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Review

5-Substituted-1*H*-tetrazoles as Carboxylic Acid Isosteres: Medicinal Chemistry and Synthetic Methods

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Abstract—5-Substituted-1*H*-tetrazoles (RCN_4H) are often used as metabolism-resistant isosteric replacements for carboxylic acids (RCO_2H) in SAR-driven medicinal chemistry analogue syntheses. This review provides a brief summary of the medicinal chemistry of tetrazolic acids and highlights some examples of tetrazole-containing drug substances in the current literature. A survey of representative literature procedures for the preparation of 5-substituted-1*H*-tetrazoles, focusing on preparations from aryl and alkyl nitriles, is presented in sections by generalized synthetic methods.

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Introduction

This review provides a summary of the medicinal chemistry aspects of 5-substituted-1*H*-tetrazoles, which have found common usage as an isosteric replacement for the carboxylic acid moiety in recent years. A discussion of the structural features of the tetrazolyl group which make it a suitable substitution for a carboxyl functionality in drug design will be presented, as well as a description of some of the metabolic liabilities of this surrogate moiety. An examination of some prominent examples of tetrazolic acid-containing drug substances from the literature will also be presented, focusing on

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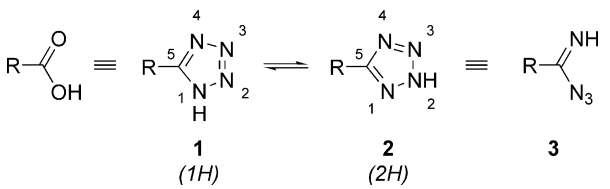
two aryl and one aliphatic compound. Practicing medicinal chemists who may be interested in an evaluation of more comprehensive tabular surveys may also consult some of the review materials listed in this bibliography.[1–5] Some representative literature procedures for the preparation of 5-substituted-1*H*-tetrazoles from aryl and alkyl nitriles will be presented, as well as some highlights from the very recent literature which involve carbon–carbon bond formations with 1-substituted-5-lithiotetrazoles.

Chemical and Pharmacological Properties

It has been long held that 5-substituted-1*H*-tetrazoles (RCN_4H) may serve as a non-classical isostere for the carboxylic acid moiety (RCO_2H) in biologically active molecules.[1–7] The term non-classical isosterism (used interchangeably with the term bioisosterism) refers to the concept in which functional groups that have similar physicochemical properties may be interchangeable, resulting in similar biological properties. Furthermore, a non-classical isostere may or may not have the same steric or electronic characteristics, nor even the number of atoms, as the substituent for which it is used as a replacement.[5,7–9] Other simple carboxylic acid surrogates include carboxamides, sulfonamides, acyl sulfonamides, sulfamides, sulfonates and phosphates. More complicated isosteres include isoxazol-3-ols, hydroxy-2-methylpyran-4-ones, 4*H*-[1,2,4]oxadiazol-5-ones, 4*H*-[1,2,4]thiadiazol-5-ones and 2,4-dihydro-[1,2,4]triazol-3-ones, to name a few.

5-Substituted tetrazoles that contain a free N–H bond are also frequently referred to as tetrazolic acids, and exist as a nearly 1:1 ratio of 1*H*- and 2*H*-tautomeric forms (Fig. 1, **1** and **2**, respectively), although it is sometimes also convenient to describe them as imidoyl azides **3**. It should be stated that tetrazolic acid structures that appear throughout this article are assumed to be mixtures of both 1*H*- and 2*H*-tautomers. Previous studies have shown that the two positional isomers **1** and **2** may be differentiated on the NMR timescale.[10] Recently, Sadlej-Sosnowska has applied calculated natural bond orbital analysis to a series of 5-substituted tetrazoles and determined that 2*H*-tautomers **2** are the more stable isomers, although they were found to have a larger degree of electron delocalization than 1*H* tautomers **1**.[11] This consideration, in combination with steric factors, may have some bearing on the observation that *N*-alkylation of tetrazolic acids often places the substituent on the N2 position.[12]

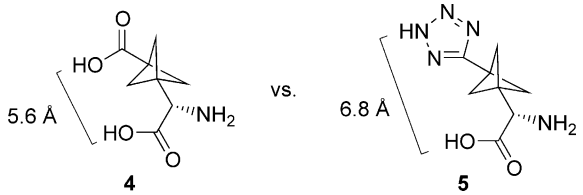


1 (1H) **2** (2H) **3**

Figure 1. Tetrazolic acids are bioisosteres of carboxylic acids.

The free N–H bond of tetrazoles makes them acidic molecules, and not surprisingly it has been shown that both the aliphatic and aromatic heterocycles have pK_a values that are similar to corresponding carboxylic acids (4.5–4.9 vs 4.2–4.4, respectively), due to the ability of the moiety to stabilize a negative charge by electron delocalization.[6,12–16] In general, tetrazolic acids exhibit physical characteristics similar to carboxylic acids and are strongly influenced by the effect of substituents at the C5-position.[6] For example, many 5-aryl tetrazoles are highly soluble in water and are best crystallized from aqueous alcoholic solvents. However 5-aliphatic analogues, while still often soluble in water, are best crystallized from solvents such as ethyl acetate or toluene/pentane mixtures.[6] The corresponding tetrazolate anionic species (RCN_4Na or RCN_4Li), which have a higher capacity for hydrogen bonding than the protic species,[17] are easily generated in hot alcohol or aqueous solutions and these intermediates are more reactive than the corresponding neutral species toward a variety of electrophiles and alkylating agents.[6] A recent review on the *N*-substitution of tetrazoles has appeared, which focuses on alkylation and electrophilic reactions at tetrazole nitrogen atoms.[12] A recent review of transformations of heterocycles into tetrazoles, and conversions of tetrazoles into other ring systems, also takes into consideration the physical properties of tetrazoles.[18]

Like their carboxylic acid counterparts, tetrazoles are ionized at physiological pH (7.4), and both exhibit a planar structure. However, Hansch has shown that anionic tetrazoles are almost 10 times more lipophilic than the corresponding carboxylates,[19] which is an important factor to bear in mind when designing a drug molecule to pass through cell membranes. Another important factor when considering a tetrazole as a replacement is the effect of delocalization of the negative charge around the tetrazole ring. The distribution of charge over a greater molecular surface area may be favorable for a receptor–substrate interaction, or may complicate the contact, depending on the local charge density available at the interface.[20] The larger size of the heterocycle (vs a carboxyl group) may also reduce the binding affinity at the active site, either by less favorable orientation of functional groups, or by steric hindering of an active conformational change of the receptor complex.[21] An interesting comparison between the effective 'length' of carboxylic acid versus tetrazole pharmacophores was recently reported by Pellicciari and coworkers (Fig. 2).[22] In a study designed to explore the SAR of propellane-derived analogues of L-glutamic acid as mGlu1 receptor agonists, the authors prepared the amino acids **4** and **5**, which contained distal carboxylic



4 **5**

Figure 2. The size of tetrazole **5** extends the distance between the two acidic pharmacophores relative to the analogous dicarboxylic acid **4**.

acid and tetrazole units, respectively. Models suggested that the distance between the acidic functional groups were such that the 2*H*-tetrazole moiety increases the distance between the pharmacophores by about 1 Å. In vitro evaluation revealed that the tetrazole **5** was 2.5-fold less potent versus **4**, which was attributed to the increased distance between the two acidic sites, indicating an unfavorable fit between two important synergistic positions.

Hydrogen bonding capability of tetrazolic anions with receptor recognition sites has recently been shown to be the key interaction for enhanced binding affinity. The finding that tetrazole substrates may form two hydrogen bonds with peptide residues in a biological target site may well explain the stronger binding interaction. For example, mutagenesis studies have indicated that the tetrazolate moiety of several nonpeptide antagonists interact with a protonated lysine and a histidine in the active site of the angiotensin II receptor.[23] A short time ago an X-ray crystal structure has revealed the ionic interaction of the N1 and N2 tetrazole nitrogens of an HIV-1 integrase inhibitor with two lysine residues within the enzyme active site.[24] Lately it has been shown that a tetrazole can form two hydrogen bonds to an *N,N'*-disubstituted benzamidine, although with a considerably smaller association constant versus the corresponding carboxylate-amidine interaction.[25]

In the design of drug molecules, one advantage of tetrazolic acids over carboxylic acids is that they are resistant to many biological metabolic degradation pathways. Some of the earliest findings showed that tetrazole-derived nicotinic acid analogues that were administered to dogs were excreted essentially unchanged over a 24-h period, whereas nicotinic acid itself was rapidly metabolized.[26] As in these cases, it is often seen that the resistance of tetrazolic drug substances to metabolism may result in a longer duration of action versus carboxylic acids, although just as often a corresponding lack of potency is also observed.

When drug substances enter the body, a host of processes take action in order to render these xenobiotics into more polar substrates for elimination. While both carboxylic acids and tetrazoles may act as ligand binding functionality for CYP450-derived oxidative metabolic processes, tetrazoles may exhibit an advantage over carboxylic acids in terms of escaping most biotransformations by Phase II (a.k.a. conjugation) reaction pathways. Benzoic acid substrates often undergo covalent bond formation with transferase enzymes such as Coenzyme-A to form activated acyl (thio)esters, which then undergo subsequent conjugation transformations by a variety of pathways.[8] However, the analogous activation process does not occur with aromatic or aliphatic tetrazoles,[7] and so this moiety will not undergo glycine conjugation, incorporation into lipids, or degradation by β-oxidation.[27]

On the other hand, tetrazolic acids have been shown to undergo conjugation reactions to form β-*N*-glucuronides, a metabolic fate that often befalls aliphatic carboxylic acids to form *O*-β-glucuronic acid conjugates (Fig. 3).[28] Glucuronidation of xenobiotics is an important pathway for the biological clearance of drug compounds, and involves the transfer of the glucuronic acid functionality of the cofactor uridine-5′-diphospho-α-D-glucuronic acid (UDPGA) to the nucleophilic atom of a substrate (e.g., carboxylate or tetrazolic anion). This transformation is mediated by an isoform of the enzyme UDP-glucuronosyltransferase (UDPGT), and the resultant inversion of the α-stereochemistry at the pyranose anomeric center by a nucleophile results in a β-product.[8] Both tetrazole tautomers may serve as substrates for *N*-glucuronidation, and indeed both structural variations are known. In 1980, Nohara identified the first tetrazole N1 glucuronide **8** in the urine stream of several animals orally dosed with a chromone-derived tetrazole,[29] which was identified as the exclusive isomer by synthesis and NMR studies. Several more recent studies have shown that the N2-product **7** is the preferred metabolite of biphenyltetrazole substrates, as determined by NMR and X-ray crystal structures,[30,31] and the N2-glucuronide of an aliphatic drug candidate has also recently been reported.[32] Some authors have attributed the long half-life of a number of orally administered tetrazolic acid drugs to enterohepatic recirculation mechanisms.[31] While *N*-glucuronide formation and subsequent biliary excretion of a tetrazolic acid may remove the drug from circulation, reabsorption of the metabolite may result in hydrolysis by microflora in the intestinal mucosa, thereby allowing additional assimilation of the parent drug in a second pass. Indeed similar reprocessing phenomena have long been implicated as a mechanism for unexpectedly long drug half-lives of other drug substances.

Tetrazole compounds which also contain an additional basic functionality in the molecule may exist as zwitterions, which can result in poor absorption properties for a potential drug candidate. In some cases a prodrug approach has been developed, similar to the strategy developed for carboxylic acids to enhance oral bioavailability.[33] Derivatization of polar molecules into compounds in which the acidic tetrazole N–H bond has been masked (protected by a moiety that can be removed under physiological conditions) results in a more lipophilic molecule of neutral charge that can exhibit greater biomembrane transport ability. This tactic has been used to improve the physicochemical

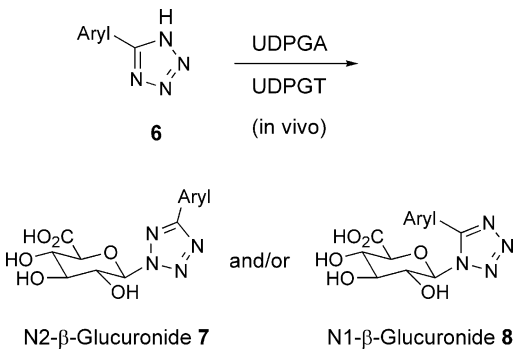


Figure 3. *N*-Glucuronidation is the major metabolic pathway for physiological clearance of aryl tetrazolic acids.

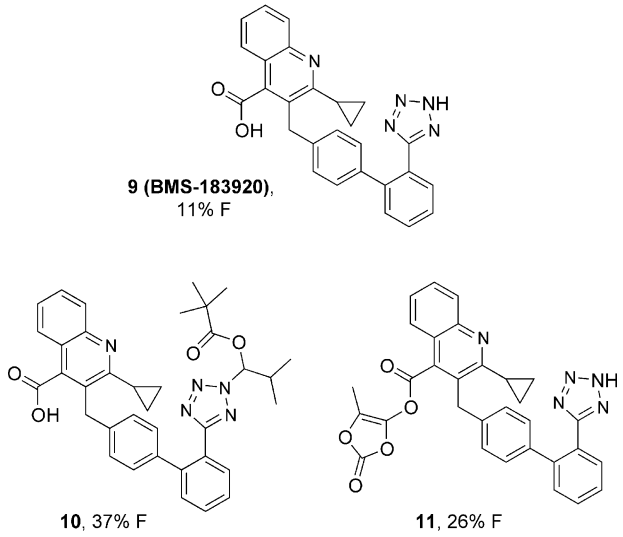


Figure 4. A tetrazole prodrug approach to mask BMS-183920 (**9**) as **10** increased bioavailability (% F) by better than 3-fold.

properties of the angiotensin II receptor antagonist BMS-183920 (diacidic structure **9**) as the prodrug **10** (Fig. 4).[34] By *N*-'bioreversible' protection of the poorly absorbed tetrazole with a pivaloylisobutyl moiety, the bioavailability in rats was increased from 11% for **9** to 37% for **10**. Interestingly, prodrug protection of the carboxylic acid instead of the tetrazole moiety (**11**) did not increase oral availability to better than 26%.

A word of caution: the pharmacological effects of bioisosteric replacement of carboxylates with tetrazoles in a potential drug candidate are not necessarily predictable, as the wealth of medicinal chemistry literature points out. In fact, diverse examples from the literature show that the pharmacological effects can be enhanced, reduced or eliminated completely when compared to carboxylic acid analogues. The next section will showcase a few examples in which the application of the surrogate strategy has advanced research toward both aromatic and aliphatic tetrazole-containing commercial drugs and drug contenders.

Before moving onto some case histories, it is also worth noting that an emerging field of research has begun to accumulate evidence that 1,5-disubstituted tetrazoles are effective bioisosteres for *cis*-amide bonds in peptidomimetics (Fig. 5). Marshall and Zabrocki have shown that peptides which contain a 1,5-disubstituted tetrazole unit, as in **13**, may be effective conformational mimics for the corresponding peptides that prefer to adopt a *cis*-amide bond conformation, or which need to preorganize the amide bonds to act as enzyme substrates, as in **12**.[35,36] A synthetic probe of this type can be important when investigating the role of peptide bond *cis–trans* isomerism in the geometry of molecular recognition. Through synthesis and conformational study of an analogue of bradykinin, it was shown that peptides containing a tetrazole in place of an amide bond were able to adopt most of the conformations available to the parent compound.[35,36] This applies to peptides that contain a free N–H bond, as well as for

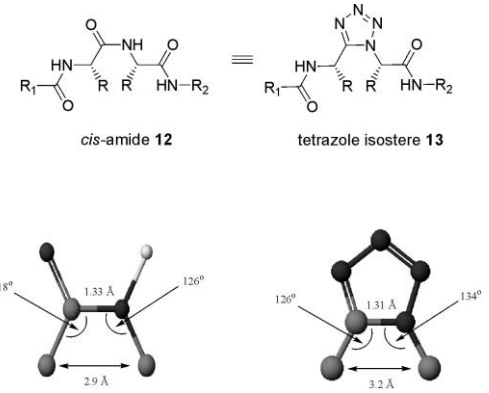


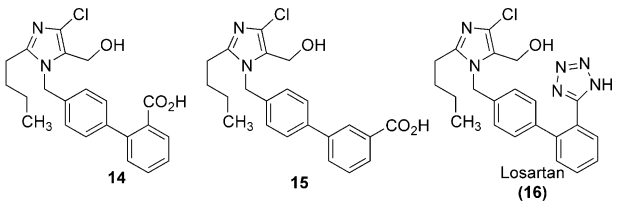
Figure 5. 1,5-Disubstituted tetrazoles as *cis*-amide surrogates.

N-methyl amino acids. In a recent report, Rodziewicz-Motowidlo and coworkers conducted a two-dimensional NMR study of the conformational constraint imparted to scyliorhinin I when a 1,5-tetrazole ring was introduced between positions 7 and 8.[37] Much more can be said of this interesting field of study, and readers should refer to the citations listed here.

Three Medicinal Chemistry Case Histories

The following examples were taken from the literature to represent the various classes of aryl and aliphatic tetrazole-containing analogues that emerged from research efforts. Often clinically advanced or commercial tetrazolic acid drugs were identified as isosteres prepared to investigate the binding energy of carboxylic acid lead compounds. In addition to these examples, several reviews have appeared which evaluate tetrazolic acid by disease state in tabular formats.[1–5] Readers are also encouraged to peruse publications of other current research efforts[38] as well as to review some lead articles for the preparation of tetrazole analogues of amino acids and peptides.[39]

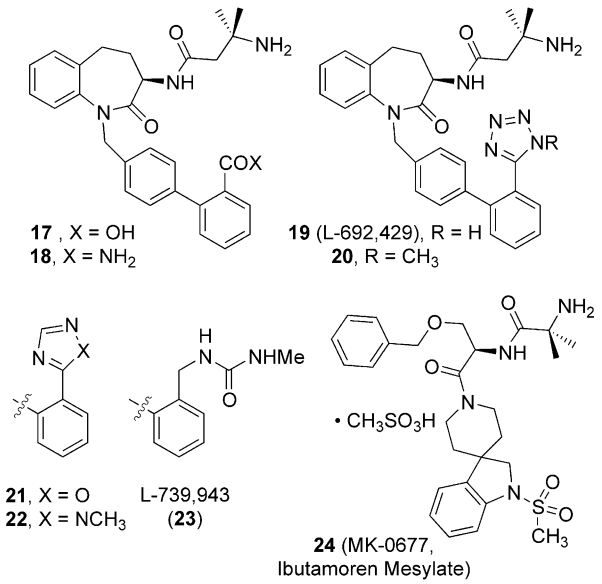
A comprehensive search of the patent literature shows that the majority of tetrazolic acid-based drug substances are aryl tetrazoles. In fact, a great part of these structures contain the biphenyl tetrazole motif, many of which are structural derivations of DuPont's non-peptidic selective angiotensin II receptor antagonist Losartan (**16**, Fig. 6), a drug launched in 1994 to treat



	14	15	Losartan (16)
IC_{50}	0.23 μM	0.49 μM	0.02 μM
EC_{30} iv	3 mg/kg	45 mg/kg	0.8 mg/kg
EC_{30} po	11 mg/kg	(inactive)	0.6 mg/kg
pKa	4.5	4.5	5.0
logP	1.2	1.4	4.5

Figure 6. Comparison of Losartan (**16**) data with early 2- and 3-carboxybiphenyl analogue leads.

hypertension.[40–42] While investigating a new series of analogues derived from a biphenyl scaffold, it was found that compound isomers **14** and **15** were both active by intravenous injection into renal hypertensive rats. Unfortunately, the effect was minimized upon oral administration. In an effort by the research team to find compounds of greater potency and bioavailability, a series of carboxylic acid isosteres were prepared. Interestingly, no carboxamide or sulfonamide compounds were found to improve the oral activity, but when tetrazole was introduced at the C2-position, a dramatic enhancement in binding affinity and oral potency were observed. The authors felt that the increase in receptor binding was due to the greater ability of the heterocycle to distribute a negative charge at physiological pH, allowing for better interaction (vs carboxylate) with the positive charge at the receptor.[23a] This early hypothesis has more recently been borne out by conformational analysis utilizing theoretical calculations and NMR spectroscopy.[43] As well, the longer spatial distance of the N–H bond into the receptor may be the optimal depth for receptor binding. Better oral bioavailability (33% for Losartan) may be due to the greater lipophilicity of tetrazole **16** versus **14** and **15**, a property which can be evaluated by a comparison of log *P* values. The major metabolite of Losartan has been identified as the N2-glucuronide,[30] which has also been implicated in the long duration of action, perhaps by an enterohepatic reprocessing mechanism.[31] Since the introduction of Losartan to the literature, a great number of papers have been published regarding potential analogues of Losartan (**16**), as well as a variety of other biphenyl tetrazolic acid structures for other indications.[23a,44]



	17*	18	19	20	21
ED_{50}	3,000 nM	80 nM	60 nM	500 nM	N/A
EC_{50}	N/A	N/A	125 nM	N/A	30 nM
%F	N/A	N/A	2%	N/A	N/A

	22	23	24	GHRP-6
ED_{50}	N/A	1 nM	1 nM	10 nM
EC_{50}	265 nM	N/A	1 nM	10 nM
%F	N/A	24%	>60%	<1%

*racemic compound

Figure 7. The role of L-692,429 (**19**) as a mid-stream success early in the development of MK-0677 (**24**).

An interesting tetrazole semi-success story can be told about Merck & Company's non-peptidyl growth hormone secretagogue L-692,429 (**19**, Fig. 7). In 1988, a program was started to identify small molecule peptidomimetics of the growth hormone releasing hexapeptide His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 (GHRP-6), from which the biphenyltetrazole **17** was designated as a lead compound.[45] Replacement of the 2′-carboxylic acid group with a series of isosteric replacements singled out primary carboxamide **18** and tetrazole **19** (L-692,429), which provided an increased in vitro potency from the micromolar range to low nanomolar concentrations. On the other hand, acidic isosteres such as sulfonamides and acyl sulfonamides were found to be only micromolar in potency.[46] At this point L-692,429 (**19**) was nominated for participation in clinical trial studies, and has progressed as far as Phase I.[47] (Note: ED_{50} is defined as the effective dose at which a 50% maximal growth hormone response was achieved in vitro. EC_{50} is defined as the effective concentration at which 50% of maximal growth hormone release was induced.) A short time later, SAR studies conducted by chemists at Novo-Nordisk determined that other heterocycles without an acidic functionality were even more potent than **19** (e.g., **21**, **22** and *N*-methyltetrazole **20**), leading the researchers to conclude that the relevant ionic interaction with the receptor involved a hydrogen bond acceptor functionality on the drug molecule.[48] This makes intuitive sense that tetrazole **19**, being slightly less acidic than carboxylic functionality of **17**, would conversely be a better hydrogen bond acceptor. It was around this time that Merck researchers became aware of some serious problems with the oral bioavailability of candidate **19**, which was determined to be about 2% as studied in beagle dogs.[49] The acidic tetrazolic functionality in presence of a basic primary amine causes **19** to be zwitterionic in nature, resulting in poor oral absorption properties that contribute to low oral efficacy. Based on this complicating factor, ongoing work to develop appropriate functional group compatibility had concurrently identified the *N*-methylurea candidate **23** (L-739,943). This neutral compound was even more potent at 1 nM (GHRP-6 has a potency of 10 nM) and was found to have a greatly enhanced oral bioavailability of 24% (hexapeptide GHPR-6 has an availability by oral dose at less than 1%).[50] Ultimately, however, Merck has progressed the candidate MK-0677 (**24**; L-163,191; Ibutamoren Mesylate) into Phase II clinical studies for the treatment of growth hormone deficiency.[51] Based on the 'privileged structure' approach[52] to discover leads for G-protein coupled receptors, the researchers grafted the spiroindane moiety onto the peptide portion of **19**, resulting in the potent growth hormone secretagogue **24**. Oral dosing with MK-0677 was shown to elevate levels of growth hormone in dogs as low as 0.125 mg/kg, and its oral bioavailability was

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