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ORIGINAL ARTICLE

SYNTHESIS AND EVALUATION OF THE ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY OF NOVEL DIBENZOTHIEPINES

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Abstract

Considering the anti-infective potential of compounds containing the dibenzothiepine scaffold, we set out to obtain new compounds bearing this structure. The synthesized compounds were characterized by spectral studies and elemental analysis and screened for their microbiostatic/microbicidal and antibiofilm properties against reference and clinical microbial strains. The new compounds exhibited a broad spectrum of antimicrobial activity, which was more intensive for the S-oxidized compounds. Some of the compounds also inhibited the ability of the investigated strains to form biofilms on the inert substratum.

Rezumat

Având în vedere potențialul antiinfecțios al compușilor cu structură dibenzotiepinică, în prezenta lucrare ne-am propus să obținem noi derivați care au la bază acest nucleu. Compușii sintetizați au fost caracterizați prin analize spectrale și analiză elementală și au fost determinate proprietățile antimicrobiene și antibiofilm față de tulpini microbiene de referință și clinice. Noii compuși au prezentat activitate antimicrobiană cu spectru larg, superioară pentru compușii S-oxidați. Unii dintre compuși au inhibat, de asemenea, capacitatea tulpinilor microbiene de a forma biofilm pe substratul inert.

Keywords: dibenzothiepines, sulfones, antimicrobial, antibiofilm

Introduction

The development and approval of new anti-infective agents efficient against multidrug-resistant, extended-drug and pan-drug resistant pathogens has not kept up with the evolution of drug resistance in these micro-organisms. World Health Organization reports show that every year, antimicrobial resistant infections caused 700,000 deaths, with 10 million deaths predicted by 2050 if this trend continues. The prognosis is worsened by the formation of bacterial biofilms, represented by microbial communities adhered to a surface or an interface, embedded in a self-secreted polymeric matrix, which exhibit a highly resistant phenotype to different antimicrobial agents, host defence mechanisms and other limiting conditions. With biofilms being involved in up to 80% of the total number of infections, often with chronic evolution, the need for antibiofilm compounds has become obvious. Antibiofilm compounds can act either on the cellular component of microbial biofilms or by disrupting the biofilm matrix, rendering the free cells susceptible to antibiotics and/or to the immune system effectors.

It is well known that development of novel compounds is time-consuming and requires immense resources. For the past few decades the number of newly approved antibiotics has decreased significantly. For these threats and challenges to be countered, there is an urgent need to identify alternative strategies to obtain new active compounds to control infectious diseases [1, 2, 4, 6, 13, 19-21].

An alternative approach for drug discovery is the screening of large chemical libraries using high-throughput technique. This technique has gained widespread popularity over the last two decades and has become a standard method for drug discovery in the pharmaceutical industry [9, 18].

High-throughput screening led to the identification of active anti-infective agents with dibenzothiepine scaffold. Dibenzothiepine compounds proved to exhibit various activities, such as antibacterial, antifungal, antiprotozoal [5, 12, 14], some compounds having efficacy against biofilms [15].

In our previous researches we highlighted the anti-bacterial, antifungal and antibiofilm activity of some new compounds with dibenzothiepine scaffold [7, 17]. Based on all above considerations and as an extension

of our research in development of new compounds with anti-infective properties, we have designed and synthesized new dibenzothiepine derivatives which were evaluated for their antibacterial and anti-biofilm activities.

Materials and Methods

Chemistry

All starting materials, reagents and solvents were purchased from commercial suppliers (Merck, Sigma-Aldrich or Fluka) and used without further purification unless otherwise specified. All melting points were measured in open capillary tubes on an Electrothermal 9100 apparatus and are uncorrected. The elemental analyses were performed on a Perkin-Elmer 2400 Series II CHNS/O Elemental Analyzer (Shelton, CT, USA). The NMR spectra were recorded on a Gemini 300 BB instrument (Varian, Palo Alto, CA, USA) at room temperature, operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C, using deuterochloroform ($CDCl_3$) as solvent. The chemical shifts were recorded as δ values in parts *per* million (ppm) relative to tetramethylsilane (TMS), used as internal standard. The coupling constants (J) values are reported in Hertz and the splitting patterns are abbreviated as following: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), b (broad). The ^{13}C-NMR data are reported in the following order: chemical shifts (ppm), the signal/atom attribution, the coupling constant (J) in some cases; Cq-quaternary carbon.

The IR spectra were recorded on a Bruker Vertex 70 spectrometer, with horizontal device for attenuated reflectance and diamond crystal, on a spectral window ranging from 4000 to 400 cm^{-1}. The spectra were recorded without any sample preparation and were processed with OPUS 5.5 program (Bruker). The IR bands were given as: w (weak), m (medium), s (strong), vs (very strong).

Synthesis

The new compounds were synthesized by the general method, outlined in Figure 1, starting from phtalide **1** and potassium thiophenolate **2** (X = -H) or potassium *p*-thiocresolate **2** (X = $-CH_3$).

In the first stage we obtained acids **3**, which were transformed into the corresponding ketones **4** in the presence of polyphosphoric acid and then converted to the corresponding oximes **5** by treatment with hydroxylamine hydrochloride in the presence of pyridine. Dibenzo[*b*,*e*]thiepines **TH** and **TM** were prepared by acylation of the corresponding oximes **5** with various aromatic acid chlorides, in dry benzene/toluene and in the presence of anhydrous pyridine as a proton acceptor. Sulfones **SH** and **SM** were prepared by the oxidation of dibenzo[*b*,*e*]thiepines **TH** respectively **TM**, with 30% aqueous hydrogen peroxide in glacial acetic acid, at boiling temperature.

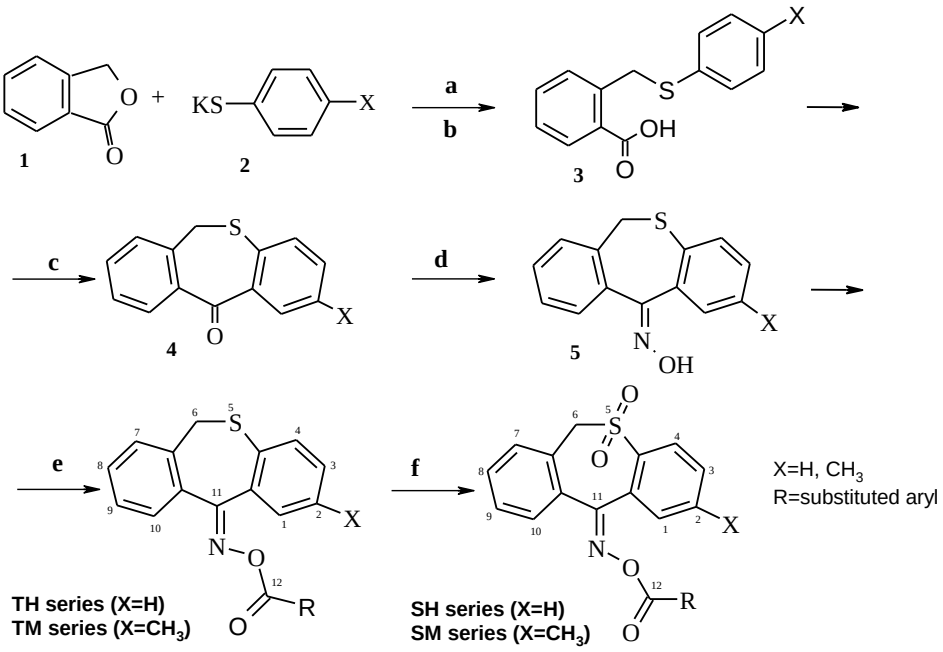


Figure 1.

The synthetic pathway of the dibenzo[*b*,*e*]thiepines TH and TM and dibenzo[*b*,*e*]thiepine-5,5-dioxides, SH and SM
Reagents and conditions: (a) xylene, reflux; 10% sodium hydroxide; (b) hydrochloric acid 1 M; (c) polyphosphoric acid 140 - 150ºC, 3 h; (d) hydroxylamine hydrochloride, pyridine, reflux, 24 h; (e) aromatic acid chlorides, benzene, pyridine, reflux, 2 h; (f) 30% aqueous hydrogen peroxide, glacial acetic acid, reflux, 3 h

Acids **3**, ketones **4** and oximes **5**, were prepared in good yields (85 - 91%) according to the previously described procedures [7, 16, 17].

*General procedure for the synthesis of the new derivatives of **TH** series*

To a solution of 10 mmol 11-hydroximino-6,11-dihydrodibenzo[b,e]thiepine in anhydrous toluene was added dropwise a solution of 11 mmol appropriated chloride acid in anhydrous toluene and 0.8 mL (10 mmol) dry pyridine. The reaction mixture was refluxed for three hours, cooled, the precipitate was filtered off and the solvent removed under reduced pressure. The resulting crude product was recrystallized from isopropanol.

*General procedure for the synthesis of the new derivatives of **SH** series*

To a solution of 10 mmol compound TH, in glacial acetic acid, 2 mL of 30% aqueous hydrogen peroxide were added dropwise. The mixture was heated for three hours and then left overnight at room temperature. The reaction mixture was diluted with water and extracted with chloroform. The combined organic layer was dried over calcium chloride and after filtration, the solvent removed under reduced pressure. The resulting crude product was recrystallized from ethanol.

*Synthesis of [(5,5-dioxo-6,11-dihydrodibenzo[b,e]-thiepin-2-methyl-11-ylidene)amino] 3,4,5-trimethoxybenzoate (**SM22**)*

To a solution of 10 mmol 2-methyl-11-hydroximino-6,11-dihydrodibenzo[b,e]thiepine **5** (X = -CH_3) in anhydrous benzene was added dropwise a solution of 11 mmol 3,4,5-trimethoxybenzoyl chloride in anhydrous benzene and 0.8 mL (10 mmol) dry pyridine. The reaction mixture was refluxed for three hours, cooled, the precipitate was filtered off and the solvent removed under reduced pressure. The resulting crude product was recrystallized from ethanol, and afterward oxidized using a solution of 30% aqueous hydrogen peroxide according to the protocol described above. The resulting crude product was recrystallized from glacial acetic acid.

Antimicrobial activity evaluation against planktonic and biofilm embedded cells

The antimicrobial properties of the new compounds were investigated using the broth micro-dilution assay. The compounds were solubilized in dimethyl sulfoxide (DMSO) and further diluted two-fold in 96 well plates containing Muller Hinton Broth (MHB). Microbial suspensions of Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 27853) and Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 6538) and *Candida parapsilopsis* ATCC 22019 with a density of 10^6 CFU/mL were also prepared from 24 h solid cultures. We have also used clinical isolates consisting of methicillin resistant *S. aureus* (MRSA), uropathogenic *E. coli* (UPEC), *Acinetobacter baumanii* and *P. aeruginosa*. The microbial suspensions were then inoculated on each microtiter well containing the two-fold dilutions of the tested compounds. A sterility control was added with 100 µL of MHB. The experiments were done in triplicate. The wells were incubated for 18 - 24 h in aerobic conditions, at 37ºC. After incubation, the minimum inhibitory concentration (MIC) value was determined spectrophotometrically at 620 nm.

To investigate the influence of the tested compounds on the ability of the tested microbial strains to colonize the inert substratum, a microtiter plate method was performed. The microplates used for the MIC assay were emptied and further washed three times with phosphate buffered saline. The biofilm formed on the plastic wells wall was fixed for 5 min with cold methanol, coloured by violet crystal solution (15 min) and finally re-suspended in 33% acetic acid solution. Microbial cell density measurement was done by reading the optical density of the coloured solution at 490 nm. The minimal biofilm eradication concentration (MBEC) values were considered as the lowest concentration of the tested compound that inhibited the development of biofilm on the plate wells [11].

Results and Discussion

Chemistry

Following the aforementioned synthesis procedure, we obtained new O-acyl-oximino-dibenzo[b,e]thiepines (TH**)** and sulfones (SH), solid, crystalline, white compounds that were characterized by NMR and IR spectra. The compounds purity was certified by elemental analyses, the results being within ± 0.4 of the theoretical values.

The chemical structures of the new compounds, dibenzothiepine derivatives of the TH and SH series are depicted in Table I. The structure of the already reported compound SM22 is included in the table.

The melting point (m.p.), the reaction yield, spectral data and elemental analysis for O-acyl-oximino-dibenzo[b,e]thiepins **TH** and their corresponding 5,5-dioxides **SH** are presented here. For compound SM22, whose synthesis and characterization have already been reported, the main chemical data are presented.

*[(6,11-dihydrodibenzo[b,e]thiepin-11-ylidene)-amino] 2,3,4-trimethoxybenzoate (**TH47**)*

m.p.: 119 - 120ºC; white solid; yield 68%.

^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 7.85 (dd, 1.4; 7.7; 1H, H-1); 7.12 - 7.42 (m, 8H, H-arom); 6.60 (d, 8.8, 1H, H-17); 4.70 (bs, 2H, H-6); 3.87 (s, 3H, OCH_3^{19}); 3.82 (s, 3H, OCH_3^{21}); 3.65 (s, 3H, OCH_3^{20}).

^{13}C-RMN ($CDCl_3$, δ ppm): 166.50 (C-12); 162.83 (C-11); 157.63 (Cq); 155.11 (Cq); 143.02 (Cq); 136.87 (Cq); 135.02 (Cq); 133.88 (Cq); 131.84 (CH); 130.54 (CH); 130.31 (CH); 129.18 (Cq); 128.01 (CH); 127.40 (CH); 127.34 (CH); 127.03 (CH); 126.46 (CH); 125.09 (CH); 116.19 (Cq-13); 106.99 (CH-18); 61.60 (OCH_3^{19}); 61.06 (OCH_3^{21}); 56.17 (OCH_3^{20}); 33.40 (C-6).

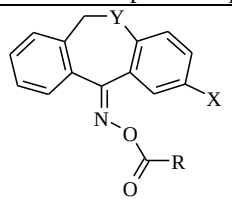
The spectra results show that only one stereoisomer is present.

FT-IR (ATR in solid, ν cm^{-1}): 3056w; 2966w; 2932w; 2834w; 1752vs; 1594m; 1495w; 1461m; 1416m; 1255vs; 1209w; 1171w; 1127m; 1091s; 1019s; 986w; 932w; 895m; 777w; 731w; 698w; 587w.

Elemental analysis: Calcd. for $C_{24}H_{21}NO_5S$ (435.50 g/mol): C, 66.19; H, 4.86; N, 3.22; S, 7.36. Found: C, 66.31; H, 5.10; N, 3.03; S, 7.16.

Table I

The chemical structures of the dibenzothiepine compounds from the three series: TH, SH and SM



Series	Experimental compound code	X	Y	R
TH	TH47	H	S	2,3,4-$(H_3CO)_3$-C_6H_2
	TH48	H	S	2,3,4-$(F)_3$-C_6H_2
	TH49	H	S	3-F_3C-C_6H_4
SH	SH47	H	SO_2	2,3,4-$(H_3CO)_3$-C_6H_2
	SH48	H	SO_2	2,3,4-$(F)_3$-C_6H_2
	SH49	H	SO_2	3-F_3C-C_6H_4
SM	SM22	CH_3	SO_2	3,4,5-$(H_3CO)_3$-C_6H_2

*[(6,11-dihydrodibenzo[b,e]thiepin-11-ylidene)amino] 2,3,4-trifluorobenzoate (**TH48**)*

m.p.: 168 - 170ºC; white solid; yield 69%.

^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 7.81 (dd, 7.7, 1.6, 1H, H-1); 7.58 (m, 1H, H-18); 7.20 - 7.50 (m, 5H, H-arom); 7.18 (td, 1.7, 7.7, 1H, H-2); 7.14 (dd, 1.4, 7.9, 1H, H-4); 6.98 (m, 1H, H-17); 4.64 (bs, 1H, H-6); 3.55 (bs, 1H, H-6').

^{13}C-RMN ($CDCl_3$, δ ppm, *J* Hz): 168.21 (C-12); 161.44 (C-11); 137.25 (Cq); 134.80 (Cq); 133.24 (Cq); 131.78 (CH); 130.84 (CH); 130.62 (CH); 128.62 (Cq); 128.20 (CH); 127.42 (CH); 127.30 (CH); 126.51 (CH); 126.40 (CH); 125.14 (CH); 112.50 (dd, 17.6, 3.4, CH-17); 33.42 (C-6).

The spectra results show that only one stereoisomer is present. The presence of the three fluorine atoms in the aromatic ring makes impossible the description of corresponding ^{13}C-NMR signals. This is due to the very close chemical shifts (~ 160 ppm) and also due to the long range couplings with the other fluorine atoms.

FT-IR (ATR in solid, ν cm^{-1}): 3054w; 2910w; 1736vs; 1621m; 1506m; 1472s; 1424w; 1314m; 1282s; 1191vs; 1102s; 1072w; 1044 m; 982w; 933w; 858m; 827w; 771m; 722w; 698w; 638w; 592w; 468w.

Elemental analysis: Calcd. for $C_{21}H_{12}F_3NO_2S$ (399.39 g/mol): C, 63.15; H, 3.03; N, 3.51; S, 8.03. Found: C, 63.36; H, 3.23; N, 3.45; S, 8.14.

*[(6,11-dihydrodibenzo[b,e]thiepin-11-ylidene)amino] 3-(trifluoromethyl)benzoate **(TH49)***

m.p.: 132 - 134ºC; white solid; yield 72 %.

^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 7.92 (bd, 7.7, 1H, H-18); 7.86 (bs, 1H, H-14); 7.77 (dd, 1.5, 7.7, 1H, H-1); 7.69 (bd, 7.7, 1H, H-16); 7.44 (t, 7.7, 1H, H-17); 7.05 - 7.45 (m, 7H, H-arom); 4.55 (bs, 1H, H-6); 3.52 (bs, 1H, H-6').

^{13}C-RMN ($CDCl_3$, δ ppm, *J* Hz): 167.78 (C-12); 162.15 (C-11); 137.20 (Cq); 135.19 (Cq); 133.08 (Cq); 132.94 (CH); 131.79 (CH); 131.40 (Cq); 131.2 (q, 32.5, C-15); 130.82 (CH); 130.61 (CH); 129.93 (CH); 129.47 (Cq); 129.32 (CH); 128.47 (Cq); 127.26 (CH); 127.14 (CH); 126.71 (CH); 126.60 (CH); 125.16 (CH); 123.49 (q, 267.8, CF_3) 33.50 (C-6).

The spectra results show that only one stereoisomer is present.

FT-IR (ATR in solid, ν cm^{-1}): 3097w; 3069w; 2963w; 2331w; 2116w; 1985w; 1930w; 1754vs; 1592m; 1474w; 1419w; 1327s; 1259w; 1223vs; 1173m; 1121s; 1055vs; 979m; 923w; 882m; 817w; 765w; 736m; 693m; 639w; 594w.

Elemental analysis: Calcd. for $C_{22}H_{14}F_3NO_2S$ (413.42 g/mol): C, 63.92; H, 3.41; N, 3.39; S, 7.76. Found: C, 64.04; H, 3.49; N, 3.48; S, 7.87.

*[(5,5-dioxo-6,11-dihydrodibenzo[b,e]thiepin-11-ylidene)amino] 2,3,4-trimethoxybenzoate (**SH47**)*

m.p.: 200 - 202ºC; white solid; yield 82%.

^{1}H-RMN ($CDCl_3$, δ ppm, J Hz): 8.04 (dd, 7.2, 1.6, 1H, H-4); 7.91 (dd, 7.7, 1.6, 1H, H-1); 7.69 (td, 7.2, 1.6, 1H, H-3); 7.64 (td, 7.7, 1.6, 1H, H-2); 7.35 - 7.55 (m, 4H, H-arom); 7.32 (d, 8.8, 1H, H-18); 6.61 (d, 8.8, 1H, H-17); 5.05 (bs, 1H, H-6); 4.20 (bs, 1H, H-6'); 3.88 (s, 3H, OCH_3); 3.82 (s, 3H, OCH_3); 3.67 (s, 3H, OCH_3).

^{13}C-RMN ($CDCl_3$, δ, ppm): 163.55 (C-12); 162.32 (C-11); 158.09 (Cq); 155.30 (Cq); 143.11 (Cq); 141.85 (Cq); 135.37 (Cq); 132.85 (CH-2); 132.27 (CH-3); 131.43 (CH); 130.34 (Cq); 130.07 (CH); 129.21 (CH); 128.15 (CH); 127.14 (CH-18); 126.20 (CH-4); 124.37

(Cq); 115.39 (Cq-13); 107.12 (C-17); 61.65 (OCH_3); 61.09 (OCH_3); 58.58 (C-6); 56.24 (OCH_3).

The spectra results show that only one stereoisomer is present.

FT-IR (ATR in solid, ν cm^{-1}): 3065w; 2998w; 2938w; 2834w; 2113w; 1994w; 1908w; 1756w; 1725vs; 1585m; 1488w; 1459m; 1408m; 1295s; 1259vs; 1209m; 1156w; 1106vs; 1016m; 979w; 929w; 888w; 819w; 785m; 740w; 684w; 636w; 604w; 523m.

Elemental analysis: Calcd. for $C_{24}H_{21}NO_7S$ (467.50 g/mol): C, 61.66; H, 4.53; N, 3.00; S, 6.86. Found: C, 61.48; H, 4.61; N, 3.14; S, 6.76.

*[(5,5-dioxo-6,11-dihydrodibenzo[b,e]thiepin-11-ylidene)amino] 2,3,4-trifluorobenzoate (**SH48**).*

m.p.: 213 - 215ºC; white solid; yield 81%.

^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 8.05 (dd, 7.4, 1.7, 1H, H-4); 7.87 (dd, 7.4, 1.6, 1H, H-1); 7.40 - 7.75 (m, 7H, H-arom); 7.02 (m, 1H, H-17); 5.06 (bs, 1H, H-6); 4.38 (bs, 1H, H-6').

^{13}C-RMN ($CDCl_3$, δ ppm): 165.26 (C-12); 141.92 (C-11); 134.62 (Cq); 132.87 (CH); 132.55 (CH); 131.41 (CH); 131.23 (CH); 129.91 (CH); 129.71 (Cq); 129.21 (CH); 128.07 (CH); 126.86 (CH); 126.34 (CH); 123.92 (Cq); 112.75 (d, 17.3, C-17); 58.55 (C-6).

Quaternary atoms due to fluorine couplings and over several links disappear into the base line of the spectrum.

The spectra results show that only one stereoisomer is present.

FT-IR (ATR in solid, ν cm^{-1}): 3073w; 2965w; 2925w; 2361w; 1748vs; 1614m; 1509m; 1477s; 1423w; 1305s; 1283s; 1193s; 1159m; 1128m; 1095m; 1066w; 990w; 933m; 880w; 828w; 785m; 722w; 682w; 635w; 605w; 566w; 521m; 484w.

Elemental analysis: Calcd. for $C_{21}H_{12}F_3NO_4S$ (431.39 g/mol): C, 58.47; H, 2.80; N, 3.25; S, 7.43. Found: C, 58.39; H, 2.87; N, 3.16; S, 7.51.

*[(5,5-dioxo-6,11-dihydrodibenzo[b,e]thiepin-11-ylidene)amino] 3-(trifluoromethyl)benzoate (**SH49**)*

m.p.: 187 - 189ºC; white solid; yield 84%.

^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 8.06 (dd, 7.7, 1.7, 1H, H-4); 8.00 (bd, 7.7, 1H, H-18); 7.93 (bs, 1H, H-14); 7.91 (dd, 7.7, 1.7, 1H, H-1); 7.81 (bd, 7.7, 1H, H-16); 7.40 - 7.75 (m, H-arom); 5.08 (bs, 1H, H-6); 4.40 (bs, 1H, H-6').

^{13}C-RMN ($CDCl_3$, δ ppm, *J* Hz): 164.95 (C-12); 161.73 (C-11); 141.96 (Cq); 134.54 (Cq); 130.04 (CH); 132.91 (CH); 132.56 (CH); 131.31 (CH); 131.37 (Cq-15, *J* = 32.3); 130.34 (q, *J* = 2.8, CH); 130.00 (CH); 129.62 (Cq); 129.53 (CH); 129.07 (CH); 128.86 (Cq); 127.85 (CH); 127.03 (q, *J* = 270, CF_3); 126.64 (q, *J* = 3.0, CH-16); 126.32 (CH); 124.31 (Cq); 58.60 (C-6).

The spectra results show that only one stereoisomer is present.

Elemental analysis: Calcd. for $C_{22}H_{14}F_3NO_4S$ (445.42 g/mol): C, 59.32; H, 3.17; N, 3.14; S, 7.20. Found: C, 59.04; H, 3.35; N, 3.43; S, 7.40.

*[(5,5-dioxo-6,11-dihydrodibenzo[b,e]thiepin-2-methyl-11-ylidene)amino] 3,4,5- trimethoxybenzoate (**SM22**)*

$C_{25}H_{23}NO_7S$ (481.53 g/mol); m.p.: 228 - 231ºC; white solid; yield 64%.

In the ^{1}H-NMR spectra of the new dibenzo[*b,e*]thiepines TH the dibenzothiepine scaffold is characterized by the methylene group CH_2S (H-6) which gave an AB coupling system, with two broad singlets at 3.52 - 3.55 ppm and 4.55 - 4.70 ppm. The magnetically non-equivalent diastereotopic protons H-6 and H-6' form an AB system. The *S*-oxidation to the corresponding dioxides (compounds SH) induces as direct effect the deshielding (approximately 1 ppm) of the two protons of the methylenic group, which appear in sulfones in the range 4.20 - 4.40 ppm and 5.05 - 5.08 ppm. At 303 K the signals appear broadened due to the slow exchange between the two positions (a flip between two conformers).

In the ^{13}C-NMR spectra of the new dibenzo[*b,e*]-thiepines TH the methylene group (C-6) is characterized by a signal at 33.40 - 33.50 ppm. The S-oxidation to the corresponding sulfone derivatives induces as direct effect a strongly deshielding (approximately 25 ppm) of the carbon of the methylenic group, which appear in sulfones SH in the range 58.55 - 58.60 ppm. The signal corresponding to the C-11 atom appears in the range 141.92 - 162.83 ppm and the signal of C-12 appears in the range 163.55 - 168.21 ppm. The other signals are in good agreement with the structure.

Owing to the asymmetry induced by sulphur in di-benzothiepine nucleus, the compounds belong to the two series TH and SH, may have *syn* or *anti* configuration. NMR spectral data showed the presence of only one of the stereoisomers in all newly synthesized compounds, most probably the *anti*-isomer.

In the IR spectra the characteristic bands for the new compounds are (cm^{-1}): υCH_2 1327 - 1424; υCH_2 2834 - 2963; -O-C=O (υC=O: 1725 - 1754; υC-O: 1156 - 1223); υC=N: 1585 - 1621; aromatic rings (υ=C-H: 3054 - 3073; υC=C: 1585 - 1621); for compounds SH: υSO_2 sym 1156 - 1191, υSO_2 asym 1305 - 1327.

Bioevaluation

The antimicrobial and antibiofilm activity of the novel compounds was tested against reference strains of *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida parapsilosis* as well as clinical isolates including *Acinetobacter baumannii*, *P. aeruginosa*, methicillin resistant *S. aureus* (MRSA) and uropathogenic *E. coli* (UPEC) (Figures 2 and 3).

The quantitative assay revealed the presence of anti-microbial activity for all tested compounds, with MIC values of 1.25 - 10 mg/mL. The lowest MIC values (1.25 - 2.5 mg/mL) were recorded against the Gram-negative bacterial strains (*P. aeruginosa*, *E. coli* reference strain and UPEC clinical isolate, *Acinetobacter*

baumannii) and *C. parapsilosis*. Our results showed that in case of the majority of the clinical strains, the S oxidized compound SH47 was more active compared to its precursor TH47, exhibiting MIC values two times lower than the corresponding non-oxidized compound. However, in the case of reference strains this trend was observed only in case of *P. aeruginosa* (Figures 2A, 2B and 3A, 3B).

For the fluorinated compounds (TH48, TH49, SH48, SH49) this trend was observed only in three cases for the TH49/SH49 couple, respectively against the *S. aureus*, *P. aeruginosa* and *E. faecalis* reference strains.

The majority of microbial infections involve biofilm development on natural, intact or damaged tissues as well as on artificial medical devices and are characterized by chronic evolution, middle intensity symptoms, and most importantly resistance to antimicrobial compounds. Many research efforts are done in order to provide novel agents acting as inhibitors of biofilm formation that act by disrupting the biofilm cells connection, rendering the microbial cells susceptible to usual therapeutic doses of antibiotics [3, 8, 10].

The experimental set up we employed within this study to test antibiofilm activity uses mini volumes and multiple well plastic plates, hence allowing the simultaneous testing of a large spectrum of concentrations.

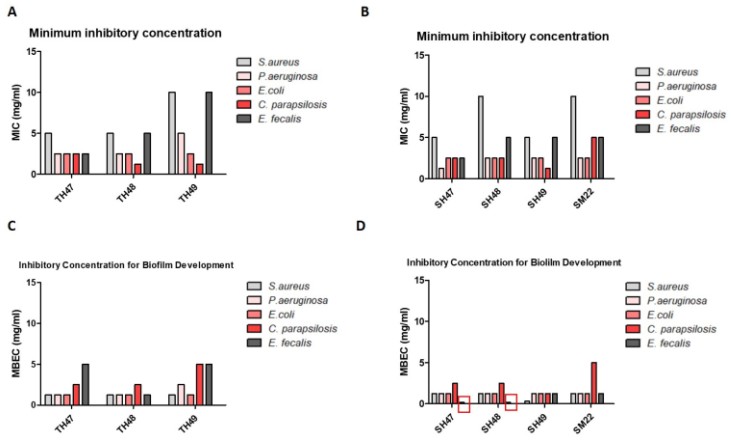


Figure 2.

Antimicrobial (A, B) and antibiofilm (C, D) activity of the novel dibenzothiepines against ATCC strains

MIC – minimum inhibitory concentration; MBEC – minimal biofilm eradication concentration. The MIC was determined as the minimum amount of the tested compounds that inhibited the microbial growth in liquid medium after 24 h treatment. The minimal biofilm eradication concentration was determined to be the lowest concentration of the tested compounds at which the decrease in absorbance value, measured at 490 nm, was observed in comparison to the positive control. Concentrations expressed as mg/mL and are presented as the average of three different experiments.

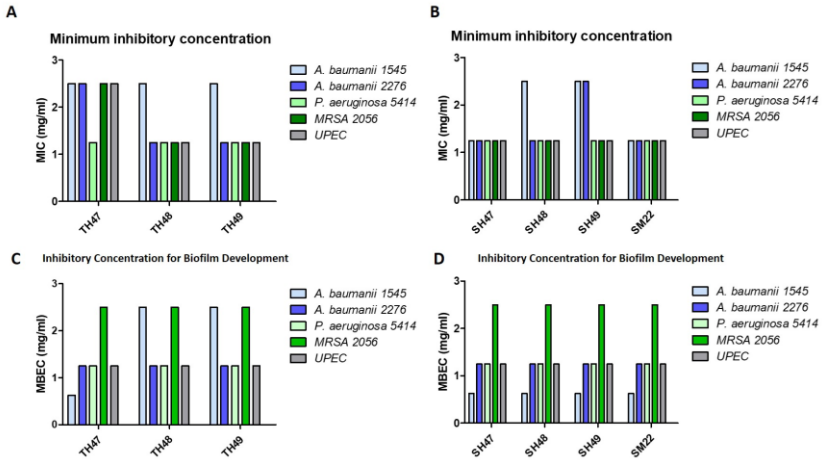


Figure 3.

Antimicrobial (A, B) and antibiofilm (C, D) activity of the novel dibenzothiepines against clinical isolates

MIC – minimum inhibitory concentration; MBEC – minimal biofilm eradication concentration. The MIC was determined as the minimum amount of the tested compounds that inhibited the microbial growth in liquid medium 24 h after the treatment. The minimal biofilm eradication concentration was determined to be the lowest concentration of the tested compounds at which the decrease in absorbance value, measured at 490 nm, was observed in comparison to the positive control. Concentrations expressed as mg/mL and are presented as the average of three different experiments.

It is worth to mention that, despite the well-known high bofilm resistance to different antimicrobial agents, in our case, the analysis revealed that the effect on biofilm development on inert substrata was generally higher that recorded on the planktonic cells, for the majority of the tested compounds, the MBEC values being in some cases up to 16 times lower than the corresponding MIC values (Figures 2C, 2D and 3C, 3D). Importantly, the oxidized compounds SH47/ SH48/SH49 harboured a more intensive anti-biofilm activity than their precursors (TH47, TH48, TH49) against one (the reference *E. faecalis* strain)/ two (the reference *E. faecalis* and the *A. baumanii* clinical strain 1545) / five (the *A. baumannii* clinical strain 1545 and all reference strains, except *E. coli*) strains.

Taken together, the obtained results revealed that the S-oxidized compound SH47 exhibited a much intensive antimicrobial activity against most of the tested planktonic microbial strains as compared to its precursor TH47, while the S-oxidized compounds SH48 and SH49 exhibited an improved antibiofilm effect compared to their precursors.

SH47 and SH48 exhibited the best anti - *E. faecalis* biofilm activity (with a MBEC of 0.156 mg/mL), while SH49 the best anti - *S. aureus* biofilm activity (with an MBEC of 0.31 mg/mL), proving their potential for the further development of antibiofilm agents against Gram-positive infections.

Conclusions

A series of novel dibenzothiepines were synthesized characterized using spectral and elemental analysis and screened for their microbicidal and antibiofilm properties against standard microbial strains and clinical isolates. The new compounds exhibited a broad spectrum of antimicrobial activity, improved for the S-oxidized compounds (SH). Some of the compounds inhibited the ability of these strains to form biofilms on the inert substratum. Further molecular modelling has to be undertaken in order to improve antimicrobial and antibiofilm effect.

Conflict of interest

The authors declare no conflict of interest.

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