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Synthesis and antimicrobial activity of azepine and thiepine derivatives

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Abstract: A series of new pyridobenzazepine and pyridobenzothiepine derivatives was synthesized by Pd-catalyzed formation of C–N and C–S bonds. All synthesized compounds were tested for their *in vitro* antimicrobial activity. The pyridobenzazepine derivatives showed better antibacterial and antifungal activity than the corresponding dipyridoazepine analogue. Among the synthesized azepines, derivative **8** displayed potent activity against the tested bacteria (*MIC* ranged 39–78 μg mL^{-1}), while azepine **12** showed promising antifungal activity (*MIC* ranged 156–313 μg mL^{-1}). The synthesized thiepine derivatives exhibited weak antibacterial activity, but showed pronounced antifungal activity.

Keywords: azepines; thiepines; heterocycles; palladium; antibacterials; antifungal.

INTRODUCTION

The tricyclic moieties of 5*H*-dibenz[*b*,*f*]azepine (**1**)[1] and dibenzo[*b*,*f*]thiepine (**2**)[2] are important heterocyclic pharmacophores in a number of drugs. Carbamazepine (**3**) and opipramol (**4**) belong to the dibenzazepine group of heterocyclic compounds. Carbamazepine (**3**) is an anticonvulsant used to treat seizures, nerve pain and bipolar disorder,[1a] while opipramol (**4**) is a tricyclic antidepressant (TCA) and is used to treat generalized anxiety disorders.[1b] The dibenzothiepine zotepine (**5**) is an atypical antipsychotic, and it is used to treat schizophrenia (Fig. 1).[3]

Over the past few decades, several different strategies were developed for the synthesis of 5*H*-dibenz[*b*,*f*]azepines[4] and dibenzo[*b*,*f*]thiepines.[2,4e,5] The use of palladium-catalyzed reactions is an efficient procedure for the synthesis of 5*H*-

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-dibenz[*b*,*f*]azepines,[6] and methods based on the double *N*-arylation reaction are of particular relevance.[7] Hitherto, only one method employing a Pd-catalyzed reaction for the construction of the dibenzothiepine core has been reported.[8] The Mizoroki–Heck cyclisation of the corresponding diaryl thioether was used for the synthesis of dibenzothiepine **2**.

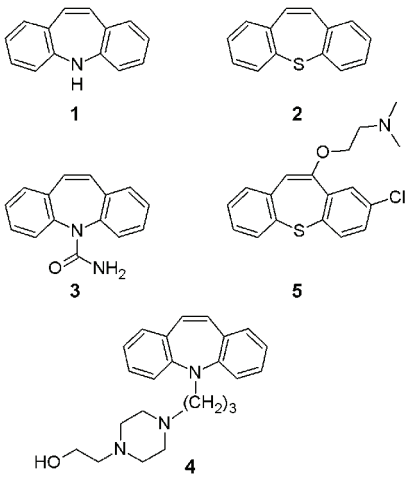
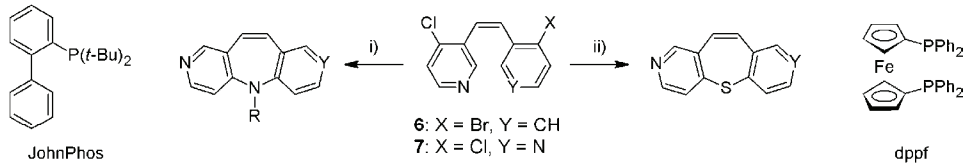


Fig. 1. Tricyclic 5*H*-dibenz[*b*,*f*]azepines and dibenzo[*b*,*f*]thiepines.

Recently, a simple and efficient Pd-catalyzed method was developed for the synthesis of 5*H*-pyrido[4,3-b:3',4'-f]benzazepine and 5*H*-dipyrido[4,3-b][1]azepine compounds (Scheme 1).[9]



Reagents and conditions: i) $Pd(OAc)_2$ (5 mol%), JohnPhos (10 mol%), RNH_2, NaO*t*-Bu, PhMe, 100 °C; ii) $Pd(OAc)_2$ (5 mol%), dppf (10 mol%), KSAc, NaO*t*-Bu, PhMe, 175 °C, μW.

Scheme 1. Pd-catalyzed synthesis of 5*H*-pyrido[4,3-b][1]benzazepine and 5*H*-dipyrido[4,3-b:3',4'-f]azepine compounds.

The protocol is based on a Pd-catalyzed double amination reaction of the corresponding stilbenes. Additionally, as an expansion of the methodology, for the first time Pd-catalyzed formation of C–S bonds was applied to the ring closure of a thiepine derivatives from the corresponding stilbene precursors and an *S*-nucleophile (Scheme 1). Formerly, the synthesized azepines and thiepines are shown in Fig. 2.

Herein, the synthesis of some new pyridobenzazepine and pyridobenzothiepine derivatives using the previously described methodology is presented. All synthesized compounds were evaluated for their *in vitro* antimicrobial activity against eight bacterial and three fungal pathogenic strains.

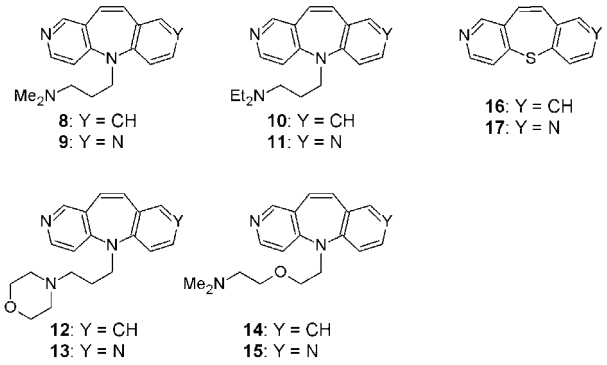
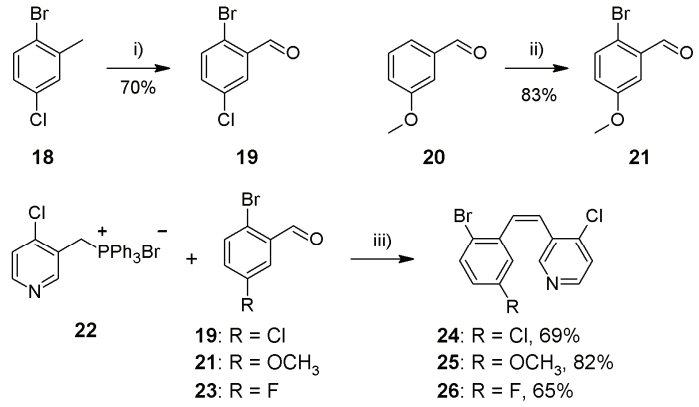


Fig. 2. Structures of the synthesized azepine and thiepine derivatives.

RESULTS AND DISCUSSION

Chemistry

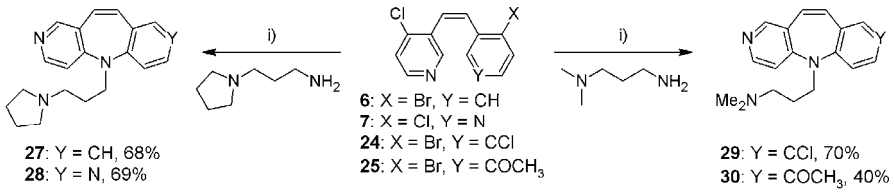
The Wittig reaction between phosphonium salt **22**[9] and aldehydes **19**[10] and **21**[11] provided the corresponding *Z*-stilbenes **24** and **25**, respectively, in high yield. For the preparation of ethylene derivative **26**, commercially available 2-bromo-5-fluorobenzaldehyde **23** was used (Scheme 2).



Reagents and conditions: i) a) CrO_3, Ac_2O, AcOH, H_2SO_4; b) MeOH, H_2O, H_2SO_4, Δ; ii) a) Br_2, AcOH, r.t.; b) $Na_2S_2O_3$, H_2O; iii) KO*t*-Bu, THF, r.t.

Scheme 2. The synthesis of *Z*-stilbenes **24**–**26**.

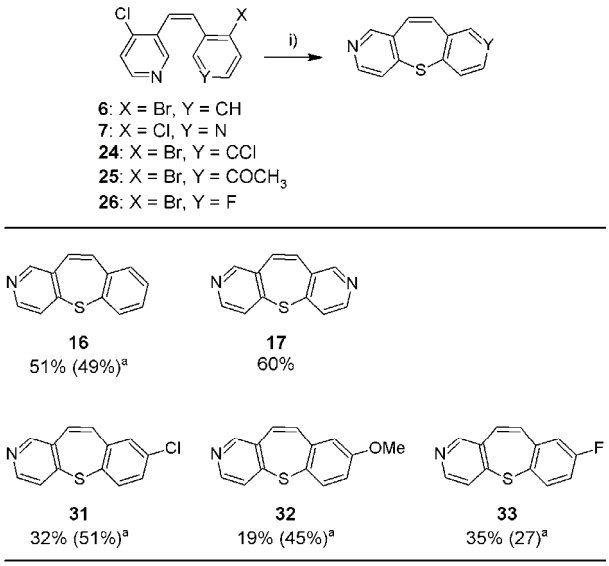
The syntheses of new iminostilbene compounds **27**–**30** were achieved using the previously described reaction conditions for Pd-catalyzed double amination reactions (Scheme 3).[9]



Reagents and conditions: i) $Pd(OAc)_2$ (5 mol%), JohnPhos (10 mol%), amine (3 equiv), NaO*t*-Bu (2.8 equiv), PhMe, 100 °C

Scheme 3. The synthesis of new pyridobenzazepine and dipyridoazepine compounds.

The reactions of *Z*-stilbenes **6**, **7** and **24**–**26** with potassium thioacetate (1.2 equiv.) in the presence of a catalyst composed from $Pd_2(dba)_3$ (5 mol %) and dppf (10 mol %) under microwave-mediated heating afforded the thiepine derivatives **16**, **17** and **31**–**33** in moderate yields (Scheme 4). It should be noted that higher proportions of KSAc (2.4 eqiuv.) resulted in significantly better yields of **31** and **32**, whereas the yield of thiepines **16** and **33** did not improve. In the case of stilbene **7**, the higher load of KSAc resulted in a complex reaction mixture.

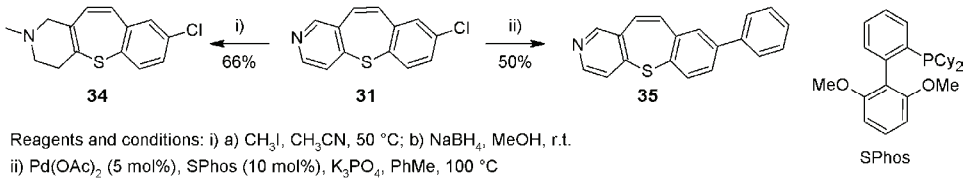


Reagents and conditions: i) Pd_2dba_3 (5 mol%), dppf (10 mol%), KSAc (1.2 equiv), NaO*t*-Bu (1.2 equiv), PhMe, 170 °C, μW

[a]2.4 equiv KSAc

Scheme 4. The synthesis of thiepine derivatives.

In the next synthetic step, the thiepine compound **31** was chemically transformed into its tetrahydro and biphenyl derivatives **34** and **35**, respectively. After N-methylation, and $NaBH_4$ reduction, the tetrahydro derivative **34** was obtained in 66% yield. The Suzuki–Miyaura reaction on thiepine **31** with phenylboronic acid gave derivative **35** in moderate yield (Scheme 5). The coupling reaction was performed with the catalytic system $Pd(OAc)_2$/SPhos–K_3PO_4 in toluene. These transformations of thiepine **31** opened up new possibilities for the preparation of structurally diverse substituted thiepines.



Scheme 5. The transformations of thiepine compound **31**.

Antimicrobial activity

The synthesized azepine derivatives were screened for their antibacterial and antifungal activities against five Gram-negative bacteria (*Escherichia coli*, *Proteus hauseri*, *Pseudomonas aeruginosa*, *Salmonella enterica* subsp. *enterica* serovar Enteritidis and *Klebsiella pneumoniae*), three Gram-positive bacteria (*Staphylococcus aureus*, *Micrococcus luteus* ATCC 10240 and *M. luteus* ATCC 4698) and three fungal strains (*Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus brasiliensis*). Amikacin (AMK) and chloramphenicol (CHL) were used as standard antibacterials, and nystatin (NYT) and fluconazole (FLC) were used as antifungal reference compounds. The minimum inhibitory concentration (*MIC*) was determined as the lowest concentration of the compound that resulted in inhibition of bacterial, respectively fungal growth, using a broth microdilution method.

The results of antibacterial activities of azepine derivatives are given in Table I. The azepines **8**–**15** and **27**–**30** exhibited lower antibacterial activity with respect to amikacin (Table I). Compound **8** was more potent than chloramphenicol against three Gram-negative bacteria (*E. coli*, *P. hauseri* and *P. aeruginosa*) and one Gram–positive bacteria (*M. luteus* ATCC 4698).

All pyridobenzazepine derivatives (**8**, **10**, **12**, **14** and **27**) showed higher inhibitory activity than the corresponding dipyridoazepine analogues (**9**, **11**, **13**, **15** and **28**) against all bacteria. Azepine **8** with an *N*,*N*-dimethyl substituent was 4 times more potent than the corresponding *N*,*N*-diethyl substituted analogue **10**. Additionally, **27**, which incorporates the side chain nitrogen in pyrrolidine ring was more potent than **10**, but less active than **8**. The results of the antibacterial screening for compounds **8** and **14** revealed that the introduction of an oxygen

atom in the side chain significantly decreased the antibacterial activity. The substituted azepines **29** and **30** showed lower antibacterial potency than **8** against all the screened bacteria.

TABLE I. Antibacterial minimal inhibitory concentrations (*MIC* / μg mL^{-1}) of the azepine derivatives

Cmpd.	Gram-negative bacteria					Gram-positive bacteria		
	E. coli	*P. hauseri*	*P. aerugi-nosa*	*S. enterica*	*K. pneu-moniae*	*S. aureus*	*M. luteus* ATCC 10240	*M. luteus* ATCC 4698
8	39	78	78	78	78	39	78	39
9	1250	1250	1250	1250	625	1250	1250	2500
10	156	313	313	625	625	156	313	313
11	1250	1250	1250	1250	1250	1250	1250	1250
12	39	313	313	313	313	39	156	78
13	1250	2500	1250	1250	1250	2500	1250	2500
14	313	625	625	625	625	313	625	313
15	1250	1250	1250	1250	1250	1250	1250	1250
27	78	156	313	313	313	156	156	156
28	625	625	625	625	625	625	625	625
29	78	156	313	313	313	156	313	156
30	156	313	313	313	625	313	313	313
AMK	5	7	50	8	8	11	2	2
CHL	62	125	250	43	62	15	31	125

Finally, among the synthesized azepines, derivative **8** was the most active one and showed a broad spectrum of antibacterial activity (MIC ranged 39–78 μg mL^{-1}).

The minimum inhibitory concentrations (*MIC*) of the synthesized azepines against three fungal strains are presented in Table II. Compounds **12** and **27** showed excellent activity (*MIC* = 156 μg mL^{-1}) against *C. albicans* and *S. cerevisiae*; they were more potent than nystatin and fluconazole. Compounds **12** and **27** are 16 times more active than nystatin against *C. albicans*, and 8 times more potent against *S. cerevisiae* than nystatin. In addition, compound **8** was more potent than nystatin and fluconazole against *S. cerevisiae*, while derivative **29** was more active than the reference compounds against the *C. albicans* strain.

Compounds **12** and **30** showed a four-fold greater potency (*MIC* = 313 μg mL^{-1}) than nystatin in inhibiting the growth of the *A. brasiliensis* strain, but were less active when compared to fluconazole. Again, as with the antibacterial activity, it was observed that the pyridobenzazepine derivatives (**8**, **10**, **12**, **14** and **27**) showed better antifungal activity than the corresponding dipyridoazepine analogues (**9**, **11**, **13**, **15** and **28**).

The synthesized thiepines were screened for their antibacterial and antifungal activities against four Gram-negative bacteria (*E. coli*, *P. hauseri*, *P. aeruginosa* and *Salmonella enterica* subsp. *enterica* serovar Enteritidis), four Gram-

-positive bacteria (*Clostridium sporogenes*, *S. aureus*, *M. luteus* ATCC 10240 and *Kocuria rhizophila*) and three fungal strains (*C. albicans*, *S. cerevisiae* and *A. brasiliensis*), using a disk diffusion method. Amikacin (AMK) was used as the standard antibacterial drug, and nystatin (NYT) was used as the antifungal reference compound. The antimicrobial activity was evaluated based on the diameter of the zone of inhibition.

TABLE II. Antifungal minimal inhibitory concentrations (*MIC* / μg mL^{-1}) of the azepine derivatives

Cmpd.	*C. albicans*	*S. cerevisiae*	*A. brasiliensis*
8	2500	156	1250
9	2500	1250	1250
10	625	625	625
11	1250	1250	1250
12	156	156	313
13	2500	1250	1250
14	625	313	625
15	1250	1250	1250
27	156	156	625
28	625	625	1250
29	156	313	625
30	313	313	313
NYT	2500	1250	1250
FLC	313	313	156

The results of antimicrobial activities of thiepine derivatives (Table III) revealed that all the tested thiepines displayed weak antibacterial activity with inhibition zones of 10–20 mm.

TABLE III. Antibacterial activity expressed as inhibition diameter zones in millimetres (mm) of thiepine derivatives

Cmpd.[a]	Gram–negative bacteria				Gram–positive bacteria			
	E. coli	*P. hauseri*	*P. aeruginosa*	*S. enterica*	*C. sporogenes*	*S. aureus*	*M. luteus* ATCC 10240	*K. rhizophila*
16	N.A.[b]	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
17	14	10	N.A.	10	16	N.A.	N.A.	N.A.
31	14	N.A.	N.A.	N.A.	20	N.A.	N.A.	10
32	14	10	N.A.	10	14	N.A.	N.A.	N.A.
33	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
34	10	14	N.A.	N.A.	10	N.A.	N.A.	N.A.
35	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
AMK[c]	26	26	25	25	24	24	27	23

[a] Compounds concentration 1 mg disk^{-1}; [b] N.A.: no activity (inhibition zone <10 mm); [c] AMK concentration 30 μg disk^{-1}

On the other hand, with the exception of **31**, all compounds exhibited pronounced antifungal activity against the three fungal strains (Table IV). The investigation of antifungal screening revealed that at a concentration of 1000 μg disk^{-1}, compounds **16**, **17**, **32**, **33**, **34** and **35** were very potent, and showed complete growth inhibition against the *C. albicans* and *S. cerevisiae* strains. Among synthesized thiepines, compound **32** showed excellent antifungal activity particularly on the *C. albicans* strain with an inhibition zone of 50 mm at 250 μg disk^{-1}, 28 mm at 125 μg disk^{-1} and 14 mm at 62.5 μg disk^{-1} concentrations. In addition, compound **32** at a concentration 125 μg disk^{-1} displayed moderate activity against the *A. brasiliensis* and *S. cerevisiae* strains (growth inhibition zones 12–18 mm). All the tested thiepine derivatives were completely inactive against the fungal strains at a concentration 31.3 μg disk^{-1}.

TABLE IV. Antifungal activity of the thiepine derivatives expressed as diameter of the inhibition zones in millimetres (mm)

Cmpd.	*C. albicans*[a]				*S. cerevisiae*[b]				*A. brasiliensis*[a]			
	Concentration, μg disk^{-1}											
	1000	500	250	125	1000	500	250	125	1000	500	250	125
16	C.I.[c]	12	10	N.A.[d]	C.I.	18	15	11	18	14	12	10
17	C.I.	C.I.	30	N.A.	C.I.	20	16	12	C.I.	16	12	10
31	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
32	C.I.	C.I.	50	28	C.I.	30	22	18	C.I.	30	20	12
33	C.I.	14	N.A.	N.A.	C.I.	22	16	12	12	10	N.A.	N.A.
34	C.I.	C.I.	10	N.A.	C.I.	26	18	N.A.	C.I.	10	N.A.	N.A.
35	C.I.	14	N.A.	N.A.	C.I.	16	10	N.A.	12	N.A.	N.A.	N.A.

[a]Nystatin concentration 30 μg disk^{-1}, 30 mm including disk; [b]nystatin concentration 30 μg disk^{-1}, 54 mm including disk; [c]complete inhibition; [d]no activity (inhibition zone <10 mm)

EXPERIMENTAL

Instrumentation

Microwave reactions were performed in a Biotage Initiator 2.5 microwave reactor. Melting points were determined using a Boetius PMHK apparatus (Carl Zeiss, Germany) and are not corrected. The IR spectra were recorded on a Perkin-Elmer spectrophotometer FTIR 1725X. The NMR spectra were recorded on a Bruker Ultrashield Advance III spectrometer (500 MHz) using TMS as the internal standard. The chemical shifts are expressed in ppm (δ) values and coupling constants (J) in Hz. The ESI–MS (HRMS) spectra were acquired on an Agilent Technologies 1200 Series instrument equipped with a Zorbax Eclipse Plus C18 column and a DAD detector in combination with a 6210 Time-of-Flight LC/MS instrument in the positive ion mode. The samples were dissolved in MeOH. GC/MS spectra were acquired on an Agilent Technologies 7890A instrument equipped with a DB-5 MS column and 5975C MSD and FID detector. Lobar LichroPrep Si 60 or LichroPrep RP-18 columns (Merck, Germany), coupled to a Waters RI 401 detector, were used for preparative column chromatography. Thin-layer chromatography was performed on pre-coated Merck silica gel 60 F254 and Merck RP-18 F254 plates. The solution MeOH (NH_3) stands for a combination MeOH/ /NH_3 aq. = 9:1. The compounds were analyzed for purity using an Agilent 1200 HPLC system

equipped with Quat pump (G1311B) and DAD detector 1260 VL (other details are presented in the Supplementary material to this paper). All compounds were >95 % pure.

Chemistry

(2-Bromo-5-chlorophenyl)methanediyl diacetate. A mixture of 1-bromo-4-chloro-2--methylbenzene (1.0 g, 4.9 mmol), acetic anhydride (6.4 mL), acetic acid (5.0 mL) and concentrated sulphuric acid (1.5 mL) was cooled to 0 °C in an ice bath. Then an acetic acid (5.0 mL) solution of CrO_3 (1.8 g, 18.0 mmol) was dropwisely added into the stirred mixture over 1.5 h. The mixture was stirred for the next 2 h at 0 °C. The product was filtered, washed with water (50 mL) and dried under reduced pressure.

*2-Bromo-5-chlorobenzaldehyde (**19**).* (2-Bromo-5-chlorophenyl)methanediyl diacetate (1.5 g, 4.7 mmol) was refluxed in MeOH–H_2O (15 mL, 1/1 *V*/*V*) containing H_2SO_4 (1.6 mL) for 30 min. The reaction mixture was then diluted with H_2O (15 mL) and extracted with EtOAc (3×20 mL). The combined organic layers were washed with H_2O (20 mL) and brine (20 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue and 1 M hydrochloric acid (4.0 mL) were heated for 3 h in THF (15 mL) under reflux. The solvent was removed under reduced pressure. The residue was purified by column chromatography (SiO_2, hexane/EtOAc = 95/5). Yield: 0.84 g, 83 %.

*2-Bromo-5-methoxybenzaldehyde (**21**).* To the solution of *meta*-anisaldehyde (3.03 g, 22.3 mmol) in AcOH (5.0 mL), Br_2 (1.4 mL, 26.8 mmol, 1.2 eq.) was drop wisely added, and the reaction mixture was stirred for 36 h at room temperature. Upon completion, the reaction was quenched with a saturated solution of Na_2SO_3 (25 mL), then poured into water (10 mL), and extracted with EtOAc (3×25 mL). The combined organic layers were washed with water (3×20 mL) and brine (15 mL), dried (Na_2SO_4), and concentrated to give the desired 2-bromo--5-methoxybenzaldehyde (**21**, 3.99 g, 83 %).

*3-[(Z)-2-(2-Bromo-5-chlorophenyl)ethenyl]-4-chloropyridine (**24**).* To a suspension of phosphonium salt **22** (0.55 g, 1.2 mmol) in THF (12 mL) KO*t*-Bu (0.16 g, 1.4 mmol) was added. After 30 min, a solution of 2-bromo-5-chlorobenzaldehyde (0.26 g, 1.2 mmol) in THF (3 mL) was added over 5 min. The reaction mixture was stirred at room temperature for 16 h, when it was quenched with sat. aqueous soln. of $NaHCO_3$. The aqueous phase was separated and extracted with EtOAc (3×25 mL). The organic extracts were combined, dried over Na_2SO_4, concentrated under vacuum and purified by column chromatography (SiO_2, hexane/EtOAc = 95/5) to yield the *Z*-isomer (270 mg, 69 %).

*3-[(Z)-2-(2-Bromo-5-methoxyphenyl)ethenyl]-4-chloropyridine (**25**).* To a suspension of phosphonium salt **22** (0.14 g, 0.30 mmol) in THF (1.6 mL) was added KO*t*-Bu (40 mg, 0.36 mmol). After 30 min, a solution of 2-bromo-5-methoxybenzaldehyde (65 mg, 0.30 mmol) in THF (2 mL) was added over 5 min. The reaction mixture was stirred at room temperature and after 18 h, it was quenched with $NaHCO_3$. The aqueous phase was separated and extracted with EtOAc (3×10 mL). The organic extracts were combined, dried over Na_2SO_4, concentrated under vacuum and purified by preparative column chromatography (RP, MeOH/H_2O = = 8:2) to yield the *Z*-isomer (60 mg, 82 %).

*3-[(Z)-2-(2-Bromo-5-fluorophenyl)ethenyl]-4-chloropyridine (**26**).* To a suspension of phosphonium salt **22** (0.45 g, 0.96 mmol) in THF (12 mL) was added KO*t*-Bu (0.13 g, 1.2 mmol). After 30 min, a solution of 2-bromo-5-fluorobenzaldehyde (0.19 g, 0.96 mmol) in THF (2 mL) was added over 5 min. The reaction mixture was stirred at room temperature and after 18 h, it was quenched with saturated aqueous solution of $NaHCO_3$ (15 mL). The aqueous phase was separated and extracted with EtOAc (3×20 mL). The organic extracts were com-

bined, dried over Na_2SO_4, concentrated under vacuum and purified by preparative column chromatography (RP, MeOH/H_2O = 8:2) to yield compound **26** (196 mg, 65 %).

General procedure for Pd-catalyzed amination

A reaction tube containing a stirring bar was evacuated and backfilled with argon. The tube was then charged with $Pd(OAc)_2$ (5 mol %), JohnPhos (10 mol %) and NaO*t*-Bu (2.8 eq.) and filled with argon. Toluene was added. After stirring at room temperature for 5 min, an aryl halide (1 eq.) and amine (3 eq.) were added, the tube was filled with argon and capped. Reaction mixture was heated to 100 °C and stirred at the same temperature. Products were purified by preparative column chromatography: SiO_2, CH_2Cl_2/MeOH(NH_3) = 9/1.

*5-[3-(Pyrrolidin-1-yl)propyl]-5*H*-pyrido[4,3-b][1]benzazepine (**27**).* Following the general procedure, a mixture of 3-[(*Z*)-2-(2-bromophenyl)ethenyl]-4-chloropyridine (24 mg, 0.080 mmol), 3-(pyrrolidin-1-yl)propan-1-amine (31 µL, 0.24 mmol), sodium *tert*-butoxide (22 mg, 0.23 mmol), $Pd(OAc)_2$ (0.9 mg, 5 mol %), JohnPhos (2.4 mg, 10 mol %) and toluene (1.5 mL) was stirred at 100 °C for 48 h. Yield: 17 mg, 68 %.

*5-[3-(Pyrrolidin-1-yl)propyl]-5*H*-dipyrido[4,3-b:3′,4′-f]azepine (**28**).* Following the general procedure, a mixture of 3,3′-(*Z*)-ethene-1,2-diylbis(4-chloropyridine) (20 mg, 0.080 mmol), 3-(pyrrolidin-1-yl)propan-1-amine (31 µL, 0.24 mmol), sodium *tert*-butoxide (22 mg, 0.23 mmol), $Pd(OAc)_2$ (0.9 mg, 5 mol %), JohnPhos (2.4 mg, 10 mol %) and toluene (1.5 mL) was stirred at 100 °C for 24 h. Yield: 17 mg, 69 %.

*3-(8-Chloro-5*H*-pyrido[4,3-b][1]benzazepin-5-yl)*-N,N-*dimethylpropan-1-amine (**29**).* Following the general procedure, a mixture of 3-[(*Z*)-2-(2-bromo-5-chlorophenyl)ethenyl]-4--chloropyridine (26 mg, 0.080 mmol), 3-(dimethylamino)-1-propylamine (30 µL, 0.24 mmol), sodium *tert*-butoxide (22 mg, 0.23 mmol), $Pd(OAc)_2$ (0.9 mg, 5 mol %), JohnPhos (2.4 mg, 10 mol %) and toluene (1.5 mL) was stirred at 100 °C for 48 h. Yield: 18 mg, 70 %.

*3-(8-Methoxy-5*H*-pyrido[4,3-b][1]benzazepin-5-yl)*-N,N-*dimethylpropan-1-amine (**30**).* Following the general procedure, a mixture of 3-[(*Z*)-2-(2-bromo-5-methoxyphenyl)ethenyl]--4-chloropyridine (26 mg, 0.080 mmol), 3-(dimethylamino)-1-propylamine (30 µL, 0.24 mmol), sodium *tert*-butoxide (22 mg, 0.23 mmol), $Pd(OAc)_2$ (0.9 mg, 5 mol %), JohnPhos (2.4 mg, 10 mol %) and toluene (1.5 mL) was stirred at 100 °C for 48 h. Yield: 10 mg, 40 %.

General procedure for the synthesis of the thiepine derivatives

A reaction tube containing a stirring bar was evacuated and backfilled with argon. The tube was charged with tris(dibenzylideneacetone)dipalladium (Pd_2dba_3, 5 mol %), dppf (10 mol %), NaO*t*-Bu (1.2 eq.), aryl halide (1 eq.) and $KSCOCH_3$ (1.2 eq.) and evacuated and backfilled with argon. The flask was capped with a rubber septum, and toluene was added. The reaction mixture was heated in a Biotage initiator 2.5 microwave at 170 °C for 60 min. After completion of the reaction, the mixture was cooled to room temperature. The products were purified by column chromatography: SiO_2, hexane/EtOAc = 8/2.

*[1]Benzothiepino[3,2-c]pyridine (**16**).* Following the general procedure, a mixture of 3-[(*Z*)-2-(2-bromophenyl)ethenyl]-4-chloropyridine (35 mg, 0.12 mmol), $KSCOCH_3$ (16 mg, 0.14 mmol), sodium *tert*-butoxide (14 mg, 0.14 mmol), Pd_2dba_3 (5.4 mg, 5 mol %), dppf (6.6 mg, 10 mol %) and toluene (1.5 mL) was heated in a Biotage Initiator 2.5 microwave at 170 °C for 60 min. Yield: 13 mg, 51 %.

*Pyrido[3′,4′:6,7]thiepino[3,2-c]pyridine (**17**).* Following the general procedure, a mixture of 3,3′-(*Z*)-ethene-1,2-diylbis(4-chloropyridine) (30 mg, 0.12 mmol), $KSCOCH_3$ (16 mg, 0.14 mmol), sodium *tert*-butoxide (14 mg, 0.14 mmol), Pd_2dba_3 (5.4 mg, 5 mol %), dppf (6.6

mg, 10 mol %) and toluene (1.5 mL) was heated in a Biotage initiator 2.5 microwave at 170 °C for 60 min. Yield: 15 mg, 60 %.

*8-Chloro[1]benzothiepino[3,2-c]pyridine (**31**).* Following the general procedure, a mixture of 3-[(*Z*)-2-(2-bromo-5-chlorophenyl)ethenyl]-4-chloropyridine (29 mg, 0.088 mmol), $KSCOCH_3$ (12 mg, 0.11 mmol), sodium *tert*-butoxide (10 mg, 0.11 mmol), Pd_2dba_3 (4.0 mg, 5 mol %), dppf (4.9 mg, 10 mol %) and toluene (1.1 mL) was heated in a Biotage initiator 2.5 microwave at 170 °C for 60 min. Yield: 6.8 mg, 32 %.

*8-Methoxy[1]benzothiepino[3,2-c]pyridine (**32**).* Following the general procedure, a mixture of 3-[(*Z*)-2-(2-bromo-5-methoxyphenyl)ethenyl]-4-chloropyridine (60 mg, 0.18 mmol), $KSCOCH_3$ (25 mg, 0.22 mmol), sodium *tert*-butoxide (21 mg, 0.22 mmol), Pd_2dba_3 (8.5 mg, 5 mol %), dppf (10 mg, 10 mol %) and toluene (2.3 mL) was heated in a Biotage initiator 2.5 microwave at 170 °C for 60 min. Yield: 8.4 mg, 19 %.

*8-Fluoro[1]benzothiepino[3,2-c]pyridine (**33**).* Following the general procedure, a mixture of 3-[(*Z*)-2-(2-bromo-5-fluorophenyl)ethenyl]-4-chloropyridine (30 mg, 0.096 mmol), $KSCOCH_3$ (13 mg, 0.12 mmol), sodium *tert*-butoxide (11 mg, 0.12 mmol), Pd_2dba_3 (4.4 mg, 5 mol %), dppf (5.3 mg, 10 mol %) and toluene (1.2 mL) was heated in a Biotage Initiator 2.5 microwave at 170 °C for 60 min. Yield: 7.6 mg, 35 %.

*8-Chloro-2-methyl-1,2,3,4-tetrahydro[1]benzothiepino[3,2-c]pyridine (**34**).* A solution of 8-chloro[1]benzothiepino[3,2-*c*]pyridine (17 mg, 0.068 mmol) in MeCN (3 mL) was refluxed with an excess of methyl iodide (25 μL, 0.27 mmol, 4 eq.). After 2 h, the solvent was removed under reduced pressure. The resulting yellow solid was dissolved in dry methanol (3 mL) and $NaBH_4$ (6.0 mg, 0.13 mmol) was added under an inert atmosphere at room temperature. After 15 min, the MeOH was removed under reduced pressure. The crude residue was dissolved in EtOAc and washed with H_2O. The organic layer was dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by column chromatography (SiO_2, EtOAc/MeOH = 1:1) to yield the product (11.8 mg, 66 %).

*8-Phenyl[1]benzothiepino[3,2-c]pyridine (**35**).* A reaction tube containing a stirring bar was evacuated and backfilled with Ar. The tube was then charged with $Pd(OAc)_2$ (0.9 mg, 5 mol %), SPhos (3.2 mg, 10 mol %), phenylboronic acid (12 mg, 0.095 mmol, 1.2 eq.) and anhydrous K_3PO_4 (34 mg, 0.16 mmol, 2.0 eq.). The tube was capped with a rubber septum and filled with argon. Dry toluene (1.0 mL) was added through the septum and the resulting mixture was stirred at room temperature for 2 min. 8-Chloro[1]benzothiepino[3,2-*c*]pyridine (20 mg, 0.079 mmol) was added and the tube was sealed. The reaction mixture was heated at 100 °C for 18 h. The reaction mixture was allowed to cool to room temperature. The product was purified by column chromatography (SiO_2, hexane/EtOAc = 8/2). Yield: 11.5 mg, 50 %.

Antimicrobial evaluation

Microbroth dilution method. The antimicrobial activity was evaluated using a broth microdilution method according to NCCLS (National Committee for Clinical Laboratory Standards (2000) Approval standard document M7-A5, Villanova, PA, USA). The following Gram-negative bacterial strains used were: *Escherichia coli* (ATCC 25922), *Proteus hauseri* (ATCC 13315), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enterica* subsp. *enterica* serovar Enteritidis (ATCC 13076) and *Klebsiella pneumoniae* (ATCC 10031). The Gram-positive bacterial strains used were: *Staphylococcus aureus* (ATCC 6538), *Micrococcus luteus* (ATCC 10240) and *M. luteus* (ATCC 4698). The employed fungal species were: *Candida albicans* (ATCC 10231), *Saccharomyces cerevisiae* (ATCC 9763) and *Aspergillus brasiliensis* (ATCC 16404). *MIC* determination was performed by a serial dilution method in sterile 96-well microtitre plates. Fresh Mueller–Hinton broth (for bacteria) and Sabouraud dextrose

broth (for fungi) were used. Stock solutions of the compounds were prepared in dimethyl sulphoxide (DMSO), and then serial dilutions of the compounds were made in the concentration range from 10,000 to 4.9 μg mL^{-1}. Amikacin (AMK) and chloramphenicol (CHL) were used as positive controls for the bacteria, while nystatin (NYT) and fluconazole (FLC) were used as positive controls for the fungi. The solvent (DMSO) served as negative control. In each well of the plate, ten microlitres of bacterial cultures (10^6 cells mL^{-1}) for antibacterial activity and 10 mL of fungal cultures (10^5 spores mL^{-1}) were inoculated. The microtiter plates were incubated at 37 °C for 24 h for the bacteria or at 28 °C for 48 h for the fungi. The *MIC* was determined as the lowest concentration that resulted in inhibition of bacterial or fungal growth.

Disk diffusion method. Antimicrobial activity was evaluated using a disk diffusion method according to NCCLS (National Committee for Clinical Laboratory Standards (1997) Approval standard document M2-A6 Performance standards for antibacterial disk susceptibility test, Wayne, PA, USA).

Antibacterial activity. The antibacterial activity was evaluated using four different strains of Gram-negative bacteria: *E. coli* (ATCC 25922), *P. hauseri* (ATCC 13315), *P. aeruginosa* (ATCC 9027) and *S. enterica* subsp. *enterica* serovar Enteritidis (ATCC 13076), and four different strains of the Gram-positive bacteria: *Clostridium sporogenes* (ATCC 19404), *S. aureus* (ATCC 6538), *M. luteus* (ATCC 10240) and *Kocuria rhizophila* (ATCC 9341). The determination of antibacterial activity was performed using the disk diffusion method. In each Petri dish (90 mm diameter), 22 mL of nutrient agar and 100 μL of bacterial suspension were added. The test substances were dissolved in CH_2Cl_2 (1 mg 100 μL^{-1}) and then 100 μL of solution was applied to a filter paper disk (8 mm in diameter) and the solvent was evaporated. The loaded disks were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Amikacin 30 μg per filter paper disk (8 mm in diameter) was used as the positive control, while the disks of the same diameter impregnated with 100 μL of CH_2Cl_2 were used as the negative control. The plates were incubated for 24 h at 37 °C. The zones of inhibition were recorded in millimetres.

Antifungal activity. The antifungal activity was tested against three different strains: *C. albicans* (ATCC 10231), *S. cerevisiae* (ATCC 9763) and *A. brasiliensis* (ATCC 16404). Sabouraud dextrose agar was prepared according to the manufacture's instruction. Into each sterile Petri dish (90 mm diameter), 22 mL of previously prepared agar suspension was poured and 100 μL of fungi was added. The test compounds were dissolved in CH_2Cl_2 and applied on filter paper disk (8 mm in diameter) at final concentrations 1000, 500, 250, 125, 62.5 and 31.3 μg/disk. Nystatin (30 μg $disk^{-1}$) was used as a positive control while a disk impregnated with CH_2Cl_2 was used as the negative control. Petri dishes were incubated for 48 h at 28 °C. The zone of inhibition was measured in millimetres, including the disk.

CONCLUSIONS

New pyridobenzazepine and pyridobenzothiepine derivatives were synthesized using a methodology for Pd-catalyzed formation of C–N and C–S bonds.[9] Additionally, the successful transformations of thiepine **31** to tetrahydro and biphenyl derivatives opened up new possibilities for the preparation of structurally diverse substituted derivatives. All newly and previously synthesized compounds were evaluated for their *in vitro* antimicrobial activity against eight bacterial and three fungal pathogenic strains. All pyridobenzazepine derivatives

showed better antibacterial and antifungal activity than the corresponding dipyridoazepine analogues. Among the synthesized azepines, derivative **8** was the most active and showed a broad spectrum of antibacterial activity (*MIC* ranged 39–78 μg mL^{-1}). The synthesized thiepine derivatives exhibited weak antibacterial activity but, on the other hand, with the exception of **31**, all thiepines showed pronounced antifungal activity.

SUPPLEMENTARY MATERIAL

Analytical and spectral data of the compounds, as well as copies of the corresponding ^{1}H-NMR and ^{13}C-NMR spectra of the products and HPLC purity chromatograms are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА И АНТИМИКРОБНА АКТИВНОСТ АЗЕПИНСКИХ И ТИЕПИНСКИХ ДЕРИВАТА

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Синтетисана је серија пиридобензазепинских и пиридобензотиепинских деривата формирањем C–N и C–S веза помоћу катализатора на бази Pd и испитана је њихова *in vitro* антимикробна активност. Синтетисани пиридобензазепински деривати показују већу антибактеријску и антифунгалну активност у поређењу са одговарајућим дипиридоазепинским дериватима. Азепин **8** показао је највећу антибактеријску активност (*MIC* у опсегу 39–78 μg mL^{-1}), а азепин **12** показао се као најактивнији дериват према испитаним сојевима гљива (*MIC* у опсегу 156–313 μg mL^{-1}). Синтетисани тиепински деривати имају слабу антибактеријску активност, али са друге стране имају добру антифунгалну активност.

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