Bioorganic & Medicinal Chemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design and total synthesis of (-)-codonopsinine, (-)-codonopsine and codonopsinine analogues by *O*-(2-oxopyrrolidin-5-yl)trichloroacetimidate as amidoalkylating agent with improved antimicrobial activity via solid lipid nanoparticle formulations

Ahmed O.H. El-Nezhawy[a,b,*], Majed Alrobaian[c], Ahmed Khames[c,d], Mohamed F. El-Badawy[e,f], Sayed F. Abdelwahab[e,g]

[a] Department of Pharmaceutical Chemistry, College of Pharmacy, Taif University, Taif 21974, Saudi Arabia
[b] Department of Chemistry of Natural and Microbial Products, National Research Center, Dokki 12622, Cairo, Egypt
[c] Department of Pharmaceutics and Industrial Pharmacy, College of Pharmacy, Taif University, Taif 21974, Saudi Arabia
[d] Department of Pharmaceutics and Industrial Pharmacy, Beni-Suef University, Beni-Suef 62521, Egypt
[e] Division of Pharmaceutical Microbiology, Department of Pharmaceutics and Industrial Pharmacy, College of Pharmacy, Taif University, Taif 21974, Saudi Arabia
[f] Department of Microbiology and Immunology, Faculty of Pharmacy, Misr University for Science and Technology, Al-Motamayez District, P.O. Box 77, 6th of October City 12568, Egypt
[g] Department of Microbiology and Immunology, Faculty of Medicine, Minia University, Minia 61511, Egypt

ARTICLE INFO

Keywords:
(-)-Codonopsinine
(-)-Codonopsine
Codonopsinine analogues
(*D*)-tartaric acid
α-Amidoalkylation
Trichloroacetimidate
Antimicrobial activity
Solid lipid nanoparticles

ABSTRACT

A general strategy towards total synthesis of (-)-codonopsinine, (-)-codonopsine and codonopsinine analogues has been developed from (*D*)-tartaric acid *via* the intermediate (3S,4R)-1-methyl-2-oxo-5-(2,2,2-trichloroacetamido)pyrrolidinediacetate (**7**). α-amidoalkylation studies of **7** with electron rich benzene derivative **8a-g** as *C*-nucleophiles afforded (aryl derivatives) **9a-g**. The target compounds **1, 2** and **13c-g** were readily obtained from **10a-g** *via* Grignard addition to the homochiral lactam which was produced by deoxygenation using Lewis-acid followed by deacetylation. The synthesized compounds were loaded onto solid lipid nanoparticle formulations (SLNs) prepared by hot emulsification-ultrasonication technique using Compritol as solid lipid and Pluronic f68 as surfactant. SLNs were fully evaluated and the permeation of synthesized compound from SLNs was assayed against non-formulated compounds through dialysis membranes using Franz cell. The data indicated good physical characteristics of the prepared SLNs, sustaining of release profiles and significant improvement of permeation ability when compared to the non-formulated compounds. The antibacterial and antifungal activities of **1, 2** and **13c-g** were determined by disc diffusion and microbroth dilution method to determine the minimum inhibitory concentrations (MIC) against seven microorganisms (*Staphyloccus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa, Acinetobacter baumannii* and *Candida albicans*). The most active compounds against the Gram positive *S. aureus* were **1, 13C**, **13d**, and **13g**. Also, **13c**, **13d**, and **13e** had antibacterial activity but not **13f** against some Gram negative organisms (*E. coli*, and *P. mirabilis*). MIC concentrations against *P. aeruginosa,* and *K. pneumoniae* were ≥512 μg/ml, while that against *A. baumannii* was ≥128 μg/ml except for nanoformulae of **13e** and **13f** that were 16 and 64 μg/ml, respectively. No antifungal activity against *Candida albicans* was recorded for all compounds and their nanoformulae (MIC > 1024 μg/ml). SLNs were found to decrease the MIC values for some of the compounds with no effect on the antifungal activity. In conclusion, we demonstrated a novel, straight-forward and economical procedure for the total synthesis of (-)-codonopsinine **1**, (-)-codonopsine **2** and codonopsinine analogues **13c-g** from simple and commercially available starting materials; D-tartaric acid; with antimicrobial activities against Gram positive and Gram-negative organisms that were improved by SLNs formulations.

* Corresponding author at: Department of Pharmaceutical Chemistry, College of Pharmacy, Taif University, Saudi Arabia.
E-mail address: ah.omer@tu.edu.sa (A.O.H. El-Nezhawy).

https://doi.org/10.1016/j.bmc.2019.02.021
Received 10 December 2018; Received in revised form 28 January 2019; Accepted 12 February 2019
0968-0896/ Published by Elsevier Ltd.

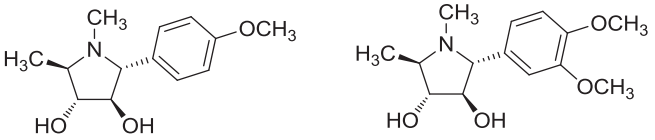


Figure 1. Structure of (-)-codonopsinine **1** and (-)-codonopsine **2.**

1. Introduction

Design and total synthesis of natural products have played important roles in many fields especially organic chemistry and medicinal chemistry. However, five membered-ring alkaloids as natural polyhydroxylated pyrrolidines (or iminosugars) and their derivatives acquired significant values in the last decade due to their promising pharmacological activities as antimicrobial, antiviral, antidiabetic and α-glucosidase inhibition.[1–10] Many polyhydroxylated pyrrolidine alkaloids exhibit potent and selective pharmacologically active compounds such as steviamine, swainsonine, casuarine uniflorine, and hyacinthacine.[11–16] Polyhydroxylated pyrrolidine alkaloids, which carry methyl group on position 5 and aromatic substituent on the 2 position of the pyrrolidine ring, are unusual and important class that is found in nature.[17] (-)-Codonopsinine **1** and (-)-codonopsine **2** (Fig. 1) separated from *Codonopsis clematidea* in 1969 by Russian scientists[18,19] are two types of complex pyrrolidine alkaloids. (-)-Codonopsinine **1** and (-)-codonopsine **2** display antibiotic activity as well as hypotensive activity without effects on the central nervous system.[20,21] Structural characterization of (-)-codonopsinine **1** and (-)-codonopsine **2**[18,22] showed a novel class of simple pyrrolidine alkaloids that possess 1,2,3,4,5 penta-substituted pyrrolidine structures with four adjacent stereo-genic centers, that are located in all *trans* positions. The absolute configuration of the (-)-codonopsinine antibiotic **1** was established as (2*R*,3*R*,4*R*,5*R*).[21,23] This was additionally verified by X-ray crystallography of (-)-codonopsine **2** separated by chromatography.[24] Due to the high biological activity and unique structural features, these pyrrolidine alkaloids **1** and **2** have gained substantial interest by several organic chemists. Numerous total syntheses of codonopsinine **1** were described: most of these methods are *enantio*-specific, starting from either D-tartaric acid[25], L-tartaric acid[26] D-ribose[27], D-alanine,[22,24] L-pyroglutamic acid,[28] (S)-3-chloropropan-1,2-diol,[29] via gold-mediated tandem-catalyzed pyrrole synthesis,[30] or by ring-closing iodoamination of homoallylic amines.[31]

Synthesis of substituted pyrrolidine has been studied via creation of quaternary stereocenters by the addition of allyltributylin or methyl silyloxyfuran to chiral *N*-acyliminium ions.[32,33] Glycosidic bond formation is usually carried out by the transformation of *O,O*- and *N,O*-hemiacetals into trichloroacetimidoyl derivatives and their acid-catalyzed activation (Fig. 2).[34,35]

Total synthesis of natural (-)-lentiginosine *via* *O*-(2-oxopyrrolidin-5-yl)trichloro acetimidate as amidoalkylating agent [36] promote us to use this concept in the synthesis of (-)-codonopsinine **1**, (-)-codonopsine **2** and codonopsinine analogues **13c-g** in an enantiomerically pure form from D-tartaric acid. The retrosynthesis (Fig. 3) shows that (3*R*,4*R*)-4-

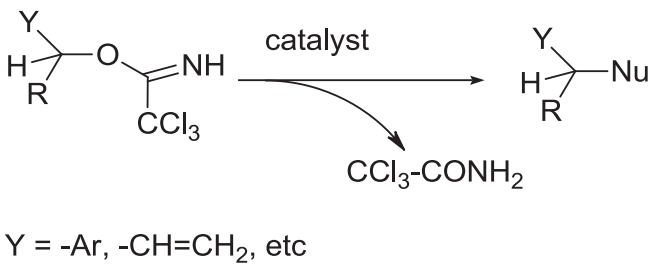


Figure 2. Alkylation of nucleophiles by acid-catalysis with *O*-alkyl trichloroacetimidate derivatives.

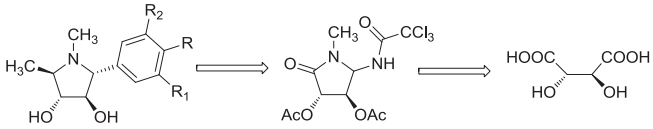


Figure 3. Retrosynthesis of (-)-codonopsinine **1**, (-)-codonopsine **2** and codonopsinine analogues **13a-g.**

(acetyloxy)-1-methyl-5-trichloroacetimidoyloxy-2-oxopyrrolidin-3-yl acetate intermediate (**7**) can be readily available from D-tartaric acid and can provide the target compounds (**1**, **2** and **13c-g**) in an efficient manner.

Nanotechnology was recently applied either for developing new therapeutic pathways and/or optimizing the traditionally available ones.[37] Solid lipid nanoparticles (SLNs) are modified colloidal carrier systems based on physiologically compatible solid lipids dispersed within an aqueous surfactant solution to obtain nano-particulate systems with a diameter in the range of 100–150 nm. They have high drug loading capability, extended stability, resist drug leak out of the lipid phase and high physiological tolerability. They can control and sustain the entrapped drug release as well as targeting to a specified tissue. They can, also, be prepared on a large scale. The lipophilic nature and small particle size of these systems allow efficient penetration through physiological membranes and increase cellular drug concentration with subsequent significant improvement of therapeutic action.[38]

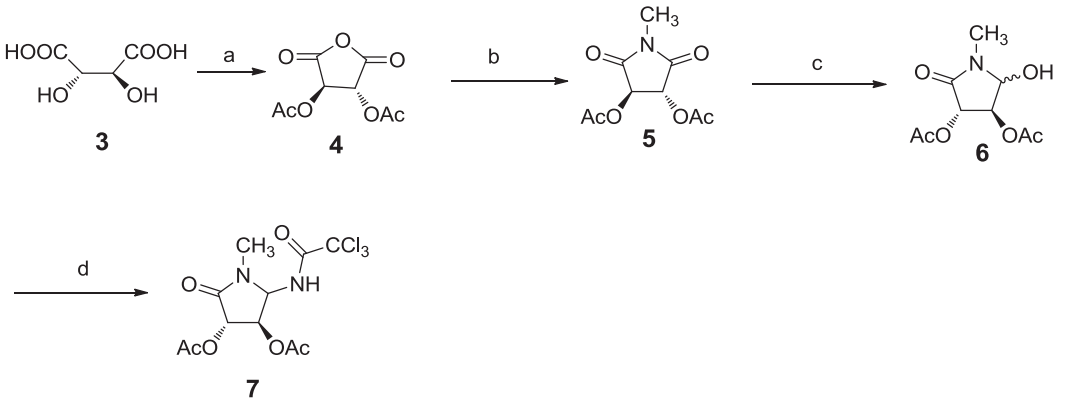
There is an enduring need for the discovery of new antibiotics. As a continuation of efforts of drug discovery[39–44], we report herein an effective methodology for constructing (-)-codonopsinine **1**, (-)-codonopsine **2** and codonopsinine analogues in enantiomerically pure form from cheap D-tartaric acid via a simple synthetic route with high yields. These compounds had antimicrobial activities against Gram positive and Gram negative organisms that was improved by SLN formulation.

2. Results and discussion

2.1. Chemistry

In this study, a novel methodology for synthesis of (-)-codonopsinine **1**, (-)-codonopsine **2** and codonopsinine analogues **13c-g** was utilized to obtain these target compounds from simple and commercially available starting material; D-tartaric acid (**3**), which was transformed into the anhydride **4** by reflux with acetyl chloride for 30 h then the anhydride **4** was refluxed with methylamine in acetonitrile to give imide **5**.[25,36] The reduction of **5** with sodium borohydride in methanol afforded (3R,4R)-2-hydroxy-1-methyl-5-oxopyrrolidine-3,4-diyl diacetate (**6**). The reaction of 5-hydroxy pyrrolidinone derivative **6** with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) as catalytic base furnished (3R,4R)-1-methyl-2-oxo-5-(2,2,2-trichloroacetamido)pyrrolidine-3,4-diyl diacetate (**7**) in high yield (Scheme 1).

Only one diastereoisomer of **7** was recovered with enantiomeric excess (100% *ee*) as confirmed by NMR. This was based on the $J_{4,5}$ coupling constant of 2.4 Hz might be the (5*R*)-isomer. Trichloroacetimidate **7** was used for the α-amidoalkylation experiments with different *C*-nucleophile. Reaction of (3R,4R)-1-methyl-2-oxo-5-(2,2,2-trichloroacetamido)pyrrolidine-3,4-diyl diacetate (**7**) with electron-rich anisole, 1,2-dimethoxybenzene, 1-fluoro-3-methoxybenzene, (methoxymethyl)benzene, benzene, toluene or 1-fluoro-3,4-dimethoxy-2-methylbenzene (**8a-g**) as the *C*-nucleophile in the presence of trimethylsilyl trifluoromethane sulfonate (TMSOTf) as catalyst led to compounds **9a-g** in good yields (scheme 2). Only one stereoisomer of each of **9a-g** was produced in high yield (100% *ee*) as confirmed by NMR. This was based on the $J_{4,5}$-coupling constant of ca. 5 Hz the (*R*)-configuration was assigned to *C*-5. Nucleophilic addition of methylmagnesium bromide to 2-pyrrolidinone **9a-g** at −78 °C afforded the labile quaternary α-hydroxy pyrrolidine **10a-g**, which equilibrated to



Scheme 1. Synthesis of trichloroacetimidate **7**. Reagents and conditions: (a) acetyl chloride, reflux 30 h (b) CH_3NH_2, THF, AcCl, reflux, **5**: 88% (c) $NaBH_4$, MeOH, −7 °C, 12 min, **6**: 89% (d) CCl_3CN, DBU, 0 °C, 10 min. **7**: 72%.

the open keto form **11a-g**. The mixture was submitted to reductive deoxygenation[45] with Et_3SiH in the presence of $BF_3.OEt_2$, cleanly leading to **12a-g** as a single stereoisomer (100% *ee*) in good yield. Deacetylation of the pyrrolidine derivative **12a-g** in the presence of NaOMe in methanol gave the corresponding (-)-codonopsinine **1**, (-)-codonopsine **2** or codonopsinine analogues **13c-g** in high yield (Scheme 2). The NMR, IR and mass spectra and microanalysis of the synthezsised (-)-codonopsinine **1** were in agreement with the published data of natural (-)-codonopsinine.[24]

In summary, easily obtained trichloroacetimidates **7** are outstanding precursors for α-amidoalkylation reactions with electron rich *C*-nucleophiles. This concept can be employed to the synthesis of substituted pyrrolidine alkaloids, as exhibited in the synthesis of (-)-codonopsinine **1**, (-)-codonopsine **2** or codonopsinine analogues **13c-g**.

2.2. Characterization of the prepared SLN formulations

2.2.1. Entrapment efficacy

Table 1 shows the characterization results for the prepared SLN formulations. All prepared formulations showed good percentage of entrapment efficacy for all tested compounds with small difference ranging from 70.8% (F7) to 86.9% (F8). It could be noted that the percentage of entrapment efficacy was dependent on the synthesized compound's solubility and molecular weight, where the higher the lipophilicity of the tested compound, the higher was its solubility within the applied lipid core and hence more amount was entrapped within the SLN formula.[38]

2.2.2. Particle size determination and size distribution

Data in Table 1 shows that all prepared SLN formulae had acceptable particle size ranging from 75 to 126 nm with satisfying narrow particle size distribution as indicated by the small Polydispersity Index (PDI) values (0.21 ± 0.015 to 0.37 ± 0.10). Particle size of SLN formulations is a complicated process that is affected by many factors including lipid type, concentration, emulsifying properties, surfactant type (HLB), crystallization conditions including homogenization/sonication time and speed.[38] Despite fixed lipid concentration and constant preparation conditions that resulted in proximity of recorded particle sizes for different formulation, it could be noted that drug content of the different formulations had positive effect on particle size and this could be explained by the increase of viscosity of formula mixture with increasing drug content.

2.2.3. Zeta potential measurement

During the preparation of SLN formulae, high shearing conditions are usually applied to affect particle size reduction that results in higher surface free energy values at the lipid/water interface and affects the physical stability of the prepared SLNs due to the induced particle agglomeration.[46] These repulsive forces are described in terms of electrical surface properties and expressed by Zeta potential (Z.P.). SLNs with Z.P. of ± 30 mV are usually physically stable formula.[47] The value of zeta potential of the prepared SLN formulations are usually controlled by surfactant addition during formulation that, also, lowers the interfacial tension at the lipid/water interface with subsequent stabilizing effect on the prepared formulae.[38] The data presented in Table 1 indicate that the recorded Z.P. values for all prepared formulae were acceptable (−21.5 to −34.7 mV) with small differences mainly due to fixation of preparation conditions (homogenization and sonication speed and time). Increasing particle size results in higher Z.P. values as a result of increasing the charge density with increasing surface area of the particle.

2.2.4. Release studies

In-vitro drug release data from the prepared SLN formulations were studied using dialysis bag diffusion technique in comparison to non-formulated compound release profiles. The release data are shown in Fig. 4. The release profiles of non-formulated compounds showed faster, complete dissolution within 2 to 3 h depending on the compound solubility in dissolution medium, where compounds **13e** and **13f** showed faster, and complete drug dissolution within two hours in comparison to 95.2%, 96.7%, 91.8% and 87.8% for compounds **1**, **13c**, **13d** and **2**, respectively, within the same time period. Compound **13g** showed a slower dissolution profile; where only 78.2% of the compound was dissolved after two hours. These data are in accordance with the solubility characteristics of the synthesized compounds at the tested experimental conditions. SLN formulations showed slow, and extended release profiles of the synthesized compounds over a 12 h period, where the percentage released reached 93.3%, 93.2%, 90.2%, 92.1%, 91.2%, 90.1%, and 92.2% from SLN formula (F)1 to F7, respectively (Fig. 4). The release profile from the prepared SLN formulations followed biphasic pattern with an initial burst high release during the first three hours, followed by a slower and sustained release within the remaining 12 h. This release pattern could be correlated to the matrix nature of the prepared SLNs, where the initial burst release results from the dissolution of the drug adsorbed on the particle surface and solubilized within particle outer most layers.[48,49] By time, the release rate slowed down depending on dissolution medium diffusion rate through deeper lipid layers and/or biodegradation and surface erosion of the matrix resulting in a slower and more extended dissolution process.[38]

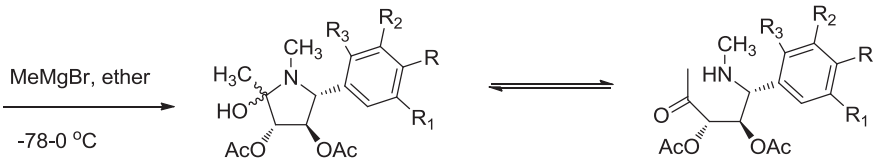
2.2.5. In-vitro permeation studies

The lipophilic characteristics and nano-size of SLNs increase its permeation through membranes especially those of lipid nature and this causes significant accumulation of the loaded drug within the targeted

7

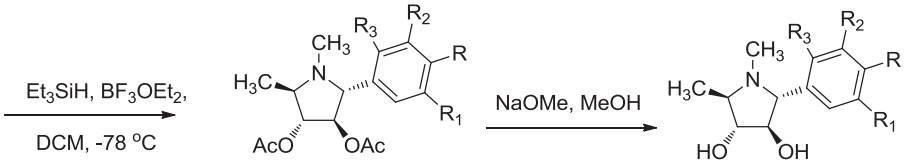
8a. R = OCH_3, R_1 = R_2 = R_3 = H
8b. R = OCH_3, R_1 = OCH_3, R_2 = R_3 = H
8c. R = OCH_3, R_1 = R_2 = H, R_3 = F
8d. R = CH_2OCH_3, R_1 = R_2 = R_3 = H
8e. R = R_1 = R_2 = R_3 = H
8f. R = CH_3, R_1 = R_2 = R_3 = H
8g. R = R_1 = OCH_3, R_2 = CH_3, R_3 = F

9a. R = OCH_3, R_1 = R_2 = R_3 = H
9b. R = OCH_3, R_1 = OCH_3, R_2 = R_3 = H
9c. R = OCH_3, R_1 = R_2 = H, R_3 = F
9d. R = CH_2OCH_3, R_1 = R_2 = R_3 = H
9e. R = R_1 = R_2 = R_3 = H
9f. R = CH_3, R_1 = R_2 = R_3 = H
9g. R = R_1 = OCH_3, R_2 = CH_3, R_3 = F



10a. R = OCH_3, R_1 = R_2 = R_3 = H
10b. R = OCH_3, R_1 = OCH_3, R_2 = R_3 = H
10c. R = OCH_3, R_1 = R_2 = H, R_3 = F
10d. R = CH_2OCH_3, R_1 = R_2 = R_3 = H
10e. R = R_1 = R_2 = R_3 = H
10f. R = CH_3, R_1 = R_2 = R_3 = H
10g. R = R_1 = OCH_3, R_2 = CH_3, R_3 = F

11a. R = OCH_3, R_1 = R_2 = R_3 = H
11b. R = OCH_3, R_1 = OCH_3, R_2 = R_3 = H
11c. R = OCH_3, R_1 = R_2 = H, R_3 = F
11d. R = CH_2OCH_3, R_1 = R_2 = R_3 = H
11e. R = R_1 = R_2 = R_3 = H
11f. R = CH_3, R_1 = R_2 = R_3 = H
11g. R = R_1 = OCH_3, R_2 = CH_3, R_3 = F



12a. R = OCH_3, R_1 = R_2 = R_3 = H
12b. R = OCH_3, R_1 = OCH_3, R_2 = R_3 = H
12c. R = OCH_3, R_1 = R_2 = H, R_3 = F
12d. R = CH_2OCH_3, R_1 = R_2 = R_3 = H
12e. R = R_1 = R_2 = R_3 = H
12f. R = CH_3, R_1 = R_2 = R_3 = H
12g. R = R_1 = OCH_3, R_2 = CH_3, R_3 = F

1. R = OCH_3, R_1 = R_2 = R_3 = H
2. R = OCH_3, R_1 = OCH_3, R_2 = R_3 = H
13c. R = OCH_3, R_1 = R_2 = H, R_3 = F
13d. R = CH_2OCH_3, R_1 = R_2 = R_3 = H
13e. R = R_1 = R_2 = R_3 = H
13f. R = CH_3, R_1 = R_2 = R_3 = H
13g. R = R_1 = OCH_3, R_2 = CH_3, R_3 = F

Scheme 2. Synthesis of target compounds. Reagents and conditions: (a) CH_2Cl_2, TMSOTf, r.t., 30–120 min. **9a**: 68%, **9b**: 75%, **9c**: 55%, **9d**: 69%, **9e**: 74%, **9f**: 69%, **9 g**: 57% (b) MeMgBr, ether, −78–0 °C (c) Et_3SiH, BF_3OEt_2, DCM, −78 °C, **12a**: 62%, **12b**: 68%, **12c**: 66%, **12d**: 69%, **12e**: 75%, **12f**: 75%, **12 g**: 73% (d) NaOMe, MeOH, 0 °C, **1**: 90%, **2**: 92%, **13c**: 87%, **13d**: 93%, **13e**: 88%, **13f**: 90%, **13 g**: 93%

Table 1
Characterization of the prepared SLN formulations of the synthesized compounds.

Formula Code	Original compound	E.E.*(%)	P.S.(nm)	Z.P.(mV)	PDI
F1	**1**	78.09 ± 2.85	88 ± 2.63	− 24.1	0.33 ± 0.053
F2	**2**	83.96 ± 1.95	118 ± 3.13	29.8 -	0.27 ± 0.071
F3	**13c**	77.98 ± 1.53	94 ± 2.19	23.8 -	0.21 ± 0.015
F4	**13d**	81.14 ± 3.15	109 ± 2.73	25.7 -	0.31 ± 0.019
F5	**13e**	70.83 ± 4.01	77 ± 1.17	21.5 -	0.32 ± 0.075
F6	**13f**	74.61 ± 3.86	75 ± 1.81	21.3 -	0.22 ± 0.054
F7	**13 g**	86.94 ± 3.87	126 ± 3.93	34.7 -	0.37 ± 0.10

* EE, entrapment efficiency; P.S., particle size; Z.P. Zeta potential; DPI, Polydispersity Index

tissue. The *in vitro* permeation of the prepared SLN formulations through simulated cellular (cellophane) membrane was studied by measuring the percentage of drug accumulation in the acceptor chamber of Franz cell. The data shown in Fig. 5 showed the cumulative percentage of permeated compound against time (hours) for 6 h. The synthesized compounds showed complete permeation from the prepared SLN formulations within 6 h, while the percentages of compounds permeated were 60.7%, 67.2%, 59.1%, 62.7%, 49.8%, 56.3% and 72.9% for non-formulated compounds **1, 2, 13c, 13d, 13e, 13f** and **13g**, respectively. Further investigation showed that the synthesized compound lipophilicity had a positive effect on the permeation results, where **13g** and **2** showed higher permeation results when compared to the other synthesized compounds. These data confirm the ability of the prepared SLN formulations to increase the permeation through membranes and improve cellular uptake of the loaded compound with an

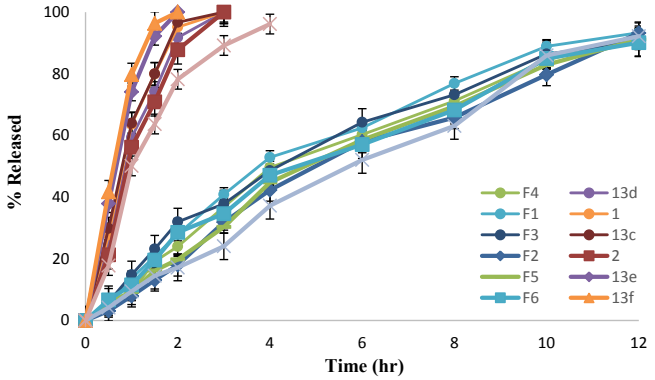


Figure 4. Release profiles for formulated and non-formulated synthesized compounds in phosphate buffer (pH 6.8).

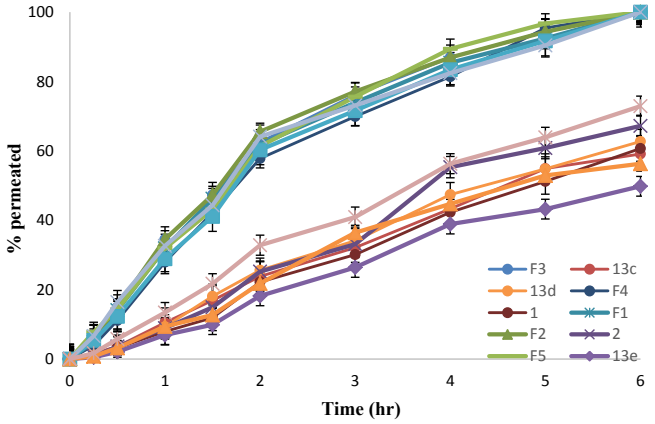


Figure 5. Permeation profiles for formulated and non-formulated synthesized compounds after 6 h.

expected increase in the antibacterial action.

2.3. Antimicrobial activity testing:

2.3.1. Antimicrobial susceptibility screening by modified Kirby-Bauer disc diffusion method:

As a preliminary screening step, we tested the synthesized compounds against seven microorganisms including Gram positive, and Gram negative organisms and yeast by disc diffusion method as described in the Experimental section. The data showed that compounds **1** and **13c** were the most effective codonopsinine derivatives against *S. aureus* (a Gram positive organism), where the inhibition zone diameters for these compounds were 32 and 30 mm, respectively. Also, compounds **2**, **13d** and **13g** showed obvious activity against *S. aureus* as shown in Figure 6 (and also in Table 2 as shown below). Apart from *S. aureus*, the current disc diffusion data showed that compound **13e** was the most effective codonopsinine derivative against Gram negative isolates, where the inhibition zone diameters for *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa* and *A. baumannii* were 40, 22, 34, 18 and 32 mm, respectively (data not shown). On the other hand, none of the codonopsinine derivatives were found to have antifungal activity against *Candida albicans* (not shown).

2.3.2. Determination of minimum inhibitory concentration of the synthesized compounds and their nanoformulae by microbroth dilution method:

The minimum inhibitory concentration (MIC) of the tested derivatives and their nanoformulae are presented in Table 2. As shown, the MIC values for the nano-formula of compound **1** against *E. coli* and *P. mirabilis* decreased from 128 μg/ml to 64 μg/ml, each, when compared

Figure 6. An example of antimicrobial susceptibility testing by disc diffusion method against *S. aureus* for the synthesized compounds.

to the parent compounds. On the other hand, the MIC value for *A. baumannii* for the same compound decreased from > 1024 μg/ml to 256 μg/ml. A similar *decrease* in MIC was seen for compounds **13e** and **13g** against *P. mirabilis* and *A. baumannii* and for **13e** against *P. mirabilis*, *P. aeruginosa* and *A. baumannii*. Regarding compound **13g**, the present study revealed that the nano-formulation of such compound was found to *decrease* the MIC values for *E. coli*, *P. mirabilis* and *A. baumannii* isolates from 64, 1024 and 512 μg/ml to 16, 128 and 256 μg/ml, respectively (Table 2). With respect to compound **13d**, nano-formulation was found to *decrease* the MIC value for *P. mirabilis* from 16 to 8 μg/ml. On the other hand, the nano-formulation for that compound didn't affect the MIC values for the other tested isolates except for *K. pneumoniae*, for which the MIC was *increased* from 256 to 512 μg/ml. These data show that the **13e** nano-formulation exhibited a great effect on the MIC value for *A. baumannii* in which the MIC *decreased* from 128 to 16 μg/ml.

For compound **13f**, it was found that nano-formulation for such compound was able to *decrease* the MIC value for *A. baumannii* and *S. aureus* from 256 and > 1024 μg/ml to 64 and 1024 μg/ml, respectively (Table 2). Finally, nano-formulation of compounds **1** and **13f** was found to *decrease* the MIC values for *K. pneumonaie* from > 1024 and 1024 μg/ml to 1024 and 512 μg/ml, respectively. On the other hand, the nano-formulation for all tested codonopsinine derivatives didn't affect or improve the antifungal activity against *C. albicans* indicating lack of antifungal activity of such compounds. Interestingly, the MIC of **13d**, and **13g** against *K. pneumoniae* was *increased*. The same applies for **2**, **13c**, and **13f** against *E. coli* (Table 2).

In summary, the data presented above suggest that **1**, **13d** and **13g** were the most potent derivatives against the Gram-positive bacterium; *S. aureus*. On the other hand, **13c**, **13d**, and **13e** are the most potent antibacterial compounds against Gram negative bacteria particularly *E. coli* and *P. mirabilis*. These compounds had MIC values ≤ 16 μg/ml either in the pure and/or the nano-formulae. In addition, nano-formulations improved the antibacterial activity of the synthesized compounds against some microorganisms while it had a deteriorating effect on others. As known, the genetic makeup of the bacterium greatly impacts its susceptibility to different antimicrobial agents. This could have an impact on our results. As indicated above, nano-formulations improved the activity of certain codonopsinine derivatives while it did not affect or deteriorated the activity of others. We speculate that the increased activity by nano-formulation can be attributed to increased uptake of the formulated compounds by facilitating the entry of the compounds to the bacterial cells or up regulation of membrane transporters of such compounds. Also, formulation could enhance the binding of such

Table 2
MIC values for non-nano-formulated and nano-formulated codonopsinine derivatives against Gram positive, Gram negative microorganisms and yeast. The MIC of the test compounds against seven microorganisms were determined as described in the Experimental section and the MIC values for the non-nano-formulated and nano-formulated compounds are shown in μg/ml.

CPD	Gram Positive		Gram Negative										Yeast	
	S. aureus ATCC 29213		*E. coli* ATCC 25922		*K. pneumoniae* ATCC 700603		*P. mirabilis* ATCC 14153		*P. aeruginosa* ATCC 27853		*A. baumannii* Clinical Isolate		*C. Albicans* 10231	
	Non-Nano	Nano	Non-Nano	Nano	Non-Nano	Nano	Non-Nano	Nano	Non-Nano	Nano	Non-Nano	Nano	Non-Nano	Nano
1	≤0.5	≤0.5	128	64	>1024	1024	128	64	1024	>1024	>1024	256	>1024	>1024
2	32	32	64	256	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024
13c	≤0.5	≤0.5	4	16	>1024	1024	>1024	>1024	>1024	>1024	512	512	>1024	>1024
13d	2	2	8	8	256	512	16	8	>1024	>1024	>1024	>1024	>1024	>1024
13e	1024	1024	16	16	1024	>1024	4	2	1024	512	128	16	>1024	>1024
13f	>1024	1024	128	512	1024	512	1024	512	1024	1024	256	64	>1024	>1024
13g	4	4	64	16	512	>1024	1024	128	1024	>1024	512	256	>1024	>1024

compounds to their target sites. On the other hand, the deteriorating effect of nano-formulation on the MIC of some of the codonopsinine derivatives may be attributed to down regulation of certain transport proteins at the cell envelope of different bacteria or the up regulation of certain efflux pumps that decrease the intracellular effective concentration of the compounds. To this end, the differential impact of nano-formulations on the MIC may be attributed to the interaction of these compounds in their native or formulated conditions with the bacterial envelope and the permeation, retention and/or efflux of these compounds by the different bacterial strains.

Finally, one the drawbacks of this study is that we did not examine the antibacterial activity of (+)-codonopsinine or its analogues and compare it with the (-)-codonopsinine and its analogues. This is currently under investigation.

3. Conclusion

In conclusion, we have demonstrated a novel, simple and efficient procedure for the total synthesis of (-)-codonopsinine **1**, (-)-codonopsine **2** and codonopsinine analogues **13c-g** from simple and commercially available starting materials (*D*-tartaric acid) via the reaction of intermediate trichloroacetamidate **7** with electron rich compounds as *C*-nucleophiles. Loading of the synthesized compounds on SLNs showed sustaining of release for 12 h and also significant improvement of permeation ability when compared to non-formulated compounds. Importantly, the synthesized compounds had antimicrobial activities against Gram positive and Gram-negative organisms that were improved by SLNs formulation.

4. Experimental

4.1. Chemistry

All chemicals were obtained from Fluka and Sigma-Aldrich (Germany) and were used as is. All reactions were carried out under inert gas and dry solvents. Also, all reactions were checked by thin layer chromatography (TLC) using Merck's silica gel-coated plastic sheets (60 F254; E. Merck, layer thickness 0.2 mm) under UV light. Detection was accomplished by treatment with either a solution of 20 g of ammonium molybdate and 0.4 g of cerium (IV) sulfate in 400 ml of 10% H_2SO_4 or with 15% H_2SO_4 and heating at 150 °C. Melting points were measured on a Gallenkamp melting point apparatus and were uncorrected. Optical rotations were measured by a Perkin-Elmer 241 MC polarimeter in a 1-dm cell. Infra-red spectra were determined on a JASCO FT/IR-4600 and reported as cm^{-1}. Bruker Unit 400 (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) instrument was used with $CDCl_3$ as a solvent and tetramethylsilane as an internal standard. Thermo Finnigan LCQ Advantage spectrometer in ESI mode, spray voltage 4.8 kV was used to perform mass spectra. Analytical analysis of carbon, hydrogen and nitrogen were carried at the Microanalytical Center of Cairo University, Egypt.

4.1.1. (3R,4R)-1-methyl-2,5-dioxopyrrolidine-3,4-diyl diacetate (5)

D-tartaric acid (10 g, 66.7 mmol) and acetyl chloride (50 ml, 0.7 mol) mixture was stirred under reflux for 25 h till the reaction mixture became homogeneous. Distillation was used to remove excess acetyl chloride at 760 mmHg, and the remaining amounts were removed by vacuum. The crude product was dissolved in tetrahydrofuran (100 ml) and methylamine (10 ml, 324 mmol) was added gradually. After stirring for two hours, the solution was concentrated *in vacuo* and the residue was kept under reflux with acetyl chloride (50 ml, 0.7 mol) for another 4 h. After the reaction mixture concentration *in vacuo*, the residue was purified using column chromatography (petroleum ether-ethyl acetate, 1:1) to give **5** (yield 87%) as a pale yellow oil. $[\alpha]_D = -51.5$ (c = 4, MeOH). IR (neat) ν 2950, 1740, 1690, 1610 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.05 (s, 6H, CH_3CO), 2.93 (s, 3H, NCH_3), 5.40 (s, 2H, CHOAc). ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 24.9, 72.4, 169.4, 169.7. MS (EI): *m/z* (%) = 229 (12) [M^+], 187 (68), 169 (1 0 0), 145 (60), 127 (65), 116 (26), 101 (92); Analytical Calculation for $C_9H_{11}NO_6$:C, 47.16; H, 4.83; N, 6.11. Found: C, 47.20; H, 4.90; N, 6.12.

4.1.2. (3R,4R)-2-hydroxy-1-methyl-5-oxopyrrolidine-3,4-diyl diacetate (6)

To a solution of **5** (4.57 mmol) in 125 ml of methanol was cooled to −10 °C in an ice-salt bath, (875 mg, 23.1 mmol) of sodium borohydride were added in one portion. The reaction mixture was stirred for 15 min and partitioned between 150 ml of CH_2Cl_2, 150 ml of saturated aqueous Na_2CO_3 and 100 ml of water. The aqueous phase was separated and extracted with three 100 ml fractions of dichloromethane. The organic phase was dehydrated using $MgSO_4$ and concentrated *in vacuo* to give **6** as a white solid (88%) mp 75–76 °C. The product was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 1:1). $[\alpha]_D$ = -24.9 (c = 1.8, MeOH). IR (neat) ν 3379, 2950, 1740, 1690, 1610 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.06 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.84 (s, 3H, NCH_3), 4.91 (br, s 1H, OH, exchangeable with D_2O), 5.02 (d, J = 2.8 Hz, 1H CHOAc), 5.05–5.16 (m, 2H, CHOAc and NCHO). ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.3, 20.5, 26.5, 73.8, 78.7, 84.9, 167.0, 170.1, 170.2. MS (EI): *m/z* (%) = 230 (10) [M^+], 170 (60), 128 (1 0 0), 111 (40), 101 (70), 70 (30), 59 (80); Analytical Calculation for $C_9H_{13}NO_6$: C, 46.75; H, 5.67; N, 6.05. Found: C, 46.55; H, 5.57; N, 6.01.

4.1.3. (3S,4R)-1-methyl-2-oxo-5-(2,2,2-trichloroacetamido)pyrrolidine-3,4-diyl diacetate (7)

A solution of **6** (2.5 mmol) in dry dichloromethane (20 ml) and trichloroacetonitrile (25 mmol) was treated with DBU (70 μl) at −10–0 °C and the reaction mixture was stirred for 12 min. The solvent

was evaporated and column chromatography was used to purify the product using triethylamine (TEA) in toluene 5% to give **7**; which is pale yellow oil (75%). $[\alpha]_D$ = + 50.5 (c = 1.9, MeOH). IR (neat) ν 3350, 2950, 1740, 1690, 1610 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.02 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.90 (s, 3H, NCH_3), 5.32 (m, 2H, 3-H, 4-H), 6.20 (d, J = 2.5 Hz, 1H, 5-H), 8.64 (br, s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 20.5, 26.9, 74.1, 78.6, 85.1, 162.2, 167.9, 170.1, 170.3.

*4.1.4. General procedure for the synthesis of (**9a–g**)*

A solution of trichloroacetimidate **7** (1.4 mmol) and the nucleophile anisole, 1,2-dimethoxybenzene, 1-fluoro-3-methoxybenzene, (methoxymethyl)benzene, benzene, toluene or 1-fluoro-3,4-dimethoxy-2-methylbenzene (**8a-g**) (1.4 mmol) in dry CH_2Cl_2 (20 ml) were treated with TMSOTf (0.16 ml) and then stirred for 20–100 min. Addition of solid sodium bicarbonate quenched the reaction, which was diluted with CH_2Cl_2. The reaction mixture was filtered and concentrated to give **9a-g**.

*4.1.4.1. (2R,3R,4S)-2-(4-methoxyphenyl)-1-methyl-5-oxopyrrolidine-3,4-diyl diacetate (**9a**)*. The colorless oil (68%) was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 1:1). $[\alpha]_D$ = -21.68 (c = 1.9, MeOH). IR (neat) ν 2940, 1742, 1694, 1610 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.03 (s, 3H, CH_3CO), 2.11 (s, 3H, CH_3CO), 2.62 (s, 3H, NCH_3), 3.80 (s, 3H, OCH_3), 4.70 (d, J = 5.0 Hz, 1H, 5-H), 5.42 (dd, J = 5.1 and 4.9 Hz, 1H, 4-H), 5.50 (d, J = 5.1 Hz, 1H, 3-H), 6.95–7.31 (m, 4H, Ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 27.9, 55.3, 62.2, 74.6, 76.5, 111.0, 114.4, 120.8, 123.6, 128.8, 130.2, 157.6, 167.8, 169.6, 169.8. MS (EI): m/z (%) = 320 (30) [M^+], 260 (60), 218 (1 0 0), 201 (30), 149 (50). Analytical Calculation for $C_{16}H_{19}NO_6$: C, 59.81; H, 5.96; N, 4.36. Found: C, 59.89; H, 6.06; N, 4.43.

*4.1.4.2. (2R,3R,4S)-2-(3,4-dimethoxyphenyl)-1-methyl-5-oxopyrrolidine-3,4-diyl diacetate (**9b**)*. Th colorless oil (75%) was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 1:1). $[\alpha]_D$ = –32.46 (c = 1.9, MeOH). IR (neat) ν 2950, 1740, 1690, 1620 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.12 (s, 3H, CH_3CO), 2.13 (s, 3H, CH_3CO), 2.65 (s, 3H, NCH_3), 3.81 (s, 6H, OCH_3), 4.73 (d, J = 5.1 Hz, 1H, 5-H), 5.38 (dd, J = 5.1 and 4.9 Hz, 1H, 4-H), 5.53 (d, J = 5.0 Hz, 1H, 3-H), 6.91 (s, 1H, ph-H), 7.08–7.20 (m, 2H, Ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.3, 21.0, 28.9, 56.7, 62.2, 70.6, 75.5, 112.0, 116.9, 121.6, 124.7, 128.9, 130.8, 156.9, 168.3, 169.9, 170.2. MS (EI): m/z (%) = 351 (45) [M^+], 235 (35), 220 (1 0 0); Analytical Calculation for $C_{17}H_{21}NO_7$: C, 58.11; H, 6.02; N, 3.99. Found: C, 58.29; H, 6.09; N, 4.13.

*4.1.4.3. (2R,3R,4S)-2-(2-fluoro-4-methoxyphenyl)-1-methyl-5-oxopyrrolidine-3,4-diyl diacetate (**9c**)*. The colorless oil (55%) was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 5:1). $[\alpha]_D$ = –22.26 (c = 1.9, MeOH). IR (neat) ν 2946, 1745, 1684, 1615 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.14 (s, 3H, CH_3CO), 2.15 (s, 3H, CH_3CO), 3.30 (s, 3H, NCH_3), 3.89 (s, OCH_3), 4.53 (d, J = 5.1 Hz, 1H, 5-H), 5.20 (dd, J = 5.1 and 4.9 Hz, 1H, 4-H), 5.43 (d, J = 5.1 Hz, 1H, 3-H), 7.02–7.07 (m, 2H, Ph-H), 7.18 (s, 1H, Ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.1, 20.3, 30.9, 54.6, 56.0, 70.6, 79.5, 124.9, 130.9, 138.8, 140.8, 157.1, 160.6, 168.3, 169.9, 170.2. MS (EI): m/z (%) = 339 (50) [M^+], 221 (35), 208 (1 0 0); Analytical Calculation for $C_{16}H_{18}FNO_6$: C, 56.63; H, 5.35; F, 5.60; N, 4.13. Found: C, 56.69; H, 5.41; F, 5.52; N, 4.23.

*4.1.4.4. 4.1.4.4.(2R,3R,4S)-2-(4-(methoxymethyl)phenyl)-1-methyl-5-oxopyrrolidine-3,4-diyl diacetate (**9d**)*. The colorless oil (69%) was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 1:1). $[\alpha]_D$ = –32.16 (c = 1.9, MeOH). IR (neat) ν 2943, 1742, 1690, 1610 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.11 (s, 3H, CH_3CO), 2.12 (s, 3H, CH_3CO), 3.35 (s, 3H, NCH_3), 3.48 (s, 3H, OCH_3), 4.86 (s, 2H, $phCH_2O$), 4.67 (d, = J 5.0 Hz, 1H, 5-H), 5.19 (dd, = J 5.0 and 4.9 Hz, 1H, 4-H), 5.49 (d, J = 5.1 Hz, 1H, 3-H), 6.90–7.16 (m, 4H, ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 21.3, 30.9, 56.3, 58.9, 70.2, 74.8, 85.5, 125.9, 126.4 130.9, 131.1, 138.8, 140.8, 168.7, 169.8, 171.6. MS: (EI) m/z (%) = 335 (30) [M^+], 219 (65), 204 (1 0 0); Analytical Calculation for $C_{17}H_{21}NO_6$: C, 60.89; H, 6.31; N, 4.18. Found: C, 60.96; H, 6.49; N, 4.23.

*4.1.4.5. (3S,4R,5R)-1-methyl-2-oxo-5-phenylpyrrolidine-3,4-diyl diacetate (**9e**)*. The colorless oil (74%) was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 10:1). $[\alpha]_D$ = –22.26 (c = 1.9, MeOH). IR (neat) ν 2940, 1742, 1694, 1610 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.10 (s, 3H, CH_3CO), 2.11 (s, 3H, CH_3CO), 3.35 (s, 3H, NCH_3), 4.62 (d, J 5.0 Hz, 1H, 5-H), 5.29 (dd, J = 5.0 and 5.2 Hz, 1H, 4-H), 5.48 (d, J = 5.0 Hz, 1H, 3-H), 7.26–7.54 (m, 5H, ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.3, 21.2, 31.3, 56.9, 75.8, 86.9, 125.9, 126.4, 129.5, 130.1, 130.9, 131.1, 168.5, 169.0, 172.1. MS (EI): m/z (%) = 291 (40) [M^+], 173 (1 0 0), 160 (75); Analytical Calculation for $C_{15}H_{17}NO_5$: C, 61.85; H, 5.88; N, 4.81. Found: C, 61.95; H, 5.92; N, 4.99.

*4.1.4.6. (3S,4R,5R)-1-methyl-2-oxo-5-(p-tolyl)pyrrolidine-3,4-diyl diacetate (**9f**)*. The colorless oil (69%) was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 10:1). $[\alpha]_D$ = -30.20 (c = 1.9, MeOH). IR (neat) ν 2950, 1745, 1690, 1614 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.12 (s, 3H, CH_3CO), 2.13 (s, 3H, CH_3CO), 2.45 (s, 3H, CH_3), 3.38 (s, 3H, NCH_3), 4.60 (d, J = 5.1 Hz, 1H, 5-H), 5.35 (dd, J = 5.1 and 5.1 Hz, 1H, 4-H), 5.44 (d, J = 5.1 Hz, 1H, 3-H), 7.26 (d, J = 12.2 Hz, 2H, ph-H), 7.34 (d, J = 11.9 Hz, 2H, ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.3, 20.6, 21.2, 30.8, 76.5, 85.8, 125.9, 126.1, 129.9, 130.1, 130.9, 131.1, 168.2, 169.1, 172.6. MS (EI): m/z (%) = 305 (20) [M^+], 189 (1 0 0), 174 (90); Analytical Calculation for $C_{16}H_{19}NO_5$: C, 62.94; H, 6.27; N, 4.59. Found: C, 62.85; H, 6.62; N, 4.68.

*4.1.4.7. (2R,3R,4S)-2-(2-fluoro-4,5-dimethoxy-3-methylphenyl)-1-methyl-5-oxopyrrolidine-3,4-diyl diacetate (**9 g**)*. The colorless oil (57%) was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 10:1). $[\alpha]_D$ = –32.18 (c = 1.9, MeOH). IR (neat) ν 2940, 1740, 1690, 1610 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.10 (s, 3H, CH_3CO), 2.12 (s, 3H, CH_3CO), 2.45 (s, 3H, CH_3), 3.35 (s, 3H, NCH_3), 3.85 (s, OCH_3), 3.87 (s, OCH_3), 4.63 (d, J = 5.2 Hz, 1H, 5-H), 5.44 (dd, J = 5.1 and 5.2 Hz, 1H, 4-H), 5.44 (d, J = 5.0 Hz, 1H, 3-H), 7.18 (s, 1H, ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.7, 20.9, 21.2, 30.8, 49.2, 56.2, 60.1, 76.9, 86.8, 116.6, 117.9, 129.7, 145.4, 150.9, 160.1, 168.7, 169.7, 171.9. MS (EI): m/z (%) = 383 (30) [M^+], 265 (1 0 0), 252 (60); Analytical Calculation for $C_{18}H_{22}FNO_7$: C, 56.39; H, 5.78; F, 4.96; N, 3.65. Found: C, 56.49; H, 5.82; F, 5.03; N, 3.78.

*4.1.5. General procedure for the synthesis of (**10a–g**)*

Methyl magnesium bromide (0.28 ml, 0.84 mmol, 1.2 equiv. as a 3 M solution in diethyl ether) was slowly added to a cooled (-78 °C) solution of **9a-g** (0.6 mmol) in diethyl ether (10 ml). Stirring continued for 2 h at −78 °C for 2 h. The reaction mixture was quenched with saturated $NaHCO_3$ (4 ml), washed with water (2 × 4 ml) and brine (3 ml), dried over $MgSO_4$, filtered and concentrated *in vacuo*, then used as crude in the next step.

*4.1.6. General procedure for the synthesis of (**12a–g**)*

To a solution of hydroxy lactam **10a-g** (0.35 mmol) in CH_2Cl_2 (5 ml), under an argon atmosphere at −78 °C, Et_3SiH (0.15 ml, 1.05 mmol) was added followed by $BF_3.OEt_2$ (0.08 ml, 0.070 mmol) dropwise. Stirring continued for 20–30 min at 0 °C. The reaction was quenched with H_2O (10 ml) and extracted with dichloromethane (3 × 20 ml). The organic phase was washed with brine (5 ml), dried over $MgSO_4$, and the solvent was removed under reduced pressure to

give **12a-g**.

*4.1.6.1. (2R,3R,4R,5R)-2-(4-methoxyphenyl)-1,5-dimethylpyrrolidine-3,4-diyl diacetate (**12a**).* The colorless oil (319 mg, 62%) was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 1:1). $[\alpha]_D$ = -31.60 (c = 2.0, MeOH), IR (neat) ν 2945, 1745, 1610 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.23 (d, J = 6.5 Hz, 3H, CH_3), 2.11 (s, 3H, CH_3CO), 2.13 (s, 3H, CH_3CO), 2.28 (s, 3H, NCH_3), 3.59 (s, 3H, OCH_3), 3.69–3.73 (m, 1H, 5- H), 4.13 (d, J = 6.5 Hz, 1H, 2-H), 4.45 (dd, J = 4.4, 4.0 Hz, 1H, 4-H), 5.04 (dd, J = 6.4 and 4.4 Hz, 1H 3-H), 6.90 (d, J = 8.9 Hz, 2H, ph-H), 7.26 (d, J = 8.9 Hz, 2H, Ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 20.9, 21.1, 38.1, 56.2, 56.8, 66.1, 75.1, 76.7, 114.2, 128.9, 129.2, 158.4, 170.1, 170.3. MS (EI): m/z (%) = 321 (40) [M^+], 203 (1 0 0), 190 (80); Analytical Calculation for $C_{17}H_{23}NO_5$: C, 63.54; H, 7.21; N, 4.36. Found: C, 63.60; H, 7.32; N, 4.38.

*4.1.6.2. (2R,3R,4R,5R)-2-(3,4-dimethoxyphenyl)-1,5-dimethylpyrrolidine-3,4-diyl diacetate (**12b**).* The colorless oil (68%) was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 1:1). $[\alpha]_D$ = –22.31 (c = 2.0, MeOH), IR (neat) ν 2950, 1740, 1600 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.20 (d, J = 6.8 Hz, 3H, CH_3), 2.10 (s, 3H, CH_3CO), 2.12 (s, 3H, CH_3CO), 2.20 (s, 3H, NCH_3), 3.85 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.66–3.70 (m, 1H, 5- H), 4.22 (d, J = 6.9 Hz, 1H, 2-H), 4.40 (dd, J = 4.6, 4.2 Hz, 1H, 4-H), 5.14 (dd, J = 6.9 and 4.6 Hz, 1H 3-H), 6.75–6.82 (m, 2H, ph-H), 6.95 (s, 1H, Ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.6, 21.1, 21.3, 38.8, 55.3, 56.7, 65.1, 76.3, 76.9, 113.7, 129.9, 130.8, 159.2, 170.4, 170.9. MS (EI): m/z (%) = 351 (30) [M^+], 235 (70), 220 (1 0 0); Analytical Calculation for $C_{18}H_{25}NO_6$: C, 61.52; H, 7.17; N, 3.99. Found: C, 61.66; H, 7.18; N, 3.88.

4.1.6.3. 4.1.6.3.(2R,3R,4R,5R)-2-(2-fluoro-4-methoxyphenyl)-1,5-dimethylpyrrolidine-3,4-diyldiacetate (12c). The colorless oil (66%) was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 2:1). $[\alpha]_D$ = –32.21 (c = 2.0, MeOH), IR (neat) ν 2955, 1744, 1610 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.12 (d, J = 6.6 Hz, 3H, CH_3), 2.11 (s, 3H, CH_3CO), 2.13 (s, 3H, CH_3CO), 2.26 (s, 3H, NCH_3), 3.61–3.63 (m, 1H, 5- H), 3.87 (s, 3H, OCH_3), 4.18 (d, J = 6.7 Hz, 1H, 2-H), 4.47 (dd, J = 4.6, 4.0 Hz, 1H, 4-H), 5.09 (dd, J = 6.7 and 4.0 Hz, 1H 3-H), 6.92–6.98 (m, 2H, ph-H), 7.15 (s, 1H, Ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.0, 20.2, 31.3, 55.0, 56.0, 71.2, 80.3, 125.5, 130.1, 139.2, 141.3, 157.3, 160.9, 169.9, 170.1. MS (EI): m/z (%) = 339 (40) [M^+], 221 (1 0 0), 208 (70); Analytical Calculation for $C_{17}H_{22}FNO_5$: C, 60.17; H, 6.53; F, 5.60; N, 4.13. Found: C, 60.25; H, 6.58; F, 5.71; N, 4.18.

4.1.6.4. 4.1.6.4.(2R,3R,4R,5R)-2-(4-(methoxymethyl)phenyl)-1,5-dimethylpyrrolidine-3,4-diyl diacetate (12d). The colorless oil (69%) was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 2:1). $[\alpha]_D$ = -26.50 (c = 2.0, MeOH), IR (neat) ν 2943, 1742, 1600 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.18 (d, J = 6.7 Hz, 3H, CH_3), 2.09 (s, 3H, CH_3CO), 2.12 (s, 3H, CH_3CO), 2.20 (s, 3H, NCH_3), 3.43 (s, 3H, OCH_3), 3.59–3.63 (m, 1H, 5- H), 4.10 (d, J = 6.7 Hz, 1H, 2-H), 4.40 (dd, J = 4.6, 3.98 Hz, 1H, 4-H), 4.87 (s, 2H, OCH_2), 5.10 (dd, J = 6.7 and 4.6 Hz, 1H 3-H), 7.06 (d, J = 8.6 Hz, 2H, ph-H), 7.10 (d, J = 8.6 Hz, 2H, Ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.2, 21.1, 21.3, 39.4, 56.9, 58.8, 65.1, 75.9, 76.9, 77.8, 126.2, 126.9, 128.7, 129.1, 134.1, 138.9, 170.7, 170.9. MS (EI): m/z (%) = 335 (20) [M^+], 188 (1 0 0), 175 (60); Analytical Calculation for $C_{18}H_{25}NO_5$: C, 64.46; H, 7.51; N, 4.18. Found: C, 64.59; H, 7.62; N, 4.28.

*4.1.6.5. (2R,3R,4R,5R)-1,2-dimethyl-5-phenylpyrrolidine-3,4-diyl diacetate (**12e**).* The colorless oil (79%) was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 1:1). $[\alpha]_D$ = -46.10 (c = 2.0, MeOH), IR (neat) ν 2940, 1742, 1610 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.11 (d, J = 6.4 Hz, 3H, CH_3), 2.10 (s, 3H, CH_3CO), 2.11 (s, 3H, CH_3CO), 2.18 (s, 3H, NCH_3), 3.55–3.59 (m, 1H, 5- H), 4.15 (d, J = 6.4 Hz, 1H, 2-H), 4.47 (dd, J = 4.4, 4.0 Hz, 1H, 4-H), 5.16 (dd, J = 6.4 and 4.4 Hz, 1H 3-H), 7.26–7.34 (m, 5H, Ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.0, 21.2, 21.3, 38.3, 57.4, 66.4, 76.8, 77.1, 124.3, 126.4, 126.8, 128.2, 138.9, 140.6, 171.3, 171.5. MS (EI): m/z (%) = 291 (30) [M^+], 173 (40), 160 (1 0 0); Analytical Calculation for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.27; N, 4.81. Found: C, 65.99; H 7.28; N, 4.86.

*4.1.6.6. (2R,3R,4R,5R)-1,2-dimethyl-5-(p-tolyl)pyrrolidine-3,4-diyl diacetate (**12f**).* The colorless oil (75%) was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 4:1). $[\alpha]_D$ = -26.20 (c = 2.0, MeOH), IR (neat) ν 2940, 1743, 1600 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.13 (d, J = 6.5 Hz, 3H, CH_3), 2.11 (s, 3H, CH_3CO), 2.13 (s, 3H, CH_3CO), 2.22 (s, 3H, NCH_3), 2.35 (s, 3H, CH_3), 3.65–3.69 (m, 1H, 5- H), 4.14 (d, J = 6.5 Hz, 1H, 2-H), 4.48 (dd, J = 4.2, 3.98 Hz, 1H, 4-H), 5.20 (dd, J = 6.5 and 4.2 Hz, 1H 3-H), 7.16 (d, J = 8.8 Hz, 2H, ph-H), 7.18 (d, J = 8.8 Hz, 2H, Ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.5, 21.1, 21.3, 22.8, 39.3, 58.4, 65.4, 75.7, 76.8, 126.1, 126.4, 128.8, 129.2, 133.9, 135.5, 170.7, 170.9. MS (EI): m/z (%) = 305 (30) [M^+], 187 (40), 174 (1 0 0); Analytical Calculation for $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59. Found: C, 66.90; H 7.66; N, 4.61.

*4.1.6.7. (2R,3R,4R,5R)-2-(2-fluoro-4,5-dimethoxy-3-methylphenyl)-1,5-dimethylpyrrolidine-3,4-diyl diacetate (**12 g**).* The colorless oil (73%) was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 2:1). $[\alpha]_D$ = –32.38 (c = 2.0, MeOH), IR (neat) ν 2950, 1742, 1610 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.12 (d, J = 6.5 Hz, 3H CH_3), 2.11 (s, 3H, Ac), 2.13 (s, 3H, Ac), 2.24 (s, 3H, NCH_3), 2.34 (s, 3H, CH_3), 3.67–3.71 (m, 1H, 5- H), 3.83 (s, OCH_3), 3.85 (s, OCH_3), 4.29 (d, J = 6.5 Hz, 1H, 2-H), 4.47 (dd, J = 4.4, 4.0 Hz, 1H, 4-H), 5.24 (dd, J = 6.5 and 4.4 Hz, 1H 3-H), 7.09 (s, 1H, Ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.7, 21.1, 29.8, 49.8, 56.3, 60.8, 75.6, 85.4, 117.6, 118.5, 129.8, 144.8, 151.7, 160.0, 170.7, 171.9. MS (EI): m/z (%) = 383 (40) [M^+], 265 (50), 252 (1 0 0); Analytical Calculation for $C_{19}H_{26}FNO_6$: C, 59.52; H, 6.84; F, 4.96; N, 3.65. Found: C, 59.75; H, 6.98; F, 5.03; N, 3.75.

*4.1.7. General procedure for the synthesis of (-)-codonopsinine (**1**), (-)-codonopsine (**2**) and codonopsinine analogues (**13c–g**)*

A stirred solution of the compound **12a-g** (2.4 mmol) in dry methanol (15–20 ml) was cooled to 0 °C and then treated with a freshly prepared solution of 0.1 M sodium methoxide (3–5 ml). Stirring then continued at room temperature for 2–3 h and the solution was stirred with Amberlite IR-120 (H^+) until the solution became neutral. The resin was filtered off, and the solution was concentrated under reduced pressure to give **1, 2** and **13-c-g**.

*4.1.7.1. (2R,3R,4R,5R)-2-(4-methoxyphenyl)-1,5-dimethylpyrrolidine-3,4-diol [(-)-codonopsinine] (**1**)*[21,25,50]. The white needles (90%) mp 168–170 °C[22,35] was purified using silica gel column chromatography (ethyl acetate–methanol, 2:1). $[\alpha]_D$ = − 10.5 (c = 1.1, MeOH); IR (neat) ν 3365, 2950, 1610 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.34 (d, J = 6.8 Hz, 3H, CCH_3), 2.24 (s, 3H, NCH_3), 3.64 (s, 3H, OCH_3), 3.67–3.72 (m, 1H, 5- H), 4.06 (d, J = 6.8 Hz, 1H, 2-H), 4.39 (dd, J = 4.4 and 3.8 Hz, 1H, 4-H), 4.65 (dd, J = 6.8 and 4.4 Hz, 1H, 3-H), 5.26 (br s, OH) 6.95 (d, J = 8.7 Hz, 2H, ph-H), 7.32 (d, J = 8.7 Hz, 2H, ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 35.2, 55.5, 65.7, 74.6, 85.6, 88.0, 114.7, 130.2, 135.4, 160.1. MS (EI): m/z (%) = 237 (20) [M^+], 205 (50), 190 (1 0 0); Analytical Calculation for $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.85; H, 8.08; N, 5.95.

*4.1.7.2. (2R,3R,4R,5R)-2-(3,4-dimethoxyphenyl)-1,5-dimethylpyrrolidine-3,4-diol [(-)-codonopsine] (**2**)*[24]. The white needles (92%) mp 149–150 °C was purified using silica gel column

chromatography (ethyl acetate–methanol, 2:1). $[\alpha]_D$ = − 16 (c = 1.2, MeOH), IR (neat) ν 3360, 2955, 1610 cm^{-1} ^1H NMR (400 MHz, $CDCl_3$) δ 1.29 (d, J = 6.7 Hz, 3H, CH_3), 2.20 (s, 3H, NCH_3), 3.64–3.37 (m, 1H, 5- H), 3.77 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 4.19 (d, J = 6.7 Hz, 1H, 2-H), 4.43 (dd, J = 4.7, 4.4 Hz, 1H, 4-H), 5.14 (dd, J = 6.7 and 4.4 Hz, 1H 3-H), 5.30 (br s, OH), 6.80–6.83 (m, 2H, ph-H), 7.01 (s, 1H, Ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.0, 37.4, 56.6, 59.2, 67.2, 77.1, 78.9, 111.7, 125.1, 130.4, 152.1, 155.2. MS (EI): m/z (%) = 267 (40) [M^+], 235 (60), 220 (1 0 0); Analytical Calculation for $C_{14}H_{21}NO_4$: C, 62.90; H, 7.92; N, 5.24. Found: C, 62.96; H, 7.98; N, 5.28.

*4.1.7.3. (2R,3R,4R,5R)-2-(2-fluoro-4-methoxyphenyl)-1,5-dimethylpyrrolidine-3,4-diol (**13c**).* The colorless oil (87%) was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 2:1). $[\alpha]_D$ = –22.20 (c = 1.0, MeOH), IR (neat) ν 3365, 2950, 1610 cm^{-1} ^1H NMR (400 MHz, $CDCl_3$) δ 1.20 (d, J = 6.5 Hz, 3H, CH_3), 2.23 (s, 3H, NCH_3), 3.54–3.57 (m, 1H, 5- H), 3.79 (s, OCH_3), 4.20 (d, J = 6.5 Hz, 1H, 2-H), 4.43 (dd, J = 4.4, 4.0 Hz, 1H, 4-H), 5.11 (dd, J = 6.5 and 4.0 Hz, 1H 3-H), 5.25 (br s, OH), 7.04 (m, 2H, ph-H), 7.20 (s, 1H, Ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.7, 39.1, 59.0, 61.7, 75.8, 80.8, 103.9, 109.8, 134.2, 136.6, 158.6, 161.6. MS (EI): m/z (%) = 255 (50) [M^+], 221 (1 0 0), 208 (80); Analytical Calculation for $C_{13}H_{18}FNO_3$: C, 61.16; H, 7.11; F, 7.44; N, 5.49. Found: C, 61.17; H, 7.13; F, 7.50; N, 5.63.

*4.1.7.4. (2R,3R,4R,5R)-2-(4-(methoxymethyl)phenyl)-1,5-dimethylpyrrolidine-3,4-diol (**13d**).* The colorless oil (93%) was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 3:1). $[\alpha]_D$ = -16.50 (c = 1.0, MeOH), IR (neat) ν 3360, 2955, 1610 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.24 (d, J = 6.5 Hz, 3H, CH_3), 2.28 (s, 3H, NCH_3), 3.45 (s, 3H, OCH_3), 3.69–3.73 (m, 1H, 5- H), 4.12 (d, J = 6.5 Hz, 1H, 2-H), 4.44 (dd, J = 4.4, 4.0 Hz, 1H, 4-H), 4.79 (s, 2H, OCH_2–), 5.13 (dd, J = 6.5 and 4.4 Hz, 1H 3-H), 5.32 (br s, OH), 7.10 (d, J = 8.6 Hz, 2H, ph-H), 7.15 (d, J = 8.6 Hz, 2H, Ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.4, 39.7, 58.9, 59.8, 65.7, 74.9, 76.8, 81.7, 126.3, 126.8, 129.1, 129.7, 134.5, 135.7MS (EI): m/z (%) = 351 (40) [M^+], 219 (1 0 0), 204 (70); Analytical Calculation for $C_{14}H_{21}NO_3$: C, 66.91; H, 8.42; N, 5.57. Found: C, 66.99; H, 8.51; N, 5.67.

*4.1.7.5. (2R,3R,4R,5R)-1,2-dimethyl-5-phenylpyrrolidine-3,4-diol (**13e**).* The colorless oil (88%) was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 1:1). $[\alpha]_D$ = -26.20 (c = 1.0, MeOH), IR (neat) ν 3360, 2945, 1600 cm^{-1} ^1H NMR (400 MHz, $CDCl_3$) δ 1.21 (d, J = 6.4 Hz, 3H, CH_3), 2.28 (s, 3H, NCH_3), 3.50–3.54 (m, 1H, 5- H), 4.17 (d, J = 6.4 Hz, 1H, 2-H), 4.48 (dd, J = 4.4, 4.0 Hz, 1H, 4-H), 5.14 (dd, J = 6.4 and 4.4 Hz, 1H 3-H), 5.32 (br s, OH), 7.25–7.36 (m, 5H, ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.4, 39.4, 59.4, 68.5, 76.5, 80.1, 124.1, 125.4, 126.1, 128.5, 129.2, 138.6. MS (EI): m/z (%) = 207 (40) [M^+], 173 (50), 160 (1 0 0); Analytical Calculation for $C_{12}H_{17}NO_2$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.70; H 8.37; N, 6.81.

*4.1.7.6. (2R,3R,4R,5R)-1,2-dimethyl-5-(p-tolyl)pyrrolidine-3,4-diol (**13f**).* The colorless oil (90%) which was isolated by column chromatography on silica gel (petroleum ether/ethyl acetate, 2:1). $[\alpha]_D$ = -36.10 (c = 1.0, MeOH), IR (neat) ν 3365, 2950, 1610 cm^{-1} ^1H NMR (400 MHz, $CDCl_3$) δ 1.14 (d, J = 6.6 Hz, 3H, CH_3), 2.23 (s, 3H, NCH_3), 2.36 (s, 3H, CH_3), 3.64–3.67 (m, 1H, 5- H), 4.16 (d, J = 6.6 Hz, 1H, 2-H), 4.48 (dd, J = 4.2, 3.98 Hz, 1H, 4-H), 5.201(dd, J = 6.5 and 4.2 Hz, 1H 3-H), 5.32 (br s, OH), 7.18 (d, J = 8.7 Hz, 2H, ph-H), 7.20 (d, J = 8.7 Hz, 2H, Ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.7, 21.5, 39.8, 59.7, 66.8, 81.2, 126.3, 126.5, 128.9, 129.1, 133.7, 136.3. MS (EI): m/z (%) = 221 (40) [M^+], 187 (60), 174 (1 0 0); Analytical Calculation for $C_{13}H_{19}NO_2$: C, 70.56; H, 8.65; N, 6.33. Found: C, 70.80; H, 8.66; N, 6.42.

*4.1.7.7. (2R,3R,4R,5R)-2-(2-fluoro-4,5-dimethoxy-3-methylphenyl)-1,5-dimethylpyrrolidine-3,4-diol (**13 g**).* The colorless oil (93%) was purified using silica gel column chromatography (petroleum ether-ethyl acetate, 3:1). $[\alpha]_D$ = -12.60 (c = 1.0, MeOH), IR (neat) ν 3360, 2950, 1600 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.14 (d, J = 6.5 Hz, 3H, CH_3), 2.26 (s, 3H, NCH_3), 2.35 (s, 3H, CH_3), 3.65–3.69 (m, 1H, 5- H), 3.81 (s, OCH_3), 3.83 (s, OCH_3), 4.29 (d, J = 6.5 Hz, 1H, 2-H), 4.45 (dd, J = 4.4, 4.0 Hz, 1H, 4-H), 5.23 (dd, J = 6.5 and 4.4 Hz, 1H 3-H), 5.34 (br s, OH), 7.06 (s, 1H, Ph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.3, 18.5, 39.0, 56.4, 59.5, 60.8, 63.4, 75.0, 82.4, 100.8, 111.6, 144.8, 150.7, 160.8. MS (EI): m/z (%) = 299 (60) [M^+], 265 (70), 252 (1 0 0); Analytical Calculation for $C_{15}H_{22}FNO_4$: C, 60.19; H, 7.41; F, 6.35; N, 4.68. Found: C, 60.30; H, 7.48; F, 6.42; N, 4.73.

4.2. Preparation of solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) were prepared using Compritol 888 (Saint-Priest Cedex, France) as a solid lipid and Pluronic f68 (Merck KGaA, Darmstadt, Germany) as a surfactant using hot emulsification-ultrasonication technique.[51] For that, the specified weights of tested compounds **1**, **2** and **13c-g** were dispersed in Compritol (5%) by heating at 75 °C (above lipid melting point). The aqueous phase was prepared by dissolving Pluronic f68 in double distilled water to make a 3% solution. The melted lipid phase was dispersed in the aqueous surfactant solution at the same temperature and gently mixed to prepare a pre-emulsion. The obtained pre-emulsion was, then, homogenized at 10,000 rpm (at 75 °C) for 15 min (Kinematica Polytron PT-MR 3100 D Homogenizer, Switzerland) followed by sonication (Rivotek,probe sonicator, Mumbai, India) for 10 min to reduce the globule size of the prepared hot oil in water (o/w) emulsion. The mixture was cooled by sudden immersion in an ice bath to stimulate lipid crystallization and the formation of SLNs. The applied percentage of lipid and surfactant were selected based on preliminary data (not shown) and proposed to give the optimum SLNs characteristics.

4.2.1. Characterization of the prepared SLNs

The prepared solid lipid nanoparticles were evaluated by measuring:

4.2.1.1. Particle size (P.S.) and size distribution. Photon Correlation Spectroscopy (PCS) using a Zetasizer 4 (Nano ZS, Zen 3600, Malvern Instruments Ltd., UK) was applied to measure the average diameter (nm) and Polydispersity Index (PDI) of the prepared SLN formulae at 25 °C without dilution. Each value is the average of three measurements.

4.2.1.2. Zeta potential measurement (Z.P.). For determination of electrical surface properties of the prepared SLN formulations, Z.P. was quantified using a Zetasizer 4 (Nano ZS, Zen 3600, Malvern Instruments Ltd., UK), at 25 °C after suitable dilution with distilled water to an adequate intensity at pH 6.5–7.5. The value was measured in triplicate.

4.2.1.3. Determination of entrapment efficiency (E.E.). Undiluted sample (5 ml) of the prepared SLNs was centrifuged at 5000 rpm for an hour for complete precipitation of the solid lipid layer. The aqueous layer was, then, collected and the absorbance of the tested compound was spectrophotometrically measured at the determined λ_{max} (274–283 nm on Shimadzu UV/Vis double beam spectrophotometer) after filtration on a 0.45 µm membrane filter. The free compound concentration in the dispersion medium was calculated using an equation obtained from a previously constructed standard calibration curve. The entrapment efficiency (E.E.) percentage of the prepared SLNs was calculated as follows:

$$E.E.\ (\%) = (D_I - -D_F/D_F)100$$

where D_I = Initial compound amount added during preparation, D_F = Amount of free compound in the aqueous phase

4.2.1.4. Determination of release rate from the prepared SLN formulations. Dialysis bag diffusion technique[38] was applied to investigate the release rate profiles of the tested compounds from the prepared SLN formulations in comparison to non-formulated compounds using the USP XXIV dissolution apparatus II (UDT-804 paddle, Logan, Utah, USA). Formulae and compound suspensions (3 ml) were packed into dialysis bags (12 kDa MWCO) and immersed in 500 ml of a phosphate buffer dissolution medium (with a pH of 6.8) without enzymes and kept at 37 ± 0.5 °C with constant stirring speed (50 rpm). At pre-specified time intervals, 5 ml samples were withdrawn with replacement for 12 h and the cumulative percentage release rate of the tested compound was calculated as previously described in E.E. determination. The mean of six determinations was considered.

4.2.1.5. In-vitro permeation studies. *In-vitro* permeation of the prepared SLN formulations in comparison to non-formulated compound aqueous dispersion was studied on a dialysis membrane using glass modified Franz diffusion cell (PermeGear, Inc., Hellertown, PA, USA). The receptor cell was filled with fresh phosphate buffer (pH = 6.8) kept at 37 ± 0.5 °C with constant stirring using a magnetic stirrer. One ml of each SLN formula and the compound suspension were separately packed into the donor chamber placed in contact to the dialysis membrane and the opening of donor cell was sealed. At predetermined time intervals, samples were withdrawn from the receptor chamber with replacement for six hours. The sample absorbance was spectrophotometrically measured and the concentration was calculated using the equation generated from a standard calibration curve as described.[52]

4.3. Determination of antimicrobial activity of the prepared compounds

Antimicrobial susceptibility testing (AST) was primarily done for the tested compounds by modified Kirby-Bauer method using sterile discs loaded with 3 µl containing 380 µg of the tested compound. Seven organisms were examined in this study and included *S. aureus* (ATCC 29213, a Gram-positive organism), *E. coli (*ATCC 25922), *K. pneumoniae (*ATCC 700603), *P. mirabilis (*ATCC 14153), *P. aeruginosa (*ATCC 27853), *A. baumannii* (a clinical isolate; All are Gram-negative organisms) and *Candida albicans* (ATCC 10231, yeast).

4.3.1. Modified Kirby-Bauer method (Disc diffusion)

The assay was performed on Muller-Hinton agar (MHA) as previously described.[53] The diameter of the inhibition zones (including the diameter of the discs) were measured with a ruler under the surface of the plate and the results were recorded in mm.[54]

4.3.2. Broth microdilution method for determination of minimum inhibitory concentration

Also, AST was determined by broth microdilution method to determine the minimum inhibitory concentration (MIC) values. Stock solutions of the tested compound were prepared by dissolving 10 mg in 1 ml of DMSO to obtain an initial stock concentration of 10 mg/ml. Broth microdilution method was performed in 96 well microtiter plates using different concentrations of the investigated compounds. All columns of the sterile 96-well plate were filled with 100 µl of sterile distilled water (SDW) except column No. 1 which was filled with 159 µl of sterile distilled water to which 41 µl of tested compound stock solution (10 mg/ml) was added to obtain a final concentration of 1024 µg/ml after addition of the broth containing the tested organism. The last two rows of the 96-well plates were used for positive and negative controls, respectively. Two fold dilutions were made in the next columns to reach a final dilution of 0.5 µg/ml. After serial dilutions of the tested compounds, all wells of the plate were filled with 100 µl of double strength nutrient broth (DSNB) containing the tested isolates and mixed well, by pipetting 3–6 times. The plates were incubated at 37 °C for 18 h.[55]

4.3.3. Inoculum preparation of the test organisms (direct colony suspension method)

Direct colony suspension method was performed by suspending a few colonies of the tested isolates in sterile normal saline. The turbidity of the prepared suspension was adjusted using 0.5 McFarland standard and this is equivalent to 1–2 × 10^8 CFU/ml, which is further diluted to obtain a final inoculum concentration of 5 × 10^5 CFU in each well of the microtiter plates. This was done by adding 35 µl from 0.5 McFarland adjusted inoculum to 35 ml of DSNB. The inoculated broth was used directly within 15 min of preparation.[55]

Acknowledgment

This study was funded by the Deanship of Scientific Research, Taif University, KSA (Research Project No. 1-438-5843).

Disclosure statement.

All authors declare that they do not have any conflict of interest.

References

1. (a) Asano N. *Tetrahedron Asymm.* 2000;11:1645–1680;

   (b) Watson AA, Fleet GWJ, Asano N, Molyneux RJ, Nash RJ. *Phytochemistry.* 2001;56:265–295.
2. A.E. Stütz, Iminosugars as glycosidase inhibitors, Wiley-VCH, 1999.
3. Stocker BL, Dangerfield EM, Win-Mason AL, Haslett GW, Timmer MS. Recent Developments in the Synthesis of Pyrrolidine-Containing Iminosugars. *Eur J Org Chem.* 2010;2010:1615–1637.
4. Compain P, Martin OR. Design, synthesis and biological evaluation of iminosugar-based glycosyltransferase inhibitors. *Curr Top Med Chem.* 2003;3:541–560.
5. Johari SA, Mohtar M, Mohammad S, et al. In vitro inhibitory and cytotoxic activity of MFM 501, a novel codonopsinine derivative, against methicillin-resistant Staphylococcus aureus clinical isolates. *Biomed Res Int.* 2015;2015.
6. Watson AA, Fleet GW, Asano N, Molyneux RJ, Nash RJ. Polyhydroxylated alkaloids—natural occurrence and therapeutic applications. *Phytochemistry.* 2001;56:265–295.
7. Asano N, Nash RJ, Molyneux RJ, Fleet GW. Sugar-mimic glycosidase inhibitors: natural occurrence, biological activity and prospects for therapeutic application. *Tetrahedron Asymmetry.* 2000;11:1645–1680.
8. Jung M, Park M, Lee HC, Kang Y-H, Kang ES, Kim SK. Antidiabetic agents from medicinal plants. *Curr Med Chem.* 2006;13:1203–1218.
9. Zou W. C-glycosides and aza-C-glycosides as potential glycosidase and glycosyltransferase inhibitors. *Curr Top Med Chem.* 2005;5:1363–1391.
10. Tsou E-L, Chen S-Y, Yang M-H, Wang S-C, Cheng T-RR, Cheng W-C. Synthesis and biological evaluation of a 2-aryl polyhydroxylated pyrrolidine alkaloid-based library. *Bioorg Med Chem.* 2008;16:10198–10204.
11. Ritthiwigrom T, Nash RJ, Pyne SG. Synthesis of polyhydroxylated pyrrolizidine and indolizidine compounds and their glycosidase inhibitory activities. *Tetrahedron.* 2010;66:9340–9347.
12. Ritthiwigrom T, Willis AC, Pyne SG. Total synthesis of uniflorine a, casuarine, australine, 3-epi-australine, and 3, 7-Di-epi-australine from a common precursor. *J Organic Chem.* 2009;75:815–824.
13. El Nemr A. Synthetic methods for the stereoisomers of swainsonine and its analogues. *Tetrahedron.* 2000;56:8579–8629.
14. Bi J, Aggarwal VK. Application of furyl-stabilized sulfur ylides to a concise synthesis of 8a-epi-swainsonine. *Chem Commun.* 2007:120–122.
15. Calveras J, Casas J, Parella T, Joglar J, Clapés P. Chemoenzymatic synthesis and inhibitory activities of hyacinthacines A1 and A2 stereoisomers. *Adv Synth Catal.* 2007;349:1661–1666.
16. Michael JP. Indolizidine and quinolizidine alkaloids. *Nat Prod Rep.* 2004;21:625–649.
17. Steglich W. Slime moulds (Myxomycetes) as a source of new biologically active metabolites. *Pure Appl Chem.* 1989;61:281–288.
18. Matkhalikova C, Malikov V, Yunusov SY. The structure of codonopsine. *Chem Nat Compd.* 1969;5:528–529.
19. Matkhalikova S, Malikov V, Yunusov SY. A study of the alkaloids of Codonopsis clematidea. *Chem Nat Compd.* 1969;5:24–25.
20. Khanov M, Sultanov M, Egrova T. *Chem. Abstr.* 1972:135091r.
21. Iida H, Yamazaki N, Kibayashi C, Nagase H. Stereochemical revision and absolute configuration of codonopsinine. *Tetrahedron Lett.* 1986;27:5393–5396.
22. Reddy JS, Rao BV. A short, efficient, and stereoselective total synthesis of a pyrrolidine alkaloid:(−)-codonopsinine. *J Organic Chem.* 2007;72:2224–2227.
23. Iida H, Yamazaki N, Kibayashi C. Total synthesis of (+)-codonopsinine and its stereoisomers: stereochemical assignment of natural (-)-codonopsinine. *J Organic Chem.* 1987;52:1956–1962.
24. Wang CLJ, Calabrese JC. Decarboxylative cyclization of allylic cyclic carbamates:

applications to the total synthesis of (-)-codonopsine. *J Organic Chem.* 1991;56:4341–4343.
25. Yoda H, Nakajima T, Takabe K. Total synthesis of natural (−)-codonopsinine employing stereoselective reduction of quaternary α-hydroxypyrrolidine. *Tetrahedron Lett.* 1996;37:5531–5534.
26. Goti A, Cicchi S, Mannucci V, et al. Iterative organometallic addition to chiral hydroxylated cyclic nitrones: highly stereoselective syntheses of α, α '-and α, α-substituted hydroxypyrrolidines. *Org Lett.* 2003;5:4235–4238.
27. Kotland A, Accadbled F, Robeyns K, Behr J-B. Synthesis and fucosidase inhibitory study of unnatural pyrrolidine alkaloid 4-epi-(+)-codonopsinine. *J Organic Chem.* 2011;76:4094–4098.
28. Oliveira DF, Severino EA, Correia CRD. Heck reaction of endocyclic enecarbamates with diazonium salts. Formal enantioselective syntheses of alkaloids (−)-codonopsine and (−)-codonopsinine, and the synthesis of a new C-aryl azasugar. *Tetrahedron Lett.* 1999;40:2083–2086.
29. Choi YJ, Kim YC, Park SJ, et al. Total synthesis of (−)-codonopsinine via regioselective and diastereoselective amination using chlorosulfonyl isocyanate. *Tetrahedron.* 2017;73:4458–4463.
30. Yamaguchi M, Itagaki D, Ueda H, Tokuyama H. Total syntheses of codonopsinine and 4-epi-codonopsinine via gold-mediated tandem-catalyzed pyrrole synthesis. *J Antibiotics.* 2016;69:253.
31. Davies SG, Lee JA, Roberts PM, Thomson JE, West CJ. Ring-closing iodoamination of homoallylic amines for the synthesis of polysubstituted pyrrolidines: application to the asymmetric synthesis of (−)-codonopsinine. *Tetrahedron.* 2012;68:4302–4319.
32. Schuch CM, Pilli RA. Creation of quaternary stereocenters by the addition of allyltributyltin to chiral cyclic N-acyliminium ions. *Tetrahedron Asymmetry.* 2000;11:753–764.
33. Santos LS, Pilli RA. Studies towards the construction of alkylidene quinolizidines: the total synthesis of homopumiliotoxin 223G. *J Braz Chem Soc.* 2003;14:982–993.
34. Roussel F, Takhi M, Schmidt RR. Solid-phase synthesis of a branched hexasaccharide using a highly efficient synthetic strategy. *J Organic Chem.* 2001;66:8540–8548.
35. Abdel-Rahman AAH, Jonke S, El Ashry ESH, Schmidt RR. Stereoselective Synthesis of β-D-Mannopyranosides with Reactive Mannopyranosyl Donors Possessing a Neighboring Electron-Withdrawing Group. *Angew Chem Int Ed.* 2002;41:2972–2974.
36. El-Nezhawy AO, El-Diwani HI, Schmidt RR. O-(2-Oxopyrrolidin-5-yl) trichloroacetimidates as Amidoalkylating Agents− Synthesis of (+)-Lentiginosine. *Eur J Org Chem.* 2002;2002:4137–4142.
37. Dubald M, Bourgeois S, Andrieu V, Fessi H. Ophthalmic drug delivery systems for antibiotherapy—a review. *Pharmaceutics.* 2018;10:10.
38. Khames A. Preparation and Characterization of Sildenafil Loaded Solid Lipid Nanoparticles: Drug Delivery System Suitable for Nebulization Ahmed khames. *Der Pharmacia Lettre.* 2017;9:98–112.
39. El Nezhawy AO, Gaballah ST, Radwan MA. Studying the reactivity of (phthalazin-1 (2H)-on-2-yl) methyl trichloroacetimidate towards different C-and O-nucleophiles. *Tetrahedron Lett.* 2009;50:6646–6650.
40. Galal SA, Naem SIE, Nezhawy AOE, Ali MA, Diwani HIE. Novel Benzimidazo [2, 1-c] [1, 4] thiazinone Derivatives with Potent Activity Against HSV-1. *Arch Pharm.* 2011;344:255–263.
41. El-Nezhawy AO, Adly FG, Eweas AF, et al. Synthesis of Some Novel D-Glucuronic Acid Acetylated Derivatives as Potential Anti-Tumor Agents. *Arch Pharm.* 2011;344:648–657.
42. El-Nezhawy AO, Biuomy AR, Hassan FS, Ismaiel AK, Omar HA. Design, synthesis and pharmacological evaluation of omeprazole-like agents with anti-inflammatory activity. *Bioorg Med Chem.* 2013;21:1661–1670.
43. El-Nezhawy AO, Eweas AF, Maghrabi IA, Edalo AS, Abdelwahab SF. Design, Synthesis, and Molecular Docking of Novel Pyrrolooxazepinediol Derivatives with Anti-Influenza Neuraminidase Activity. *Arch Pharm.* 2015;348:786–795.
44. Gaballah S, El-Nezhawy A, Amer H, Ali M, Mahmoud AE, Hofinger-Horvath A. Synthesis and Antiproliferative Activities of Benzimidazole-Based Sulfide and Sulfoxide Derivatives. *Sci Pharm.* 2015;84:1–18.
45. Giovannini A, Savoia D, Umani-Ronchi A. Organometallic ring-opening reactions of N-acyl and N-alkoxycarbonyl lactams, Synthesis of cyclic imines. *J Organic Chem.* 1989;54:228–234.
46. Tanvir S, Qiao L. Surface tension of nanofluid-type fuels containing suspended nanomaterials. *Nanoscale Res Lett.* 2012;7:226.
47. Li X, Lin X, Zheng L, et al. Effect of poly (ethylene glycol) stearate on the phase behavior of monocaprate/Tween80/water system and characterization of poly (ethylene glycol) stearate-modified solid lipid nanoparticles. *Colloids Surf, A.* 2008;317:352–359.
48. Sawant KK, Dodiya SS. Recent advances and patents on solid lipid nanoparticles. *Recent Pat Drug Delivery Formulation.* 2008;2:120–135.
49. Seyfoddin A, Shaw J, Al-Kassas R. Solid lipid nanoparticles for ocular drug delivery. *Drug Delivery.* 2010;17:467–489.
50. Iida H, Yamazaki N, Kibayashi C. Synthesis of (+)-codonopsinine: determination of absolute configuration of natural (−)-codonopsinine. *Tetrahedron Lett.* 1985;26:3255–3258.
51. Battaglia LS, Gallarate M, Panciani P, et al. Techniques for the Preparation of Solid Lipid Nano and MicroparticlesApplication of Nanotechnology. *Drug Deliv.* 2014.
52. Simon A, Amaro MI, Healy AM, Cabral LM, de Sousa VP. Comparative evaluation of rivastigmine permeation from a transdermal system in the Franz cell using synthetic membranes and pig ear skin with in vivo-in vitro correlation. *Int J Pharm.* 2016;512:234–241.
53. Bauer A, Kirby W, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol.* 1966;45:493–496.
54. Vandepitte J. *Basic laboratory procedures in clinical bacteriology.* World Health Organization; 2003.
55. Cockerill FR. Performance standards for antimicrobial susceptibility testing: twenty-first informational supplement. *Clinical and Laboratory Standards Institute (CLSI).* 2011.