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The structural use of carbostyril in physiologically active substances

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ABSTRACT

Carbostyril (2-quinolinone, 2-quinolone) is an important structural component frequently used in natural products and in physiologically active substances including drugs. It is a 2-ring condensed heterocyclic compound containing several positions that can be replaced by arbitrary substituent groups and is used as a chemical building block, scaffold, fragment, and pharmacophore in drug design or discovery. Since the number of compounds that can be designed using carbostyril is exceedingly large, the steric structures of carbostyril derivatives can be adjusted to the unique, spatially oriented shape of, for example, the active sites of pharmaceutical target molecules. Moreover, the internal amide of the carbostyril unit exhibits distinctive features because of the fixed *cis* form of the lactam amide group. Because carbostyril has been used as a component in drugs and other bioactive compounds over time, carbostyril derivatives may improve absorption, distribution, metabolism, excretion, and toxicity (ADMET). Therefore, carbostyril derivatives have enormous potential. In this review, the potential and advantages of the use of carbostyril and its related molecular skeletons, such as 3,4-dihydrocarbostyril, are discussed by focusing on the physiologically active substances in which they are incorporated.

Carbostyril (2-quinolinone, 2-quinolone) (Fig. 1) is the structural component present not only in secondary metabolic products, such as natural compounds, but also in artificial substances including physiologically active substances (e.g., drugs) and fluorescent materials. Furthermore, related molecular skeletons belonging to carbostyril analogues, such as 3,4-dihydrocarbostyril (Fig. 1), are also used in drugs and other bioactive compounds. The use of the carbostyril skeleton as a building block, fragment, or scaffold in molecules allows for the design of diverse and complex carbostyril-derived compounds. As a result, the compounds' steric structures can be constructed to fit many different spatially oriented structures, such as the active sites of pharmaceutical target molecules including proteins and nucleic acids. In some cases, carbostyril units themselves become part of pharmacophores. In fact, the carbostyril unit is a time-tested structural component of drugs and other biologically active compounds. This suggests that carbostyril derivatives potentially exhibit good absorption, distribution, metabolism, excretion, and toxicity (ADMET) features. Thus, carbostyril is a highly attractive component in drug design or drug discovery. The syntheses of carbostyril skeletons from the corresponding quinoline *N*-oxides formed the basis of the pioneering synthetic studies performed by Eiji Ochiai about half a century ago.[1–6] Since then, synthetic studies on carbostyril derivatives have largely expanded, explaining the variety of compounds that has been developed and evaluated for their biological activity. In this review, I introduce the use of carbostyril in physiologically active substances to inform on its potential and advantages.

Characterization of carbostyril: Carbostyril is a 2-ring condensed heterocyclic compound composed of a benzene ring and a six-membered aromatic lactam ring whose lactam nitrogen is tethered to the benzene ring. 2-Quinolinone can be tautomerized to 2-quinolinol based on amide tautomerism (Fig. 1). 3,4-Dihydrocarbostyril, which is the reduced form of carbostyril, is also often used as a structural component of drugs. The benzene ring of carbostyril can interact with the aromatic rings of peptides or

Abbreviations: *c*log*P*, the calculated log octanol/water partition coefficient; tPSA, topological polar surface area; C=O, carbon—oxygen double bond; $LiAlH_4$, lithium aluminum hydride; HDAC, histone deacetylase; DprE1, decaprenylphosphoryl-β-D-ribose 2′-epimerase; Mtb, *Mycobacterium tuberculosis*; MIC, minimum inhibitory concentration; Asp, aspartic acid; Tyr, tyrosine; MAO, monoamine oxidase; CNS, central nervous system; COX, cyclooxygenase; PDK1, 3-phosphoinositide-dependent kinase 1; Ser, serine; Thr, threonine; PKB, protein kinase B; PKC, protein kinase C; S6K, p70 ribosomal S6 kinase; SGK, serum- and glucocorticoid-induced protein kinase; PI3K, phosphatidylinositol 3-kinase; NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; GTP, guanosine-5′-triphosphate; cGMP, 3,5-cyclic guanosine monophosphate; NOS, nitric oxide synthase; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase; PFT, farnesyltransferase; TNKS, tankyrase; PARP, poly-ADP-ribose polymerase; TRF1, telomeric repeat-binding factor 1; TSA, thermal shift assay; DSF, differential scanning fluorometry; CRTAase, calreticulin transacetylase; GST, glutathione *S*-transferase.

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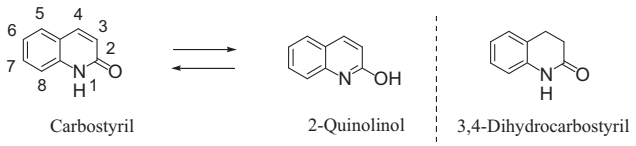

Figure 1. The structures and tautomerism of carbostyril.

nucleic acids through π–π stacking to form noncovalent interactions. Moreover, the internal amide of carbostyril is the fixed *cis* form of the lactam amide group and can form hydrogen bonds with peptides, nucleic acids, or water molecules. However, the amide bonds of most peptides in a living organism are in the *trans* form although about 10% of proline residues exists in the *cis* form.[7] Nevertheless, the *cis* configuration induced by some of these proline residues is isomerized to the *trans* form by peptidylprolyl isomerase (PPIase) (Fig. 2).[8] *Trans* form amides are thermodynamically more stable than their *cis* counterparts. Thus, the *cis* conformations of peptidyl amide groups are rare and the internal *cis* form of the amide of carbostyril is, therefore, uncommon in an organism and may show distinctive features, different from other skeletons such as naphthalenes, other quinolones, and naphthoquinones which lack amide bonds. Furthermore, 3,4-dihydrocarbostyril is more hydrophobic than carbostyril. In drug design, various physicochemical parameters (*c*log*P*, tPSA, aqueous solubility, etc.) of the whole molecule are important. However, these factors can be tuned not only by introducing or deleting some functional groups but also by creatively using carbostyril and 3,4-dihydrocarbostyril.

Metabolic processing of carbostyril and its related compounds: The ester bonds of small molecules, such as prodrugs, are hydrolyzed by esterases. From a metabolic point of view, the internal amide of carbostyril could interacts with proteases, the ubiquitin–proteasome system, or other metabolic systems. The ubiquitin–proteasome system degrades polyubiquitin-tagged proteins into oligopeptides through the cleavage of amide bonds. Because carbostyril derivatives are probably not tagged with a polyubiquitin chain, the internal amide of carbostyril is not cleaved by the ubiquitin–proteasome system. As for nucleophilic addition to the carbonyl carbon atom of ketone, a nucleophile can attack the carbonyl carbon atom from the carbon atom side at about a 107° angle to the C=O bond plane according to the Bürgi–Dunitz trajectory[9], which is expanded to the carbonyl carbon atom of the amide, aldehyde, and ester (Fig. 3). However, nucleophilic addition to the carbonyl carbon atom of the fixed *cis* form of the lactam amide of carbostyril or 3,4-dihydrocarbostyril might be difficult because of the steric hindrance resulting from the methine or methylene at position 3 and 4 and the benzene ring. Nevertheless, the amide of 3′,4′-dihydro-spiro(cyclohexane-1,4′-carbostyril) was reduced to the corresponding amine with $LiAlH_4$ in a quantitative yield (Fig. 4).[10] This suggested that small molecules, such as reagents, were able to attack the carbonyl carbon atom of the amide of carbostyril or 3,4-dihydrocarbostyril. However, large molecules, including proteins such as proteases, could not approach this carbonyl carbon atom because of steric hindrance. Proteases are categorized into four groups on the basis of their reaction mechanisms:

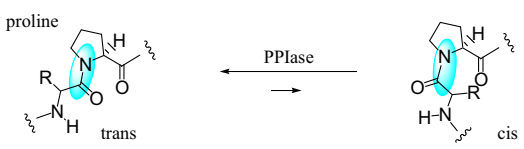

Figure 2. The *trans* and *cis* amide conformations and the conformation change of proline residues by peptidylprolyl isomerase (PPIase).

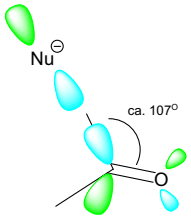

Figure 3. Bürgi–Dunitz trajectory. A nucleophile attacks the carbonyl carbon atom at about a 107° angle to the C=O bond.

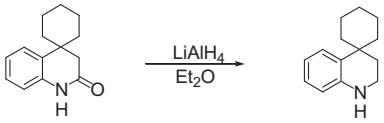

Figure 4. The reduction of carbostyril with $LiAlH_4$ in a quantitative yield.

cysteine proteases,[11] serine proteases,[12] metalloproteases,[13,14] and aspartic acid proteases.[15] These four proteases catalyze the cleavage of the amide bond via a tetrahedral transition state. The formation of a tetrahedral transition state between the proteases and the amide of carbostyril or 3,4-dihydrocarbostyril in a tight space might be thermodynamically highly unstable, preventing proteolytic cleavage of the amide bond. Indeed, 6-hydroxycarbostyril-6-glucuronide was detected in urine after carbostyril was fed to rabbits.[16] On the other hand, carbostyril was decomposed at the C—N bond between the nitrogen atom and the benzene ring by metabolic processing in bacteria (Fig. 5).[17] Biologically, this implied that the amide bond of carbostyril, which is different from the external amide bonds of some other compounds, was not enzymatically cleaved. Furthermore, this internal amide is relatively tolerant to chemical alkaline hydrolysis. Thus, the carbostyril skeleton is metabolically stable and its ring structure cannot be opened, particularly at the amide bond, in a living organism. Therefore, toxic aniline metabolites from carbostyril and related analogues such as 3,4-dihydrocarobostyril cannot be formed through the cleavage of the internal amide bond. This may be one of the reasons why carbostyril derivatives show good ADMET.

Biological activity of carbostyril derivatives used as chemical building blocks: The use of carbostyril in physiologically active substances is illustrated by actual examples. First, chemical building blocks are the composition elements of spatial domains within molecules and are connected to the main structures by a covalent bond or linker unit. In drug design and discovery, building blocks should be able to freely interact with the pharmaceutical target proteins or other substances by hydrogen bonding or π–π stacking. Since replacing a building block with other ones creates numerous novel compounds, building blocks are often used in combinatorial chemistry. Carbostyril and its analogues are representative of such building blocks (Fig. 6).

Histone deacetylases (HDACs) catalyze the deacetylation of lysine residues on histone and non-histone proteins to epigenetically control transcriptional regulations and, thereby, play an important role in proliferation, differentiation, cell cycle arrest, and/or apoptosis of tumor cells. HDAC inhibitors are known to have anticancer effects. The 18 HDAC isozymes are subdivided into four classes: Class I (HDACs 1–3 and 8), class IIa (HDACs 4, 5, 7, and 9), class IIb (HDACs 6 and 10), class IV (HDAC11), and class III HDACs (sirtuins 1–7). Compound **1** (Fig. 6), which possesses a 6-substituted 4,4-dimethyl-3,4-dihydrocarbostyril as the cap group, had a more than 10-fold stronger inhibitory activity against each human HDAC isozyme in vitro than that of suberoylanilide hydroxamic acid (SAHA) (IC_{50} values of **1**: HDAC1, 0.0038 μM; HDAC2,