Bioorganic & Medicinal Chemistry Letters 26 (2016) 1512–1515

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

# One-pot synthesis and antimicrobial evaluation of novel 3-cyanopyridine derivatives of (−)-β-pinene

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ARTICLE INFO

*Article history:*
Received 10 December 2015
Revised 6 February 2016
Accepted 10 February 2016
Available online 10 February 2016

*Keywords:*
β-Pinene
Nopinone
Synthesis
3-Cyanopyridine
Antimicrobial

ABSTRACT

A series of novel 3-cyanopyridine derivatives of (−)-β-pinene were designed and synthesized by one-pot four-component domino reactions. The targeted compounds were evaluated for their antimicrobial activity against four bacteria (*Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*) and a fungus (*Candida albicans*). The results showed that most of the minimal inhibitory concentrations (MICs) of these 3-cyanopyridine derivatives against the tested strains was in the range of 15.6–125 mg/L. Among these 3-cyanopyridine derivatives, the MICs of compound **5h** against *S. epidermidis* and *C. albicans* were 15.6 mg/L, which revealed that compound **5h** featured double fluoro substituents at *meta*- and *para*-position was the most active compound. In addition, the preliminary structure–activity relationship analysis indicated that the change of substituents on the pyridine ring and benzene ring of 3-cyanopyridine derivatives was an important factor for inducing antimicrobial activity. This research would promote the development of heterocyclic derivatives of β-pinene with antimicrobial activity.



Turpentine oil, an important natural resource, had received much attention for its various biological activity. Using turpentine oil to develop chemical derivatives with biological activity was of great interest to the researchers in the field.[1] β-Pinene was a main component of turpentine oil, former researches showed that β-pinene obtained a variety of biological activity such as antibacterial,[2] antiviral,[3] anticancer,[4] and antidepressant-like activity,[5] etc. Meanwhile, the derivatives of β-pinene also exhibited many biological activity, including antibacterial activity,[6–9] human ORL1 receptors antagonist,[10] prostaglandin D2 receptor antagonist.[11] Therefore, using β-pinene to exploit derivatives with biological activity had wide prospects.

Recent researches showed that the derivatives of pinene with heterocycle groups exhibited good antimicrobial activity. Wei et al. found that some pinanyl-2-aminopyrimidines (Fig. 1a) and pinanyl isoxazolines (Fig. 1b) exhibited good antibacterial activity against *Escherichia coli* and *Bacillus subtilis*.[12] Dhar et al. synthesized a pinanyl-β-lactam derivative (Fig. 1c) which obtained excellent antimicrobial activity against *Candida albicans* and *Staphylococcus aureus*.[13] However, most of the heterocyclic derivatives of pinene were synthesized from α-pinene, rather than from β-pinene. Hence, the synthesis and antimicrobial activity evaluation of heterocyclic derivatives of β-pinene were interesting and needed.

Among the heterocyclic compounds, 3-cyanopyridine was known to have multiple biological activity, such as antibacterial,[14] antiviral[15] and anti-inflammatory activity.[16] Considering that both the β-pinene and 3-cyanopyridine exhibited good biological activity, it was of great significance to synthesize the 3-cyanopyridine derivatives of β-pinene. To the best of our knowledge, the study on the synthesis and antimicrobial activity of 3-cyanopyridine derivatives of β-pinene had not been reported before.

In this study, a series of novel 3-cyanopyridine derivatives of (−)-β-pinene were synthesized by one-pot four-component domino reactions. The structures of these synthesized compounds were characterized by FT-IR, $^{1}$H NMR, $^{13}$C NMR and ESI-MS. The antimicrobial activity of these 3-cyanopyridine derivatives were evaluated against four bacteria *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and a fungus *Candida albicans*.

(−)-β-Pinene was an olefin which could not be directly applied to the mentioned four-component domino reaction. Hence, (−)-β-

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http://dx.doi.org/10.1016/j.bmcl.2016.02.024
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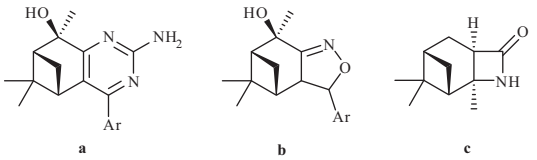


**Figure 1.** Heterocyclic derivatives of pinene.

pinene was in advance transferred into (+)-nopinone, which was tolerated to the four-component domino reaction. Although several synthetic methods were available for the transformation of (−)-β-pinene into (+)-nopinone,[17–19] oxidation using $KMnO_4$ as oxidant under solvent condition was one of the commonly used method.[18] Compared with other methods, the oxidation with $KMnO_4$ had many merits, including mild reaction conditions, considerable yield and high selectivity.

In this work, the oxidation of (−)-β-pinene was performed with $KMnO_4$ in acetone. The reaction sequence for the synthesis of (+)-nopinone (**2**) was presented in Figure 2. (−)-β-Pinene (**1**) was firstly oxidized into nopinic acid (**1-a**) by $KMnO_4$, then nopinic acid (**1-a**) was further oxidized by $KMnO_4$ to form the (+)-nopinone (**2**). The IR spectrum of (+)-nopinone showed that the $\nu$(C=C—H), $\nu$(C=C) and $\nu$(C=$CH_2$) bands of (−)-β-pinene disappeared and the $\nu$(C=O) band appeared; the $^1$H NMR spectrum showed that the signals at 4.59–4.66 ppm attributed to the olefinic protons disappeared, while a signal at 2.56 ppm assigned to the protons in the carbons next to the carbonyl group appeared. These characterization results, which agreed with earlier reported data,[19] indicated that (+)-nopinone (**2**) was synthesized by the oxidation with $KMnO_4$ in acetone.

One-pot four-component domino reaction between ketone, aldehyde, ammonium acetate and ethyl cyanoacetate (or malononitrile) was an efficient method for the synthesis of 3-cyanopyridine compounds. The one-pot four component domino reactions for the synthesis of 3-cyanopyridines could be carried out under various reaction conditions.[20–23] The catalytic reaction condition using rare earth as catalysts was commonly applied for the synthesis of 3-cyanopyridines, due to its mild reaction condition and considerable yield of product.[23] In this work, twenty-five 3-cyanopyridine derivatives (**5a**–**y**) were synthesized by the one-pot four-component domino reactions between (+)-nopinone (**2**), benzaldehydes (**3a**–**n**), ethyl cyanoacetate (or malononitrile) (**4**) and ammonium acetate under $Yb(OTf)_3$ catalysis (Fig. 3). When the ethyl cyanoacetate was chose as starting material, the products were 2-hydroxy-3-cyanopyridines (**5a**–**5m**), and when the malononitrile was used, the products were 2-amino-3-cyanopyridines (**5n**–**5y**).

According to the literature,[23] the possible mechanism of this four-component domino reaction was proposed. Taking compound **5a** as an example, the synthesis mechanism of 3-cyanopyridine derivatives was depicted in Figure 4. Firstly, (+)-nopinone (**2**) might react with ammonium acetate to form an enamine (**2-b**), meanwhile, the benzaldehyde (**3a**) and ethyl cyanoacetate (**4**) were condensed to form an ethyl (*E*)-2-cyano-3-phenylacrylate (**3a′**). Then, the enamine (**2-b**) reacted with the ethyl (*E*)-2-cyano-3-phenylacrylate (**3a′**) to give an intermediate (**4b′**). After cycloaddition, isomerization, and aromatization, the compound **5a** was obtained.

The structures of these 3-cyanopyridine derivatives were characterized by IR, $^1$H NMR, $^{13}$C NMR and ESI-MS. Taking compound **5j** as an example, in IR spectrum the absorption band at 3352 $cm^{-1}$ was assigned to —OH group, the absorption bands at 2914 $cm^{-1}$ and 2867 $cm^{-1}$ were attributed to —$CH_2$—, and —CH—, the absorption bands at 2220 $cm^{-1}$ and 1650–1460 $cm^{-1}$ were represented the existence of —CN and aromatic ring, respectively. In $^1$H NMR spectrum of compound **5j**, the signal appearing as a singlet at about 13.8 ppm was assigned to the proton of hydroxyl group in the pyridine ring. The signals attributed to the aromatic protons of benzene rings with different substituent groups were observed in the range of 6.8–7.8 ppm. The other signals were assigned to the protons in the pinane skeleton. In $^{13}$C NMR spectrum of compound **5j**, the characteristic C=N peaks related to pyridine ring were observed at 170–150 ppm, the signals of aromatic carbons appeared at 140.00–110.00 ppm, the characteristic C—Cl peaks related to benzene ring were observed at ~134 ppm. The signals of —CN group appeared at ~116 ppm. The signals of aliphatic groups appeared between 50.00 ppm and 20.00 ppm. In ESI-MS spectrum of compound **5j**, the signals at 325.1 $[M+1]^+$ and 347.1 $[M+23]^+$ indicated that the molecular weight of **5j** was 324.1. These results showed that the characterization data were in full agreement with the proposed structures.

The minimal inhibition concentrations (MICs) of the 3-cyanopyridine derivatives of (−)-β-pinene were determined by serial two-fold broth dilution method. The antimicrobial activity of the 3-cyanopyridine derivatives against four bacteria (two Gram-positive and two Gram-negative bacteria) and one fungus were summarized in Table 1. For the Gram-negative bacteria *K. pneumoniae* and *E. aerogenes*, the MICs of all the 3-cyanopyridine derivatives were greater than or equal to 62.5 mg/L, while the MICs of kanamycin were 2 mg/L and 3.9 mg/L, respectively. The results indicated that these 3-cyanopyridine derivatives exhibited weak antibacterial activity against *K. pneumoniae* and *E. aerogenes*. For the Gram-positive bacteria *S. aureus* and *S. epidermidis*, the MICs of most of the 3-cyanopyridine derivatives were in the range between 15.6 and 125 mg/L, while the MICs of kanamycin were 2 mg/L and 3.9 mg/L, respectively. These results revealed that these 3-cyanopyridine derivatives showed moderate antibacterial activity against *S. aureus* and *S. epidermidis*. For the fungi *C. albicans*, the MICs of the 3-cyanopyridine derivatives were within the range of 15.6–125 mg/L and the MICs of rifampicin was 3.9 mg/L, which revealed that these derivatives exhibited moderate antifungal activity against *C. albicans*. Furthermore, among these 3-cyanopyridine derivatives, compound **5h** with the MICs of 15.6 mg/L against *S. epidermidis* and *C. albicans* was the most active compound.

The preliminary structure–activity relationship was analyzed to understand the influence of structures of these 3-cyanopyridine derivatives on their antimicrobial activity. It can be seen from Table 1 that 3-cyanopyridine derivatives exhibited better antimicrobial activity than (+)-nopinone, which suggested that the introduction of 3-cyanopyridine scaffold into the (+)-nopinone molecule improved the antimicrobial activity against the tested

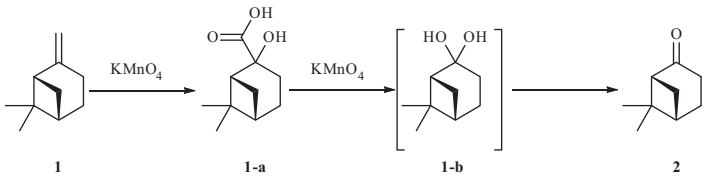


**Figure 2.** Synthesis of (+)-nopinone (**2**) from (−)-β-pinene (**1**) by oxidation with $KMnO_4$; reaction conditions: 3 equiv $KMnO_4$, acetone, rt, 8 h.