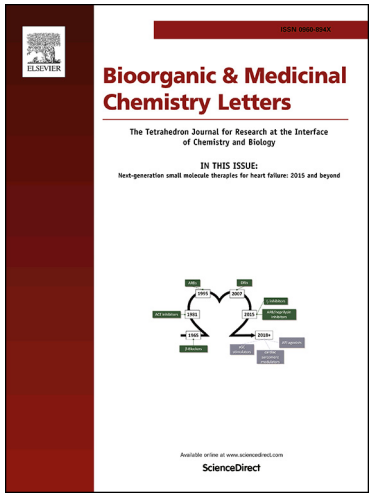
Accepted Manuscript



Discovery of niclosamide and its *O*-alkylamino-tethered derivatives as potent antibacterial agents against carbapenemase-producing and/or colistin resistant *Enterobacteriaceae* isolates

Jimin Xu, María Eugenia Pachón-Ibá ñez, Tania Cebrero-Cangueiro, Haiying Chen, Javier Sánchez-Céspedes, Jia Zhou

PII: S0960-894X(19)30171-4
DOI: https://doi.org/10.1016/j.bmcl.2019.03.032
Reference: BMCL 26346

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 17 January 2019
Revised Date: 18 March 2019
Accepted Date: 22 March 2019

Please cite this article as: Xu, J., Pachón-Ibá ñez, M.E., Cebrero-Cangueiro, T., Chen, H., Sánchez-Céspedes, J., Zhou, J., Discovery of niclosamide and its *O*-alkylamino-tethered derivatives as potent antibacterial agents against carbapenemase-producing and/or colistin resistant *Enterobacteriaceae* isolates, *Bioorganic & Medicinal Chemistry Letters* (2019), doi: https://doi.org/10.1016/j.bmcl.2019.03.032

Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

Discovery of niclosamide and its *O*-alkylamino-tethered derivatives as potent antibacterial agents against carbapenemase-producing and/or colistin resistant *Enterobacteriaceae* isolates

Jimin Xu[a,†], María Eugenia Pachón-Ibáñez[b,†], Tania Cebrero-Cangueiro[b,†], Haiying Chen[a], Javier Sánchez-Céspedes[b,*], and Jia Zhou[a,*]

[a] *Department of Pharmacology and Toxicology, University of Texas Medical Branch, 301 University Blvd, Galveston, Galveston, Texas 77555, United States*
[b] *Unit of Infectious Diseases, Microbiology and Preventive Medicine, Institute of Biomedicine of Seville (IBiS), University Hospital Virgen del Rocío/CSIC/University of Seville, E41013 Seville, Spain*

ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
Niclosamide derivatives
Antibacterial agents
Carbapenemase-producing *Enterobacteriaceae*
Colistin resistance
Synergistic effects

ABSTRACT

Carbapenemase-producing *Enterobacteriaceae* (CPE) represents the most worrisome evolution of the antibiotic resistance crisis, which is almost resistant to most of available antibiotics. This situation is getting even worse particularly due to the recent emergence of colistin resistance. Herein, niclosamide, an FDA-approved traditional drug, and its novel *O*-alkylamino-tethered derivatives were discovered as new and potent antibacterial agents against carbapenemase-producing and/or colistin resistant *Enterobacteriaceae* isolates. Among these molecules, compound **10** (**HJC0431**) with 4-aminobutyl moiety showed the broad antibacterial activities, effective against 6 strains. *In vitro* checkerboard and time-kill course studies demonstrated the synergistic effects of the screened compounds with colistin against the corresponding strains with various degrees.

The development of antibiotics for the treatment of fatal infections is considered one of the major breakthroughs of modern medications. However, the acquisition of resistance towards their antimicrobial activity has inevitably been happening. The emergence and dissemination of antibiotic resistant pathogens is becoming a serious threat to human health. The "ESCAPE" pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species*) are responsible for the majority of nosocomial infection and capable of escaping the biocidal action of antimicrobial agents[1-3]. In particular, carbapenemase-producing *Enterobacteriaceae* (CPE, mostly contributed by *K. pneumonia*) represents the most worrisome evolution of the antibiotic resistance crisis, which is almost resistant to all available antibiotics, and the mortality rates associated with CPE infections are intolerably high[4, 5]. Currently, the clinically most important carbapenemases in *Enterobacteriaceae* include the class A enzymes of the *Klebsiella pneumoniae* carbapenemases (KPC) type, the zinc-dependent class B metallo-β-lactamases (MβLs) of the VIM, IMP, and NDM types and the plasmid-expressed class D carbapenemases of the OXA-48 type[6]. These types of CPE are structurally and mechanistically different from each other, and this makes it challenging to identify an agent that is active against all types of CPE. Colistin and tigecycline have been reported as the remaining therapeutic options against various CPE infections[7-9]. Nevertheless, over the past few years, the colistin resistant CPE cases have been sporadically reported from various parts of the world, such as South Korea, Israel, Singapore and United States[10]. Thus, there is an urgent need for the screening and development of new antimicrobial agents to keep up with the evolution of the drug resistance mechanisms in the *Enterobacteriaceae* family.

Niclosamide, an FDA-approved drug, has been traditionally used in the clinic for the treatment of tapeworm infections for several decades, and it is well tolerated with extremely high acute oral LD_{50} values of > 1000 mg/kg[11, 12]. Its mechanism of anticestodal action has been reported to involve uncoupling oxidative phosphorylation and stimulating adenosine triphosphatase activity in the mitochondrial[12-15]. Over the past several years, accumulated studies showed that niclosamide is a multifunctional drug that can regulate multiple signaling pathways and biological processes including Wnt/β-catenin, mTORC1, STAT3, NF-κB, Notch, NS2B-NS3 protease, pH,

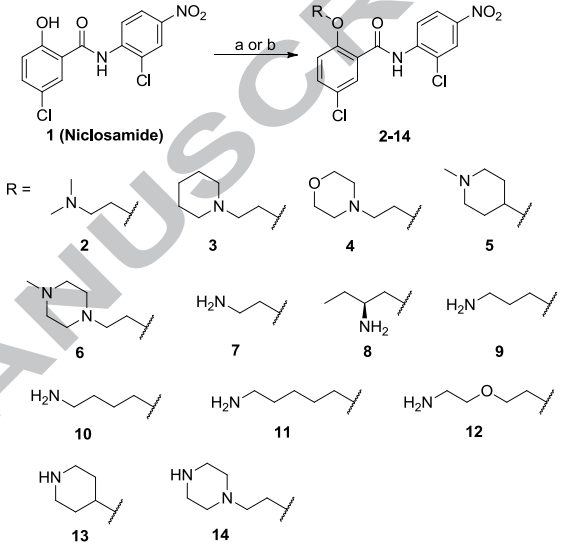
* Corresponding authors. Jia Zhou, Tel.: +1-409-772-9748; fax: +1-409-772-9648; e-mail: jizhou@utmb.edu. Javier Sánchez Céspedes, Tel.: +34 955 923 100; fax: +34 955 923 101; email: jsanchez-ibis@us.es.

† These authors contributed equally to the manuscript.

etc[16-25]. Due to its various biological properties, niclosamide has the great potential being repurposed to treat other human conditions, such as cancer, viral infections and tuberculosis, more than just helminthic diseases[17]. To improve the poor aqueous solubility and bioavailability of niclosamide, our group discovered a series of *O*-alkylamino-tethered derivatives as potent and orally bioavailable anticancer agents[26]. Herein we reported the screening and studies of these niclosamide derivatives with enhanced drug-like properties against different clinical carbapenemase-producing and/or colistin (COL) resistant *Enterbacteriaceae* isolates.

The synthesis of the selected compounds was summarized in **Scheme 1**. Analogues **2-6** were easily prepared by Mitsunobu reaction of niclosamide with corresponding amino alcohols. Mitsounobu coupling of niclosamide with *N*-Boc-protected amino alcohols was followed by Boc-deprotection to afford compounds **7-14**. The structures and purity of all synthesized compounds were confirmed by ^{1}H and ^{13}C NMR, HR-MS and HPLC analysis[26, 27]. Sixteen clinical carbapenemase-producing and/or colistin resistant (COLR) *Enterbacteriaceae* isolates were selected for screening including carbapenemase-producing *Klebsiella pneumoniae* (KPC) (4 strains), standard KPC (CECT997), carbapenemase-producing NDM-1 COL-susceptible (2 strains) and Enterobacter COL-resistant (9 strains). As shown in **Table 1**, five compounds including niclosamide were discovered with antibacterial activities at the concentration of 50 μg/mL[28, 29]. Niclosamide (**1**) showed inhibitory activities against two COL resistant strains: *E. cloacae* 280 and *E. cloacae* 297. Compound **8** with *S*-2-aminobutyl moiety inhibited the growth of *E.coli* NDM-1. Excitingly, compound **10** with 4-aminobutyl moiety showed the broad antibacterial activities, effective against 6 strains: KPC CECT-997, KPC-28, KPC NDM-1, *E.coli* NDM-1, *E. cloacae* 280 and *E. cloacae* 297. Compound **11** with 5-aminopentyl moiety was effective against KPC-28, KPC NDM-1 and *E.coli* NDM-1. Interestingly, compound **12** with 2-(2-aminoethoxy) ethyl moiety also exhibited inhibitory activities against two strains: *E.coli* NDM-1 and *E. cloacae* 297. However, Compounds **7** with 2-aminoethyl moiety and **9** with 3-aminopropyl were found to have no effects. The active compounds **8** and **10-12** all bear a terminal amino side chain at the phenol moiety significantly improving their aqueous solubility, and the four carbon length side chain (**10**) displayed good and broad antibacterial activities. Meanwhile, compound **10** showed excellent aqueous solubility, with a saturated concentration of 650 μg/mL, which was determined by an HPLC analysis method[30]. Other amino derivatives **2-6** and **13-14** with no terminal NH_2 moiety displayed no inhibitory activities against the sixteen selected strains at 50 μg/mL.

Scheme 1[a]



[a]Reagents and conditions: (a) ROH, Ph_3P, DIAD, THF, r.t, **2-6**. (b) i. Boc-ROH, Ph_3P, DIAD, THF, r.t; ii. TFA, CH_2Cl_2, 0°C to r.t, **7-14**.

Table 1. Inhibition screening of niclosamide and its *O*-aminoalkyl-tethered derivatives against selected clinical strains of carbapenemase-producing *Enterobacteriaceae* isolates and / or colistin resistant [a]

Compd	KPC					*E.coli*	KPC	*E. aerogenes*		*E. cloacae*						
	CECT -997	07	21	28	29	NDM-1	NDM-1	3	53	32	142	190	263	272	280	297
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	+	-	-	+	-	+	+	-	-	-	-	-	-	-	+	+
11	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] All compounds were screened at 50 µM; + Inhibition of bacterial growth; - No inhibition of bacterial growth; KPC: *Klebsiella pneumoniae* carbapenemases; KPC-07 (VIM-1) (COL susceptible (S) MIC COL= 0.5 µg/mL); KPC-21 (VIM-1, DHAI) (COL resistant (R) MIC COL= 64 µg/mL); KPC-28 (OXA-48, CTX-M-15) (COLS MIC COL= 0.5 µg/mL); KPC-29 (OXA-48, CTX-M-15) (COLR MIC COL= 32 µg/mL); KPC NDM-1 (MIC COL= 0.5 µg/mL); *E.coli* NDM-1 (MIC COL= 0.5 µg/mL); *E. aerogenes* 3 (MIC COL= 32 µg/mL); *E. cloacae* 32 (MIC COL > 256 µg/mL); *E. aerogenes* 53 (MIC COL= 32 µg/mL); *E. cloacae* 142 (MIC COL= 64 µg/mL); *E. cloacae* 190 (MIC COL= 32 µg/mL); *E. cloacae* 263 (MIC COL= 8 µg/mL); *E. cloacae* 272 (MIC COL= 16 µg/mL); *E. cloacae* 280 (MIC COL= 32 µg/mL); *E. cloacae* 297 (MIC COL= 3 µg/mL).

The minimal inhibitory concentration (MIC) values of the selected *in vitro* active derivatives were further evaluated[31, 32]. As listed in **Table 2**, the MIC values of compound **10** against KPC CTCT-997, KPC-28 and *E.coli* NDM-1 were 15 µg/mL. Compound **11** also showed good inhibitory activity against KPC-28 strain with an MIC of 15 µg/mL, while all other MIC values were higher than 15 µg/mL.

Table 2. Minimal inhibitory concentration (MIC; µg/mL) of the *in vitro* active derivatives[a]

Compd	KPC		*E. coli*	KPC	*E. cloacae*	
	CECT-997	28	NDM-1	NDM-1	280	297
1	-	-	-	-	>15	>15
8	-	-	>15	-	-	-
10	15	15	15	>15	>15	>15
11	-	15	>15	>15	-	-
12	-	-	>15	-	-	>15

[a] KPC: *Klebsiella pneumoniae* carbapenemases; - No inhibition.

Considering the drawbacks of colistin and other potential active antibiotics in monotherapy, combination therapy has been raised as an interesting strategy to overcome those limitations as a single agent. *In vitro* synergy against CPE has been documented with some agents such as colistin-rifampin and colistin-tigecycline[33, 34]. We also investigated *in vitro* synergisms of these active derivatives together with COL using checkerboard assay and time-kill kinetic assays. As depicted in **Table 3**, these active compounds displayed various degrees of synergistic effects with COL against the selected strains. Some of the time-kill curves also confirmed these synergistic effects. The combination of niclosamide (**1**) and COL was bactericidal against *E. cloacae* 280 since 2 hrs and synergistic since 8 hrs (**Figure 1A**). Compound **10** and COL showed bactericidal effect against *E. coli* NDM-1 since 2 hrs, while their combination was bactericidal since 2 hrs as well (**Figure 1B**). Compound **10** had a synergistic effect with COL against KPC-28 since 8 hrs, and the combination was bactericidal from 4 hrs to 8 hrs (**Figure 1C**). The combination of **11** and COL also had a bactericidal and synergistic activity against KPC-28 since 2 hrs (**Figure 1D**).

Table 3. Checkerboard assay of the *in vitro* active derivatives[a]

Compd	Strains	Concentrations	FICI
1	*E. cloacae* 280 *E. cloacae* 297	1/8 COL+ 1/8 compound **1**	0.25
8	*E. coli* NDM-1	1/4 COL+ 1/4 compound **8**	0.50
10	KPC[b] NDM-1	1/4 COL+ 1/4 compound **10**	0.50
	E. coli NDM-1	1/8 COL+ 1/8 compound **10**	0.25
11	*E. coli* NDM-1	1/4 COL+ 1/8 compound **11** 1/8 COL+ 1/4 compound **11**	0.38

[a] The FICI was interpreted as follow: synergism = FICI ≤ 0.5; indifferent > 0.5 to ≤ 4; antagonism = FICI > 4. The experiments were performed in triplicate.

[b] KPC: *Klebsiella pneumoniae* carbapenemases.

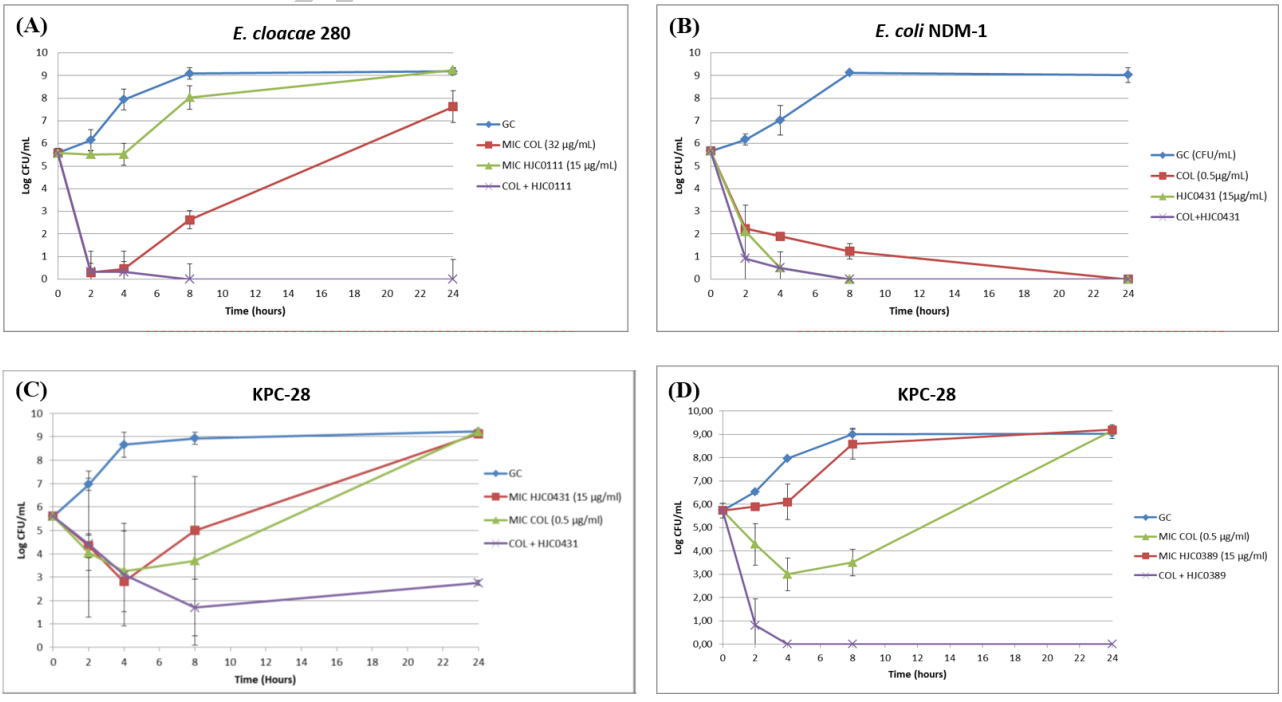


Figure 1. Time-kill curves. (A) Compound **1** (**HJC0111**) and COL alone and in combination against *E. cloacae* 280. (B) Compound **10** (**HJC0431**) and COL alone and in combination against *E. coli* NDM-1. (C) Compound **10** (**HJC0431**) and COL alone and in combination against KPC-28 (OXA-48, CTX-M-15). (D) Compound **11** (**HJC0389**) and COL alone and in combination against KPC-28 (OXA-48, CTX-M-15).

In summary, niclosamide and its novel *O*-alkylamino-tethered derivatives (**8**, **10**, **11** and **12**) were discovered with antibacterial activities against carbapenemase-producing and/or colistin resistant *Enterobacteriaceae* isolates. Among these compounds, **10** (**HJC0431**) with 4-aminobutyl moiety showed the broad antibacterial activities, effective against 6 strains. *In vitro* checkerboard and time-kill course studies demonstrated the synergistic effect of the screened compounds with COL against the corresponding strains with various degrees. These promising data support the further optimization potential of *O*-alkylamino-tethered derivatives as new and unique antibiotics against carbapenemase-producing and/or colistin resistant *Enterobacteriaceae* isolates.

Acknowledgements

This work was supported by UTMB Technology Commercialization Program, and Sanofi Innovation Awards (iAwards), John Sealy Memorial Endowment Fund, and Institute for Translational Sciences (ITS) at UTMB, *Plan Nacional de* I + D + i 2013-2016 and *Instituto de Salud Carlos III, Subdirección General de Redes y Centros de Investigación Cooperativa, Ministerio de Economía, Industria y Competitividad*, Spanish Network for Research in Infectious Diseases (REIPI RD16/0016/0009), cofinanced by European Development Regional Fund "A way to achieve Europe", Operative program Intelligent Growth 20142020, the Instituto de Salud Carlos III, Proyectos de Investigación en Salud (PI15/00489) and Proyectos de Desarrollo Tecnológico en Salud (DTS17/00130), the Spanish Adenovirus Network (AdenoNet, BIO2015/68990-REDT), and the program "Nicolás Monardes" (C-0059-2018) Servicio Andaluz de Salud, Junta de Andalucía.

References and notes

1. Rice, L. B. Progress and challenges in implementing the research on ESKAPE pathogens. *Infect Control Hosp Epidemiol.* **2010**; *31 Suppl 1*: S7-10.
2. Rice, L. B. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. *J Infect Dis.* **2008**; *197*(8): 1079-1081.
3. Pendleton, J. N.; Gorman, S. P..Gilmore, B. F. Clinical relevance of the ESKAPE pathogens. *Expert Rev Anti Infect Ther.* **2013**; *11*(3): 297-308.
4. Croft, A. C.; D'Antoni, A. V..Terzulli, S. L. Update on the antibacterial resistance crisis. *Med Sci Monit.* **2007**; *13*(6): RA103-118.
5. Nordmann, P.; Naas, T..Poirel, L. Global spread of Carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis.* **2011**; *17*(10): 1791-1798.
6. Tzouvelekis, L. S.; Markogiannakis, A.; Psichogiou, M.; Tassios, P. T..Daikos, G. L. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. *Clin Microbiol Rev.* **2012**; *25*(4): 682-707.
7. Biswas, S.; Brunel, J. M.; Dubus, J. C.; Reynaud-Gaubert, M..Rolain, J. M. Colistin: an update on the antibiotic of the 21st century. *Expert Rev Anti Infect Ther.* **2012**; *10*(8): 917-934.
8. Sader, H. S.; Castanheira, M.; Flamm, R. K.; Mendes, R. E.; Farrell, D. J..Jones, R. N. Tigecycline activity tested against carbapenem-resistant Enterobacteriaceae from 18 European nations: results from the SENTRY surveillance program (2010-2013). *Diagn Microbiol Infect Dis.* **2015**; *83*(2): 183-186.
9. Kumar, M. Colistin and Tigecycline Resistance in Carbapenem-Resistant Enterobacteriaceae: Checkmate to Our Last Line Of Defense. *Infect Control Hosp Epidemiol.* **2016**; *37*(5): 624-625.
10. Marchaim, D.; Chopra, T.; Pogue, J. M., et al. Outbreak of colistin-resistant, carbapenem-resistant Klebsiella pneumoniae in metropolitan Detroit, Michigan. *Antimicrob Agents Chemother.* **2011**; *55*(2): 593-599.
11. Andrews, P.; Thyssen, J..Lorke, D. The biology and toxicology of molluscicides, Bayluscide. *Pharmacol Ther.* **1982**; *19*(2): 245-295.
12. Al-Hadiya, B. M. Niclosamide: comprehensive profile. *Profiles Drug Subst Excip Relat Methodol.* **2005**; *32*: 67-96.
13. Weinbach, E. C..Garbus, J. Mechanism of action of reagents that uncouple oxidative phosphorylation. *Nature.* **1969**; *221*(5185): 1016-1018.
14. Williamson, R. L..Metcalf, R. L. Salicylanilides: a new group of active uncouplers of oxidative phosphorylation. *Science.* **1967**; *158*(3809): 1694-1695.
15. Frayha, G. J.; Smyth, J. D.; Gobert, J. G..Savel, J. The mechanisms of action of antiprotozoal and anthelmintic drugs in man. *General Pharmacology: The Vascular System.* **1997**; *28*(2): 273-299.
16. Li, Y.; Li, P.-K.; Roberts, M. J.; Arend, R. C.; Samant, R. S..Buchsbaum, D. J. Multi-targeted therapy of cancer by niclosamide: A new application for an old drug. *Cancer Lett.* **2014**; *349*(1): 8-14.
17. Chen, W.; Mook, R. A.; Premont, R. T..Wang, J. Niclosamide: Beyond an antihelminthic drug. *Cell Signal.* **2018**; *41*: 89-96.
18. Fonseca, B. D.; Diering, G. H.; Bidinosti, M. A., et al. Structure-activity analysis of niclosamide reveals potential role for cytoplasmic pH in control of mammalian target of rapamycin complex 1 (mTORC1) signaling. *J Biol Chem.* **2012**; *287*(21): 17530-17545.
19. Ippolito, J. E.; Brandenburg, M. W.; Ge, X., et al. Extracellular pH Modulates Neuroendocrine Prostate Cancer Cell Metabolism and Susceptibility to the Mitochondrial Inhibitor Niclosamide. *PLoS One.* **2016**; *11*(7): e0159675.
20. Balgi, A. D.; Fonseca, B. D.; Donohue, E., et al. Screen for chemical modulators of autophagy reveals novel therapeutic inhibitors of mTORC1 signaling. *PLoS One.* **2009**; *4*(9): e7124.
21. Ren, X.; Duan, L.; He, Q., et al. Identification of Niclosamide as a New Small-Molecule Inhibitor of the STAT3 Signaling Pathway. *ACS Med Chem Lett.* **2010**; *1*(9): 454-459.
22. Chen, M.; Wang, J.; Lu, J., et al. The anti-helminthic niclosamide inhibits Wnt/Frizzled1 signaling. *Biochemistry.* **2009**; *48*(43): 10267-10274.
23. Jin, Y.; Lu, Z.; Ding, K., et al. Antineoplastic mechanisms of niclosamide in acute myelogenous leukemia stem cells: inactivation of the NF-kappaB pathway and generation of reactive oxygen species. *Cancer Res.* **2010**; *70*(6): 2516-2527.
24. Wang, A. M.; Ku, H. H.; Liang, Y. C.; Chen, Y. C.; Hwu, Y. M..Yeh, T. S. The autonomous notch signal pathway is activated by baicalin and baicalein but is suppressed by niclosamide in K562 cells. *J Cell Biochem.* **2009**; *106*(4): 682-692.
25. Li, Z.; Brecher, M.; Deng, Y.-Q., et al. Existing drugs as broad-spectrum and potent inhibitors for Zika virus by targeting NS2B-NS3 interaction. *Cell Res.* **2017**; *27*: 1046.
26. Chen, H.; Yang, Z.; Ding, C., et al. Discovery of O-Alkylamino Tethered Niclosamide Derivatives as Potent and Orally Bioavailable Anticancer Agents. *ACS Med Chem Lett.* **2013**; *4*(2): 180-185.
27. Spectra data of the representative compound: (*S*)-2-(2-Aminobutoxy)-5-chloro-*N*-(2-chloro-4-nitrophenyl)benzamide (**8**). Pale yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.51 (d, *J* = 9.1 Hz, 1H), 8.43 (d, *J* = 2.4 Hz, 1H), 8.28 (dd, *J* = 9.1, 2.6 Hz, 1H), 7.92 (d, *J* = 2.8 Hz, 1H), 7.64 (dd, *J* = 8.9, 2.8 Hz, 1H), 7.38 (d, *J* = 9.0 Hz, 1H), 4.24 (dd, *J* = 10.2, 5.0 Hz, 1H), 4.06 (dd, *J* = 10.1, 7.5 Hz, 1H), 3.02 (s, 1H), 1.56 – 1.48 (m, 1H), 1.32 – 1.26 (m, 1H), 0.91 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 162.7, 155.6, 143.3, 141.2, 133.4, 130.4, 125.1, 124.8,

124.5, 123.4, 123.3 (2C), 116.4, 74.8, 51.0, 26.5, 10.1. HRMS (ESI) calcd for $C_{17}H_{18}Cl_2N_3O_4$ 398.0674 $(M + H)^+$, found 398.0670. HPLC analysis conditions: Waters μBondapak C18 (300 × 3.9 mm); flow rate 0.5 mL/min; UV detection at 270 and 254 nm; linear gradient from 10% acetonitrile in water (0.1% TFA) to 100% acetonitrile (0.1% TFA) in 20 min followed by 30 min of the last-named solvent; t_R = 17.07 min; purity (by peak area) 96%.

28. Torres, N. S.; Abercrombie, J. J.; Srinivasan, A.; Lopez-Ribot, J. L.; Ramasubramanian, A. K..Leung, K. P. Screening a Commercial Library of Pharmacologically Active Small Molecules against Staphylococcus aureus Biofilms. *Antimicrob Agents Chemother.* **2016**; *60*(10): 5663-5672.
29. The activity screening studies were performed as follows: All compounds were screened at 15 μg/mL in 96-well plates to identify which produced inhibition of growth of the 16 clinical *Enterobacteriaceae* strains. Briefly, 50 μL of 1 × 10^6 Colony Forming Unit (CFU) were inoculated in 50 μL Müeller Hinton Broth (MHB) medium in each well containing the different derivatives. Then, plates were incubated at 37 °C with humidity for 18 hrs. For each tested compound and strain, if after the incubation visual growth was observed in the wells the compound activity was considered negative. If no visual growth was observed in the wells the compound activity was considered positive. The screening was performed in duplicate.
30. Vogel, H. G.; Hock, F. J.; Maas, J..Mayer, D. Determination of solubility by hyphenated HPLC methods. *Drug discovery and evaluation: safety and pharmacokinetic assays.* New York: Springer; **2006**: 400-402.
31. Cebrero-Cangueiro, T.; Alvarez-Marin, R.; Labrador-Herrera, G., et al. In vitro Activity of Pentamidine Alone and in Combination With Aminoglycosides, Tigecycline, Rifampicin, and Doripenem Against Clinical Strains of Carbapenemase-Producing and/or Colistin-Resistant Enterobacteriaceae. *Front Cell Infect Microbiol.* **2018**; *8*: 363.
32. For the determination of the Minimal Inhibitory Concentration (MIC) a broth microdilution method was used (cation-adjusted Müeller Hinton broth; Becton Dickinson, Cockeysville, Md.), in accordance with the CLSI guidelines (Clinical and Laboratory Standards Institute, 2012). The compounds for which inhibition of the bacterial growth was observed in the screening studies were tested (6 clinical strains). The MIC value was the lowest concentration of derivative compound that completely inhibited the bacterial growth. All assays were performed in triplicate to ensure reproducibility.
33. Zavascki, A. P.; Bulitta, J. B..Landersdorfer, C. B. Combination therapy for carbapenem-resistant Gram-negative bacteria. *Expert Rev Anti Infect Ther.* **2013**; *11*(12): 1333-1353.
34. Daikos, G. L.; Tsaousi, S.; Tzouvelekis, L. S., et al. Carbapenemase-producing Klebsiella pneumoniae bloodstream infections: lowering mortality by antibiotic combination schemes and the role of carbapenems. *Antimicrob Agents Chemother.* **2014**; *58*(4): 2322-2328.

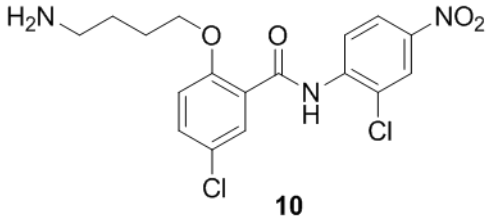
Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Discovery of niclosamide and its *O*-alkylamino-tethered derivatives as potent antibacterial agents against carbapenemase-producing and/or colistin resistant *Enterobacteriaceae* isolates

Leave this area blank for abstract info.

Jimin Xu, María Eugenia Pachón-Ibáñez,Tania Cebrero-Cangueiro, Haiying Chen, Javier Sánchez-Céspedes, and Jia Zhou



10

KPC CETT-997
KPC-28
E.coli NDM-1
} MIC = 15 μg/mL

KPC NDM-1
E. cloacae 280
E. cloacae 297
} MIC >15 μg/mL