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Synthesis and Insecticidal Activities of Novel Solanidine

Derivatives

Rémi Beaulieu^{a,b}, Eric Grand^a, Imane Stasik^a, Jacques Attoumbre^{b,c}, Quentin Chesnais^c,
Virginie Gobert^b, Arnaud Ameline^c, Philippe Giordanengo^d, José Kovensky^{a*}

^a Laboratoire de Glycochimie, des Antimicrobiens et des Agroressources (LG2A) CNRS UMR 7378, and Institut de Chimie de Picardie FR 3085, Université de Picardie - Jules Verne, 80039 Amiens, France.

^b Semences, Innovation, Protection, Recherche et Environnement (SIPRE), rue des Champs Potez, 62217 Achicourt, France.

^c Ecologie et Dynamique des Systèmes Anthropisés (EDYSAN) CNRS UMR 7058, Université de Picardie Jules Verne, 1 rue des Louvels 80037 Amiens, France.

^d Université de Picardie Jules Verne, Amiens, France – Institut Sophia Agrobiotech, CNRS 7254 – INRA 1355 – Université de Nice Sophia Antipolis, Sophia Antipolis, France.

1 **Abstract**

2

3 **BACKGROUND:** Potato (*Solanum tuberosum*) is the fourth culture in the world and is
4 widely used in the agri-food industries. They generate by-products where α -chaconine and α -
5 solanine, the two major solanidine based glycoalkaloids of potato, are present. As secondary
6 metabolites, they play an important role in the protecting system of potato and are involved in
7 plant protection against insects. To add value to these by-products, we described herein new
8 glycoalkaloids that could have phytosanitary properties.

9 **RESULTS:** Solanidine, as a renewable source, was modified with an azido linker and coupled
10 by Copper catalyzed alkyne azide cycloaddition (CuAAC) to alkynyl derivatives of the
11 monosaccharides found in the natural potato glycoalkaloids: D-glucose, D-galactose and L-
12 rhamnose. The efficacy of our compounds was evaluated on the potato aphid *Macrosiphum*
13 *euphorbiae*. The synthetic compounds have stronger aphicidal properties against nymphs than
14 unmodified solanidine. They also showed strong aphicidal activities on adults and a negative
15 impact on fecundity.

16 **CONCLUSION:** Our synthetic neoglycoalkaloids affected *Macrosiphum euphorbiae* survival
17 at the nymphal stage as well as at the adult stage. Furthermore, they induced a decrease of
18 fecundity. Our results show that chemical modifications of by-products may afford new
19 sustainable compounds for crop and plant protection.

20

21 **Keywords**

22 glycoalkaloid, insecticide, solanidine, synthesis, solanum, potato.

23

24 Running Title: Solanidine Derivatives

25

26 **1 Introduction**

27

28 Plants constitute an important source of bioactive compounds, and one of the best alternatives
29 to fossil resources in the development of sustainable chemistry. Potato is the fourth world
30 agricultural production with about 380 million metric tons (FAOSTAT,
31 <http://www.fao.org/faostat/en/#data/QC>). The consumption per capita increases each year and
32 goes along with the consumption of processed products, to the detriment of fresh potato. In
33 the last decades, the development of industrial potato-based food products led to a large
34 amount of by-products (mainly skin and tuber). Their management is an environmental and
35 economic challenge. Nowadays, these by-products are partially used as farm animal feed, in
36 the production of fuel-grade ethanol and in anaerobic digesters. However, they may be a
37 source of bioactive compounds such as phenolic compounds and glycoalkaloids¹ which may
38 be considered as high added-value residues for industrial purposes.

39 Glycoalkaloids are secondary metabolites found in solanaceae species. α -Chaconine and α -
40 solanine are the main glycoalkaloids found in potato. They have the same aglycone,
41 solanidine, but they differ in their saccharide moiety, chacotriose and solatriose respectively
42 (Figure S1). The glycoalkaloids content is highly dependent on the varieties of potatoes. They
43 are located all over the plant: tubers, sprouts or aerial parts as reported by Friedman² and
44 analyzed by mass spectrometry.³ Their amounts vary from a few milligrams per kilogram of
45 fresh matter in the inner part of the tuber to several grams per kilogram of fresh matter in the
46 sprouts.

47 As secondary metabolites, α -chaconine and α -solanine play an important role in the immune
48 system of potato and are involved in plant protection against insects.⁴ The potato leafhopper
49 (*Empoasca fabae*) is sensitive to glycoalkaloids. α -Chaconine and α -solanine at 0.09% (about

50 1 mM) have a toxic effect with a mortality of 59% and 8%, respectively. When the
51 concentration is increased to 0.27%, mortality was higher than 80% for both compounds.⁵ A
52 toxic effect was also observed against beetle (*Trogoderma granarium*). This pest of stored
53 grains and cereal products is drastically affected after 96 h, by a topical application of α -
54 chaconine (LD₅₀ = 18.1 μ g/mg insect) and α -solanine (LD₅₀ = 22.5 μ g/mg insect) as shown by
55 Nenaah.⁶ α -Chaconine or α -solanine containing phyto extracts also affect the development
56 and reproduction of *Drosophila melanogaster*.⁷ On the contrary, colorado potato beetle
57 (*Leptinotarsa decemlineata*) survival is not affected by the two glycoalkaloids.⁸

58 Aphids can be harmful to a lot of cultures because of their phytophagous activity, but also as
59 carriers of pathogens leading to plant diseases. The survival of *Schizaphis graminum*, an
60 aphid infested to *Poaceae* plants, is affected by either α -chaconine or α -solanine by
61 ingestion. After 24 h on an artificial diet, 48% of aphids died with α -chaconine at 250 μ M and
62 61% with α -solanine at 250 μ M.⁹ Similarly, an extract of potato glycoalkaloids at 160 mg.L⁻¹
63 increased mortality of the green peach aphid, *Myzus persicae*.¹⁰ The authors also
64 demonstrated that glycoalkaloids alter life history traits of this aphid by reducing diet uptake
65 and fecundity. These results have been partially confirmed for another aphid species, the
66 potato aphid *Macrosiphum euphorbiae*.¹¹ α -Chaconine at 250 ppm allowed the reproduction
67 to decrease while α -solanine did not. None of these two glycoalkaloids induced mortality,
68 even at high concentration. Nevertheless, the authors demonstrated a toxic effect of solanidine
69 (the aglycone of α -chaconine and α -solanine) at 250 ppm.

70 The toxicity of potato glycoalkaloids is mainly due to three mechanisms of action. They are
71 cytotoxic (affecting the cell membranes containing sterols) with a synergistic action,¹² they
72 disturb the ionic flux,¹³ and they inhibit cholinesterases.¹⁴

73 Considering the literature, it is possible to evaluate more precisely the influence of the
74 saccharide moiety. Inhibition of cholinesterases by solanidine glycoalkaloids is slightly
75 dependent on the saccharide moiety. α -Chaconine and α -solanine inhibited acetyl or
76 butyrylcholinesterase to the same level, few more than β_2 -chaconine.¹⁵ The cytotoxicity of
77 solanidine glycoalkaloids is more dependent on the saccharide moiety, as it has been shown
78 on liposome models.¹⁶ α -Chaconine showed significant lytic activity on liposomes while α -
79 solanine or β_2 -chaconine did not. As a consequence, the toxicity of the solanidine based
80 glycoalkaloids can be affected by the sugar composition. α -Chaconine and β_1 -chaconine were
81 more toxic on frog embryos than α -solanine, β_2 -chaconine or γ -chaconine.¹⁷ The same trend
82 was observed for teratogenicity. α -Chaconine and β_1 -chaconine were more teratogenic for
83 frog embryos than β_2 -chaconine or γ -chaconine.

84 Synthetically modified solanidine derivatives could be the base of a new sustainable crop and
85 plant protection strategy. Herein, we describe the synthesis of new glycoalkaloids starting
86 from solanidine as a renewable source of hemisynthetic bioactive molecules. We combined
87 solanidine and the monosaccharides found in the natural potato glycoalkaloids (chacotriose
88 and solatriose): D-glucose, D-galactose and L-rhamnose. A spacer arm was incorporated
89 between the aglycon and the saccharide moiety. The efficiency of our compounds was
90 evaluated on the aphid *Macrosiphum euphorbiae*. As this insect is polyphagous, found
91 worldwide and a main pest of many cultivated plants such as potato, tomato, peas, etc., it is a
92 good model for testing new sustainable pesticides. The insecticidal activity of our compounds
93 was compared to that of the solanidine in order to evaluate the effects of structural
94 modifications.

95

96 **2 Materials and Methods**

97 **2.1 Chemicals and Instruments**

98 Solanidine was extracted from potato as described previously.¹⁸ Skin and sprouts from
99 *S. tuberosum* cv. Pompadour was supplied by the Comité Nord Plants de Pomme de Terre and
100 was used as starting material. All purchased materials were used without further purification.
101 Dichloromethane was distilled from calcium hydride and tetrahydrofuran over sodium and
102 benzophenone. Analytical thin-layer chromatography (TLC) was carried out using Merck
103 D.C.-Alufolien Kieselgel 60 F₂₅₄. Flash chromatography (FC) was performed on a Reveleris
104 iES System supplied by Grace (USA) using pre-packed silica cartridges and ELSD/UV
105 detection.

106

107 **2.2 Synthetic procedures**

108 **2.2.1 Procedure A - 1,3-dipolar cycloaddition (Meldal conditions).** The azido solanidine
109 derivative (1 mmol) and the peracetylated propargyl sugar derivative (1.2 mmol) were
110 dissolved in toluene (25 mL). N,N-Diisopropylethylamine (DIPEA) (1.2 mmol) and CuI (0.2
111 mmol) were added and the reaction mixture was stirred for 8 h at 110 °C. After filtration
112 through celite, the solvent was evaporated under reduced pressure. The crude product was
113 purified by flash chromatography (1:0 to 1:1 cyclohexane-EtOAc containing 0.1% Et₃N). The
114 purified product was finally dried in a desiccator overnight.

115

116 **2.2.2 Procedure B – Deacetylation with sodium methanolate.** Sodium (1 mmol) was added
117 to MeOH (45 mL). The sodium methanolate solution obtained was then added to a solution of
118 the acetylated compound (1 mmol) in MeOH (45 mL). The reaction mixture was stirred at
119 room temperature for 4 h. Acid resin Amberlite® IR120 [H⁺] was added until a pH value of 5-
120 6 was reached. The reaction mixture was stirred at room temperature for 30 min and then
121 filtered to remove the resin. The solvent was removed under reduced pressure.

122

123 **2.2.3 Propargyl 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside (2).** 1,2,3,4,6-penta-*O*-acetyl-
124 β -D-glucopyranose (**1**, 25.62 mmol, 10.0 g) was dissolved in anhydrous CH₂Cl₂ (200 mL)
125 under argon. Propargyl alcohol (30.72 mmol, 1.82 mL) was added followed by BF₃.Et₂O
126 (102.48 mmol, 12.64 mL). The mixture was stirred for 2 h at room temperature. Then,
127 potassium carbonate (38.43 mmol, 5.31 g) was added and the reaction stirred for 30 min at
128 room temperature. After filtration, the mixture was washed with distilled water (2 x 200
129 mL). The aqueous layers were combined and extracted with CH₂Cl₂ (2 x 100 mL). The
130 organic layers were combined, dried over MgSO₄, filtered and the solvent was removed
131 under reduced pressure. The crude product was dissolved in a minimum volume of CH₂Cl₂,
132 then cyclohexane was added until the precipitation started. The mixture was stirred for 20
133 min at room temperature. Compound **2** was obtained after filtration through sintered glass,
134 with 84% yield (8.3 g) as a white solid.

135

136 **2.2.4 Propargyl 2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranoside (4).** 1,2,3,4,6-penta-*O*-acetyl-
137 β -D-galactopyranose (**3**, 12.81 mmol, 5.0 g) and silver trifluoroacetate (19.21 mmol, 4.28 g)
138 were dissolved in anhydrous CH₂Cl₂ (150 mL). Propargyl alcohol was added (19.21 mmol,
139 1.13 mL) followed by SnCl₄ (38.43 mmol, 4.5 mL). The reaction mixture was stirred for 1.5
140 h at room temperature. CH₂Cl₂ (400 mL) was added and the mixture was washed with,
141 successively, saturated solution of NaHCO₃ (300 mL), distilled water (3 x 300 mL) and
142 brine (300 mL). The organic layer was dried over MgSO₄, filtered and the solvent was
143 removed under reduced pressure. The crude product was purified by flash chromatography
144 (1:0 to 1:1 cyclohexane-EtOAc). Compound **4** was obtained in 87% yield (4.3 g) as a white
145 solid.

146

147 **2.2.5 1,2,3,4-tetra-O-acetyl-L-rhamnopyranose (6).** L-rhamnose (**5**, 109.0 mmol, 20.0 g)
148 was dissolved in pyridine (80 mL). Acetic anhydride (80 mL) was then added and the reaction
149 mixture was stirred for 15 h at room temperature. After evaporation of the solvent, the residue was
150 dissolved in EtOAc (250 mL) and washed with distilled water (3 x 100 mL). The organic layer
151 was dried over MgSO₄, filtered, and the solvent was removed under reduced pressure.
152 Compound **6** was obtained in 98% yield (35.1 g) as a colorless syrup (α/β : 86/14).

153

154 **2.2.6 Propargyl 2,3,4-tri-O-acetyl- α -L-rhamnopyranoside (7).** Compound **6** (11.14 mmol,
155 3.8 g) was dissolved in anhydrous CH₂Cl₂ (50 mL) under argon. Propargylic alcohol
156 (13.72 mmol, 0.81 mL) was added followed by BF₃.Et₂O (45.74 mmol, 5.65 mL). The
157 mixture was stirred for 2 h at room temperature. Then, potassium carbonate (17.15 mmol,
158 2.37 g) was added and the reaction stirred for 30 min at room temperature. After filtration,
159 the solution was diluted with CH₂Cl₂ (100 mL) and washed with distilled water (2 x 100
160 mL). The aqueous layers were combined and extracted with CH₂Cl₂ (2 x 50 mL). The
161 organic layers were combined, dried over MgSO₄, filtered and the solvent was removed
162 under reduced pressure. The crude product was purified by flash chromatography (1:0 to 3:2
163 cyclohexane-EtOAc). Compound **7** was obtained in 79% yield (2.9 g) as a white solid.

164

165 **2.2.7 3-azidopropan-1-ol (9).** 3-chloropropan-1-ol (**8**, 0.11 mol, 10.0 g) was dissolved in
166 distilled water (45 mL). Sodium azide (0.21 mmol, 13.78 g) was added and the reaction
167 mixture was stirred for 15 h at 80°C. After cooling to room temperature, the mixture was
168 extracted with diethyl ether (3 x 50 mL). The organic layers were combined and dried over
169 MgSO₄. After filtration, the solvent was removed under reduced pressure. Compound **9** was
170 obtained in 99% yield (11.0 g) as a colorless liquid.

171

172 **2.2.8 3-azidopropyl *p*-toluenesulfonate (10).** Compound **9** (0.11 mol, 11.5 g) was dissolved in
173 CH₂Cl₂ (110 mL). 4-Dimethylaminopyridine (DMAP) (23.0 mmol, 2.78 g) and Et₃N
174 (0.17 mol, 23.8 mL) were added and the reaction mixture was placed at 0°C. A solution of *p*-
175 Toluenesulfonyl chloride (0.17 mol, 32.41 g), in CH₂Cl₂ (60 mL) was added and the mixture
176 was stirred at room temperature for 15 h. Then, the mixture was diluted with CH₂Cl₂ (200
177 mL) and washed with, successively, a saturated solution of NaHCO₃ (200 mL), a 10% solution
178 of HCl (200 mL) and distilled water (200 mL). The organic layer was dried over MgSO₄ and
179 the solvent was removed under reduced pressure. The crude product was purified by flash
180 chromatography (1:0 to 3:2 cyclohexane-Et₂O). Compound **10** was obtained in 76% yield
181 (21.4 g) as a colorless liquid.

182

183 **2.2.9 3-*O*-(3-azidopropyl)solanidine (12).** Solanidine (**11**, 0.25 mmol, 100 mg) was
184 dissolved in anhydrous THF (4 mL) and the solution was placed under argon. NaH 95%
185 (1.26 mmol, 33 mg) was added and the mixture stirred for 30 min at room temperature.
186 Compound **10** (1.26 mmol, 0.32 g) was then added and the reaction mixture stirred for 48 h at
187 60 °C. After cooling to room temperature, the solvent was removed under reduced pressure.
188 The residue was dissolved in chloroform and filtered through celite. After concentration under
189 reduced pressure, the crude product was purified by flash chromatography (1:0 to 9:1 CHCl₃-
190 MeOH). The product was finally recrystallized in acetonitrile. Compound **12** was obtained in
191 71% yield (85 mg) as a white solid.

192

193 **2.2.10 3-*O*-{3-[4-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxymethyl)-1,2,3-triazol-1-
194 yl]propyl}solanidine (13).** Compound **13** was prepared from compound **12** (0.34 mmol, 165
195 mg) and compound **2** (0.72 mmol, 170 mg) according to the procedure A and obtained in 73%
196 yield (217 mg) as a white solid.

197

198 **2.2.11** *3-O*-{3-[4-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyloxymethyl)-1,2,3-triazol-1-
199 **yl]propyl}solanidine (**14**). Compound **14** was prepared from compound **12** (0.21 mmol, 100
200 mg) and compound **4** (0.25 mmol, 96 mg) according to the procedure A, and obtained in 72%
201 yield (129 mg) as a white solid.**

202

203 **2.2.12** *3-O*-{3-[4-(2,3,4-tri-*O*-acetyl- α -L-rhamnopyranosyloxymethyl)-1,2,3-triazol-1-
204 **yl]propyl}solanidine (**15**). Compound **15** was obtained from compound **12** (0.66 mmol, 320
205 mg) and compound **7** (0.80 mmol, 262 mg) according to the procedure A and obtained in 71%
206 yield (379 mg) as a white solid.**

207

208 **2.2.13** *3-O*-{3-[4-(β -D-glucopyranosyloxymethyl)-1,2,3-triazol-1-yl]propyl}solanidine
209 (**16**). Compound **16** was prepared from compound **13** (0.14 mmol, 125 mg) according to the
210 procedure C and purified by flash chromatography (1:0 to 4:1 CHCl₃-MeOH containing 0.1%
211 Et₃N). Compound **16** was obtained in 68% yield (68 mg) as a white solid.

212

213 **2.2.14** *3-O*-{3-[4-(β -D-galactopyranosyloxymethyl)-1,2,3-triazol-1-yl]propyl}solanidine
214 (**17**). Compound **17** was synthesized from compound **14** (0.15 mmol, 129 mg) according to
215 the procedure C purified by flash chromatography (1:0 to 4:1 CHCl₃-MeOH containing 0.1%
216 Et₃N). Compound **17** was obtained in 72% yield (76 mg) as a white solid.

217

218 **2.2.15** *3-O*-{3-[4-(α -L-rhamnopyranosyloxymethyl)-1,2,3-triazol-1-yl]propyl}solanidine
219 (**18**). Compound **18** was prepared from compound **15** (0.53 mmol, 430 mg) according to the
220 procedure C and purified by flash chromatography (1:0 to 9:2 CHCl₃-MeOH containing 0.1%
221 Et₃N). Compound **18** was obtained in 77% yield (279 mg) as a white solid.

222

223 **2.3 Insects and feeding assays**

224 *Macrosiphum euphorbiae* was mass-reared on potato plants (*Solanum tuberosum* cv Désirée)
225 in environmental chambers maintained at $20 \pm 1^\circ$ C, $60 \pm 5\%$ relative humidity, and a
226 photoperiod of 16:8 h (L:D) to induce parthenogenesis. The colony was initiated from a single
227 virginiparous female supplied by INRA/INSA Villeurbanne (France) in 2004 and initially
228 collected in 1995 from an eggplant field in the Rhône Alpes region (southern France). A
229 standard artificial diet, diet pouches and feeding chambers were prepared as described on the
230 literature.^{19,20} It used as a carrier for natural glycoalkaloids (α -chaconine, α -solanine,
231 solanidine) and synthesis derivatives (**16**, **17**, **18**) dilution and as a control. The concentrations
232 of the above compounds incorporated into the artificial diet were 0 (control), 2, 20 and 200
233 μ M. For nymphal survival, pools of synchronized first instar nymphs (less than 24 h old) were
234 obtained from parthenogenetic females placed on pouches of control diet. Groups of five first
235 instar nymphs were then transferred on pouches of each diet with a sample of 50 nymphs per
236 conditions. Survival was recorded every 2 days for 10 days (until nymphs become adults). For
237 adult experiments, the development of synchronized first instar nymphs until adult state was
238 done in control diet for 10 days. Then, groups of five young adults were transferred on
239 pouches of each diet with a sample of 40 nymphs per conditions. Survival and fecundity was
240 recorded every 2 days for 16 days. Pouches of artificial diet were changed every 2 days to
241 avoid bacterial or fungal contamination.^{21,22}

242

243 **2.4 Statistical analyses**

244 Mean values are given with their standard error of the mean (SEM). The effect of compounds
245 and different concentrations on aphid survival (after 10 for nymphs and 16 days for adults)
246 that were not normally distributed was analyzed using a Kruskal–Wallis one-way analysis of

247 variance (H), followed by multiple comparison tests using the R package “nparcomp” (type:
248 Dunnet for comparison to control). Aphid survival was analyzed using a Cox- proportional
249 hazards model. The effect of compounds and different concentrations on aphid fecundity that
250 was not normally distributed was analyzed using a Kruskal–Wallis one-way analysis of
251 variance (H), followed by multiple comparison tests using the R package “PMCMR”
252 (pairwise comparison Dunn test). All statistical analyses were carried out using the statistical
253 program “R” (R 3.2.2—R Development Core Team, 2015).

254

255 **3 Results and Discussion**

256 ***3.1 Synthetic route of new glycoalkaloids***

257 The hemisynthesis of our new glycoalkaloids was designed with a convergent strategy.
258 Solanidine (isolated by extraction of potato sprouts) was first functionalized with an azido-
259 propyl chain by S_N2 substitution on the 3-OH position. Nucleophilic substitution has been
260 previously used to prepare 3-*O*-steroid derivatives.^{23,24} Among the synthetic options, a leaving
261 group have been placed on the linker, the 3-OH acting as the nucleophile, was chosen in order
262 to prevent inversion/epimerization of the chiral carbon 3 atom.

263 Then, the azido-solanidine derivative was linked to a propargyl glycoside by copper(I)-
264 catalyzed alkyne-azide cycloaddition (CuAAC) affording a triazole type spacer arm. CuAAC
265 has several advantages such as regioselectivity and the lack of secondary reactions. The 1,2,3-
266 triazole is stable in biological conditions and, as far as we know, is biocompatible. The use of
267 a triazole as a spacer between an aglycon (diosgenin) and chacotriose has been already
268 described to study the effect on the cytotoxic activity.²⁵ This modification was conducted by
269 click reaction of an 1-azidochacotriosyl with a propargyl-diosgenin or by the reaction of
270 propargyl chacotrioside with an azido/azidoalkyl diosgenin derivative.

271 The synthesis had to be simple and efficient. Consequently we chose, as saccharide moiety,
272 the three different monosaccharide units present in the natural potato glycoalkaloids (β -D-
273 glucose, β -D-galactose and α -L-rhamnose). By addition of only one monosaccharide, we
274 could evaluate their individual impact on the activity.

275 For cytotoxicity activity, the mechanism of lytic action is based on the interaction of steroids
276 alkaloids with sterol containing in membranes.²⁶ This interaction is followed by the formation
277 of intermolecular hydrogen bonds between saccharide moieties which induces the destruction
278 of membranes. In our strategy, we wanted to balance the lack of some saccharide units by
279 adding a flexible spacer arm which could enhance intermolecular hydrogen bond formation.
280 The presence of the triazole ring, resulting from the click reaction, could result in additional
281 H-bond or CH/ π interactions, and the flexible propyl spacer arm could facilitate the
282 interactions between solanidine and the binding site on cholinesterases.

283

284 **3.2 Synthesis**

285 First, alkynyl glycosides were synthesized. The reaction of 1,2,3,4,6-penta-*O*-acetyl- β -D-
286 glucopyranose **1** with propargyl alcohol, in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ at room temperature,
287 following a reported procedure,²⁷ afforded the glycoside **2** (84% yield). For the galactoside
288 derivative **4**, only Praly's conditions²⁸ with SnCl_4 and $\text{CF}_3\text{CO}_2\text{Ag}$ allowed to obtain β -anomer
289 exclusively, in 87% yield. The propargyl α -L-rhamnoside **7** was obtained in 71% overall yield
290 via the peracetate **6** in the conditions used for the glucose derivative.

291 On the other hand, to obtain the azido-solanidine derivative by nucleophilic substitution, we
292 had to synthesize an appropriate linker with an azido group and a tosylate as leaving group. 3-
293 chloropropanol was azidated with NaN_3 in water, and the resulting 3-azidopropanol was
294 tosylated²⁹ with 75% overall yield for **10** from **8** (Figure 1).

295

296 Then, we could put the different moieties together. The first step was the reaction of
297 solanidine **11** (extract from potato sprout) with 5 equivalents of **10**, afforded the azido-derived
298 solanidine **12** in 71% yield (Figure 1). We were able to purify it using 1:0 to 9:1 CHCl₃-
299 CH₃OH containing 0.1% of Et₃N, followed by precipitation in acetonitrile. Acetyl protected
300 propargyl glycosides **2**, **4** and **7** were reacted with solanidine derivative **12** by CuAAC in the
301 presence of CuI and DIPEA, to give the protected coupled derivatives **13** (73%), **14** (72%)
302 and **15** (71%), respectively (Figure 2). Deacetylation of compounds **13**, **14** and **15** afforded
303 the final adducts **16** (68%), **17** (72%) and **18** (77%), respectively.³⁰

304

305 **3.3 Effect of α -solanine, α -chaconine and solanidine on nymphal survival**

306 The effect of the natural compounds α -solanine, α -chaconine and solanidine on *Macrosiphum*
307 *euphorbiae* was studied using a standard artificial diet as a carrier containing the compound at
308 three different concentrations: 2, 20 and 200 μ M. At 10 days, nymphal survival was
309 significantly affected by treatments (Kruskal-Wallis test, $H = 41.751$, $df = 9$, $P < 0.001$)
310 (Figure 3a). Compared to the control, nymphal survival was significantly reduced at the 200
311 μ M threshold for α -chaconine (54% survival), α -solanine (64%) and solanidine (52%)
312 (multiple nonparametric comparisons, type: Dunnet; $P < 0.001$, $P < 0.01$ and $P < 0.001$,
313 respectively). At 20 μ M, the reduction was significant only for solanidine with 64% of
314 survival ($P < 0.01$).

315

316 When comparing the different treatments during all the experiment length (supporting
317 information, figure S2), nymphal survival was significantly affected by the added compound
318 (ANOVA Cox model, $\chi^2 = 6.923$, $df = 2$, $P < 0.05$) and by the concentration (ANOVA Cox
319 model, $\chi^2 = 27.955$, $df = 2$, $P < 0.001$) indicating a dose dependent effect.

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Solanidine at a concentration of 200 μM has been thus used as a positive control in the evaluation of synthetic compounds.

3.4 Effect of synthetic compounds 16, 17 and 18 on nymphal survival.

Synthetic derivatives **16-18** were also evaluated at three concentrations: 2, 20 and 200 μM in comparison of solanidine at 200 μM selected from the above experiment. After 10 days, nymphal survival was significantly affected by treatments (Kruskal-Wallis test, $H = 125.55$, $df = 9$, $P < 0.001$) (Figure 3b). Compared to solanidine at 200 μM (nymphal survival 52%), the glucose-containing molecule **16** displayed higher aphicidal properties at 20 μM and at 200 μM , with 25% of nymphal survival for both concentration (multiple nonparametric comparisons, type: Dunnet; $P < 0.05$). The galactosyl derivative **17** at 2 and 20 μM showed similar effects than solanidine at 200 μM (64% and 34% survival respectively). However, at 200 μM , a significantly higher aphicidal effect (24% survival) was observed (multiple nonparametric comparisons, type: Dunnet; $P < 0.05$). Finally, compared to solanidine at 200 μM , compound **18** did not show any significant difference at 20 μM but showed stronger aphicidal activity at 200 μM with 6% of nymphal survival (multiple nonparametric comparisons, type: Dunnet; $P < 0.001$).

In conclusion, at 20 μM only the glucose-containing molecule **16** exhibited higher activity than solanidine at 200 μM . At equal concentration (200 μM), the three synthetic molecules were significantly more active than solanidine. The rhamnose-containing molecule **18** at 200 μM shows the most significant activity on nymphal survival.

344 When comparing the different treatments during all the experiment length (supporting
345 information, figure S3), nymphal survival was significantly affected by the compound
346 (ANOVA Cox model, $\chi^2 = 24.579$, $df = 3$, $P < 0.001$) and by the concentration (ANOVA Cox
347 model, $\chi^2 = 132.978$, $df = 2$, $P < 0.001$) indicating a dose dependent effect. As observed for α -
348 chaconine, α -solanine and solanidine, a severe reduction in nymphal survival was observed
349 within the first 4 days of treatment.

350

351 ***3.5 Effect of synthetic compounds 16, 17 and 18 on adult survival.***

352 Synthetic derivatives **16-18** (at 2, 20 and 200 μM) were evaluated on *Macrosiphum*
353 *euphorbiae* adults (Figure 4a). Compared to the control, adult survival was significantly
354 affected by treatments (Kruskal-Wallis test, $H = 130.95$, $df = 9$, $P < 0.001$) after 16 days. The
355 glucose-containing molecule **16** and the galactosyl derivative **17** exhibited significant
356 aphicidal activity at the 200 μM threshold, 46% and 54% adult survival respectively (multiple
357 nonparametric comparisons, type: Dunnet; $P < 0.001$ for both compounds). The rhamnosyl
358 derivative **18** showed strong aphicidal properties at 20 μM (ca. 67% survival, multiple
359 nonparametric comparisons, type: Dunnet; $P < 0.05$) and at 200 μM (ca. 7% survival, multiple
360 nonparametric comparisons, type: Dunnet; $P < 0.001$).

361
362 When comparing the different synthetic glycoalkaloids treatments (Figure 4b), adult survival
363 was significantly affected by the added compound (ANOVA Cox model, $\chi^2 = 12.367$, $df = 2$,
364 $P < 0.01$) and by the concentration (ANOVA Cox model, $\chi^2 = 101.299$, $df = 2$, $P < 0.001$)
365 indicating a dose dependent effect. Globally, adult survival was significantly lower for the
366 strongest concentrations. The rhamnose-based compound **18** at 200 μM showed a significant
367 decreased of aphid survival compared to all the other treatments (pairwise comparisons using
368 least-squares means, $P < 0.05$).

369

370 ***3.6 Effect of synthetic compounds 16, 17 and 18 on reproduction.***

371 The presence of synthetic glycoalkaloids in the artificial diet affected reproduction (Kruskal-
372 Wallis test, $H = 130.95$, $df = 9$, $P < 0.001$) (Figure 5). Overall, reproduction was more
373 affected by the three synthetic compounds at the highest concentrations. At low concentration
374 (2 μM), only the glucosyl derivative **16** decreased significantly the fecundity (pairwise
375 comparison Dunn test, $P < 0.05$), whereas compounds **17** and **18** had no effect ($P > 0.05$). At
376 20 μM , all three compounds decreased significantly the fecundity to a similar extent,
377 compared to the control. At 200 μM , the three molecules **16**, **17** and **18** had a strong effect.
378 The rhamnosyl derivative **18** showed the strongest activity, significantly higher than **16** and
379 **17** ($P < 0.05$), and with a dose-dependent response.

380

381 **4 Conclusion**

382 All these results show that our approach to obtain strong aphicidal activity with modified
383 glycoalkaloids is promising. Starting from solanidine, a simple and efficient synthetic strategy
384 allowed to click different sugars in a few steps. The analogs have been designed to include a
385 triazole-containing spacer between the sugar and the alkaloid moieties. This modification

386 increases the flexibility of the molecule and might facilitate interactions with biological
387 targets. The aphicidal activity of our synthetic neoglycoalkaloids containing only one
388 monosaccharide unit was proven. They affected *Macrosiphum euphorbiae* survival at the
389 nymphal stage as well as at the adult stage. Furthermore, they induced a decrease of
390 fecundity. In addition, our results show the influence of the monosaccharide structure on the
391 activity, as the rhamnosyl derivative **18** is clearly more active than the glucosyl and the
392 galactosyl conjugates **16** and **17** on adult survival and on reproduction. Further studies could
393 shed light on the specific mechanism of these new glycoalkaloid derivatives and to study their
394 effects on other pests. In perspective, it could also be interesting to investigate the feeding
395 behavior of aphids in the presence of our compounds using the electrical penetration graph
396 (EPG) technique.³¹ Anyway, our results show that the effect of natural glycoalkaloids can be
397 amplified through structural modifications performed by chemical synthesis, leading to new
398 sustainable compounds for crop and plant protection.

399

400 **5 Acknowledgments**

401 We thank SIPRE, the Comité Nord Plant de Pommes de Terre, the Conseil Régional de
402 Picardie, the Ministère de l'Enseignement Supérieur et de la Recherche, and the Centre
403 National de la Recherche Scientifique for financial support.

404

405 **6 Supporting Information**

406 Characterization of compounds **2** to **18**. ¹H and ¹³C NMR spectra of compounds **12-18**.
407 Names and chemical structures of natural hydrolysis products of α -chaconine and α -solanine
408 (Figure S1). Cox graphic representations of the nymph survival of *Macrosiphum euphorbiae*
409 reared on diets (Figures S2 and S3). Tables corresponding to graphics (Tables S1, S2, S3).

410

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497 peach aphid, *Myzus persicae* (Sternorrhyncha: Aphididae). *Afr J Agric Res* **9**:1344-1352
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499 **Figure captions**

500

501 Fig. 1. Reagents: (a) NaN₃, H₂O, 80 °C, 15 h, 99%; (b) TsCl, Et₃N, DMAP, CH₂Cl₂, 15 h, 0
502 °C → rt, 76%; (c) NaH, THF, 60 °C, 24 h, 71%.

503

504 Fig. 2. Reagents: (a), **12**, CuI, DIPEA, toluene, 8 h, 110 °C; (b) NaMeO, MeOH, 4 h, rt.

505

506 Fig. 3. (a) Nymphal survival of *Macrosiphum euphorbiae* reared on diets containing 2, 20 or
507 200 μ M of α -chaconine (light gray), α -solanine (gray) and solanidine (black) after 10 days of
508 treatment. (b) Nymphal survival of *Macrosiphum euphorbiae* reared on diets containing 2, 20
509 or 200 μ M of **16** (light gray), **17** (gray) and **18** (black) after 10 days of treatment. Asterisks

510 indicate statistically significant differences between the control (solanidine at 200 μM) and
511 the treatment (multiple nonparametric comparisons, type: Dunnet; * $P < 0.05$; ** $P < 0.01$;
512 *** $P < 0.001$).

513

514 Fig. 4. (a) Adult survival of *Macrosiphum euphorbiae* reared on diets containing 2, 20 or 200
515 μM of compounds **16** (light gray), **17** (gray) and **18** (black) after 16 days of treatment.

516 Asterisks indicate statistically significant differences between the control and the treatment
517 (multiple nonparametric comparisons, type: Dunnet; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

518 (b) Adult survival of *Macrosiphum euphorbiae* reared on diets containing 2 μM (full line), 20
519 μM (dashed line) or 200 μM (dotted line) of compounds **16** (light gray), **17** (gray) and **18**
520 (black).

521

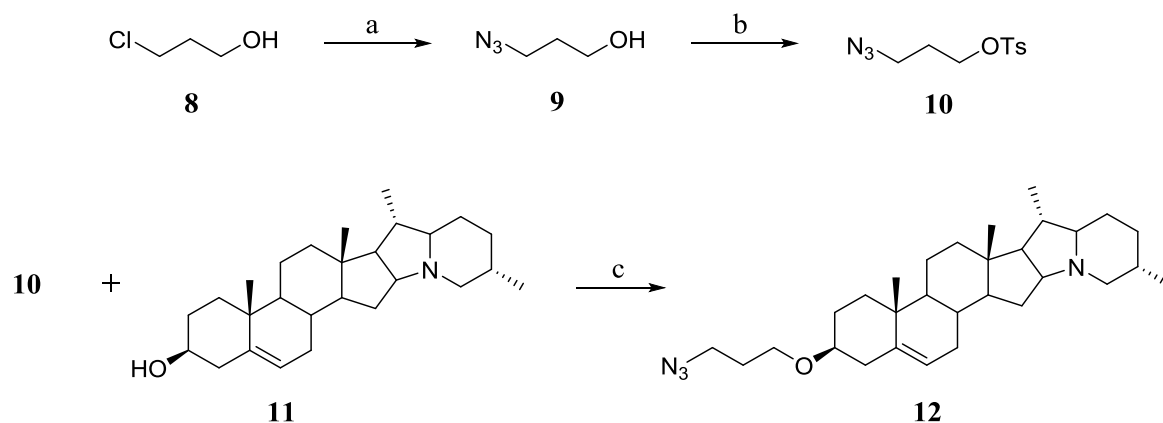
522 Fig. 5. Total fecundity (\pm SEM) of *Macrosiphum euphorbiae* reared on diets containing 2 μM ,
523 20 μM or 200 μM of compounds **16** (light gray), **17** (gray) and **18** (black) and control (white).

524 Letters indicate significant differences between treatments associated with Dunn test.

525

526 Figure graphics

527



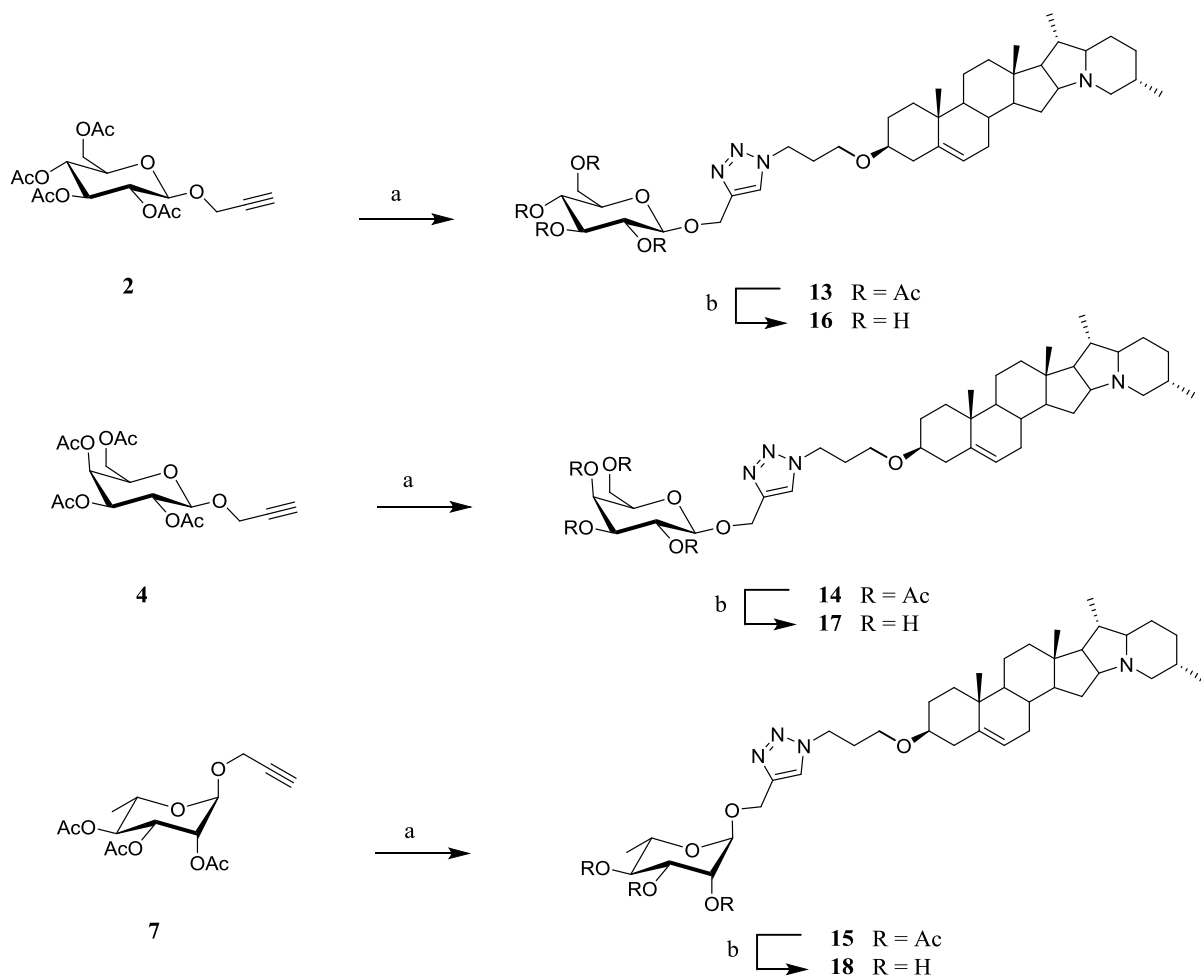
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Fig. 1.

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Fig. 2.

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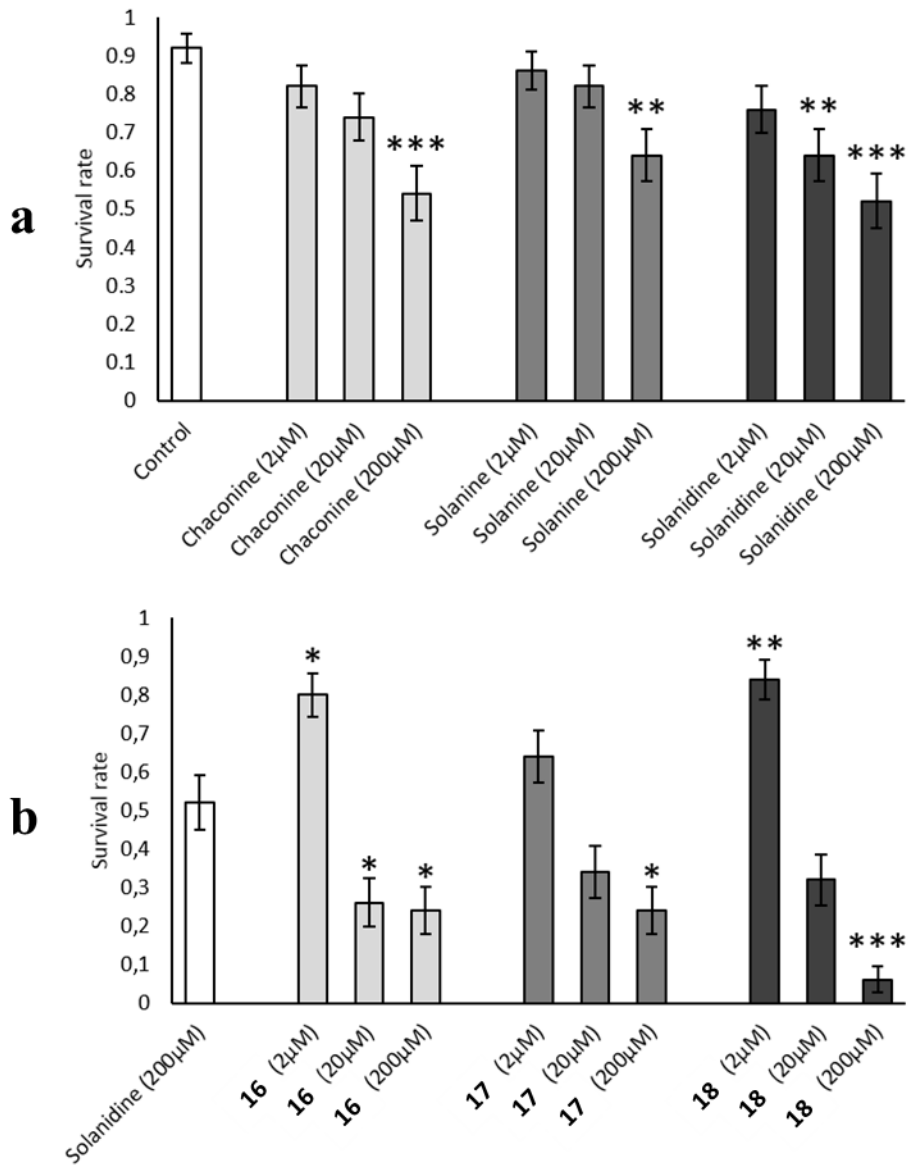


Fig. 3.

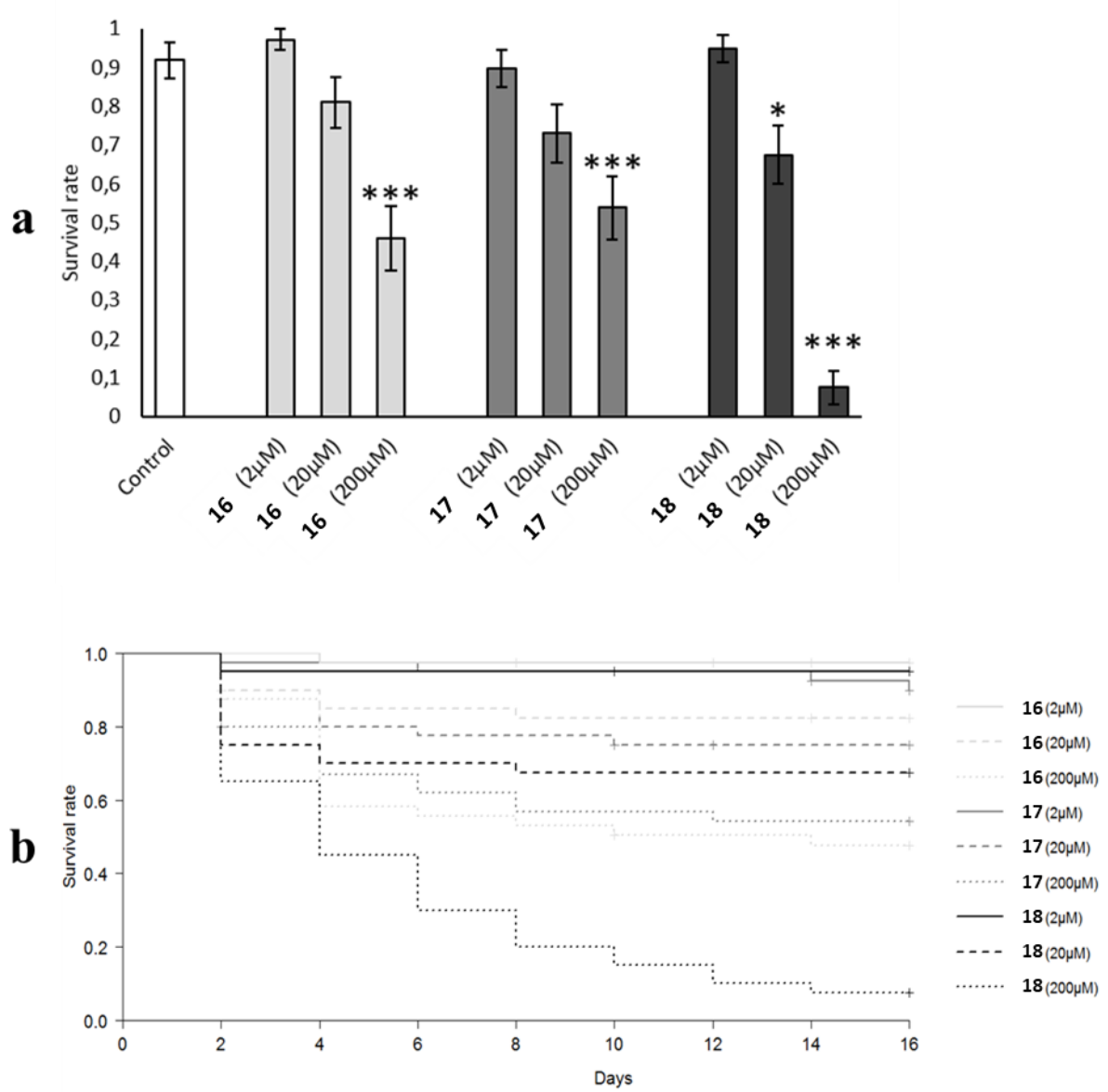
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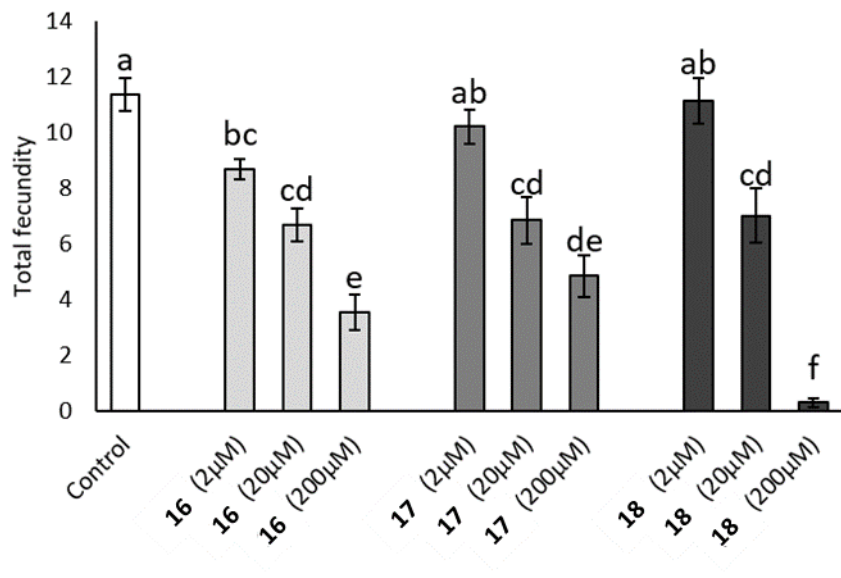
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Fig. 4.



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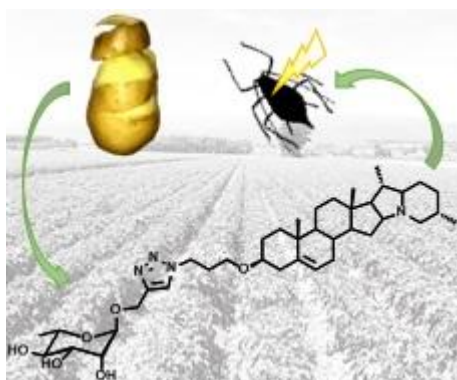
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Fig. 5.

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549 **Graphical abstract**

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Synthesis and Insecticidal Activities of Novel Solanidine Derivatives

Rémi Beaulieu, Eric Grand, Imane Stasik, Jacques Attoumbré, Quentin Chesnais, Virginie Gobert, Arnaud Ameline, Philippe Giordanengo, José Kovensky*

553

554 This article describes the synthesis of new glycoalkaloids starting from potato solanidine. The

555 synthetic neoglycoalkaloids showed aphicidal activities against *Macrosiphum euphorbiae*

556 nymphs and adults.