the compounds were more potent against microbial biofilms, exhibiting the potential to prevent the biofilm formation by *S. aureus, E. faecalis* and *C. albicans.* We observed no significant inhibition of microbial adherence of Gram-negative strains, thus suggesting that these compounds may act specifically against Gram-positive bacteria and fungi. The results are indicating that the investigated compounds may hold promise in the development of novel anti-biofilm agents.

Acknowledgement

This research was financially supported by "Carol Davila" University of Medicine and Pharmacy Bucharest, Romania, through Contract no. CNFIS-FDI-2022-0253, funded by the Romanian Ministry of Education.

Conflict of interest

The authors declare no conflict of interest.

References

1. Anu K, Suman B, Sunil K, Neha S, Vipin S, Schiff bases: a versatile pharmacophore. *J Catal.*, 2013; 2013: 893512: 1-13.
2. Avram S, Udrea AM, Nuta DC, Limban C, Balea AC, Caproiu MT, Dumitrascu F, Buiu C, Bordei AT, Synthesis and bioinformatic characterization of new Schiff bases with possible applicability in brain disorders. *Molecules*, 2021; 26(14): 4160: 1-13.
3. Bordei (Telehoiu) AT, Nuță DC, Mușat DG, Missir AV, Căproiu MT, Dumitrașcu F, Zarafu I, Ioniță P, Bădiceanu CD, Limban C, Ozon EA, Microwave assisted synthesis and spectroscopic characterization of some novel Schiff bases of carprofen hydrazide. *Farmacia*, 2019; 67(6): 955-962.
4. Chakraborty A, Saha C, Podder G, Chowdhury BK, Bhattacharyya P, Carbazole alkaloid with antimicrobial activity from *Clausena heptaphylla*. *Phytochemistry*, 1995; 38: 787-789.
5. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standard Institute; 2020.
6. Cuong NM, Wilhelm H, Porzel A, Arnold N, 1-O-Substituted derivatives of murrayafoline A and their antifungal properties. *Prod Res.*, 2008; 22: 1428-1432.
7. Dziedzic A, Kubina R, Wojtyczka RD, Kabała-Dzik A, Tanasiewicz M, Morawiec T, The antibacterial effect of ethanol extract of polish propolis on mutans *Streptococci* and *Lactobacilli* isolated from saliva, *Evid Based Complement Alternat Med.*, 2013; 2013: 681891: 1-12.
8. Encio I, Migliaccio M, Martinez V, Synthesis and cytotoxic activity of lipophilic sulphonamide derivatives of the benzo[b]thiophene 1,1-dioxide. *Bioorg Med Chem.*, 2004; 12: 963-968.
9. Guma (Tanasiev) N, Bordei (Telehoiu) AT, Limban C, Sogor C, Manoliu L, Avram S, Bioinformatics and cheminformatics study of carbazole derivatives - p3c7, p3c7-a20 p3c7-s243, and *de novo* 2,5-disubstituted 1,3,4-oxadiazole derivatives. *Farmacia*, 2020; 68(4): 665-671.
10. Hagiwara H, Choshi T, Fujimoto H, Sugino E, Hibino S, A novel total synthesis of antibiotic carbazole alkaloid carbazomycin G. *Tetrahedron*, 2000; 56: 5807-5811.
11. Husain A, Parscha V, Khan SA, Varshney M, Ahmad A, Synthesis and biological evaluation of new hydrazide-Schif bases. *Bangladesh J Pharmacol.*, 2015; 10(1): 555-561.
12. Joshi S, Vagdevi H, Vaidya V, Gadaginamath G, Synthesis of new 4-pyrrol-l-yl benzoic acid hydrazide analogs and some derived oxadiazole, triazole and pyrrole ring systems: a novel class of potential antibacterial and antitubercular agents. *Eur J Med Chem.*, 2008; 43: 1989-1996.
13. Mathew B, Vakketh SS, Kumar SS, Synthesis, molecular properties and anthelmintic activity of some Schiff bases of 1,3,4 thiadiazole derivatives. *Der Pharma Chem.*, 2010; 2(5): 337-343.
14. Montoia A, Rocha E, Silva LF, Torres ZE, Costa DS, Henrique MC, Lima ES, Vasconcellos MC, Souza RC, Costa MR, Grafov A, Grafova I, Eberlin MN, Tadei WP, Amorim RC, Pohlit AM, Antiplasmodial activity of synthetic ellipticine derivatives and an isolated analog. *Bioorg Med Chem Lett.*, 2014; 24(12): 2631-2634.
15. Murtaza S, Akhtar MS, Kanwal F, Abbas A, Ashiq S, Shamim S, Synthesis and biological evaluation of Schiff bases of 4-aminophenazone as an anti-inflammatory, analgesic and antipyretic agent. *J Saudi Chem Soc.*, 2017; 21(1): S359-S372.

16. Nayak GS, Poojary B, Synthesis of novel Schiff bases containing arylpyrimidines as promising antibacterial agents. *Heliyon*, 2019; 5(8): E02318
17. Pandey S, Lakshmi V, Pandey A, Biological activity of Mannich bases. *Indian Pharm Sci.*, 2003; 65: 213-222.
18. Patel OPS, Mishra A, Maurya R, Saini D, Pandey J, Taneja I, Raju KSR, Kanojiya S, Shukla SK, Srivastava MN, Wahajuddin M, Tamrakar AK, Srivastava AK, Yadav PP, Naturally occurring carbazole alkaloids from *Murraya koenigii* as potential antidiabetic agents. *J Nat Prod.*, 2016; 79(5): 1276-1284.
19. Patrinoiu G, Calderón-Moreno JM, Chifiriuc CM, Saviuc C, Birjega R, Carp O, Tunable ZnO spheres with high anti-biofilm and antibacterial activity *via* a simple green hydrothermal route. *J Colloid Interface Sci.*, 2016; 462: 64-74.
20. Pollo LAE, Martin EF, Machado VR, Cantillon D, Wildner LM, Bazzo ML, Waddell SJ, Biavatti MW and Sandjo LP, Search for Antimicrobial Activity Among Fifty-Two Natural and Synthetic Compounds Identifies Anthraquinone and Polyacetylene Classes That Inhibit *Mycobacterium tuberculosis*. *Front Microbiol.*, 2021; 11: 622629: 1-11.
21. Prodan AM, Iconaru SL, Chifiriuc CM, Bleotu C, Ciobanu CS, Motelica-Heino M, Sizaret S, Predoi D, Magnetic properties and biological activity evaluation of iron oxide nanoparticles. *J Nanomater.*, 2013; 2013: 893970: 1-7.
22. Rajakumar P, Sekar K, Shanmugaiah V, Mathivanan N, Synthesis of novel carbazole based macrocyclicamides as potential antimicrobian agents. *Eur J Med Chem.*, 2009; 44: 3040-3045.
23. Ruegg UT, Burgess GM, Staurosporine, K-252 and UCN-01: potent but nonspecific inhibitors of protein kinases. *Trends Pharmacol Sci.*, 1989; 10: 218-220.
24. Sahoo A, Swain SS, Behera A, Sahoo G, Mahapatra PK, Panda SK, Antimicrobial peptides derived from insects offer a novel therapeutic option to combat biofilm: A review. *Front Microbiol.*, 2021; 12: 661195: 1-29.
25. Shaheen MA, Feng S, Anthony M, Tahir MN, Hussan M, Seo SY, Ahmad S, Iqbal M, Saleem M, Lu C, Metal-based scaffolds of Schiff bases derived from naproxen: synthesis, antibacterial activities, and molecular dockinh studies. *Molecules*, 2019; 24(7): 1237: 1-12.
26. Sharma D, Kumar N, Pathak D, Synthesis, characterization and biological evaluation of some newer carbazole derivatives. *J Serb Chem Soc*., 2014; 79(2): 125-132.
27. The United States Pharmacopeial Convention, with Supliment, USP 30, Washington D.C., 2007.
28. Vandana J, Munira M, Laddha K, Murraya Koenigii: An updated review. *J Ayu Her Med*., 2012; 2(4): 607-627.
29. Venugopal K, Jayashree B, Microwave-induced synthesis of Schiff bases of aminothiazolylbromocoumarins as antibacterials. *Indian J Pharm Sci.*, 2008; 70: 88-91.
30. Xcalibur™ Software, Thermo Fisher Scientific, 168 3rd Avenue, Waltham, MA USA, www.thermofisher.com.
31. Zarafu I, Pătrașcu B, Măruțescu L, Bleotu C, Limban C, Tatibouët A, Chifiriuc MC, Nuță DC, Ioniță P, Bioevaluation of the antimicrobial and anti-proliferative potential of some derivatives of 3,5-dinitro-4-methoxyamino-benzoic acid. *Farmacia*, 2020; 68(1): 8-14.
32. Zhang FF, Gan LL, Zhou CH, Synthesis, antibacterial and antifungal activities of some novel carbazole derivatives. *Bioorg Med Chem.*, 2010; 20: 1881-1884.