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Cyclopenta[*g*]quinazoline-Based Antifolates: The Effect of the Chirality at the 6-Position on the Inhibition of Thymidylate Synthase (TS)

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Abstract—Cyclopenta[*g*]quinazoline-based inhibitors of thymidylate synthase (TS) possess a chiral centre at the 6-position of the molecule. The effect of this chirality on the inhibition of TS was investigated by synthesising compounds 6*S*-**1a–c**, 6*R*-**1a–c**. It was shown, in particular with the diastereoisomers 6*S*-**1c**, 6*R*-**1c**, that the inhibitory activity against TS is mainly due to the 6*S* diastereoisomer rather than the 6*R* diastereoisomer, which is virtually inactive. © 2001 Elsevier Science Ltd. All rights reserved.

The thymidylate synthase (TS) enzyme that catalyses the conversion of 2′-deoxyuridine 5′-monophosphate (dUMP) to thymidine 5′-monophosphate (TMP) has been an attractive target in cancer chemotherapy for several years. A number of structurally diverse molecules have been clinically evaluated and raltitrexed (Tomudex) has been approved for the treatment of colorectal cancer.[1]

Cyclopenta[*g*]quinazoline-based antifolates constitute a new and promising class of inhibitors of thymidylate synthase that display a high inhibitory activity against this enzyme.[2–4]

The presence of the cyclopenta[*g*]quinazoline ring introduces a chiral centre at the 6-position of the molecule (Fig. 1). From the two diastereoisomers with regard to the stereochemistry at the 6-position, it was thought that the 6*S*-diastereoisomer is mainly responsible for the inhibition of TS since this stereochemistry shapes the molecule into a conformation that is favourable for binding to the enzyme.[5] To investigate this, it was necessary to prepare pure 6*S* and 6*R*-diastereoisomers for some of the most potent inhibitors of the enzyme that have been originally reported as a mixture of diastereoisomers (i.e., compounds **1a–c**, Fig. 1).[2]

The synthesis of 6*S*-**1a–c**, 6*R*-**1a–c** was achieved by coupling the appropriately protected ligand **2d–f** to 6*S*-**2** or 6*R*-**2** followed by the removal of the protecting groups (Scheme 1).[2] The acids 6*S*-**2** and 6*R*-**2** were prepared by the enzymatic hydrolysis of *N*-(4-{*N*-[(6*RS*)-2-methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoyl)-L-glutamic acid as previously described, and the enantiomeric purity was determined by chiral HPLC.[3] The absolute stereochemistry of 6*S*-**2** was established by carrying out an X-ray crystal structure determination on a compound derived from 6*S*-**2**.[3] Compounds 6*S*-**3d**, 6*R*-**3d** were prepared from the corresponding acids and **2d** via diethyl phosphorocyanidate (DEPC) carboxyl activation. To synthesise 6*S*-**3e**, 6*R*-**3e**, 6*S*-**3f**, 6*R*-**3f**, the acids 6*S*-**2** and 6*R*-**2** were first converted into their pentafluorophenyl esters which then reacted with the appropriate ligand **2e**, **2f**. Alkaline or acidic hydrolysis of the ester protecting groups afforded the final products 6*S*-**1a–c**, 6*R*-**1a–c**. These compounds were analysed by chiral HPLC (ASTEC Cyclobond I column, ASTEC Cyclobond II, or ASTEC Chirobiotic T column) and, as expected, the stereochemical integrity for each of these molecules was correlated with that of the starting materials (i.e., the acids 6*S*-**2** and 6*R*-**2**). This meant that compounds 6*R*-**1a–c** were contaminated with ~10% of the corresponding 6*S*-diastereoisomer. However, in the case of 6*R*-**1c**, it was possible to remove the undesired 6*S*-diastereoisomer by semipreparative HPLC (Chirobiotic T column (25 cm × 10 mm); mobile phase: MeOH containing 0.1% AcOH and 0.1% Et_3N; flow = 5 mL/min, $\lambda = 230$ nm). So, this compound (6*R*-**1c**) was obtained in a pure form regarding the stereochemistry at the 6-position.

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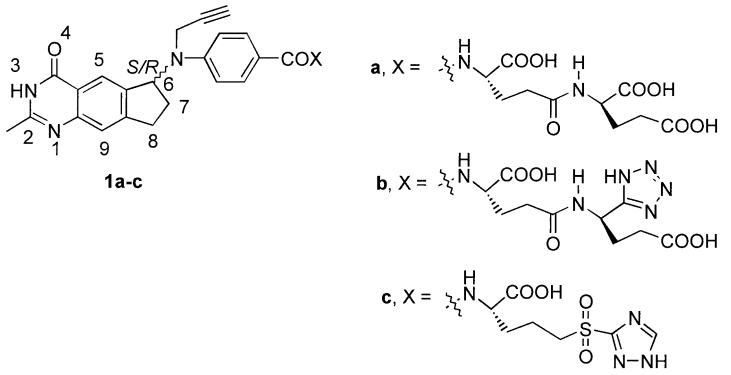
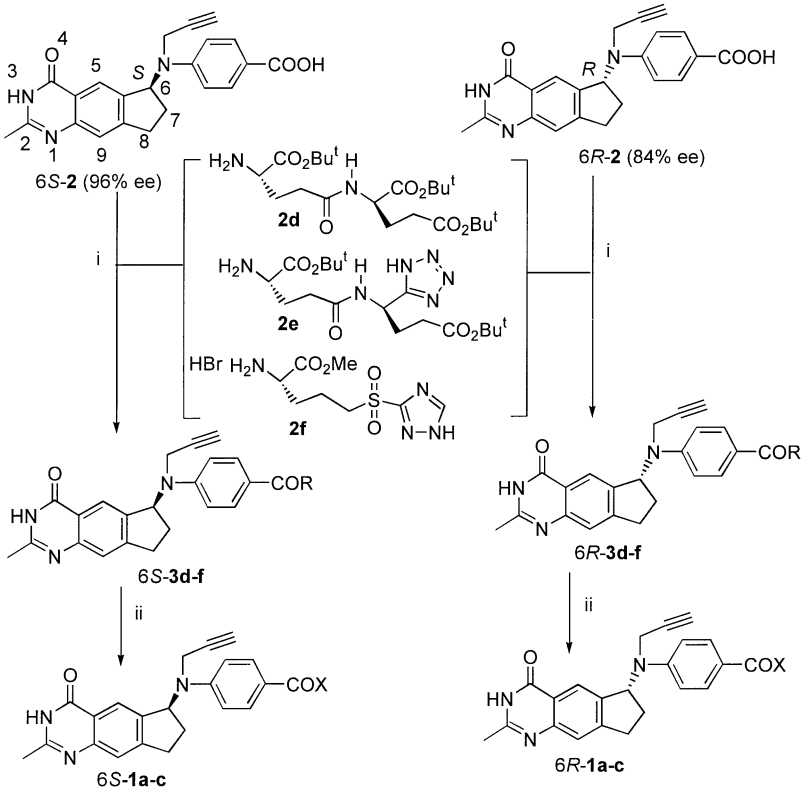


Figure 1. Cyclopenta[*g*]quinazoline-based inhibitors of TS.



Scheme 1. Reagents and conditions: (i) for 6*S*-**3d**, 6*R*-**3d**: **2d**, DEPC, Et_3N, DMF; for 6*S*-**3e**, 6*R*-**3e**: $CF_3CO_2C_6F_5$, pyridine, DMA, column chromatography then **2e**, HOBt (cat), DMF; for 6*S*-**3f**, 6*R*-**3f**: $CF_3CO_2C_6F_5$, pyridine, DMA, column chromatography then **2f**, HOBt (cat), Et_3N, DMF; (ii) for 6*S*-**1a**, 6*R*-**1a**: TFA; for 6*S*-**1b**, 6*R*-**1b**: TFA–H_2O; for 6*S*-**1c**, 6*R*-**1c**: 1 N NaOH, H_2O–MeOH.

Regarding inhibition of TS, 6*R*-**1a** was ~10-fold less potent against TS compared with 6*S*-**1a** (Table 1). Likewise compound 6*R*-**1b** was approximately ~20-fold less potent than 6*S*-**1b** (Table 1). These results indicated that indeed the TS activity is mainly due to the 6*S*-diastereoisomer, since 6*R*-**1a**, 6*R*-**1b** were contaminated with ~10% of the corresponding 6*S*-diastereoisomer. This was unequivocally shown by comparing the TS inhibitory activity of 6*R*-**1c** (devoid of any 6*S*-diastereoisomer) with that of 6*S*-**1c**. Indeed, 6*R*-**1c** was ~1800-fold less potent an inhibitor of the enzyme than 6*S*-**1c** (Table 1).

Table 1. Inhibition of thymidylate synthase

Compd	L1210TS Kiapp (nM)
6*S*-**1a**	0.33±0.16
6*R*-**1a**	3.5, 3.9
6*S*-**1b**	0.17, 0.16
6*R*-**1b**	2.1, 4.8
6*S*-**1c**	0.71±0.16
6*R*-**1c**	1200, 1460

6*R*-**1a** and 6*R*-**1b** contaminated with ~10% of 6*S*-**1a** and 6*S*-**1b**, respectively. Kiapps normalised to CB3717 (L1210TS Kiapp for CB3717 = 20 nM).[2] L1210TS Kiapp for 6*RS*-**1a** = 0.42; for 6*RS*-**1b** = 0.2; for 6*RS*-**1c** = 0.78 nM.[2]

In conclusion, cyclopenta[*g*]quinazoline-based antifolates that possess a chiral centre at the 6-position constitute a new class of potent inhibitors of TS. The effect of the stereochemistry at the 6-position on inhibiting the TS enzyme was studied by synthesising compounds 6*S*-**1a**–**c**, 6*R*-**1a**–**c**. It was shown, in particular

with the pair of compounds 6*R*-**1c**, 6*S*-**1c** that the 6*R*-diastereoisomer was virtually inactive compared with its 6*S* counterpart.

Acknowledgement

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References and Notes

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