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Tetrazolone as an acid bioisostere: application to marketed drugs containing a carboxylic acid†‡

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Matched molecular pair analysis was used to evaluate the ability of a tetrazolone group to act as a bioisostere of a carboxylic acid. Compound **7**, a tetrazolone of the anti-hypertensive drug, telmisartan **6**, was shown to be a potent AT_1 antagonist (K_b = 0.14 nM), with activity comparable to telmisartan itself (K_b = 0.44 nM). Additionally, compound **9**, a tetrazolone congener of the marketed anti-cancer agent, bexarotene **8**, was shown to be an agonist at the retinoid X receptor alpha (EC_{50} = 64 nM). Compounds containing a tetrazolone group showed similar microsomal stability and plasma protein binding to marketed acid counterparts, while also reducing the value for clog *P*. Furthermore, compound **7** displayed an improved rat pharmacokinetic profile *cf*. telmisartan **6**. Taken together, the results demonstrate that a tetrazolone group may serve as a bioisostere for a carboxylic acid.

Drugs containing a carboxylic acid (RCO_2H), comprise a significant number of approved medications, and are active against a diverse-range of biological targets.[1] The importance of carboxylic acids within medicinal chemistry has led to the identification of alternative motifs that may be similarly active, thereby acting as carboxylic acid bioisosteres.[2] Such bioisosteres comprise both "classical" and "non-classical" groups. For example, classical acid bioisosteres include sulfonic acids, phosphonic acids, and sulfonamides, whereas, non-classical acid bioisosteres include small heterocycles, such as tetrazoles, oxadiazolones, isoxazolones, and cyclic sulfonimidamides.[2–4] Mono-substituted 1,4-dihydro-5*H*-tetrazol-5-ones (tetrazolones) are another potential non-classical bioisostere of a carboxylic acid. However, the use of a tetrazolone group has received little attention from the medicinal chemistry community. For example, only one paper has described an acid-to-tetrazolone switch in any detail.[5] Although pK_a and log *P* data were consistent with an ability to act as an acid bioisostere, tetrazolone **1** was not active when assessed for an ability to lower blood glucose in db/db mice (Fig. 1).[5] An under-utilization of tetrazolones within medicinal chemistry is unfortunate, given the structural similarity to the well-known tetrazole group. Additionally, the presence of a disubstituted tetrazolone in the marketed analgesic, alfentanil **2**,[6] and advanced preclinical candidate L-770,644 **3**,[7] suggest that the tetrazolone motif may have wider potential than is currently recognized. As an extension to our study of small substituents within medicinal chemistry,[8] and as a complement to our use of disubstituted tetrazolones as kinase inhibitors,[8e–g,9] we sought to explore the potential of mono-substituted tetrazolones in further detail. In this paper, we demonstrate that a tetrazolone group can be successfully employed as a carboxylic acid bioisostere.[10] Additionally, we show that tetrazolones have similar *in vitro* and *in vivo* pharmacokinetic characteristics to their acid counterparts, while reducing the clog *P*. Together with our accompanying paper describing a one-pot synthesis of tetrazolones from fully-functionalized precursors,[10a,c] our results indicate that the tetrazolone group is an attractive motif for medicinal chemists.

We began our examination of the tetrazolone group as a potential acid bioisostere by undertaking a late-stage functionalization of acid precursors. For our studies, we chose indomethacin **4**, telmisartan **6** and bexarotene **8** as representative examples of bioactive acids (Fig. 2). As reported in an accompanying patent and paper, the acids could be converted in to tetrazolone products, in moderate-to-excellent yield, by a one-pot procedure.[10a,c] We then used a matched molecular pair analysis to compare the tetrazolone products **5** (R007), **7** (R000) and **9** (R006) against their marketed acid counterparts **4**, **6** and **8** (Table 1).[11] Compound **5**, a tetrazolone of the non-steroidal anti-inflammatory drug indomethacin **4**, was inactive as an inhibitor of cyclooxygenase, with no significant activity at COX-1, when screened at 1 μM (*cf.* indomethacin). Although this result was not encouraging, and illustrates that the success of an acid-to-tetraozolone switch may be non-predictable, and case-dependent, it is important to note that success

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†A paper detailing a one-pot synthesis of tetrazolones from fully-functionalized precursors has been submitted.

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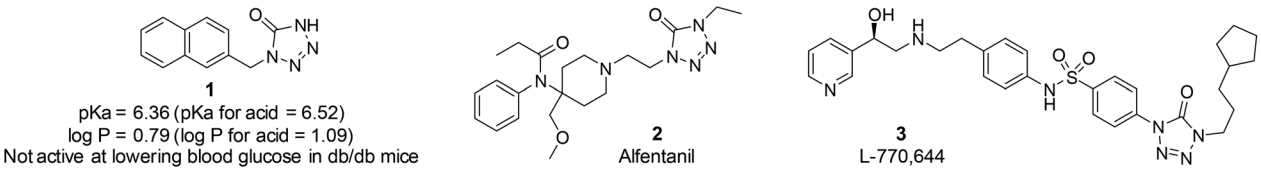

Fig. 1 Non-active glucose-lowering tetrazolone **1**, and disubstituted tetrazolones, alfentanil **2**, and L-770,644 **3**.[5–7]

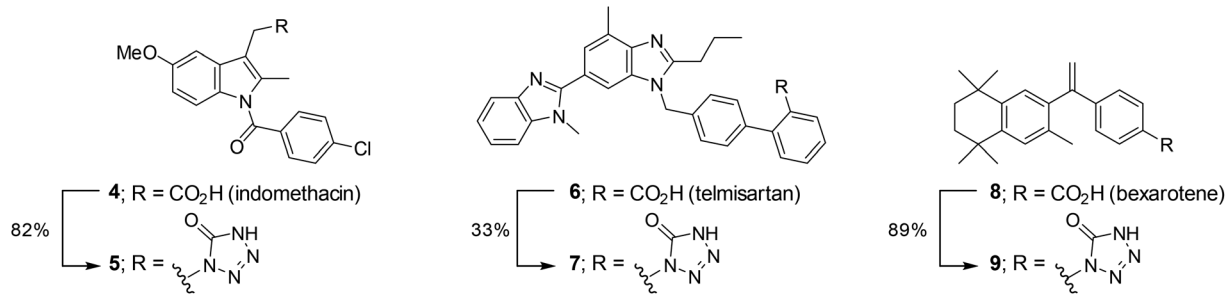

Fig. 2 Marketed drugs containing a carboxylic acid and their tetrazolone counterparts.[10]

Table 1 Biological activity, *in vitro* pharmacokinetic profile, and clog *P*, for marketed acids and tetrazolone congeners

Compound	hCOX-1 (% inhibition at 1 μM)	hAT1 IC_{50} (K_b)	hRXRα EC_{50}	Half-life human liver microsomes	Half-life rat liver microsomes	Binding to human plasma protein	clog P^a
4	88%	—	—	≥45 min	≥45 min	99.7%	4.25 ± 0.80
5	37%	—	—	≥45 min	≥45 min	99.9%	3.85 ± 0.79
6	—	5.7 nM (0.44 nM)	—	≥45 min	≥45 min	99.9%	6.48 ± 1.19
7	—	1.7 nM (0.14 nM)	—	≥45 min	≥45 min	99.9%	6.00 ± 1.17
8	—	—	<10 nM [b]	23 min	≥45 min	99.9%	6.90 ± 0.53
9	—	—	64 nM [b,c]	≥45 min	≥45 min	99.9%	5.90 ± 0.51

[a] clog *P* values calculated using ACD/log *P* version 11.02.[21,22] [b] EC_{50} for fluorobexarotene control = 9 nM. [c] Similar relative efficacy to bexarotene at maximal tested concentrations (see ESI).

with established bioisosteres, are also non-predictable, and dependent on the individual context of use. For example, replacing a carboxylic acid with a non-classical bioisostere, such as a tetrazole ring, does not always result in retention of biological activity,[12] as recent experiences with antagonists of TRPM8 illustrate.[13] We then progressed to evaluate telmisartan **6** and its tetrazolone congener **7**, as inhibitors of AT_1. We were more confident that an acid-to-tetrazolone switch may be tolerated for this class of compound, since AT_1 antagonists containing both acids, and acid bioisosteres, such as tetrazoles, are well-known in the literature.[14,15] Additionally, a knowledge of the binding models for AT_1 antagonists,[16] suggested that a tetrazolone ring could be accommodated within the AT_1 binding pocket. Gratifyingly, compound **7** was shown to be a potent antagonist at the AT_1 receptor, with a K_b value of 0.14 nM (IC_{50} = 1.7 nM). This compared favourably with telmisartan **6** in the same experiment (K_b = 0.44 nM; IC_{50} = 5.7 nM). The results of this evaluation are significant, since they show that a tetrazolone group is capable of functioning as an effective bioisostere for a carboxylic acid. Indeed, compound **7** is one of the more potent AT_1 antagonists described to-date. The results are even more impressive, when it is considered that replacement of the acid in telmisartan, with a tetrazole ring, results in a less-active analog.[17] Thus, for the case of telmisartan, a tetrazolone serves as a more effective bioisostere of a carboxylic acid, than a tetrazole counterpart. Finally, we progressed to examine the anti-cancer agent, bexarotene **8**, and its tetrazolone congener **9**, as RXRα agonists. Tetrazolone **9** was active as an RXRα agonist with an EC_{50} = 64 nM. Although compound **9** was somewhat less active than bexarotene **8** (EC_{50} < 10 nM), both compounds elicited a similar maximal response (relative efficacy). Indeed, the activity observed with compound **9** is encouraging, as an ability of a tetrazolone to replace an acid, for both agonists (RXRα), and antagonists (AT_1), was deemed to be an important milestone for showing a degree of versatility as an acid bioisostere.

We then progressed to examine the pharmacokinetic behaviour of compounds **4** to **9**. The pharmacokinetic qualities of molecules containing a tetrazolone group have been described infrequently. The marketed analgesic, alfentanil **2**, which contains a disubstituted tetrazolone, has a rapid, but short duration of action, with a human half-life of

90–111 min,[18] and a half-life in rat of approximately 23 min.[19] Interestingly, the pharmacokinetic profile of another disubstituted tetrazolone, L-770,644 **3**, was shown to be superior to imidazolidinone, or imidazolone congeners when dosed to dogs.[7] In contrast to their disubstituted counterparts, the pharmacokinetic qualities of mono-substituted tetrazolones have not been reported. Thus, we initiated studies to understand the pharmacokinetic behaviour of the tetrazolones described in Fig. 2. Initially, we examined the microsomal stability of tetrazolone final compounds, together with their marketed acid counterparts. As can also be seen from Table 1, nearly all the compounds showed robust stability with a half-life >45 min when incubated with either human or rat microsomes. This is not a surprising result, given that the starting points for our studies are established drugs that do not undergo extensive metabolism. Nevertheless, the observation that a tetrazolone group did not result in a significant metabolic liability is pleasing. Next, we examined binding to human and rat plasma protein. Again, results between tetrazolones and their parent acids were near identical, with all compounds showing significant binding to plasma proteins. In addition to *in vitro* PK, we also investigated the effect of a tetrazolone on physicochemical properties by calculating the octanol/water partition coefficient (clog *P*) for all compounds in Table 1.[20,21] Replacement of an acid with a tetrazolone group, resulted in a slight reduction to clog *P* of 0.4–1.0 log units.[22] The results of our calculations are also consistent with the observations of a previous study, which showed a reduction in the measured log *P* of approximately 0.4 log units for tetrazolone **1**, when compared with an acid counterpart.[5] Therefore, an acid-to-tetrazolone switch may result in a small deflationary outcome for clog *P*. This finding may be particularly helpful for medicinal chemists looking to optimize a bioactive acid, as replacing the acid with a tetrazolone group may be used to partially-offset any inflation in clog *P* during the optimization process.[23]

Given the favourable biological activity, and attractive *in vitro* pharmacokinetic profile for compound **7**, we also wished to investigate the *in vivo* pharmacokinetic behaviour of this compound (in rats). Again, we would compare results with those observed with the parent acid, telmisartan **6**, in an equivalent experiment. As can be seen from the i.v. pharmacokinetic profile in Table 2, tetrazolone **7** showed lower clearance (Cl), and higher exposure (AUC), when compared to telmisartan **6**. Compound **7** also had a slightly longer half-life than telmisartan (5.4 h for **7** *cf.* 3.6 h for **6**). When administered orally, at 3.5 mg kg^{-1}, tetrazolone **7** showed a significantly higher C_{max} and higher exposure (AUC), than telmisartan **6** dosed at 3.95 mg kg^{-1}. Thus, when normalizing the exposure to take account of the different doses (AUC per dose), the oral exposure for compound **7** was about 1.75 times higher than for telmisartan **6**. The results in Table 2 are encouraging, as they demonstrate that compounds containing a tetrazolone group can exhibit an attractive pharmacokinetic profile.[24] In particular, our data may suggest that replacement of an acid with a tetrazolone group could be a beneficial strategy for reducing clearance. Overall, the *in vivo* pharmacokinetic profile for compound **7** demonstrated the feasibility of an acid-to-tetrazolone bioisosteric replacement strategy.

Table 2 *In vivo* pharmacokinetics for Telmisartan **6**, and its tetrazolone congener **7**, in rat[a]

Compound	**6**	**7**
i.v.		
Dose (mg kg^{-1})	0.79	0.70
Cl (ml min^{-1} kg^{-1})	7.2 ± 1.1	4.5 ± 0.6
$AUC_{0-24\ h}$ (ng ml^{-1} h^{-1})	1830 ± 245	2490 ± 249
V_{ss} (L kg^{-1})	1.6 ± 0.1	1.7 ± 0.2
Half-life (h)	3.6 ± 0.8	5.4 ± 1.6
p.o.		
Dose (mg kg^{-1})	3.95	3.5
C_{max} (ng ml^{-1})	506 ± 158	1330 ± 515
$AUC_{0-24\ h}$ (ng ml^{-1} h^{-1})	5460 ± 1860	8420 ± 1660
F (%)	59	65

[a] Data is the mean value ± standard deviation.

In conclusion, this paper describes the use of a tetrazolone group as a bioisostere of a carboxylic acid. In favourable examples, compounds containing a tetrazolone can improve both the *in vitro* biological activity and *in vivo* pharmacokinetic profile, when compared with a marketed acid counterpart. Similar to other motifs utilized as a bioisostere of a carboxylic acid, the success of an acid-to-tetrazolone switch was found to be case-dependent. Taken with our accompanying paper on the one-pot synthesis of tetrazolones from acid chlorides, this work may help facilitate a wider investigation of the tetrazolone group within medicinal chemistry.

Abbreviations

AT_1	Angiotensin receptor subtype 1
AUC	Area under the curve
Cl	Clearance
clog *P*	Calculated log of the octanol/water partition coefficient
C_{max}	Maximum/peak concentration
COX-1	Cyclooxygenase-1 or prostaglandin-endoperoxide synthase 1
DMPK	Drug metabolism and pharmacokinetics
F	Bioavailability
i.v.	Intravenous
log *P*	Log of the octanol/water partition coefficient
PK	Pharmacokinetics
pK_a	Negative log of acid dissociation constant
p.o.	per os
RXRα	Retinoid X receptor alpha
V_{ss}	Steady state volume of distribution.

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