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Original article

Synthesis of nordihydroguaiaretic acid derivatives and their bioactivities on *S. pombe* and *K562* cell lines

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ABSTRACT

Nordihydroguaiaretic acid (NDGA) and its synthetic analogues are potentially useful in treating diseases related to cancers, diabetes, viral and bacterial infections, and inflammation. In this paper, we report the optimal synthetic methods and the bioactivity study of terameprocol **2**, NDGA derivative **3**, and its cyclized analogue **4**. The IC_{50} of these three compounds **2**, **3** and **4** on the growth metabolism of *Schizosacchromyces pombe* and *K562* cell lines were determined by microcalorimetry. The preliminary results showed that the compounds **2**, **3** and **4** possessed good inhibition activities on *S. pombe* and *K562* cell lines, and exhibited bidirectional biological effect and Hormesis effect. In particular, terameprocol **2** was found to possess the most potent inhibitory effect on *K562* cell lines.

1. Introduction

During the past century nordihydroguaiaretic acid (NDGA, **1**) (see Fig. 1), one of the naturally occurred lignins, has been an attractive research topic due to its broad range of bioactivities and potential medical applications [1–4] on the cardiovascular, immune and neurological systems, cancer, tissue engineering, etc. NDGA as a drug (generic name: masoprocol, trade name: Actinex®) was approved by Food and Drug Administration (FDA, USA) in September 1992. Actinex® is an antineoplastic drug used to treat skin growths caused by sun exposure, which contains 10% masoprocol or NDGA, is a topical cream product, developed by University of Arizona Cancer Center. However, masoprocol was discontinued in June 1996 due to its low market demand and minor side effects. Terameprocol **2** (see Fig. 1), commonly known as tetra-*O*-methyl-nordihydroguaiaretic acid, EM-1421 and M4N, is a semisynthetic natural product. Remarkably, terameprocol **2**, currently being developed by Erimos Pharmaceuticals, is a promising anticancer agent currently under Phase I/II clinical trials, which is the first NDGA derivative in clinical trials. Although NDGA has been extensively studied during the past decades, NDGA derivatives have been less explored.

Biological microcalorimetry, providing a continuous measurement of heat production, can be employed to directly determine the biological activities of a living system. Heat flux is an expression of overall metabolic flux, and the detection of small changes in heat production to respond to toxic insult will be a sensitive indicator of altered metabolism. Since microcalorimetry is a nondestructive method with high accuracy and automaticity, it is now widely applied in biological research [5] and pharmacological analysis [6].

In this paper we report the optimal synthetic methods and the anticancer activities of DNGA derivative **3** (see Fig. 2), its cyclized analogue **4** (see Fig. 2) and terameprocol **2**. Their bioactivities were determined by microcalorimetry [7–9], cytomorphology, and fluorescence probe method. The results showed that terameprocol **2**, NDGA derivative **3** and its cyclized analogue **4** exhibited good inhibitory activities on the growth metabolism of *Schizosacchromyces pombe* and *K562* cell lines. The values of IC_{50} of

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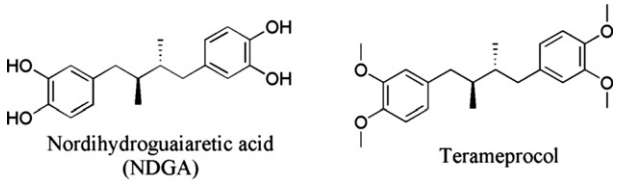

Fig. 1. Structure of NDGA **1** and terameprocol **2**.

terameprocol **2**, NDGA derivative **3** and NDGA derivative **4** on the growth metabolism of *S. pombe* cell lines were found to be 330.0, 290.0 and 350.0 mg L^{-1}, respectively. The values of IC_{50} of terameprocol **2**, NDGA derivative **3** and NDGA derivative **4** on the growth metabolism of *K562* cell lines were found to be 5.87, 8.57 and 11.23 mg L^{-1}, respectively. The inhibitory activity of these three compounds on the growth metabolism of the *S. pombe* has been observed to decrease in the order of NDGA derivative **3** > terameprocol **2** > NDGA derivative **4**. While their inhibitory activity on *K562* cell lines was found to decrease in the order of terameprocol **2** > NDGA derivative **3** > NDGA derivative **4.**

2. Results and discussions

2.1. Chemistry

NGDA derivatives **2**–**4** were synthesized using our developed procedures based on literature reports [10–12], and were outlined as in Fig. 3.

The key step to establish the stereochemistry of terameprocol **2** was the hydrogenation of tetra-substituted furan **4** to give the corresponding *cis*-tetrahydrofuran **9**. The synthesis started with veratrole **5**, which was acylated with propionyl chloride **6** using chloroform as solvent to give a higher yield (94%) of the corresponding ketone **7**. Bromination of compound **7** in refluxing chloroform then gave α-bromo-3,4-dimethoxypropiophenone **8** in 95% yield. Alkylation of **7** in liquid ammonia and sodium amide (produced in situ via adding sodium) at 33 °C with the bromoketone **8** gave racemic diketone **3** in 93%. The synthesis of compound **9** was also reported by Faid et al. [12], who used sodium amide (in situ produced), ferric chloride as catalysts and liquid ammonia as solvent. Cyclodehydration of **9** in 1% HCl–MeOH under reflux to give the corresponding furan compound **4**, followed by hydrogenation created the *cis*-tetrahydrofuran **9**, which was further converted to terameprocol **2** via catalytic hydrogenation as shown in Fig. 3.

After preparing NDGA derivatives **3**, **4** and terameprocol **2**, we next evaluate their bioactivities on *S.pombe* and *K562* cell lines (tumor cell lines) using micocalorimetry. The microcalorimetric study was performed on a 3116-2/3239 TAM Air isothermal calorimeter (Thermometric AB, Sweden).

2.2. Bioactivity

2.2.1. Microcalorimetry

The thermogenic curves (power–time curves) for the growth metabolism of *S. pombe* cells [13] treated by different concentrations of terameprocol **2**, NDGA derivative **3** and NDGA derivative **4** were recorded on the TAM Air microcalorimeter at 32.00 °C, respectively. All microcalorimetric experiments were repeated three times. The results are illustrated in Fig. 4. From Fig. 4, we can find that the thermogenic curves are similar to those of *S.pombe* treated by different concentrations of three compounds.

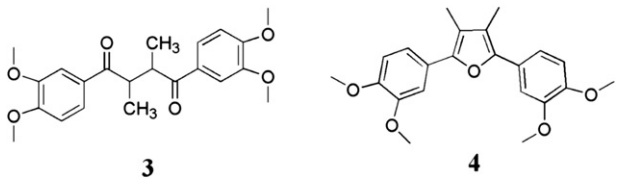

Fig. 2. Structure of NDGA derivative **3** and its cyclized analogue **4**.

2.2.1.1. The growth rate constant (κ) of S. pombe cells. As shown in Fig. 4a, the power–time curve of growth of *S. pombe* cells showed that growth of *S. pombe* cells could be divided into four phases, that is, a lag phase (AB), a log phase (BC), a stationary phase (CD) and a decline phase (DE). During the log phase, the power–time curves obeyed the following equation:

$$n_t = n_0 \exp[k(t - t_0)] \tag{1}$$

where t was the time after the start of exponential growth phase, t_0 was the start time of exponential growth phase, n_t and n_0 were the cell number at time t and t_0, respectively. If the power output of each *S. pombe* cell was one w, then

$$n_t w = n_0 w \exp[k(t - t_0)] \tag{2}$$

If $p_t = n_t w$, $p_0 = n_0 w$, then

$$p_t = p_0 \exp[k(t - t_0)] \tag{3}$$

or

$$\ln p_t = \ln p_0 - kt_0 + kt \tag{4}$$

where P_t was the heat output power of the *S. pombe* cell at time t, and k was the growth rate constant of the *S. pombe* cell at specified conditions, whose size represented growth speed. Using this equation, the growth rate constant k could be calculated and the results are shown in Table 1. It could be seen from Fig. 4 and Table 1 that the compounds **2, 3** and **4** possessed the bidirectional biological effect and Hormesis effect, i.e., these three compounds stimulated the growth of *S. pombe* at low concentration, but inhibited the growth of *S. pombe* at high concentration.

2.2.1.2. Inhibition ratio (I) and half inhibition concentration (IC_{50}). The inhibition ratio of the growth metabolism of *S. pombe* cells treated by drugs was defined as following:

$$I = (k - k_c)/k_0 \times 100\% \tag{5}$$

where k_0 was the control rate constant (without any drug inhibition) of *S. pombe* and k_c was the growth rate constant of *S. pombe* treated by an inhibitor with a concentration of c. The values of (I) are shown in Table 1. When the inhibition ratio was 50%, the drug concentration was called as the half inhibition concentration (IC_{50}). The results showed that terameprocol **2**, NDGA derivative **3** and NDGA derivative **4** possessed significantly inhibitory effect on the growth metabolism of *S. pombe* cell lines. The values of IC_{50} of terameprocol **2**, NDGA derivatives **3**, and NDGA derivatives **4** on the growth metabolism of *S. pombe* cell lines were 330.0, 290.0 and 350.0 mg L^{-1}, respectively. The inhibitory ability of these three compounds above on the growth of the *S. pombe* has been observed to decrease according to the following order: NDGA derivative **3** > terameprocol **2** > NDGA derivative **4**. The NDGA derivative **2, 3** and **4** possessed the bidirectional biological effect and Hormesis effect. In other words, they stimulated the growth of the *S.pombe* at low concentration, but inhibited the growth at high concentration.

The thermogenic curves for the growth metabolism of *K562* cell lines treated by different concentrations of the compounds