Synthesis of antitumour agents

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Abstract. A regiocontrolled total synthesis of cervinomycin A₁ and A₂ has been achieved utilizing simple and easily accessible reagents under viable reaction conditions. An approach towards the synthesis of the taxol skeleton using intramolecular Diels-Alder reaction followed by Wittig rearrangement is described.

Cancer, a complex of diseases associated with the abnormal growth of new body tissues, is a most dreadful disease in the world today. About 20% of the deaths in western countries occur due to neoplastic diseases and today, it is one of the third largest killer diseases in India. The most alarming aspects of cancer in India is that it affects the Indian 15-20 years earlier than their counterpart in western countries. This could be due to the climatic conditions. Radiation and surgery are the effective tools to combat it as long as this disease is detected early and localized. However, by the time it is detected it often spreads to other parts of the body. Chemotherapy or its combination with the radiation is the only solution. Realising the need and problem, we initiated a programme to develop chemotherapeutic agents. The synthesis of Cervinomycin 1 & 2 and Taxol 4 will be discussed.

A. CERVINOMYCIN A₁1 AND A₂2

Cervinomycin, produced by Streptomyces cervinus sp. nov., is an antibiotic possessing (ref.1) strong inhibitory activity against anaerobic bacteria and mycoplasma. Cervinomycin consists of two components A₁ 1 and A₂ 2 which are insoluble in most of the organic solvents. However, triacetyl cervinomycin 3, has high solubility and is developed as a drug because of its low toxicity and enhanced activity. Cervinomycin's unique structure, which possesses a sensitive isoquinolone moiety fused angularly with a novel and highly functionalized xanthone unit makes it a synthetically challenging target. A programme was therefore initiated on its total synthesis (ref. 2,3) with the aim to prepare chemical variants of 1 to further examine therapeutic efficacy.
Incisive architectural scrutiny of target molecule 1 unfolds that it is a heptacyclic aromatic compound with extended conjugation. ABC and EFG rings are angularly fused to central D ring, giving a shape of extended phenanthrene. ABC rings consist of the xanthone moiety of the molecule. D is the central ring, whose substitution pattern imparts an "elbow bend" to the molecule. The location of the carbonyl group in pyron F ring imposes regiospecificity to the molecule, which can be characterised as an "upward" xanthone.

Before embarking on the total synthesis of any complex molecule such as cervinomycin, it is important to conduct crucial reactions on model compounds so as to gain expertise in executing key reactions, which will not only provide an insight into the reactions and conditions to determine the sensitivity of the molecule but also help in the visualization of suitable precursors making the retrosynthetic plan easier. The major strategic concern, therefore, would be the identification of suitable methodologies for both the fragments oxazolidine 5 and xanthone 6 fragments corresponding to ABCD and DEFG rings of cervinomycins (scheme 1).

**Scheme 1**

![Scheme 1 diagram]

**Scheme 2**

![Scheme 2 diagram]

Reagents: i. Zn, BrCH₂COOEt, Benzene; ii. NaH, Me₂SO₄, DMF; iii. KOH, aq. DMSO; iv. LDA, (EtO)₂CO, THF; v. Ac₂O, Pyridine, 10% aq. NaOH, Ac₂O, HClO₄(Cat); vi. H₂NCH₂CH₂OH, MeOH

At the first instance, we planned to develop the methodology for the preparation of the oxazolidine unit which is very sensitive to acids, bases and heat. It was envisaged that this moiety can be prepared from the corresponding isocoumarin 10 by the action of 2-amino-ethanol. The isocoumarin, prepared as depicted in the scheme 2, when treated with 2-amino-ethanol in refluxing methanol produced 12 as the sole product. However, when the reaction was carried out (ref.4) at room temperature, the desired oxazolidine unit 11 could be obtained in 80% yield.

Our next attention was drawn to develop the methods for the preparation of the xanthone moiety, which was achieved by the sequence of reactions as delineated in scheme 3. Thus, the known (ref.5) 2-bromo-1,4-dimethoxynaphthalene 13 on Friedel-Crafts acylation afforded (ref.6) the 6-acetyl derivative 14 regioselectively which on oxidative demetylation gave rise to 2-bromo-naphthoquinone 15. Treatment of 15 with methyl-2-hydroxy-4,5-dimethoxybenzoate (ref. 7) in presence of K₂CO₃ produced 16 which was hydrolysed and cyclized to xanthone 18.
After establishing the methods for the preparation of the oxazolidine and xanthone moieties, we imagined that 19 would be the ideal synthon having CDE rings of cervinomycin built in it. It has a tolic acid portion for the elaboration to theoxazolidine unit and a bromosubstituent to stitch the xanthone unit. Thus, the attention was now focused to prepare the key intermediate 19.

Thus, an efficient approach for the synthesis of 19 was developed as depicted in scheme 4 starting from 14. 14 on treatment with thallium trinitrate in acidic medium produced (ref. 8) 20a which was transformed to 20c in two steps. 20c was olefinited with Ph3P=CHCOOEt and hydrogenated over Adam’s catalyst to afford 21, which after hydrolysis, was cyclized (ref.9) to 22a in overall good yield. The ester functionality was introduced in 22a to get 22b which was aromatized and methylated to get the key synthon 19.
Having the pivotal intermediate 19 in hand with the required substituents for a regiospecific annulation of the xanthone and oxazolo-isouquinolone units, the next concern was to acetylate the aromatic methyl first, then to construct the xanthone. Accordingly, 19 was converted to 23 using LDA and excess N-methoxy-N-methyl acetamide (ref 10) in THF at -78°C. Here, the oxazolidine ring was intended to be built at a later stage of the synthetic planning because it would not stand the reaction conditions employed in the preparation of the xanthone.

The ketone 23 on reduction with NaBH₄ in methanol afforded lactone 24 in 80% yield. 24 on oxidative demethylation furnished quinone 25a, which was stirred with excess methyl 2-hydroxy-4,5-dimethoxy benzoate (ref. 7) in presence of anhydrous K₂CO₃ in DMF to afford 25b in 65% yield (scheme 5). Quinone 25b on reduction with sodium dithionite and subsequent O-methylation of the resultant hydroquinone using potassium carbonate and dimethyl sulphate in an inert atmosphere furnished 26a. The ester 26a on hydrolysis with ethanolic KOH afforded a diacid, which on subsequent crystallization with PPE furnished the hexacyclic xanthone 27.

Although we had developed the method for the formation of the oxazolidine ring by reacting 2-aminoethanol with an isocoumarin, it was envisaged, keeping in view the mechanistic consideration for the formation of oxazolidine, that a ketoester would serve as a better substrate in comparison to the isocoumarin (scheme 6).

**Scheme 5**

![Scheme 5](image)

**Reagents:** i. NaBH₄, MeOH; ii. (NH₄)₂Ce(NO₃)₆, aq. CH₃CN; iii. Methyl 2-hydroxy-4,5-dimethoxybenzoate, K₂CO₃, DMF; iv. Na₂S₂O₄,(MeO)₂SO₂-K₂CO₃; v. KOH, EtOH; vi. PPE, CHCl₃; vii. KOH-EtOH, CH₂N₂-ether. PCC-CH₂Cl₂

Thus, lactone 27 was hydrolysed with alcoholic KOH and the hydroxy acid thus obtained was esterified with diazomethane. The alcohol functionality was oxidized to get the keto ester 28, which on treatment with 2-aminoethanol in refluxing MeOH in presence of K₂CO₃ afforded 29 having the complete skeleton of cervinomycin.
Having obtained 29 successfully, the only remaining task was to strip off three methyl ethers to generate cervinomycin A1, 1. When 29 was subjected to oxidative demethylation (ref. 11) using ceric ammonium nitrate in acetonitrile, the E ring methyl ethers were cleaved chemoselectively to afford O-methyl cervinomycin A2 29 in 95° yield, mp 300°C dec. [lit. (ref. 1b), mp, 300°C dec.] After several unsuccessful attempts with various reagents, Et$_3$N·BCl$_3$ was found to be an optimum reagent to cleave the methyl ether of ring C (1 eq. of boron trichloride in presence of 1.1 eq. of triethylamine in DCM at 0°C) to afford (±) cervinomycin A$_2$ 2 in 85% yield, mp 280°C dec [lit. (ref. 12) mp 279-284°C dec.]. The (±) 2 so obtained was identical (ref. 1) with an authentic sample of (-) cervinomycin A$_2$ by direct comparison. Reduction of (±) cervinomycin A$_2$ 2 with NaBH$_4$ afforded cervinomycin A$_1$ 1.

In conclusion, a regiocontrolled total synthesis of cervinomycin A$_1$ and A$_2$ has been achieved utilizing simple and easily accessible reagents under viable reaction conditions. This strategy would also facilitate the preparation of its analogues in the development of effective chemotherapeutic agents. An, effective and efficient methodology, hitherto unknown in the literature for the annulation of oxazolidine unit with a suitable treatise on mechanism of its formation was also unfurled.

B. TAXOL 4

Taxol 4, a highly oxygenated complex diterpenoid isolated (ref.12) from the bark of the western yew plant, Taxus brevifolia, is an exceptionally promising cancer chemotherapeutic agent. Taxol, apart from showing a broad spectrum of potent antileukemic and tumor inhibiting activity (ref. 13), has driven the attention (ref. 14) of scientists the world over owing to its tremendous structural complexity and also being the drug of choice for ovarian cancer.

We have started earnestly, in our laboratory, a feasible approach to the major tricyclic framework of Taxol which could pave the way ultimately to Taxol. Hitherto approaches (ref. 14, 15) were there for Taxol
which lead to an unfunctionalized or partly functionalized central eight membered ring. Our approach centres around an intramolecular Diels -Alder reaction of 30a whose retroanalysis leads to the diol 33 and the diene unit 34. After unsuccessful attempts to obtain the diene unit by the reported procedures (ref. 17) involving didebromination of 2-bromoethyl-1,2-butene with zinc dust, we looked for an alternative approach as shown in scheme 8.

Condensation of a homogenous mixture of freshly distilled acetaldehyde and ethyl acrylate using DABCO as catalyst afforded (ref. 18) the olefin ester 35 in 90% yield. Treatment of the ester with DIBAL-H DIBAL-H gave the allylic diol 36 which was subsequently treated with hydrobromic acid in refluxing benzene to furnish the dibromide 38 which can also be obtained from diol 37. Mono dehydrobromination of the dibromide by distilling with 1 eq. of HMPA gave the diene 34 in quantitative yield.

Scheme 9
Thus, selective mono O-alkylation of the diol 33 with the bromodiene 34 using one equivalent of NaH in THF gave the monoalcohol 39 in 70% yield (scheme 9). Swern oxidation of the alcohol 39 afforded a mixture of aldehydes in the ratio of 3:1. The aldehyde mixture was stirred overnight with sodium methoxide in methanol which gave a single isomer 41. Treatment of the aldehyde 41 with vinyl magnesium bromide gave the allylic alcohol 42 which was then subjected to a Swern oxidation to realize ketone 30a. IMDA reaction of the triene 30a system using diethyl aluminium chloride at 0°C for ten minutes gave the tricyclic system in 45% yield. Prior to the Wittig rearrangement the ketone was reduced with sodium borohydride to the alcohol and later protected as its silyl ether 31a. The key intramolecular Wittig rearrangement was performed with n-butyl lithium in THF to furnish 32a the desired tricyclic framework of taxol.

After obtaining the taxol skeleton through intramolecular Diels-Alder followed by Wittig rearrangement, we now aimed to make the fully functionalized taxol skeleton by using the same synthetic strategy as shown in scheme 7 and 9.

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\begin{align*}
\text{Scheme 10} \\
\begin{array}{c}
\text{2 steps} \\
\text{1)P_2O_5, ether} \\
\text{2)LAH, ether} \\
\end{array}
\end{align*}
\]

Diol 33 was mono-O-alkylated using diene bromide 45 (prepared as depicted in scheme 10), NaH and DMF, followed by Swern's oxidation, and vinyl Grignard gave 42b, which on Jones's oxidation gave 30b. Compound 30b was subjected to IMDA reaction. But all efforts to cyclize 30b (both thermal and catalytic methods under different conditions) to 43b failed. Surprisingly, we have observed an intramolecular ene reaction leading to 44.

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\begin{align*}
\text{Scheme 11} \\
\begin{array}{c}
\text{Br} \\
\text{NaH} \\
\text{DMF} \\
\end{array}
\end{align*}
\]

With the above result in hand, we have studied the ene vs. Diels-Alder reaction by preparing several substrates 47 (n=1, 2, 3, 4, 5) and subjecting them to the intramolecular reaction. In all the cases the reactants underwent the ene reaction, instead of the desired Diels-Alder reaction. Even 49 where the outer...
ring would have been an eight membered ring also underwent the ene reaction. A close scrutiny of the literature (ref. 20, 21) suggests that the substituents at $\alpha$ and $\beta$ to carbonyl of the dienophile induce the Diels-Alder reaction when the ring size formed is precisely eight membered. We, therefore, put a substituent at the $\beta$ carbon atom with respect to the carbonyl of the dienophile as in 52 which underwent the Diels-Alder reaction. Likewise, 54 also underwent the Diels-Alder reaction leading to 55. Now, we are doing theoretical calculations to rationalise these results.

We have now initiated a programme for a fully functionalized taxol skeleton employing an intramolecular Diels-Alder reaction based on the above results.

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