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Novel process for large scale synthesis of N-(4-hydroxyphenyl) retinamide

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Abstract: A method to synthesize anticancer drug N-(4-hydroxyphenyl) retinamide (4-HPR) on a large scale is described. It consists of the preferred steps of reacting all-trans retinoic acid with thionyl chloride to form retinoyl chloride and then reacting with triethylamine to generate retinoyl ammonium salt which in turn is reacted with p-aminophenol to eventually produce 4-HPR. This process can overcome many scale-up challenges that exist in the methods reported in the literature and provide an easy, mild and high yield route for large scale synthesis of 4-HPR. Moreover, the effects of the molar ratios of the reagents on the yield are examined. The best molar ratios are a 2.0 molar equivalence of thionyl chloride and a 3.0 molar equivalence of p-aminophenol to retinoic acid, and the total yield is 80.7%.

Key words: 4-HPR; large scale; synthesis; anticancer

N-(4-hydroxyphenyl) retinamide (4-HPR, see Fig. 1), known as fenretinide, is an amide derivative of the naturally occurring parent retinoid, all-trans retinoic acid (ATRA, see Fig. 1). It was developed in 1979 as a breast cancer chemopreventive and chemotherapeutic agent[1]. By further investigation, the researchers found that, in addition to breast tumor cells, 4-HPR inhibited the growth and induced apoptosis of other tumor cells including ovarian cancer cells, uterine cervix cancer cells, prostate cancer cells, bladder cancer cells, and neuroblastoma cells[2]. And the latest research indicated that 4-HPR showed great potential in treating Ewing's sarcoma[3]. As well as an antitumor agent in animal studies[4] and a chemopreventive agent for several cancers in human clinical trials[5], a formulation of fenretinide as ST602 was developed by Sirion for the treatment of ophthalmological diseases such as dry age-related macular degeneration (AMD), geographic atrophy (GA) and Stargardt's disease and is now in phase Ⅱ Clinical Trials (Press release, Sirion, Aug 16, 2006 and Dec 4, 2006).

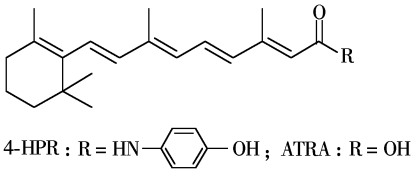

Fig. 1 Structure of retinoids

The mechanism by which 4-HPR induces cell apoptosis in tumor cells has not exactly been understood, but currently there are some theories trying to explain it. One theory holds that the effect of 4-HPR relates to changes in intracellular reactive oxygen species (ROS) and the subsequent regulation of Caspase[6-7]. In other theories, it is proposed that 4-HPR acts by selectively activated retinoid receptors which enables it to be less toxic and more efficient[8].

Several processes for preparing 4-HPR are reported in the literature. One sort of processes (see Fig. 2) uses agents such as thionyl chloride or phosphorus trichloride for acyl chloride formation[9-10]. Although these methods give acceptable yields (50% to 80%) on a small scale (nmol), they are not suitable for larger scales since the final product is purified by column chromatography and very low yields (20% to 25%) are obtained. Alternatively, Maryanoff provided an efficient and high yield process for the large scale synthesis of 4-HPR (see Fig. 3)[11]. However, a megatemperature reaction (150 to 160 ℃) is involved in this process, which results in more cost and time. Therefore, it is necessary to improve the known synthetic processes or to develop a new process.

We report herein an easy, relatively cheap and high yield process for large scale synthesis of 4-HPR (see Fig. 4).

1 Experimental

1.1 Materials

ATRA is purchased from Northeast General Pharmaceutical Factory (Liaoning, China). The dry DMF is obtained by storing over 0.4 nm sieves. Et_3N and $SOCl_2$ are purified by distillation. The other commercially available reagents and solvents are used without further purification. All reactions are conducted under N_2 atmosphere.

1.2 Retinoyl ammonium salt

In a 1-L, 3-neck round bottom flask, a mixture of 47.3 mL $SOCl_2$ (0.666 mol) and 51.5 mL dry DMF (0.666 mol) is stirred under N_2 atmosphere for 1 h. A slurry of 100 g ATRA (0.333 mol) in 500 mL dry DMF is added to the solution. After stirring at 0 ℃ for 3 h in subdued light, 92.3 mL Et_3N (0.666 mol) is added to this clear deep red retinoyl chloride solution. Following one more hour of stirring, the formed retinoyl ammonium salt solution is cooled in ice for later reaction.

1.3 N-(4-hydroxyphenyl) retinamide

In a 2-L, 3-neck round bottom flask equipped with thermometer and dropping funnel, 108.0 g p-AP (0.99 mol) in 500 mL dry DMF is cooled in ice-salt bath to 0 ℃. The fresh retinoyl ammonium salt solution protected from light is gradually added to p-AP solution while the temperature is maintained between 10 and 15 ℃ during the addition. The dark-coloured solution is stirred at room temperature until TLC analysis indicates no more products formed. The reaction is quenched with NH_4Cl water solution and extracted

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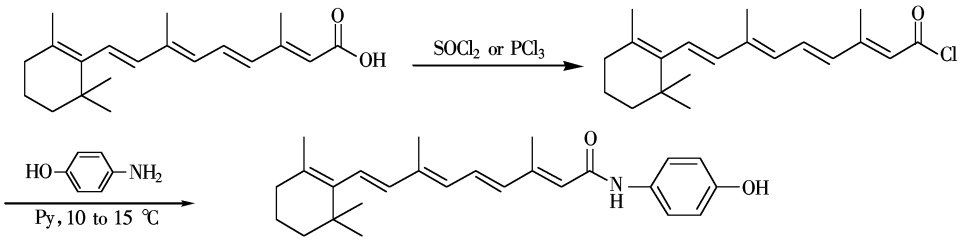

Fig. 2 Synthetic route A of 4-HPR

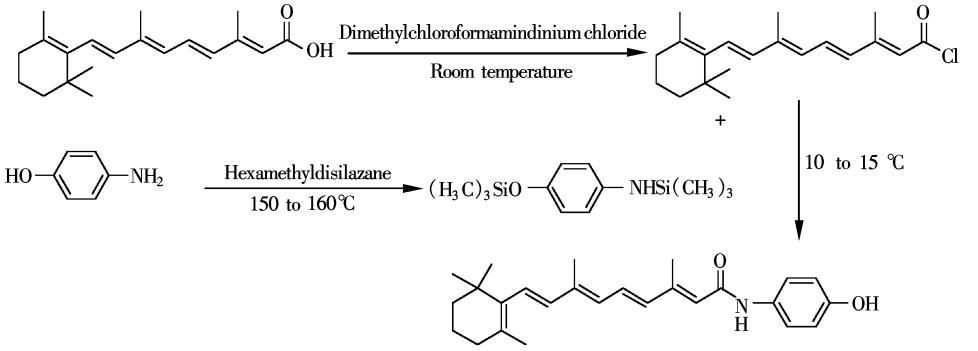

Fig. 3 Synthetic route B of 4-HPR

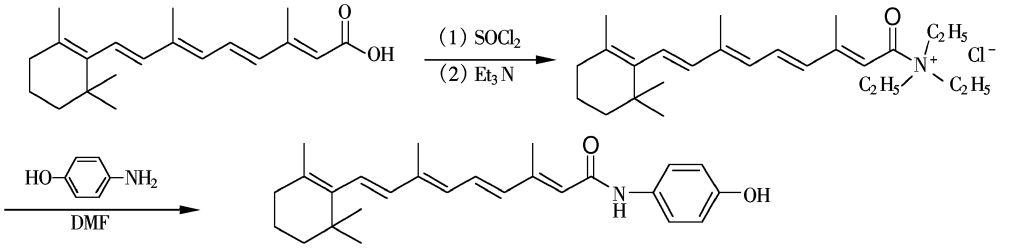

Fig. 4 Synthetic route C of 4-HPR

with CH_2Cl_2. The extracts are washed with H_2O and brine, and dried with Na_2SO_4 over night. The crude product is collected by filtration and evaporation. The product is then recrystallized from ethanol to give 105.2 g 4-HPR (80.7% yield) as a yellow solid. Mp: 170 to 172 ℃.

MS: $[M+H]^+ = 392.3$;

^{1}HNMR (300 MHz, $CDCl_3$) δ: 1.04 (s, 6H), 1.46 to 1.50 (m, 2H), 1.58 to 1.65 (m, 2H), 1.73 (s, 3H), 2.01 to 2.05 (m, 5H), 5.08 (brs, 1H), 5.79 (s, 1H), 6.12 to 6.31 (m, 4H), 6.78 to 6.80 (d, J = 8.8, 2H), 6.94 to 7.02 (dd, J_1 = 11.5, J_2 = 11.4, 1H), 7.0926 (brs, 1H), 7.37 to 7.40 (d, J = 8.9, 2H);

^{13}CNMR (75MHz, $CDCCl_3$) δ: 12.9, 13.7, 19.2, 21.7, 28.9, 33.1, 34.3, 39.6, 115.7, 121.2, 122.2, 128.5, 129.5, 129.9, 130.3, 130.9, 135.3, 137.3, 137.7, 139.2, 150.4, 152.7.

2 Results and Discussion

Our new process for the synthesis of 4-HPR is illustrated in Fig. 4. As shown in Fig. 4 the process comprises the preferred steps of reacting retinoic acid with thionyl chloride to form retinoyl chloride and then reacting with triethylamine to form retinoyl ammonium salt which in turn is reacted with p-aminophenol (p-AP) to eventually form 4-HPR.

The low yields of the process summarized in Fig. 2 on a large scale are due to the sensitivity and low selectivity between amino and hydroxyl of p-AP of the retinoyl chloride intermediate, and 4-HPR sensitivity to light, heat, oxygen and base-catalyzed decomposition[12]. The process in Fig. 3 which obtains a high yield on a large scale results from an intermediate bis-(N, O)-trimethylsilyl-p-amino-phenol (BS-pAP) prepared by reacting p-aminophenol with hexamethyldisilazane since it favors N-acylation and disfavors O-acylation. However, the megatemperature reaction of preparing BS-pAP results in more cost and complicates the process.

Our novel intermediate retinoyl ammonium salt which is produced by the mild reaction retinoyl chloride with triethylamine successfully overcomes the above shortcomings. Retinoyl ammonium salt possesses better stability and easier acylation than retinoyl chloride. In addition, no more base is added to the p-AP solution as a catalyst; the neutral condition prefers N-acylation to O-acylation strongly. Moreover, the postprocessing of our process is much easier than process B. As a result, a high yield on a large scale is obtained, which greatly decreases the cost since the reactant retinoic acid is expensive (over 10^4 yuan/kg).

Additionally, in our quest to find the optima molar ratios of reagents, reactions are carried out under various conditions (see Tab. 1). As shown in Tab. 1, the use of 2.0, 3.0 or 5.0 molar equivalence of $SOCl_2$ shows no significant difference in terms of yield. Thus, we recommend a 2.0 molar equivalence. As the same, we recommend a 3.0 molar equivalence of p-AP.

Tab. 1 Optimization of molar ratios of reagents

Entry	$n(SOCl_2)/n(ATRA)$	$n(p\text{-}AP)/n(ATRA)$	Yield/%
1	1.5	2.0	70.2
2	2.0	2.0	75.4
3	3.0	2.0	75.8
4	5.0	2.0	72.3
5	2.0	3.0	80.7
6	2.0	5.0	81.0
7	2.0	10.0	80.2

3 Conclusion

We develop a new route for preparing 4-HPR on a large scale. Particularly, retinoyl ammonium salt is an interesting intermediate and plays a significant role in the process. The best molar ratios are a 2.0 molar equivalence of $SOCl_2$ and a 3.0 molar equivalence of p-AP to ATRA. It is believed that this process is practical for industry since it obtains a high yield on a large scale and each step employs mild reaction conditions coupled with relatively cheap reagents and easy handling.

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一种大规模合成 N-(4-羟苯基)维甲酰胺的新方法

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摘要:介绍了抗肿瘤药物 N-(4-羟苯基)维甲酰胺(4-HPR)的合成新方法. 该方法包括如下反应步骤:全反式维甲酸与二氯亚砜反应生成酰氯,再与三乙胺成盐,然后将得到的酰基铵盐与对氨基苯酚发生酰胺化反应,制得 4-HPR. 该方法解决了文献报道方法在大规模合成时存在的多种问题如后处理繁琐、收率低等,具有操作简单、反应条件温和以及产率高等特点. 此外,研究了反应试剂的摩尔比对反应收率的影响,最佳摩尔比为二氯亚砜和对氨基苯酚物质的量分别是维甲酸物质的量的 2 倍和 3 倍,总收率为 80.7%.

关键词:4-HPR;大规模;合成;抗肿瘤

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