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Synthesis and antimicrobial activity of new 3,5-diarylidene-4-piperidone derivatives

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Abstract: Three series of heteroaromatic analogues (twenty seven compounds) with monoketone linkers were synthesized and evaluated for their antimicrobial activity against six microbial strains. Among them, 3,5-dibenzylidene-1-[(3,5-dichloro-2-hydroxyphenyl)sulfonyl]piperidin-4-one (**5e**) showed the best antifungal activity against *Aspergillus niger* and *A. fumigatus*. Structural elucidation of the synthesized compounds was realised based on various spectroscopic methods.

Keywords: *N*-arylsulfonyl-3,5-diarylidene-4-piperidone; *N*-alkylcarbonyl-3,5-diarylidene-4-piperidone; crotonisation; antimicrobial activity.

INTRODUCTION

The emergence of antibiotic resistance is an evolutionary process that is based on the selection of organisms that have enhanced ability to survive doses of antibiotics that would previously have been lethal.[1] Antibiotics such as penicillin[2] and erythromycin[3] that used to be considered as one-time miracle cures are now less effective because bacteria have evolved and become more resistant. While the prevalence of resistant bacteria is increasing, unfortunately, there is a marked decrease in the R&D industry. This is a cause for alarm, especially for physicians who treat infectious diseases. The pipelines of new antibiotics are drying up. Major pharmaceutical companies are losing interest in the antibiotics market because these drugs may not be profitable as drugs that treat chronic conditions and lifestyle issues. If this trend continues, effective antibiotics may not be available to treat seriously ill patients in the near future. Thus, the research to find new as well as effective drug against antibacterial and antifungal strains is vital in nature.

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Curcumin analogue is a versatile lead molecule in the pharmaceutical development and has a wide range of biological activities, especially in the 3,5-diarylidene-4-piperidone system, such as anti-microbial,[4,5] topoisomerase IIα inhibition,[6] cytotoxic activity,[7,8] HIV-I multiplication,[9] anti-carcinogenic and anti-angiogenesis agents,[10] anti-malarial,[11] anti-oxidant,[12] anti-inflammatory,[13] anti-cancer[14] and anti-mycobacterium tuberculosis.[15] In the present study, the 3,5-diarylidene-4-piperidone function with sulphonamide and peptide substitutions are introduced, which are quite stable, encouraging chemists to utilize this stable fragment in bioactive moieties to synthesize new compounds possessing biological activities.

EXPERIMENTAL

Chemistry

All the purchased reagents and solvents were of reagent grade and used without further purification. Progress of reactions was monitored by TLC on silica gel-coated aluminium sheets (Type 60 GF254). The FTIR spectra (in KBr pellets) were recorded on a Nucon infrared spectrophotometer and only characteristic peaks are reported in cm^{-1}. The melting points were measured in open capillaries and are uncorrected. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker 400/100 MHz spectrometer in DMSO-d_6 as solvent. The chemical shift values are reported in ppm and J values are given in Hz. The mass spectrometric data was determined using an Agilent 1200 (liquid chromatography) series instrument. The products were purified by flash column chromatography using silica gel (60–120 mesh). The analytical and spectral data of the synthesized compounds are included in the Supplementary material to this paper.

*General procedure for synthesis of 3,5-diarylidene-4-piperidone **3a–f***

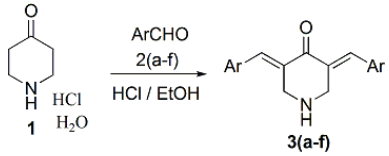
A mixture of 4-piperidone hydrochloride monohydrate (**1**, 10 mmol) and various substituted aromatic aldehydes (20 mmol) in ethanol (10 mL) was stirred at room temperature (29 °C) for 10 min. The reaction mixture was cooled to 0 °C and conc. HCl (2.0 g, 20 mmol) was added dropwise. The resulting mixture was refluxed for about 4.5 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the crude product was filtered and recrystallized from ethanol to obtain pure **3a**, **3b**, **3d** and **3f** of this series in excellent yields. The compounds **3c** and **3e** were neutralized with 10 % NaOH (8 mL), extracted twice with chloroform (2×50 mL) and dried over by sodium sulphate. The combined organic layer was concentrated under reduced pressure and the crude product was purified by column chromatography using a 5 % mixture of methanol and chloroform as the eluent to obtain pure compounds **3c** and **3e**. Detailed structures for corresponding reactants and products are shown in Table I.

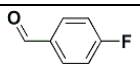
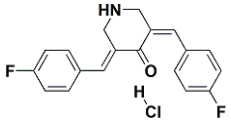
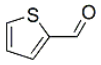
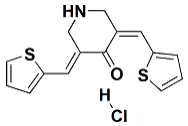
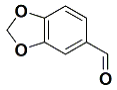
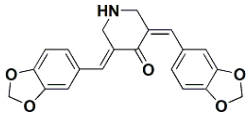
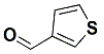
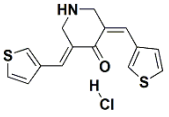
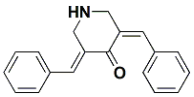
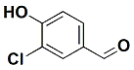
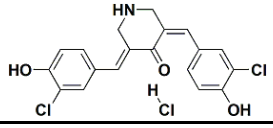
*General procedure for synthesis of **5a–q***

A mixture of 3,5-bis(4-fluorobenzylidene)piperidin-4-one hydrochloride (**3a**, 0.34 g, 1 mmol) and triethylamine (0.203 g, 2 mmol) in dichloromethane (DCM, 10 mL) was stirred at room temperature (29 °C), 3,5-dichloro-2-hydroxyphenylsulfonyl chloride (**4a**, 0.28g, 1.1 mmol) was added to the mixture and stirring was continued for 30 min. The progress of the reaction was monitored by TLC. After completion of the reaction, the resulting mixture was diluted with water and extracted twice with DCM (2×10 mL). The crude product was purified by gravity column chromatography using 3 % mixture of methanol and DCM as the eluent to

obtain 1-[(3,5-dichloro-2-hydroxyphenyl)sulfonyl]-3,5-bis(4-fluorobenzylidene)piperidin-4--one (**5a**) in excellent yield (0.49 g, 92 %). The above general method was adopted for the synthesis of compounds **5b**–**q** of this series. Compound **5f** was synthesized from **3f** without protection of the hydroxyl group. Detailed structures for corresponding reactants and products are shown in Tables II–IV.

TABLE I. Synthesis of compounds **3a**–**f**



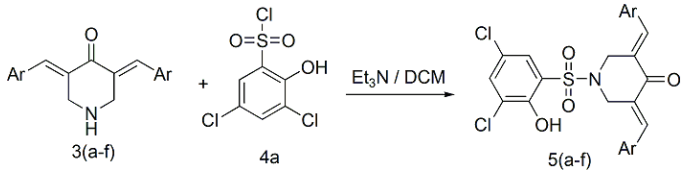
Entry	ArCHO	**3a**–**f**
3a		
3b		
3c		
3d		
3e		
3f		

*General procedure for the synthesis of **7a** and **b***

A mixture of 3,5-bis(2-thienylmethylene)piperidin-4-one hydrochloride (**3b**, 0.32 g, 1 mmol) and 2-[(*tert*-butoxycarbonyl)amino]acetic acid (**6a**, 0.17 g, 1.1 mmol) in DCM (10 mL) was stirred at room temperature (29 °C), triethylamine (0.50 g, 5 mmol) was added to the mixture and stirring was continued for 15 min. The reaction mixture was cooled to 0 °C, 50 % solution of propylphosphonic anhydride in ethyl acetate (T 3P, 1.59 g, 2.5 mmol) was added dropwise to the mixture and stirring was continued for 12 h at room temperature (29 °C). The progress of the reaction was monitored by TLC. After completion of the reaction, the resulting mixture was diluted with DCM (30 mL) and washed with water (2×50 mL). The DCM layer

was dried over sodium sulphate and concentrated under reduced pressure. The crude product was purified by chromatography using a 5 % mixture of methanol and DCM as the eluent to obtain *tert*-butyl {2-oxo-2-[4-oxo-3,5-bis(2-thienylmethylene)piperidin-1-yl]ethyl}carbamate (**7a**) in good yield (0.35 g, 78 %). The above general method was adopted for the synthesis of compound **7b** of this series. Detailed structures for the corresponding reactants and products are shown in Table V.

TABLE II. Synthesis of compounds **5a**–**f**



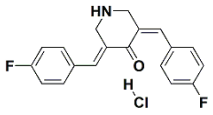
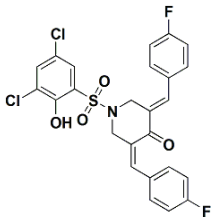
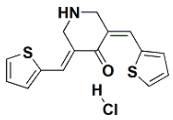
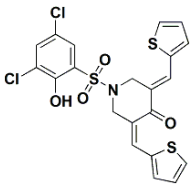
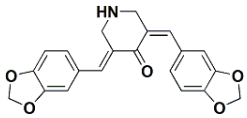
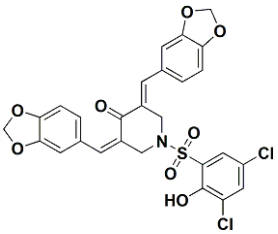
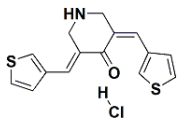
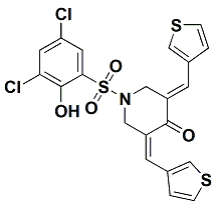
Entry	**3a**–**f**	**5a**–**f**
5a		
5b		
5c		
5d		

TABLE II. Continued

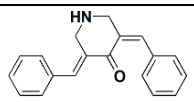
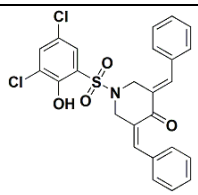
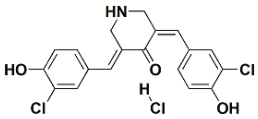
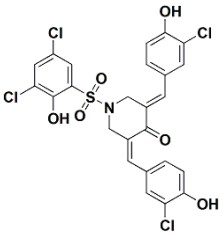
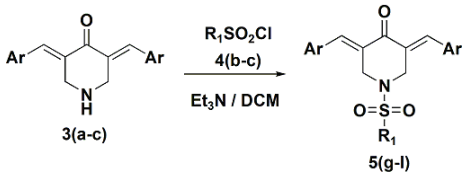
Entry	**3a–f**	**5a–f**
5e		
5f		

TABLE III. Synthesis of compounds **5g–l**



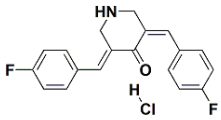
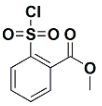
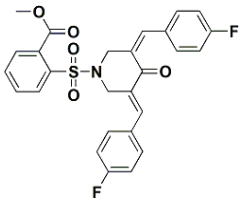
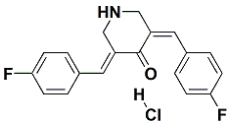
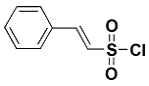
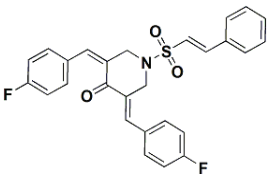
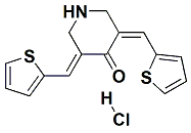
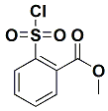
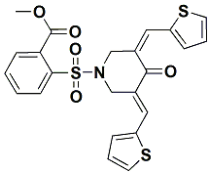
Entry	**3a–c**	R1SO_2Cl	**5g–l**
5g			
5h			
5i			

TABLE III. Continued

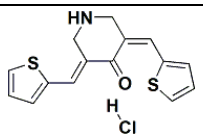
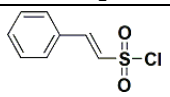
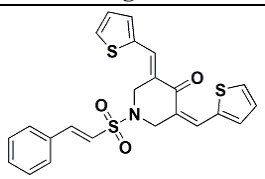
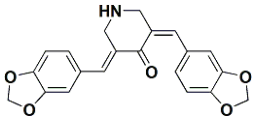
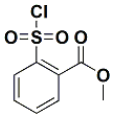
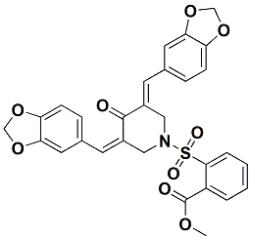
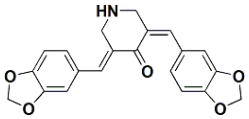
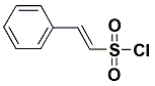
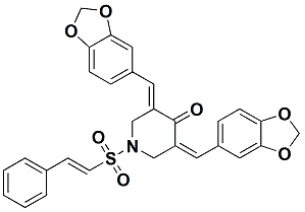
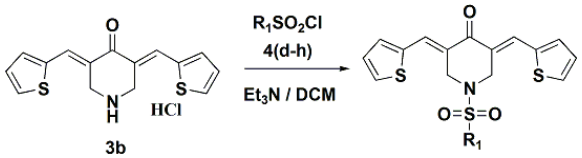
Entry	**3a–c**	$R1SO_2Cl$	**5g–l**
5j			
5k			
5l			

TABLE IV. Synthesis of compounds **5m–q**



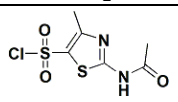
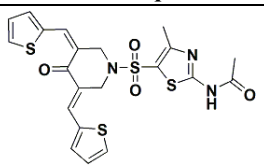
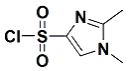
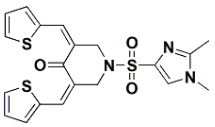
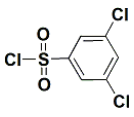
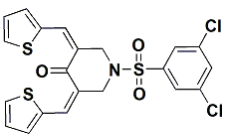
Entry	$R1SO_2Cl$	**5m–q**
5m		
5n		
5o		

TABLE IV. Continued

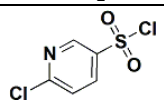
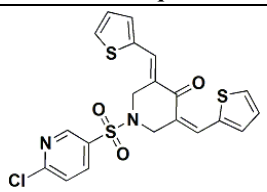
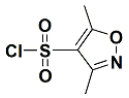
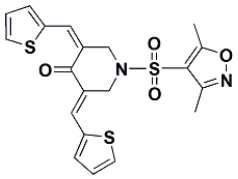
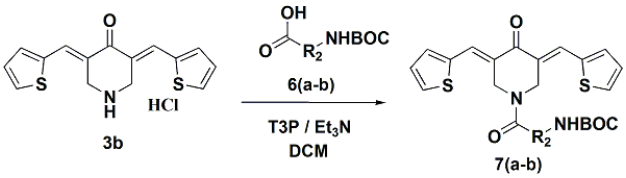
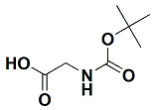
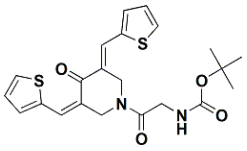
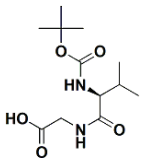
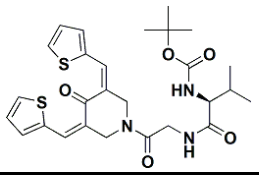
Entry	$R1SO_2Cl$	**5m–q**
5p		
5q		

TABLE V. Synthesis of compounds **7a** and **b**

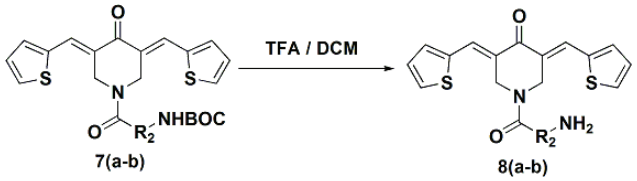


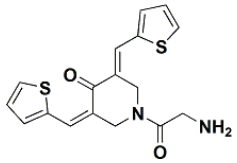
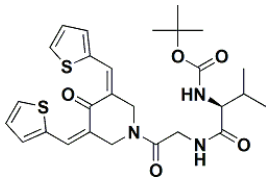
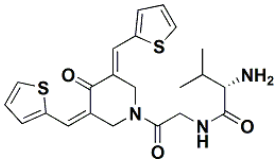
Entry	**6a** and **b**	**7a** and **b**
7a		
7b		

*General procedure for synthesis of **8a** and **b***

A mixture of *tert*-butyl {2-oxo-2-[4-oxo-3,5-bis(thiophen-2-ylmethylene)piperidin-1-yl]-ethyl}carbamate (**7a**, 0.44 g, 1 mmol) in dry DCM (10 mL) was stirred at room temperature (29 °C) for 15 min. The reaction mixture was cooled to 0 °C, trifluoroacetic acid (TFA, 0.57 g, 5 mmol) was added dropwise to the mixture and stirring was continued for 2 h at 29 °C. The progress of the reaction was monitored by TLC. After completion of the reaction, excess solvent was evaporated under reduced pressure to dryness. The crude product was washed with *n*-hexane repeatedly to remove traces of residual acid to obtain 1-(2-aminoacetyl)-3,5--bis(2-thienylmethylene)piperidin-4-one (**8a**) in excellent yield (0.42 g, 93 %). The above general method was adopted for the synthesis of compound **8b** of this series. Detailed structures for corresponding reactants and products are shown in Table VI.

TABLE VI. Synthesis of compounds **8a** and **b**



Entry	**7a** and **b**	**8a** and **b**
8a		
8b		

Antimicrobial activity assay

The antimicrobial activities of the synthesized compounds were evaluated by the agar well-diffusion method.[16,17] The diameter of the inhibition zone in mm (*IZD*) was used as a criterion for the antimicrobial activity. The antimicrobial activities were assayed biologically using diffusion plate technique. The experiments were performed by pouring a spore suspension 10^6 colony-forming units (*CFU*) per mL of the test strain to 75 mL of nutrient agar medium at 45 °C and mixing well, and pouring into a 15 cm sterile metallic Petri plate. The medium was allowed to solidify and 8 mm wells were dug with a sterile metallic borer, then a dimethyl sulfoxide (DMSO) solution of the test sample (1 mL) at 1 μg mL^{-1} was added to the respective wells. The layer was allowed to set for 30 min and incubated at 37 °C for 48 h and the results were noted. A solvent control (DMSO) was included in every experiment as a negative control. Ciprofloxacin and fluconazole were used as standard drugs for studying the potential activities of these compounds.

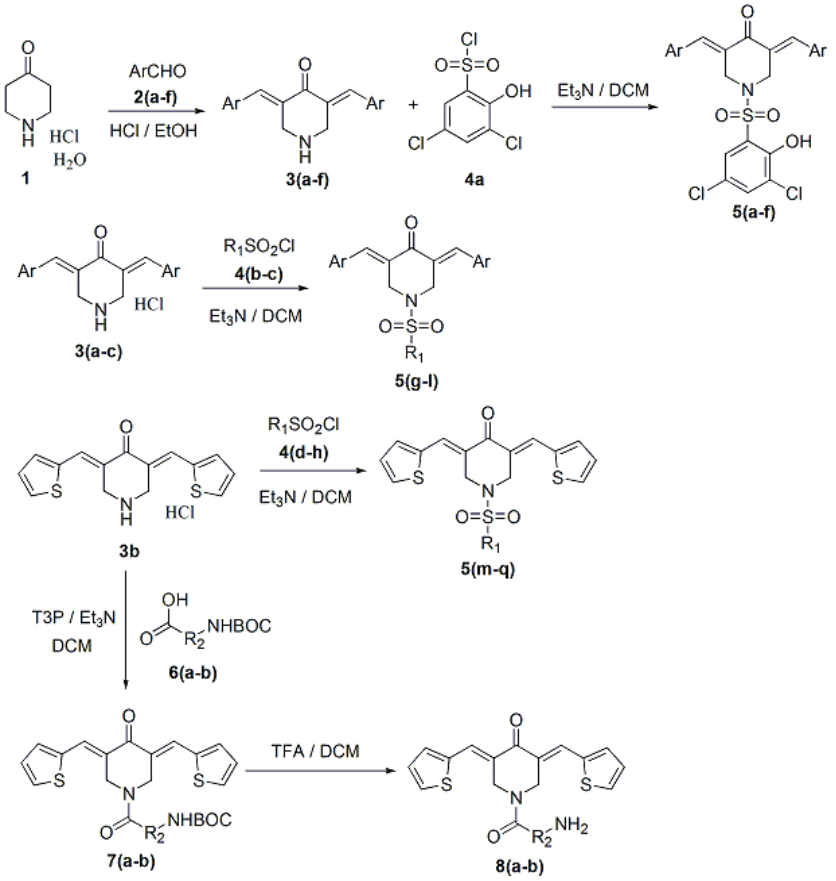
RESULTS AND DISCUSSION

Chemistry

Detailed analytical and spectral data of the synthesized compounds are given in the Supplementary material to this paper.

The reaction sequences employed for synthesis of title compounds are showed in Scheme 1. In the present work, 3,5-diarylidene-4-piperidones **3a**–**f** were synthesized from 4-piperidone hydrochloride monohydrate (**1**) and various substituted aromatic aldehydes **2a**–**f** in ethanol and aq. HCl, at reflux.[10] Simple filtration of the reaction mixture afforded the pure crystalline 3,5-diarylidene-4--piperidone hydrochloride except **3c** and **3e**. However, these two compounds

were purified further using column chromatography after neutralising with dil. NaOH. Compounds **3a**–**f** were stirred with various sulfonyl chlorides **4a**–**h** and triethylamine in DCM to obtain *N*-arylsulfonyl-3,5-diarylidene-4-piperidones **5a**–**q**. Synthesis of Boc-protected *N*-alkylcarbonyl-3,5-bis(2-thienylmethylene)-piperidines **7a**–**b** from 3,5-bis(2-thienylmethylene)piperidin-4-one hydrochloride **3b**, different Boc-protected amino acids and triethylamine was performed in DCM and propylphosphonic anhydride solution in ethyl acetate. The *N*-alkyl-carbonyl-3,5-bis(2-thienylmethylene)piperidines **8a**–**b** were obtained by dissolving **7a**–**b** in DCM and TFA with stirring at room temperature.



Scheme 1. Synthesis of 3,5-diarylidene-4-piperidone derivatives **3a**–**f**, **5a**–**q**, **7a** and **b** and **8a** and **b**.

Antimicrobial evaluation

The synthesized compounds were evaluated for their *in vitro* antimicrobial activity using agar well diffusion method against four bacterial and two fungal

pathogenic strains, *i.e.*, *Salmonella enterica* subsp. *enterica serovar Typhi* (ATCC-700931*), Vibrio cholerae* (ATCC-14033), *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* subsp. *aureus* (ATTC-9144), *A. niger* (ATCC-9029) and *Aspergillus fumigatus* (ATCC-46645). DMSO was used for dissolving the tested compounds. It showed no inhibition zones, confirming that it had no influence on the growth of the tested microorganisms. The results of testing for antibacterial and antifungal effects summarized in Table VII showed that the new derivatives tested showed variable *in vitro* antibacterial and antifungal actions.

TABLE VII. *In vitro* antimicrobial activity of the compounds tested by the well-diffusion agar assay expressed as the diameter (mm) of the inhibition zone

Tested compound	Bacteria				Fungi	
	S. enterica	*V. cholerae*	*E. coli*	*S. aureus*	*A. niger*	*A. fumigatus*
3a	13	14	8	12	18	17
3b	14	8	9	8	12	16
3c	10	8	8	7	11	13
3d	11	17	11	12	15	11
3e	12	14	15	10	17	14
3f	14	15	13	11	16	13
5a	16	15	7	11	18	15
5b	20	18	9	10	20	18
5c	16	10	7	8	17	12
5d	11	10	12	17	19	13
5e	22	18	15	21	26	24
5f	10	14	16	14	18	14
5g	14	12	9	11	15	11
5h	12	14	9	10	16	12
5i	12	10	9	10	14	13
5j	14	12	8	11	15	13
5k	8	8	9	8	12	9
5l	9	8	9	8	13	9
5m	11	11	10	10	14	11
5n	12	10	10	10	14	13
5o	12	11	10	12	14	12
5p	18	12	11	11	20	14
5q	12	10	11	10	14	14
7a	11	10	13	10	14	13
7b	10	11	12	11	13	12
8a	11	12	13	14	14	12
8b	18	14	13	12	20	16
Ciprofloxacin	25	23	22	22	–	–
Fluconazole	–	–	–	–	25	26

Considering the antimicrobial activity of compounds **3a**–**f**, most of the compounds showed moderate activity against all the microbial strains. Especially, compounds **3a**, **3b**, **3d** and **3f** were tested as HCl salt. From the screening results,

considering the antibacterial activity of compounds **5a**–**q**, it could be seen that compounds **5b** and **5e** showed moderate activity against *S. enterica* and *V. cholerae.* Similarly, compounds **5a**, **5c** and **5p** showed moderate activity against *S. enterica*. The rest of the compounds showed non-significant activity against the tested bacteria compared with the standard drugs.

Considering the antifungal activity of compounds **5a**–**q**, interestingly compound **5e** showed promising inhibitory activity against *A. niger* and *A. fumigatus* compared with fluconazole as the reference drug. Compounds **5b**, **5p** and **5d** showed moderate activity against *A. niger.* Considering the antimicrobial activity of compounds **7a** and **b** and **8a** and **b**, most of the compounds showed moderate activity against all the microbial strains. The inhibition zone diameter obtained for these compounds suggested that some of the synthesized compounds possess moderate antimicrobial activity against the tested organisms used in these assays. Whereas, 3,5-dichloro-2-hydroxyphenylsulfonyl group on the piperdine-4-one ring **5e** showed best antifungal activity against *A. niger* and *A. fumigatus*, when compared with the other compounds.

CONCLUSION

In conclusion, a general and convenient method for the synthesis of new *N*-arylsulfonyl-3,5-diarylidene-4-piperidones **5a**–**q**, *N*-alkylcarbonyl-3,5-diarylidene-4-piperidone derivatives **7a** and **b** and **8a** and **b** in moderate to excellent yields was reported. The synthesized compounds were evaluated for their *in vitro* antimicrobial activity against a representative panel of pathogenic strains using the agar well diffusion method and the results indicated that compound **5e** showed the best antifungal activity against *A. niger* and *A. fumigatus*. Hence, active compound **5e** could be considered for investigation of its mode of action and for further development.

SUPPLEMENTARY MATERIAL

Detailed analytical and spectral data of the synthesized compounds are available electronically from http://www.sbd.org.rs/JSCS/ or from the corresponding author on request.

И З В О Д
СИНТЕЗА И АНТИМИКРОБНА АКТИВНОСТ НОВИХ ДЕРИВАТА 3,5-ДИАРИЛИДЕН-4-ПИПЕРИДОНА

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Синтетисане су три серије (двадесет седам једињења) хетероароматичних аналога који садрже монокетонске линкере и испитана је антимикробна активност добијених производа према шест микробних сојева. Од испитиваних једињења, 3,5-дибензилиден-1-[(3,5-дихлор-2-хидроксифенил)сулфонил]пиперидин-4-он (**5e**) показује најбољу анти-

фунгалну активност према *Aspergillus niger* и *A. fumigatus*. Одређивање структуре синтетисаних једињења извршено је уобичајеним спектроскопским методама.

(Примљено 12. новембра 2015, ревидирано 10. априла, прихваћено 20. маја 2016)

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