INHIBITION OF TYROSINE PROTEIN KINASE BY SYNTHETIC ERBSTATIN ANALOGS

Sir:

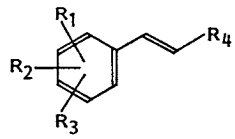
The effective synthesis of the specific tyrosine protein kinase (TPK) inhibitor, erbstatin (**1**), and its dihydroxy analogs (**11** and **12**) was reported in the preceding paper[1]. Herein, mono-, di- and tri-hydroxy analogs of **1** have been synthesized by a similar procedure and their TPK inhibiting activities were evaluated.

Mono-hydroxy analogs such as 2-hydroxy- (**2**), 3-hydroxy- (**3**), 4-hydroxy- (**4**), 5-bromo-2-hydroxy- (**5**) and 2-hydroxy-5-methoxy-compound (**6**) were prepared in high yields from corresponding aldehydes and SCHÖLLKOPF's reagent[2], diethyl(isocyanomethyl)phosphonate (**15**), as described in the preceding paper[1]. On the other hand, similar treatment of 2,4-di- or 2,3,4-tri-hydroxybenzaldehyde with the phosphonate (**15**) gave no desired products although a large number of variables including bases [$NaN(Si(CH_3)_3)_2$, BuLi and NaH] were assessed.

The protection, however, of the hydroxyl groups in the aldehydes with trimethylsilyl chloride ($(CH_3)_3SiCl$ - Et_3N in THF) gave suitable materials **13** and **14** for subsequent reaction with the reagent (**15**) to give the intermediary isocyanides (**16**) [**13**: ^1H NMR ($CDCl_3$) δ 0.30 (18H, s), 6.35~6.6 (2H, m), 7.80 (1H, d, J=8.5 Hz), 10.36 (1H, s), **14**: ^1H NMR ($CDCl_3$) δ 0.20, 0.25 and 0.28 (27H, each s), 6.62 (1H, d, J=8.5 Hz), 7.42 (1H, d, J=8.5 Hz), 10.27 (1H, s)] (Fig. 2). By acid hydrolysis (0.1 N HCl - EtOAc)[1], the isocyanides (**16**) were directly converted into the desired formamides (**7**) and (**8**) with removal of the trimethylsilyl groups in 57% and 53% overall yields [**7**: ^1H NMR (acetone-d_6) δ 6.25~6.55 (3H, m), 7.15 (1H, d, J=8.5 Hz), 7.56 (1H, dd, J=15 and 11 Hz), 8.17 (2H, s), 8.47 (1H, s), 9.1 (1H, br), **8**: ^1H NMR (acetone-d_6) δ 6.43 (1H, d, J=9 Hz), 6.47 (1H, d, J=15 Hz), 7.76 (1H, d, J=9 Hz), 7.4 (2H, br), 7.62 (1H, dd, J=15 and 10.5 Hz), 8.1 (1H, br), 8.22 (1H, s), 9.2 (1H, br)].

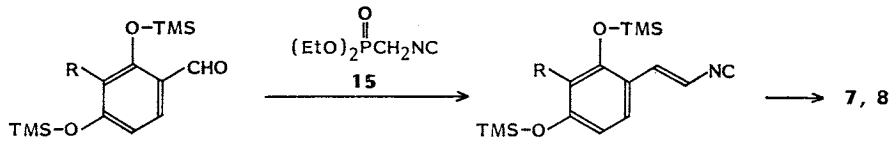
Other related compounds (**9**) and (**10**) were prepared as follows. The peracetylation (Ac_2O - Et_3N - p-dimethylaminopyridine in THF) of

Fig. 1. Erbstatin and its analogs.



1	R_1=2-OH	R_2=5-OH	R_3=H	R_4=NHCHO (erbstatin)
2	R_1=2-OH	R_2=H	R_3=H	R_4=NHCHO
3	R_1=3-OH	R_2=H	R_3=H	R_4=NHCHO
4	R_1=4-OH	R_2=H	R_3=H	R_4=NHCHO
5	R_1=2-OH	R_2=5-Br	R_3=H	R_4=NHCHO
6	R_1=2-OH	R_2=5-OCH_3	R_3=H	R_4=NHCHO
7	R_1=2-OH	R_2=4-OH	R_3=H	R_4=NHCHO
8	R_1=2-OH	R_2=3-OH	R_3=4-OH	R_4=NHCHO
9	R_1=2-OH	R_2=5-OH	R_3=H	R_4=NHAc
10	R_1=2-OH	R_2=5-OH	R_3=H	R_4=COOH
11	R_1=2-OH	R_2=3-OH	R_3=H	R_4=NHCHO
12	R_1=3-OH	R_2=4-OH	R_3=H	R_4=NHCHO

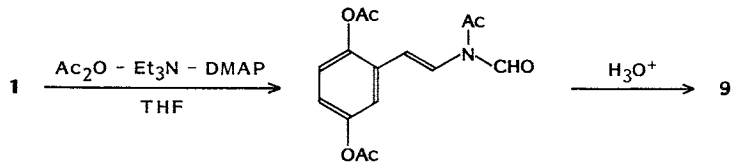
Fig. 2.



13 R = H
14 R = O-TMS

16

Fig. 3.



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DMAP: *p*-Dimethylaminopyridine.

Table 1. TPK inhibitory activities of erbstatin and its analogs.

Compounds	TPK-IC_{50} (μg/ml)
Erbstatin (**1**)	0.6
2	>100
3	>10
4	>10
5	>6.4
6	>6.4
7	>25
8	0.8
9	3.0
10	0.8
11	0.3
12	1.3

erbstatin gave triacetyl derivative (**17**) [^{1}H NMR ($CDCl_3$) δ 2.28, 2.32 and 2.43 (9H, each s), 6.9~7.3 (5H, m), 9.30 (1H, s)] (Fig. 3). Selective removal of formyl and *O*-acetyl groups (0.1 N HCl in MeOH) gave **9** [^{1}H NMR (acetone-d_6) δ 1.99 (3H, s), 6.35 (1H, d, J=15 Hz), 6.50 (1H, dd, J=9 and 3 Hz), 6.70 (1H, d, J=9 Hz), 6.82 (1H, d, J=3 Hz), 7.60 (1H, dd, J=15 and 10.5 Hz), 9.2 (1H, br)]. Compound **10** was prepared by the Wittig reaction of 2,5-di-hydroxybenzaldehyde and (carbo-*tert*-butoxymethylene)triphenylphosphorane (Ph_3P=CHCOO^{t}Bu) in benzene - THF (10:1) followed by acid hydrolysis (F_3CCOOH in CH_2Cl_2) [**10**: ^{1}H NMR (acetone-d_6) δ 6.0 (1H, br), 6.52 (1H, d, J=16 Hz), 6.82 (2H, m), 7.09 (1H, m), 8.02 (1H, d, J=16 Hz), 8.45 (1H, br)].

The TPK inhibitory activities of above derivatives (Fig. 1) are listed in Table 1. The TPK activities were assayed using the A-431 cell membrane fraction as the enzyme/substrate as described previously[3]. As shown in the table, 2-(2,3,4-trihydroxyphenyl)vinylformamide (**8**), 2,5-dihydroxycinnamic acid (**10**), 2-(2,3-dihydroxyphenyl)vinylformamide (**11**) and 2-(3,4-dihydroxyphenyl)vinylformamide (**12**) showed potent inhibitory activities comparable to erbstatin (**1**). Other biological activities and the stability of these compounds are being studied.

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