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In vitro antibacterial activities of *p*-toluenesulfonyl-hydrazinothiazoles and hydrazinoselenazoles against multi-drug resistant Gram-negative phenotypes

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Abstract

Background: Bacterial multidrug resistance (MDR) constitutes a major hurdle in the treatment of infectious diseases worldwide. The present study was designed to evaluate the antibacterial activities of synthetic *p*-toluenesulfonyl-hydrazinothiazoles against multidrug resistant Gram-negative bacteria.

Methods: The broth microdilution method was used to determine the minimal inhibitory concentrations (MIC).

Results: The results demonstrated that the best activities were obtained with hydrazinoselenazoles. *p*-Chloro-benzyliden-selenosemicarbazide, 4-methyl-2-[(4-chloro-benzyliden)-hydrazinyl]-1,3-selenazole, *p*-chloro-benzoyl-selenosemicarbazide and 4-chloromethyl-2-[(4-chlorobenzyliden)-*N*-acetyl-hydrazinyl]-1,3-selenazole were more active than the chloramphenicol on *Klebsiella pneumoniae* KP63. Tested alone, the lowest MIC value of 16 mg/L was obtained with *p*-methoxy-benzyliden-selenosemicarbazide against *Enterobacter aerogenes* ATCC13048, *K. pneumoniae* ATCC112296 and KP55. Tested in the presence of an efflux pump inhibitor, phenylalanine arginine β -naphthylamide (PA β N), the activity of *p*-chloro-benzyliden-selenosemicarbazide, 4-methyl-2-[(4-chloro-benzyliden)-hydrazinyl]-1,3-selenazole, *p*-chloro-benzoyl-selenosemicarbazide and *p*-methoxy-benzyliden-selenosemicarbazide significantly increased with MIC values below 10 mg/L obtained respectively on 43.8 %, 31.3 %, 62.5 % and 100 % of the 16 tested bacterial strains. The lowest MIC value of 0.5 mg/L in the presence of PA β N was recorded with *p*-chloro-benzoyl-selenosemicarbazide against *Escherichia coli* ATCC8739 and KP55 as well as *p*-methoxy-benzyliden-selenosemicarbazide against *E. aerogenes* KP55. *p*-Chloro-benzyliden-selenosemicarbazide and *p*-chloro-benzoyl-selenosemicarbazide contained the same pharmacophore as *p*-methoxy-benzyliden-selenosemicarbazide.

Conclusion: This study indicates that *p*-chloro-benzyliden-selenosemicarbazide, *p*-chloro-benzoyl-selenosemicarbazide and *p*-methoxy-benzyliden-selenosemicarbazide could be explored more to develop novel antimicrobial drugs to fight MDR bacterial infections.

Keywords: Antibacterial, Efflux pumps, Hydrazinoselenazoles, *p*-toluenesulfonyl-hydrazinothiazoles, Multidrug resistance

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Background

Bacterial multidrug resistance (MDR) constitutes a major hurdle in the treatment of infectious diseases worldwide. Bacterial efflux systems, especially AcrAB-TolC pumps in *Enterobacteriaceae* or MexAB-OprM in *Pseudomonas aeruginosa*, are involved in multidrug resistance of pathogenic Gram-negative bacteria [1–5]. Clinically, the continuous emergence of Gram-negative MDR bacteria drastically reduces the efficacy of the antibiotic arsenal leading globally to an increase of the frequency of therapeutic failure [6]. Consequently, new antibacterials are needed to fight these bacterial pathogens, but progress in developing them has been slow [7]. *p*-Toluenesulfonyl-hydrazinotiazoles and hydrazinoselenazoles are synthetic compounds with established anticancer, analgesic and anti-inflammatory activities [8–11]. The aim of the present work was to determine the antibacterial activities of a panel of *p*-toluenesulfonyl-hydrazinotiazoles and hydrazinoselenazoles against different bacterial strains expressing MDR phenotype. Furthermore, we highlighted possible pharmacophoric cores amongst the active compounds.

Methods

Chemicals for antimicrobial assay

Chloramphenicol ≥ 98 % (Sigma-Aldrich, St. Quentin Fallavier, France) was used as reference antibiotics (RA) against the tested bacteria. *p*-iodonitrotetrazolium chloride (INT) and phenylalanine arginine β -naphthylamide (PA β N) were used as microbial growth indicator and efflux pumps inhibitor (EPI) respectively [12, 13]. The tested synthetic compounds included eleven *p*-toluenesulfonyl-hydrazinotiazoles: 4-methyl-2-[2-(benzenesulfonyl)-*N,N*-diacetyl-hydrazino]-thiazole, C₁₅H₁₈N₃O₄S₂ (**1**; *m/z* 368.07); 5-acetyl-4-methyl-2-[2-(benzenesulfonyl)-hydrazino]-thiazole, C₁₃H₁₈N₃O₄S₂ (**2**; *m/z* 344.07); 5-acetyl-4-methyl-2-[2-(benzenesulfonyl)-*N,N*-diacetyl-hydrazino]-thiazole, C₁₇H₂₀N₃O₅S₂ (**3**; *m/z* 410.08); 4-Phenyl-2-[2-(benzenesulfonyl)-*N,N*-diacetyl-hydrazino]-thiazole, C₂₂H₂₄N₃O₂S₂ (**4**; *m/z* 426.13); 5-bromo-4-Chloromethyl-2-[2-(benzenesulfonyl)-*N,N*-diacetyl-hydrazino]-thiazole, C₁₇H₂₀BrClN₃O₂S₂ (**5**; *m/z* 475.98); *p*-toluenesulfonylthiosemicarbazide, C₁₀H₁₈N₃O₂S₂ (**6**; *m/z* 276.08); 4-methyl-2-[2-(*p*-toluenesulfonyl)-*N,N*-diacetyl-hydrazino]-thiazole, C₁₆H₂₀N₃O₄S₂ (**7**; *m/z* 382.08); 4-chloromethyl-2-[2-(*p*-toluenesulfonyl)-hydrazino]-thiazole, C₁₂H₁₅ClN₃O₂S₂ (**8**; *m/z* 332.02); 4-phenyl-2-[2-(*p*-toluenesulfonyl)-hydrazino]-thiazole, C₁₇H₁₈N₃O₂S₂ (**9**; *m/z* 360.08); 5-acetyl-4-methyl-2-[2-(*p*-toluenesulfonyl)-hydrazino]-thiazole, C₁₄H₁₈N₃O₃S₂ (**10**; *m/z* 340.07); 5-acetyl-4-methyl-2-[2-(*p*-toluenesulfonyl)-*N,N*-diacetylhydrazino]-thiazole, C₁₈H₂₂N₃O₅S₂ (**11**; *m/z* 424.10) and hydrazinoselenazoles: *p*-chloro-benzyliden-selenosemicarbazide, C₈H₈ClN₃Se (**12**; *m/z* 260.95); 4-methyl-2-[(4-chloro-

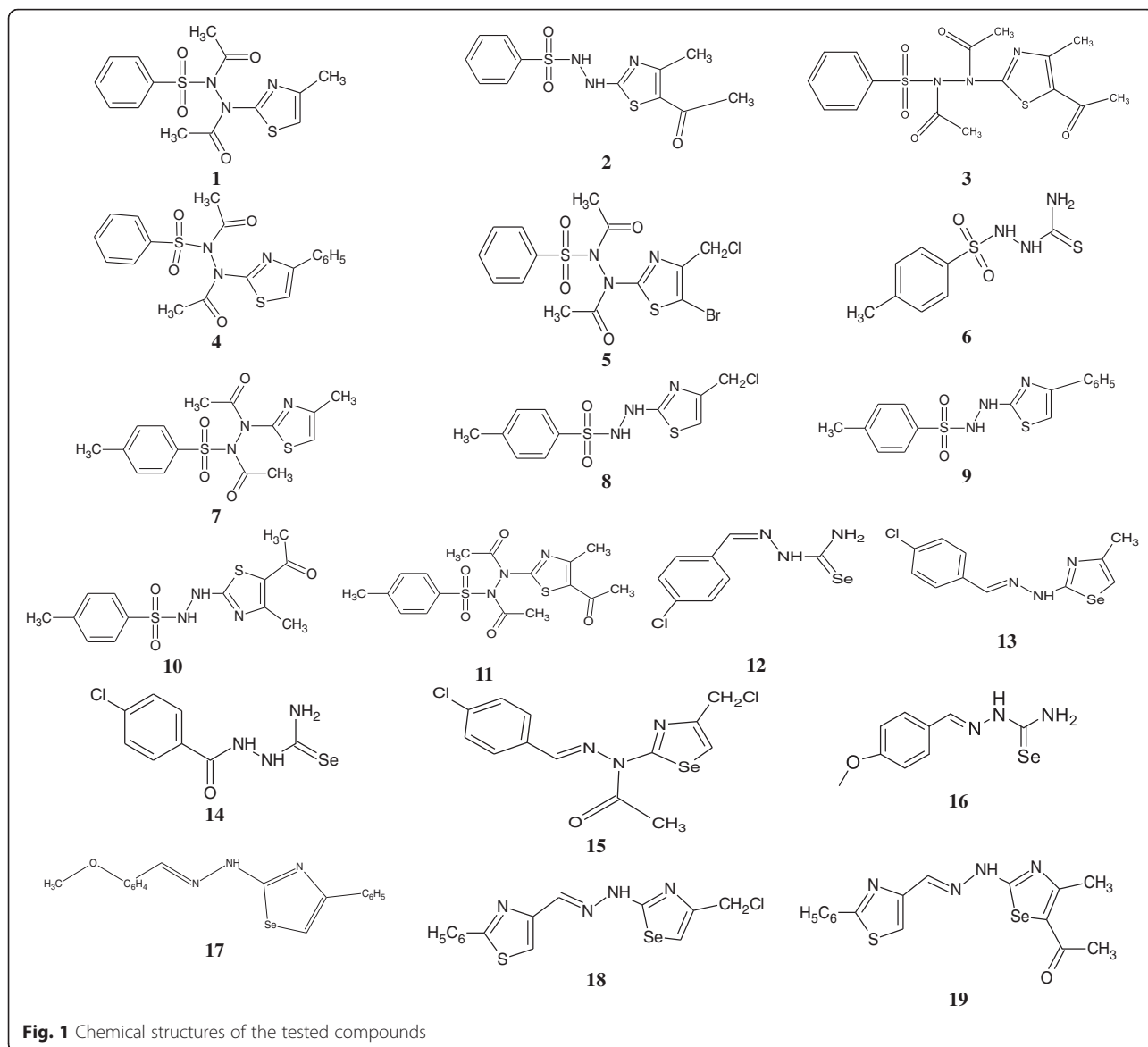
benzyliden)-hydrazinyl]-1,3-selenazole, C₁₁H₁₀ClN₃Se (**13**; *m/z* 298.97); *p*-chloro-benzoyl-selenosemicarbazide C₈H₈ClN₃OSe (**14**; *m/z* 276.95); 4-chloromethyl-2-[(4-chlorobenziliden)-*N*-acetyl-hydrazinyl]-1,3-selenazole, C₁₃H₁₁Cl₂N₃OSe (**15**; *m/z* 374.94); *p*-methoxy-benzyliden-selenosemicarbazide, C₉H₁₁N₃OSe (**16**; *m/z* 257.00); 4-phenyl-2-(4-methoxybenzyliden)-hydrazinyl]-1,3-selenazole, C₁₇H₁₅N₃OSe (**17**; *m/z* 357.03); 4-chloromethyl-2-(2-phenyl-1,3-thiazolo-4-methylidene)-hydrazinyl]-1,3-selenazole C₁₆H₁₉ClN₄SSe (**18**; *m/z* 414.01); and 4-methyl-5-acetyl-2-(2-phenyl-1,3-thiazolo-4-methylidene)-hydrazinyl]-1,3-selenazole, C₁₈H₂₂N₄OSSe (**19**; *m/z* 422.06). The synthesis of *p*-toluenesulfonyl-hydrazinotiazoles (**1–11**) [9] and hydrazinoselenazoles (**12–19**) [11] were previously reported. Their chemical structures are summarized in Fig. 1.

Microbial strains and culture media

The studied microorganisms included sensitive and resistant strains of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Escherichia coli* obtained from the American Type Culture Collection (ATCC) as well as clinical strains. Their bacterial feature were previously reported [5, 14–16]. Nutrient agar were used for the activation of the tested Gram-negative bacteria [17].

INT colorimetric assay for MIC and MBC determinations

The Minimum Inhibitory Concentration (MIC) determinations on the tested bacteria were conducted using rapid *p*-iodonitrotetrazolium chloride (INT) colorimetric assay according to described methods [12] with some modifications [18, 19]. The tested samples and RA were first of all dissolved in DMSO/Mueller Hinton Broth (MHB). The final concentration of DMSO was lower than 2.5 % and does not affect the microbial growth [20, 21]. The solution obtained was then added to Mueller Hinton Broth, and serially diluted two fold (in a 96-wells microplate). One hundred microlitre (100 μ L) of inoculum 1.5 $\times 10^6$ CFU/mL prepared in appropriate broth was then added [18, 19]. The plates were covered with a sterile plate sealer, then agitated to mix the contents of the wells using a plate shaker and incubated at 37 °C for 18 h. The assay was repeated thrice. Wells containing adequate broth, 100 μ L of inoculum and DMSO to a final concentration of 2.5 % served as negative control. The MIC of samples was detected after 18 h incubation at 37 °C, following addition (40 μ L) of 0.2 mg/mL of INT and incubation at 37 °C for 30 min. Viable bacteria reduced the yellow dye to a pink. MIC was defined as the sample concentration that prevented the color change of the medium and exhibited complete inhibition of microbial growth [12]. To evaluate the role of efflux pumps in the susceptibility



of Gram-negative bacteria, compounds were tested in the presence of PAβN (at 30 μg/mL) and MIC was determined as above.

Results

Studied compounds

Compounds tested in the present study included *p*-toluenesulfonyl-hydrazinothiazoles (1–11) [9] and hydrazinoselenazoles (12–19) [11] previously synthesized. They were tested for their antimicrobial activities on a panel of 16 bacterial strains and the results are summarized in Table 1.

Activity of *p*-toluenesulfonyl-hydrazinothiazoles

When they were tested alone at up to 256 mg/L, all *p*-toluenesulfonyl-hydrazinothiazoles generally displayed

low or no antibacterial activity. However, compound 8 displayed a best activity with detectable MIC values against 14/16 (87.5 %) bacterial strains (Table 1). This compound was also more active than chloramphenicol against *E. aerogenes* EA289, EA27 and *K. pneumoniae* KP63. No detectable MIC value was recorded with compounds 1, 2, 4, 5, 7, 9, 10 and 11 (Table 1).

Activity of hydrazinoselenazoles

The best activities were obtained with *p*-toluenesulfonyl-hydrazinothiazoles; When they were tested at up to 256 mg/L, hydrazinoselenazole displayed selective antibacterial activities. Compound 16 displayed MIC values on 14/16 (87.5 %) tested bacteria whilst compounds 12, 13, 14, 15, 19 and were active respectively on 5/16 (31.3 %), 7/16 (43.8 %), 4/16 (25.0 %), 3/16 (18.8 %) and

Table 1 Minimal inhibitory concentration (MIC) of the studied samples on the tested microbial species

Samples	<i>E. coli</i> strains and MIC* (mg/L) without PAβN (and with PAβN in parenthesis)																% of PAβN Modulating effect
	<i>E. coli</i> strains				<i>E. aerogenes</i> strains							<i>K. pneumoniae</i>			<i>P. aeruginosa</i>		
	ATCC87 39	AG100	AG100A	AG100A tet	AG102	ATCC 13048	EACM64	EA289	EA294	EA298	EA27	ATCC 11296	KP55	KP63	PA01	PA124	
<i>p</i>-Toluenesulfonylhydrazinotiazoles																	
1	i	i	i	i	i	i	i	i (64)	i (256)	i	i	i	i	i	i	i	2/16 (12.5%)
2	i	i	i	i	i	i	i	i (64)	i	i	i	i	i	i	i	i	1/16 (6.3%)
3	i (256)	i	i	i	i	i	i	i (64)	i (256)	i	i	i 256 (256)	i	i	i	i	3/16 (18.8%)
4	i	i	i	i	i	i	i	i (64)	i (32)	i	i	i	i	i	i	i	2/16 (12.5%)
5	i	i	i	i	i	i	i	i (64)	i	i	i	i	i	i	i	i	1/16 (6.3%)
6	i	i	i	i	i	i	i	i (64)	i	i	i	i 256 (256)	i	i	i	i	1/16 (6.3%)
7	i	i	i	i	i	i	i	i (64)	i (256)	i	i	i (256)	i (256)	i	i	i	4/16 (25.0 %)
8	256 (256)	256 (256)	256 (256)	128 (128)	256 (256)	256 (256)	256 (256)	256 (256)	256 (256)	i (256)	256 (256)	256 (256)	256 (256)	256 (256)	i	256 (256)	1/16 (6.25 %)
9	i	i	i	i	i	i	i	i (64)	i	i	i	i	i	i	i	i	1/16 (6.25%)
10	i	i	i	i	i	i	i	i	i	i	i	i	i	i	i	i	0/16 (0%)
11	i (256)	i	i	i	i	i	i	i	i	i	i	i (256)	i	i (256)	i	i	3/16 (18.75%)
Hydrazinoselenazoles																	
12	256 (4)	256 (64)	i (4)	i (16)	i (16)	i (256)	i (16)	256 (128)	i (8)	i (8)	i (128)	i (8)	128 (4)	128 (4)	i	i (256)	15/16 (93.8%)
13	256 (16)	128 (8)	128 (4)	128 (64)	i	i	i	i	i (8)	i (8)	i	128 (64)	128 (16)	32 (8)	i	i	9/16 (56.3%)
14	256 (0.5)	i (256)	i (1)	i (2)	i (8)	i (32)	i (8)	i (32)	i (2)	i (2)	i (64)	256 (2)	64 (0.5)	128 (1)	i (256)	i (256)	16/16 (100%)
15	i	i	i	i	i	i	i	i	i	i	i	256 (64)	32 (32)	128 (32)	i	i (256)	3/16 (18.75 %)
16	64 (2)	256 (1)	256 (2)	64 (8)	64 (8)	16 (8)	128 (8)	256 (8)	i (2)	i (2)	256 (8)	16 (2)	16 (0.5)	32(1)	128 (8)	128 (8)	16/16 (100 %)
17	i	i	i	i (256)	i	i	i	i	i (32)	i (32)	i	i 256 (16)	i (32)	i	i	i (128)	6/16 (37.5%)
18	i	i	i (128)	i	i	i	i	i	i (16)	i (32)	i	i (32)	256 (32)	i	i	i	5/16 (25.8 %)
19	i 256 (128)	i	256 (128)	256 (128)	256 (256)	i	i	i (128)	256 (32)	256 (32)	i (128)	256 (16)	256 (32)	i (256)	i	i (256)	11/16 (68.8%)
Chloramphenicol	4 (1)	4 (0.5)	0.5 (0.25)	32 (4)	32 (2)	4 (1)	256 (8)	i (128)	64 (16)	64 (16)	i (128)	4 (1)	32 (4)	i (128)	128 (8)	256 (8)	16/16 (100 %)

*The MIC of PAβN was 64 mg/L on AG100A and >256 mg/L for other *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *E. aerogenes* strains
i: sample not active up to 256 mg/L; () : sample tested in presence of PAβN at 20 mg/L final concentration; (-): not tested; In bold: significant activity

7/16 (43.8 %) and tested bacteria (Table 1). Compounds **12**, **13**, **14** and **15** were more active (lower MIC value) than the reference drug (chloramphenicol) on *K. pneumoniae* KP63. Compound **12** was also more active than the reference drug on *E. aerogenes* EA289 meanwhile this was also the case with **16** against *E. aerogenes* CM64, EA27, EA289, KP55 and KP63. Tested alone, the lowest MIC value of 16 mg/L was obtained with compound **16** against *E. aerogenes* ATCC13048, *K. pneumoniae* ATCC11296 and KP55 (Table 1).

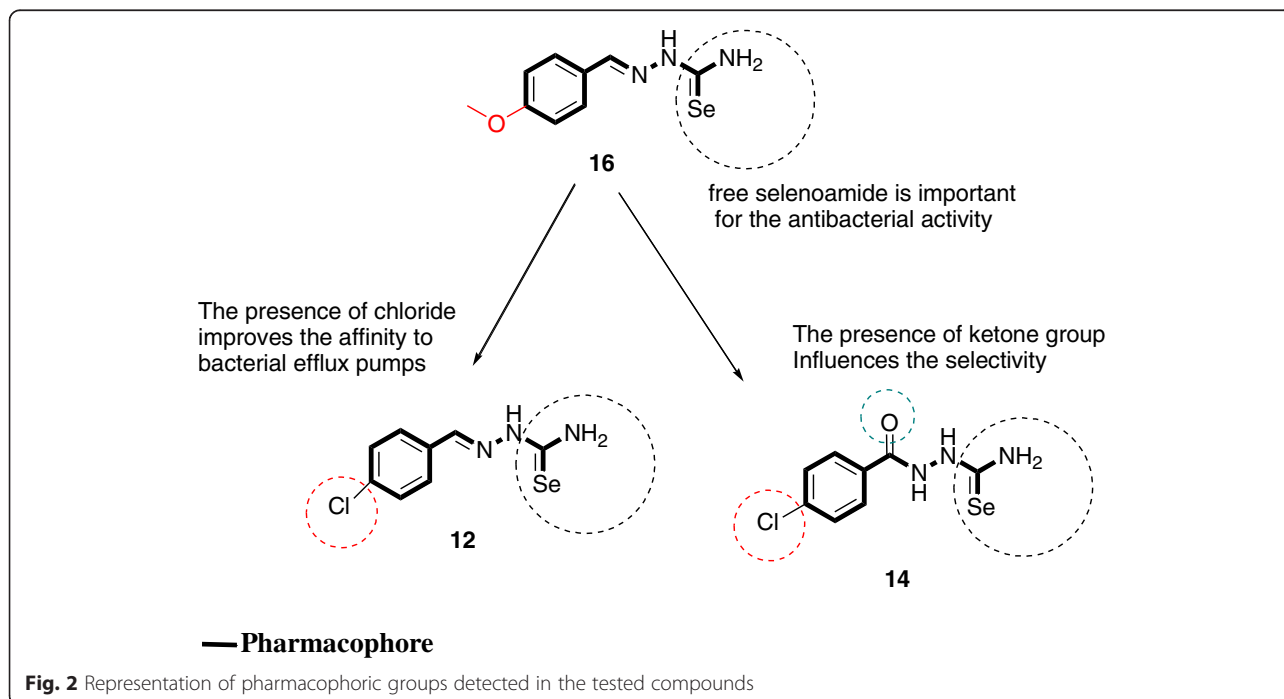
Role of efflux pumps in the susceptibility of tested bacteria: identification of a PA β N-sensitive efflux in addition to AcrAB-TolC pump

To evaluate the role of efflux on the susceptibility of bacteria to the tested compounds, they were combined with a well-known EPI, PA β N. The EPI was tested at 30 μ g/mL, a concentration lower than its MIC value in the studied bacteria. The results are summarized in brackets in Table 1 (MIC of EPI is provided as footnote). Apart from *p*-toluenesulfonyl-hydrazinotiazole **10**, PA β N improved the activity of all tested compounds on at least one tested bacteria. The most important improvements of the activity of compounds in the presence of PA β N were recorded with **12**, **14**, **16** and **19** on respectively 15/16 (93.8 %), 16/16 (100 %), 16/16 (100 %) and 11/16 (68.8 %) tested bacteria. With PA β N, the activity of hydrazinoselenazoles **12**, **13**, **14** and **16** significantly increased with MIC values below 10 mg/L obtained respectively on 7/16 (43.8 %), 5/16 (31.3 %), 10/16 (62.5 %) and 16/16 (100 %) tested bacterial strains.

The lowest MIC value of 0.5 mg/L was recorded with compounds **14** against *E. coli* ATCC8739 and KP55 as well as **16** against *E. aerogenes* KP55 (Table 1). When they were combined with EPI, hydrazinoselenazoles **12**, **13**, **14** and **16** were more active than chloramphenicol on at least two of the tested bacterial species.

Structure activity relationship and identification of pharmacophoric moiety

When regarding the structure-activity relationship, it appears that amongst the tested compounds, *p*-toluenesulfonyl-hydrazinotiazoles were less active than hydrazinoselenazoles. Within *p*-toluenesulfonyl-hydrazinotiazoles, it was also found that compound **8** scaffold is the most active and that any modification of its structure resulted to a decrease of antibacterial activity. Within hydrazinoselenazoles, it was also found that compound **16** [active on 14/16 (87.5 %) and 16/16 (100 %) tested bacteria in the absence or presence of EPI respectively; MIC values below 10 mg/L on all tested bacteria in the presence of EPI and lowest MIC value of 0.5 mg/L] can be considered as the pharmacophoric moiety. Within this moiety, the presence of a free selenoamide is primordial for the antibacterial activity, as compounds **12** and **14** (bearing a free selenoamide) were also very active, especially when they were combined with EPI (Fig. 2). The presence of chloride substituent seems to increase the affinity of hydrazinoselenazole **12** and **14** to bacteria efflux pumps. In fact in the absence of EPI, the activity of



compounds **12** and **14** (with chloride substituent) were much lower than that of **16** (with -OCH₃ substituent).

Discussion

Bacterial multidrug resistance represents a major problem in the treatment of infectious diseases. In the present study, we tested a panel of bacterial strains including both reference ATCC strains and MDR phenotypes expressing active efflux pumps [4, 5, 22]. In fact, tripartite drug efflux pumps, mainly those clinically reported as AcrAB–TolC in Enterobacteriaceae or as MexAB–OprM in *P. aeruginosa* tested in the present study, play a key role in multidrug resistance of pathogenic Gram-negative bacteria [23, 24]. *p*-Toluenesulfonyl-hydrazinothiazoles and hydrazinoselenazoles are synthetic compounds that previously displayed good anticancer, analgesic and anti-inflammatory activities [8–11]. Herein, we investigated the ability of such compounds to combat MDR Gram-negative bacteria expressing active efflux pumps. As results, it was found that hydrazinoselenazoles were much more active than *p*-toluenesulfonyl-hydrazinothiazoles. However, all the tested chemicals were substrates of bacterial efflux pumps. Interestingly, when efflux pumps were blocked by EPI, significant increase of the activity of three compounds **12**, **14** and **16** was observed. More importantly, the obtained activity was better than that of the reference drug chloramphenicol in many cases (Table 1), suggesting that the three compounds can be used in combination with PAβN to fight bacterial infections involving MDR phenotypes. The antimicrobial activity of a compound has been defined as significant when MIC is below 10 mg/L, moderate when 10 mg/L < MIC < 100 mg/L or low when MIC > 100 mg/L [25, 26]. In this study, the MIC values below 10 mg/L was noted with hydrazinoselenazoles **12**, **14** and **16** on 43.8 %, 62.5 % and 100 % tested bacterial strains when they were tested in the presence of EPI, highlighting their possible use in combination with PAβN in the control of MDR bacterial infections.

Regarding the involvement of MDR bacteria in treatment failures and the re-emergence of infectious diseases [1, 27, 28], these findings can be considered very promising. In fact, *Pseudomonas aeruginosa* is an important nosocomial pathogen highly resistant to clinically used antibiotics, causing a wide spectrum of infections and leading to substantial morbidity and mortality [29] and was found sensitive to the three compounds. MDR Enterobacteriaceae, including *K. pneumoniae*, *E. aerogenes*, *P. stuartii* and *E. coli*, have also been classified as antimicrobial-resistant organisms of concern in healthcare facilities [28, 30].

Conclusions

Regarding the medical importance of the studied microorganisms, the results obtained in this study

demonstrated that hydrazinoselenazoles are strong source of inspiration in antibacterial drug discovery. Thus, the present data showed that hydrazinoselenazoles **12**, **14** and **16** are promising antibacterial agents to combat MDR phenotypes, but are also substrates of efflux pumps. They should therefore be combined with an EPI to combat MDR phenotypes. We also found that compound **16** skeleton was the pharmacophoric moiety of the tested hydrazinoselenazoles and that the presence of a free selenoamide scaffold is necessary for their antibacterial activity. However, more compounds should be further synthesized to fully investigate the structure-activity relationship for this series of hydrazinoselenazoles. Toxicological studies will also be performed to ensure the safety of the active hydrazinoselenazoles, especially compound **16**.

Abbreviations

1: 4-methyl-2-[2-(benzenesulfonyl)-*N,N*-diacetyl-hydrazino]-thiazole; **2**: 5-acetyl-4-methyl-2-[2-(benzenesulfonyl)-hydrazino]-thiazole; **3**: 5-acetyl-4-methyl-2-[2-(benzenesulfonyl)-*N,N*-diacetyl-hydrazino]-thiazole; **4**: 4-phenyl-2-[2-(benzenesulfonyl)-*N,N*-diacetyl-hydrazino]-thiazole; **5**: 5-bromo-4-chloromethyl-2-[2-(benzenesulfonyl)-*N,N*-diacetyl-hydrazino]-thiazole; **6**: *p*-toluenesulfonylthiosemicarbazide; **7**: 4-methyl-2-[2-(*p*-toluenesulfonyl)-*N,N*-diacetyl-hydrazino]-thiazole; **8**: 4-chloromethyl-2-[2-(*p*-toluenesulfonyl)-hydrazino]-thiazole; **9**: 4-phenyl-2-[2-(*p*-toluenesulfonyl)-hydrazino]-thiazole; **10**: 5-acetyl-4-methyl-2-[2-(*p*-toluenesulfonyl)-hydrazino]-thiazole; **11**: *a*-Acetyl-4-methyl-2-[2-(*p*-toluenesulfonyl)-*N,N*-diacetyl-hydrazino]-thiazole; **12**: *p*-chloro-benzyliden-selenosemicarbazide; **13**: 4-methyl-2-[(4-chloro-benzyliden)-hydrazinyl]-1,3-selenazole; **14**: *p*-chloro-benzoyl-selenosemicarbazide; **15**: 4-chloromethyl-2-[(4-chlorobenzyliden)-*N*-acetyl-hydrazinyl]-1,3-selenazole; **16**: *p*-methoxy-benzyliden-selenosemicarbazide; **17**: 4-phenyl-[2-(4-methoxybenzyliden)-hydrazinyl]-1,3-selenazole; **18**: 4-chloromethyl-[2-(2-phenyl-1,3-thiazolo-4-methylidene)-hydrazinyl]-1,3-selenazole; **19**: 4-methyl-5-acetyl-[2-(2-phenyl-1,3-thiazolo-4-methylidene)-hydrazinyl]-1,3-selenazole; ATCC: American Type Culture Collection; CFU: Colony forming unit; DMSO: dimethylsulfoxide; EPI: Efflux pumps inhibitor; INT: *p*-iodonitrotetrazolium chloride; MDR: Multidrug resistant; MHB: Mueller Hinton Broth; MIC: Minimal inhibitory concentration; PAβN: Phenylalanine arginine β -naphthylamide; RA: Reference antibiotic.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

ATM, AGI carried out the study; ATM and VK wrote the manuscript; BN, BTN, VZ and VK supervised the work; VK designed the experiments, provided the bacterial strains and chemicals; all authors read and approved the final manuscript.

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