Tetrazole Compounds: The Effect of Structure and pH on Caco-2 Cell Permeability

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ABSTRACT: A tetrazole ring is often used in drug discovery as a replacement for the carboxylic acid group. Previous work indicates that compounds containing a tetrazole moiety show asymmetric permeability in Caco-2 cells characteristic of an efflux transporter substrate. The aim of this study is to determine which transporters are responsible for polarization of transport of tetrazole-containing compounds in Caco-2 cells. Results indicate that only select compounds with tetrazole moieties display asymmetric transport. Three compounds (two commercial drug products and one druglike structure) were selected for further studies. Losartan appears to be primarily a P-glycoprotein (P-gp) substrate, as previously reported, but MRP inhibitors such as MK-571 and rifampicin also affect the difference between apical to basolateral and basolateral to apical transport. Pemirolast and phenyltetrazole derivative C are sensitive to P-gp inhibition, but transport seems to be mediated by one or more of the MRP family of transporters. Additionally, lowering the pH from 7.4 to 4.0 eliminates the polarization of permeability in Caco-2 cells. These studies indicate that some tetrazole compounds are susceptible to efflux, therefore caution should be used when choosing an appropriate functional group to replace carboxylic acids when synthesizing a drug candidate.
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INTRODUCTION

One major consideration when developing a drug candidate is the potential for interactions with cellular efflux transporters. One such transporter, P-glycoprotein (P-gp), has been shown to significantly decrease the intestinal absorption of numerous compounds.[1] An accurate screening method early in the drug discovery/development process that can detect which candidates are susceptible to P-gp efflux is a highly valuable tool.[1,2]

The Caco-2 assay, using cells derived from a human adenocarcinoma is widely used to assess intestinal permeability, and results from this *in vitro* system compare favorably with *in vivo* and *in situ* data.[3,4] The apically (luminally) located P-gp is expressed and active in Caco-2 cells, therefore the cell line is employed to evaluate whether a drug candidate is a substrate for this transporter.[5]

Previous studies in our laboratories have shown that druglike molecules with particular chemical groups often used to improve a drug candidate's physicochemical properties can exhibit polarized transport properties that are characteristic of efflux pump substrates.[6] In particular, benzoic acid, amidine, and tetrazole moieties conferred high basolateral to apical permeability compared

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to apical to basolateral permeability, suggesting contributions of polarized transport mechanisms. We have selected tetrazole-containing compounds to study because they showed the highest degree of polarization.[6] Losartan, an angiotensin II antagonist containing a tetrazole ring (Fig. 1), has been shown to be a P-gp substrate.[7–9] Its poor oral absorption can at least partially be attributed to its affinity for P-gp, however it appears that other transporters may also be responsible for asymmetric transport of losartan in Caco-2 cells.[8] Structurally related angiotensin II antagonists show a wide variability in bioavailability that suggests a difference in efflux transporter affinity among the structures.[9]

In this study, currently marketed drugs as well as a selection of synthesized "drug-like" compounds were used to assess the effect of structure and charge on Caco-2 cell permeability for molecules containing a tetrazole moiety.

MATERIALS AND METHODS

Dulbecco's Modified Eagle's Medium (DMEM) buffered with HEPES, heat-inactivated fetal bovine serum, nonessential amino acids, and penicillin–streptomycin were obtained from Gibco Invitrogen (Carlsbad, CA). Losartan, valsartan, irbesartan, and cilostazol were purchased from

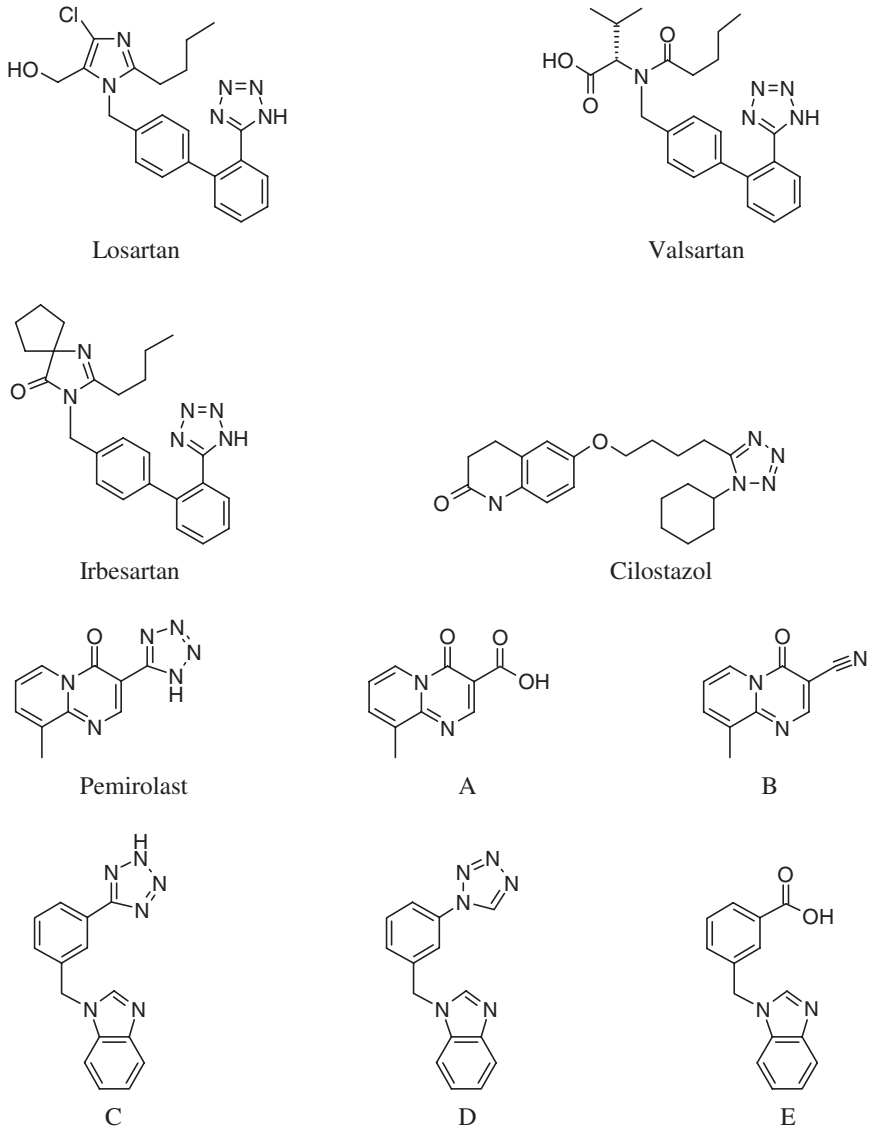

Figure 1. Structures of selected compounds.

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