SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF A NEW DERIVATIVE OF LEVOFLOXACIN

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ABSTRACT

A new levofloxacin derivative using silver triflate with antibacterial activity was synthesized and characterized. The new compound has been physicochemically characterized through elemental analysis, spectroscopic and thermal methods. All correlated experimental data suggested that the levofloxacin triflate was obtained. The antibacterial activity of the new compound was tested against six Gram-positive and Gram-negative bacteria. *In vitro,* the new compound had similar activity to levofloxacin against *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* and very closed to the minimum inhibitory concentration values of levofloxacin against *Staphylococcus aureus* MRSA, *Enterococcus faecalis,* and *Pseudomonas aeruginosa*.

Keywords: levofloxacin, triflate, silver triflate, antibacterial, antibiotic resistance.

INTRODUCTION

Increasing antibiotic resistance of bacteria has become a real threat to humanity. Worldwide many organizations and governments fight and try to align their action plans to combat this dangerous phenomenon [1]. Nowadays, only a few new antibiotics have been discovered and introduced into therapy. Unfortunately, no new class of antibiotics has been found for decades [2]. Levofloxacin (LVF) is a third-generation fluoroquinolone, the *S* stereoisomer of the racemic ofloxacin (**Figure 1**) [3].

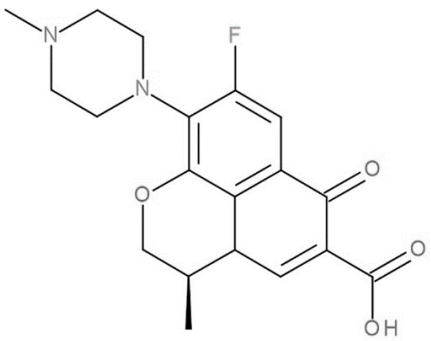

Figure 1. The chemical structure of LVF: *(S)*-9-fluoro-2,3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid.

LVF interfere with bacterial DNA synthesis by inhibiting DNA gyrase and topoisomerase IV, the two target enzymes. LVF has a broad spectrum being active on Gram-positive, Gram-negative bacteria, and atypical bacteria. LVF has been used successfully to treat a large number of severe infectious diseases [4, 5]. Researchers strive to obtain new antibacterial compounds through the design of new fluoroquinolone derivatives to combat increasing bacterial resistance [6-8]. Previously, synthesized silver complexes with LVF still had the antibacterial activity similar to that of LVF [9].

The primary purpose of the present work is to obtain a new derivative of LVF with increased antibacterial activity and therapeutically valuable new compound taking into consideration silver trifluoromethanesulfonate (silver triflate) (STF) as a chemical derivative partner.

EXPERIMENTAL

Materials, methods and instrumentation

LVF and STF were purchased from Sigma-Aldrich. All other chemicals and organic solvents used were of analytical reagent grade.

A Perkin Elmer PE 2400 (USA) analyzer was used for CHN elemental analyses. After the destruction of the obtained compound in a Berghof microwave digestion system, the silver content was checked by flame atomic absorption spectrometry (FAAS) analysis using a Shimadzu AA 6300 spectrometer. The FT-IR spectra were recorded and processed with an FT-IR Thermo Nicolet (USA) spectrometer and Omnic V.6 software. All samples were prepared as KBr pellets in the range of 400-4000 cm^{-1}. An Agilent 6410 Triple Quadrupole (Agilent Technologies, USA) mass spectrometer equipped with electrospray ionization (ESI) ion source in positive ion mode and MassHunter software was used for recorded and processed mass spectra of the obtained compound. The parameters of the ionization source were: gas flow 8 L/min, 40 psi, 4000 V, 300°C, a full scan on the field 100-1500 amu data acquisition module. Using a Jasco V650 spectrophotometer, the electronic spectra were recorded by diffuse reflectance technique in the range 200 - 800 nm with Spectralon as standard. An Analytik Jena UV-VIS Specord 210 (Germany) spectrophotometer and the software WinASPECT were used for recording the UV spectra in solution. The stock solutions were prepared in dimethyl sulfoxide (DMSO) ($1 \cdot 10^{-3}$M) and then adjusted to necessary dilutions with the same solvent. Similar molar concentrations were used to record UV spectra.

DSC 60 Shimadzu apparatus was used for Differential Scanning Calorimetry (DSC) analysis with parameters as follow: weight of the samples 3 mg, temperature increase rate of 10°C/min, and curves were recorded at the range of 40-400ºC. The melting point was determined using an Optimelt-Stanford Research System. An analyzer InoLab® pH/Cond 740 was used for determination of molar conductance for 10^{-3} M solution of the compound (in DMSO).

Obtaining method

A solution was obtained from 1.38 mmol STF and 40 mL of water and has been added into a mixture of 2.76 mmol of LVF and 40 mL methanol (2:1 molar ratio LVF: STF) and stirred in a sealed flat-bottom flask for 8 hours, protected from light. A yellowish solution was obtained and then left overnight (at room temperature). The next day, the solvent was partially removed with a rotary

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evaporator at 40 °C under vacuum until a yellowish-white precipitate appeared into the last 10 mL of the mixture. The precipitate was filtrated and slowly dried in an oven set at 40°C for 1 hour. The compound was protected from the light and kept in a desiccator above anhydrous $CaCl_2$.

Screening of the antibacterial activity

The obtained derivative of LVF was tested against three Gram-positive (*Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* MRSA 43300, *Enterococcus faecalis* ATCC 29212) and three Gram-negative (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853) bacterial strains.

The antibacterial activity was performed by the microdilution method, according to CLSI standards. All the details of the technique were presented in our previous work [10, 11]. The minimum inhibitory concentration (MIC) was considered in the last well without bacterial growth.

RESULTS AND DISCUSSION

Levofloxacin triflate (LVF-TF) was obtained in an attempt to synthesize a silver complex of LVF using STF as a silver salt. STF is useful in organometallic chemistry to activate the metal for metal-mediated processes and to catalyze some reactions as alcohol dehydration, vinyl hydrovinylation, electrophilic aromatic substitution, and the intramolecular hydroamination of alkyne [12, 13]. Due to the presence of the electron-withdrawing moiety CF_3, the triflate is known as a weakly coordinating ligand without fluorinating properties and presents a high resistance to oxidation [14]. The results of elemental analysis and other physicochemical properties for the LVF-TF are comprised in **Table 1**.

Table 1. Physicochemical properties of the obtained compound (*MW = molecular weight, **M.p. = melting point).

Physical and structural properties	**Values/Results**	
Molecular formula	$C_{19}H_{21}F_4N_3O_7S$	
***MW**	511,44 g mol^{-1}	
****M.p.**	304-309 ºC	
Appearance	a white-yellowish powder, stable to air	
Solubility (at 1 mg/mL)	soluble in boiling water, dimethylformamide (DMF), DMSO, concentrated ammonia, 10% sodium hydroxide solution and 10% hydrochloric acid solution	
Molar conductance (Λ_M)	34 Ω^{-1} cm^2 mol^{-1}	
Elemental analysis	**Found (%)**	**Calculated (%)**
% C	44.77	44.62
% H	3.80	4.14
% N	8.12	8.22

The recorded melting point of LVF-TF was 250 ºC, a value higher than that known for LVF (M.p. 225 - 227 ºC), triflic acid (trifluoromethanesulfonic acid) (M.p. 34 ºC), and lower than the one of STF (M.p. 286 ºC) [15-17]. The molar conductance value suggests that the new compound has the characteristics of a 1:1 electrolyte [18, 19]. No silver content was determined by FAAS.

FT-IR spectra analysis.

The most characteristic absorption bands of LVF are for stretching vibrations of the carboxyl groups ν(C=O) at 1724 cm^{-1} and for the pyridone ν(C=O) at 1621 cm^{-1} [20, 21]. The bands at 3500–2700 cm^{-1} correspond to the ν(C-H) stretching vibrations of a methyl radical at the N4 nitrogen atom in the piperazinyl moiety, and (or) to the ν(C-H) vibrations of methylene groups in R–O–Aryl [22]. The characteristic absorption bands of triflate anion appear in the FT-IR spectrum of LVF-TF as follows: 1262 cm^{-1}(s), $\nu_{as}(SO_3)$; 1227 cm^{-1}(s), $\nu_s(CF_3)$; 1160 cm^{-1}(s), $\nu_{as}(CF_3)$; 1036 cm^{-1}(vs), $\nu_s(SO_3)$; 760 cm^{-1} (w), $\delta_s(CF_3)$+ $\nu_s(CS)$; 637 cm^{-1} (s), $\delta_s(SO_3)$; 572 cm^{-1} (w), $\delta_{as}(CF_3)$; 517 cm^{-1}(w), $\delta_{as}(SO_3)$; (br, broad; m, medium; s, strong; v, very; w, weak) [23], [24-26]. Thereby, the obtained compound LVF-TF presents differences of the FT-IR spectra comparative to LVF (**Table 2**, **Figure 2**).

Table 2. FT-IR band assignments for LVF and LVF-TF (br, broad; m, medium; s, strong; v, very; w, weak) [9], [20-23], [27-29].

Analyzed compounds	**Band assignments**						
	ν(N-H) ν(O–H); H_2O and ν (N-H)	**ν(N⋯H); N4 piperazinic atom and ν(O–H); H_2O, ν(C–H)**	**ν(C=O) (carboxyl)**	**ν(C=O) (carbonyl)**	**ν_{as}(COO)**	**ν_s(COO)**	**C-F**
LVF	3500-3000 br	2936 w 2848 w 2691 w	1724 m	1621 vs	1594 w	1361 w	1160 s
LVF-TF	3046 w	2798 w	-	1712 s	1622 s	1422 m	1160 s

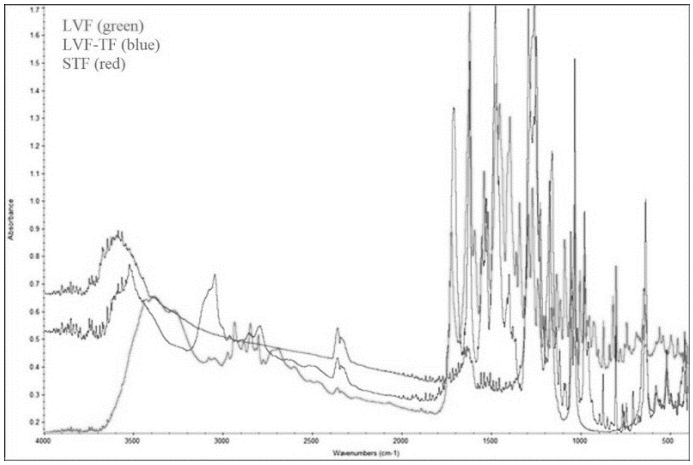

Figure 2. The overlapping FT-IR spectra of LVF, LVF-TF and STF.

MS spectra analysis

ESI-MS technique was used to determine the molecular mass of the LVF-TF, both in positive ion mode (to promote positive ion formation of LVF) and negative ion mode (to promote deprotonation of triflic acid). The known main fragmentation pattern of ofloxacin (racemic) and LVF with recorded molecular ions were previously reported [9]. As expected, the LVF presented the 362 m/z [M+1] fragment and the triflate from the STF was revealed as 148.9 m/z [M-1] (**Figure 3**). These data strongly suggest that the new obtained compound could be a salt, the LVF-TF.

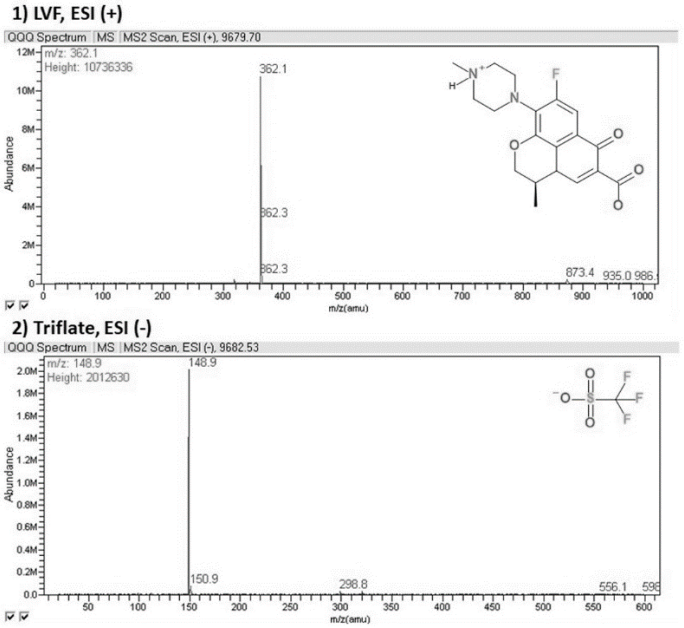

Figure 3. The recorded ESI-MS spectra of LVF-TF, 0 – 1000 m/z (amu): 1) LVF, ESI (+), 2) Triflate, ESI (-).

UV-VIS spectroscopy

The UV spectra of LVF and LVF-TF show some differences in terms of absorbance and absorption peaks (**Figure 4**). The maximum absorption peak of LVF at 304 nm presented a hyperchromic and a hipsochromic effect in the spectrum of the new compound. The absorption of LVF at 327 nm has been slightly modified in LVF-TF spectrum (**Table 3**). Electronic spectra recorded in solid-state of LVF-TF compared with the parent fluoroquinolone are presented in **Figure 5**. The LVF-TF exhibits a supplementary broad bathochromic band compared to LVF (**Table 4**). Thereby, the LVF-TF shows spectral differences recorded in the UV domain that can support the possibility of a new derivative.

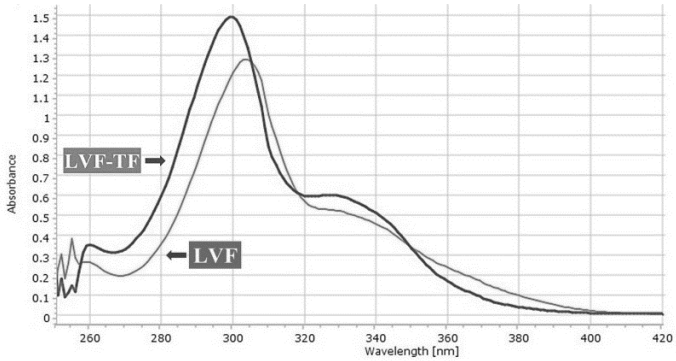

Figure 4. UV spectra of LVF-TF and LVF.

Table 3. Selective UV spectroscopic data of LVF and LVF-TF (max. = maxim).

Compound	λ (nm)	A	Assignments	Compound	λ (nm)	A	Assignments
LVF	300	1.237	n→π*	**LVF-TF**	300 (max.)	1.492	n→π*
	304 (max.)	1.280	n→π*		304	1.305	n→π*
	327	0.523			327	0.613	
	370	0.181			370	0.081	

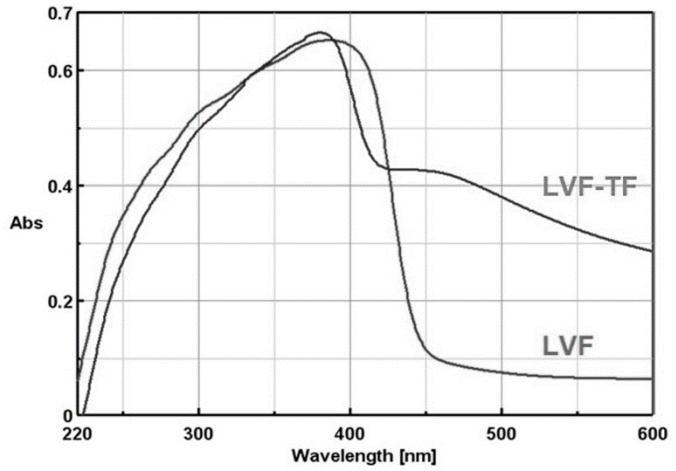

Figure 5. Electronic spectra recorded in solid-state of LVF-TF and LVF.

Table 4. Selective electronic spectra data (solid state) of LVF and LVF-TF (sh – shoulder)

Compound	λ_{max} (nm)	A (a.u.)	Assignments	Compound	λ_{max} (nm)	A (a.u.)	Assignments
LVF	266 sh	0.417	π→π*	**LVF-TF**	265 sh	0.350	π→π*
	300 sh	0.525	n→π*		297 sh	0.480	n→π*
	339 sh	0.597			334 sh	0.589	
	386	0.65			380.5	0.670	
					436.5	0.427	

DSC analysis

The thermal analysis was performed using the DSC method to assess the behaviour of LVF-TF subjected to an increasing temperature comparative to LVF and STF behaviour. The melting onset and the M.p. were recorded. Also, peak temperature at complete melting and energy of melting transition (enthalpy of the transitions) were recorded. Thus, the DCS curve of LVF-TF exhibits a melting point value of 309.39ºC (- 6.00 mW), higher than the recorded melting point of LVF, which corresponds to previously published values (endothermic peaks) (**Figure 6**) [15, 30].

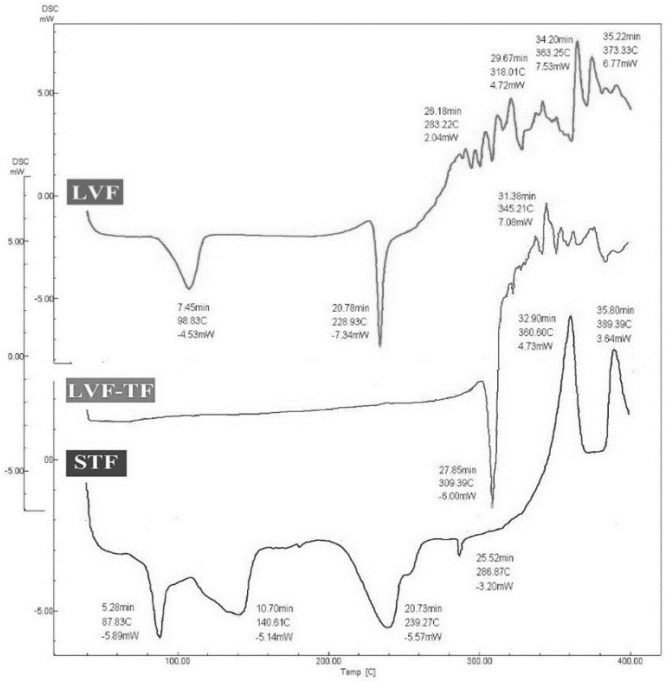

Figure 6. DSC curves of LVF-TF comparative to LVF and STF.

However, the shape of the DSC curve of STF is very characteristic, with endothermic and exothermic peaks. The endothermic peak at 239.27ºC and exothermic peak at 360.60ºC are similar to the peaks from the DSC curve of aluminium triflate used as a catalyst into a polymerization process [31]. These changes may be associated with sulphur trioxide group loss followed with the trifluoromethyl anion. It may be considered that the decomposition of LVF-TF started at 309.39ºC steadily up to 400ºC.

Chemical structure considerations

Based on previously analytical results, we suggest a chemical structure for the obtained compound (**Figure 7**). Most likely, the obtained derivative is the triflate salt of LVF. The most persuasive arguments were brought by CHN elemental analysis, molar conductivity recorded value and the spectroscopic analysis methods (FTIR, ESI-MS and UV-VIS spectroscopy). Also, DSC analysis highlights the differences between LVF/STFs and LVF-TF derivative behaviours.

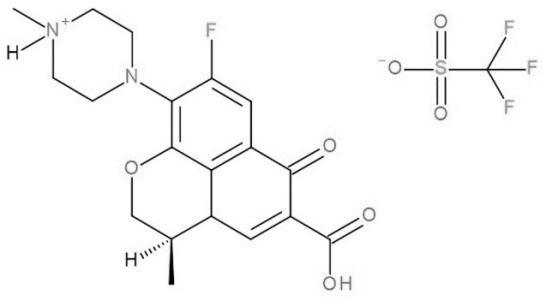

Figure 7. Proposed chemical structure of the LVF-TF compound.

Antibacterial activity

LVF and LVF-TF were tested against three Gram-positive and three Gram-negative bacterial strains. MIC values are comprised in **Table 5**.

Table 5. The antibacterial activity data for LVF and LVF-TF on the selected bacterial strains.

Bacterial strains		**MIC (μg · ml^{-1})**	
		LVF	**LVF-TF**
Gram-positive	*Staphylococcus aureus 29213*	0.12	0.12
	Staphylococcus aureus MRSA 43300	0.25	0.5
	Enterococcus faecalis 29212	0.12	0.25
Gram-negative	*Escherichia coli 25922*	0.25	0.25
	Klebsiella pneumoniae 13883	0.12	0.12
	Pseudomonas aeruginosa 27853	0.12	0.25

LVF-TF show similar MIC values with LVF regarding *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. For the other bacterial species, LVF-TF did not show an increased activity compared to LVF, as we expected. However, *in vivo* activity of the new derivative may be different than *in vitro* antibacterial activity. Also, due to the deduced chemical structure, LVF-TF may present different pharmacokinetic properties comparative to LVF, features that can positively influence the antibacterial activity. Besides, more bacterial species need to be tested to find if they are susceptible to the new compound. Also, the cytotoxicity of LVF-TF will be studied.

CONCLUSIONS

Through a simple method, a new compound with molecular formula $C_{19}H_{21}F_4N_3O_7S$ was obtained. The elemental, spectral and thermal analysis suggest most likely a structure of LVF salt with triflic acid. Regarding antibacterial potential, LVF-TF presented very similar MIC values to those of LVF on the selected bacterial strains. However, the compound could be further tested against other bacterial strains and the probability of a cytotoxic effect could also be studied.

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