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Novel *N*-thiazolyl piperazine-1-carboxamide CCR2 antagonists – investigation of an unexpected reaction with glutathione

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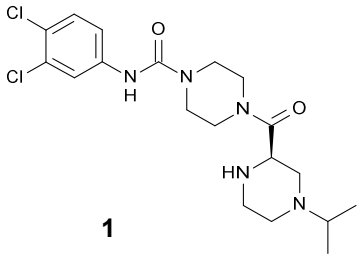
A series of CCR2 antagonists containing halothiazoles were found to undergo an unexpected reaction with glutathione. Experiments revealed the structure-reactivity relationship and calculations suggest that competition between two reactions of a tautomer of the reactant (deprotonation and nucleophilic addition) governs the rate of this reaction.

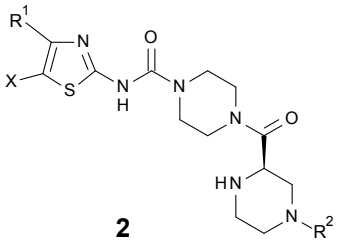
CCR2, the receptor for the chemokine CCL2 (commonly known as MCP-1) is an attractive target for drug discovery because it plays a role in a variety of diseases including inflammatory disease, metabolic disease, pain, COPD and cancer. [1-3] Previously, we reported the discovery of a novel, potent and selective series of orally bioavailable *N*-aryl piperazine-1-carboxamide CCR2 antagonists with very high hERG selectivity, exemplified by compound **1**. [4, 5]



1

Compound **1** had an IC_{50} of 5.8 nM in an assay measuring the inhibition of MCP-1-induced calcium flux mediated by human CCR2 natively expressed in THP-1 cells using FLIPR technology. [4, 5] However, its IC_{50} in a rat version of the assay (using HEK293 cells and mCCL2) was only 0.2 μM which reduced its utility as a tool compound for the study of the effects of CCR2 antagonism in rodent disease models. During the course of SAR studies around this series it was found that the 3,4-dicholorophenyl moiety in **1** could be replaced with 4-substituted-5-chloro-thiazol-2-yl and the terminal *N*-isopropyl could be replaced with *N*-tert-butyl (compounds **2**, Table 1).

Table 1. Human CCR2 mediated Ca^{2+} flux inhibition data and reactivity towards glutathione for selected compounds.



2

Cmpd	X	R^1	R^2	hCCR2 Ca^{2+} flux IC_{50} (nM)[a]	Glutathione reactivity t½ (h)[c]
2a	Cl	H	tBu	7.4[b]	nr
2b	Cl	Me	tBu	0.65	17.7
2c	Cl	Et	iPr	1.7	23.5
2d	Cl	iPr	iPr	1.2	30.4
2e	Cl	cPr	tBu	<0.45	2.8

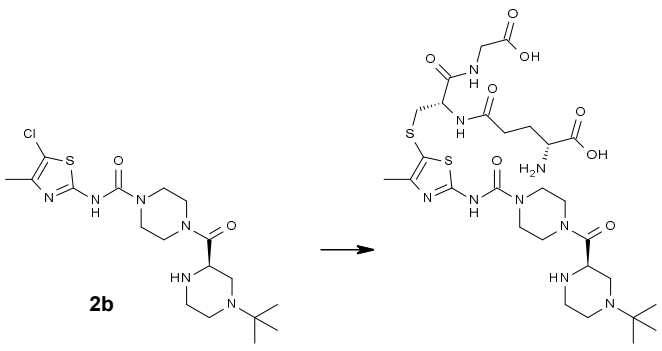
2f	Cl	CF_3	tBu	<0.45	nr
2g	Cl	tBu	iPr	2.3	33.5
2h	H	cPr	tBu	1.1	nr
2i	F	tBu	iPr	2.7	nr
2j	F	H	tBu	9.2	nr
2k	OMe	Me	tBu	41	nr
2l	Br	Me	tBu	<0.45	2.4

[a] IC_{50}s were derived from at least two independent measurements, of which standard errors were normally <5% in a given assay, unless stated otherwise. Assay to assay variability was within 2 fold based on the results of a standard compound. [b]n=1. [c]nr = no reactivity (t½ > 100 h).

The compounds were prepared using previously described synthetic routes. [4, 5] The chiral centre in the *N*-tert butyl analogues was installed with high enantioselectivity using a novel *sec*-butyl lithium/(−)-sparteine-mediated chiral deprotonation of 1-Boc-4-tert-butyl piperazine. [6] The IC_{50} values for inhibition of the calcium flux mediated by human CCR2 in the assay are shown in Table 1. The change from *N*-isopropyl to *N*-tert-butyl unexpectedly resulted in an approximately 10-fold improvement in CCR2 potency with no statistically significant change in hERG inhibition (results not shown). A variety of alkyl substituents could be tolerated in the thiazole 4-position; cyclopropyl and trifluoromethyl were particularly good for potency (**2e**, **2f**), while hydrogen in this position was less favourable (**2a**, **2j**). The 5-chloro could be replaced by fluoro (**2i**, **2j**) or bromo (**2l**) without loss of potency but replacement with methoxy led to a drop in CCR2 antagonism (**2k**).

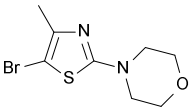
Following further profiling, compound **2b** was selected for an in vivo metabolite identification study in rats. To our surprise, the major metabolite in rat plasma was found to arise from chemical reactivity of **2b** with glutathione. A covalent adduct having a mass consistent with displacement of the chlorine at the thiazole 5-position was detected (Scheme 1).

Scheme 1. Displacement of the chlorine from the thiazole 5-position of **2b** with glutathione



The reaction between **2b** and glutathione was shown to take place in the absence of any metabolising enzymes using a simple in vitro assay originally developed to assess the reactivity of nitrile groups towards glutathione. [7]

Glutathione is a thiol-containing tripeptide, present in cells at concentrations between 1 and 10 mM. [8] It plays a major role in protecting cells from oxidative stress through reaction with electrophilic metabolites. Formation of covalent bonds between potential drug compounds and thiols is undesirable as it may lead to toxicity via oxidative stress due to glutathione depletion or from irreversible binding to proteins or DNA. In order to explore the relationship between thiazole substitution pattern and reactivity towards glutathione, and to help elucidate the reaction mechanism, the thiazole-containing CCR2 antagonists in Table 1 were tested in the in vitro glutathione assay along with probe compounds **3**, **4**, and **5**. The results are given as the half-life for the disappearance of the parent compound (50 μM starting concentration) in the presence of 5 mM glutathione in pH 7.4 phosphate buffer containing 1 mM ethylene diamine tetra-acetic acid at 37°C, as measured by LC-UV. The half-life of the positive control compound *p*-nitrobenzyl chloride was 3.6 h. In addition, the reaction products for compounds **2b**, **2e**, **2l**, and **3** were determined by mass spectrometry.



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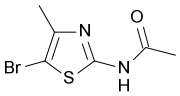

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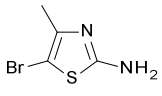


5

The reaction products were found to be solely from direct replacement of the halogen with glutathione for **2b**, **2e**, and **3** (as shown in Scheme 1 for **2b**) but for **2l** this was the minor product, with the major product being the replacement of bromine with hydrogen. This product was also was also detected when **2l** was incubated with cysteine rather than glutathione, although in this case none of the thiol adduct was observed. In contrast, incubation of **2e** with cysteine led to the displacement of the chlorine atom with cysteine. No reaction of **2l** was observed in the absence of thiol. The effect of changing the halogen and the thiazole 4-substituent can be seen by examining the half-lives in Table 1 (the terminal piperazine *N*-substituent is assumed to have no influence on the reaction rate). Keeping the halogen as chlorine it can be seen that the relative order of reaction of the 4-substituent R^1 is cPr >> Me > Et > iPr > tBu, while the compounds where R^1 is H or CF_3 do not react. By comparing **2b** and **2l** it can be seen that Br reacts more quickly than Cl, and by comparing **2g** with **2i** it can be seen that F reacts more slowly than Cl. Finally, comparison between **2l**, **3** (t½ = 8.1 h), **4** (no reaction) and **5** (t½ = 13.3 h) shows that replacing the thiazole-2-urea functionality with a secondary amine results in a small

reduction in reactivity while replacing it with *N*-acetyl results in no reactivity towards glutathione.

The effects of different 2-substituents on the reactivity of 5-halothiazoles towards displacement of the halogen by nucleophiles have been reported in the synthetic chemistry literature. In reaction with 4-chlorobenzenethiolate anion (in ethanol at reflux) 2-amino-4-methyl-5-bromothiazole was much more reactive than 2-acetamido-4-methyl-5-bromothiazole **4**, [9] which is consistent with our results since **4** showed no reaction with glutathione. Neutral chlorobenzenethiols also reacted with 2-amino-4-methyl-5-bromothiazole under these conditions and the rate was reported to be slower. In another study, it was found that the rate of reaction with sodium methoxide in methanol at 50°C was 10,000-fold higher for 2-amino-5-bromothiazole than for 5-bromothiazole, [10] demonstrating the dramatic rate-enhancing effect of the 2-amino group. Neutral piperidine also reacted with 2-amino-5-bromothiazole (in ethanol solvent with a rate around 1000-fold lower than for sodium methoxide). The reaction of 2-amino-5-halothiazoles with sodium methoxide showed second order kinetics and the ratio $k_{5\text{-Br}}/k_{5\text{-Cl}}$ was about 1, suggesting that the breaking of the carbon-halogen bond was not important in the transition state. It was proposed that the reaction proceeds via an imino tautomer with the hydrogen moved to either the C5 or N3 position on the thiazole, although a more detailed reaction mechanism was not suggested.

An explanation for the observed reactivities was sought using quantum mechanical computations. These were carried out at the B3LYP/6-31+G* level incorporating solvation effects for water via the IEFPCM formalism. [11-14] All calculations were performed in Gaussian09 and reported energies are free energies in kcal/mol. [15] The thiolate of glutathione was modelled as the anion of methane thiol; the slower rate of reaction with neutral thiols, mentioned above, [9] is assumed to be caused by the reduced concentration of the thiolate. Model compounds **6** were used in which the remote urea or amide were abbreviated to simplify the calculations. These compounds are referred to throughout as **6(1-10)** where the number in parentheses refers to the entry number in Table 2.

Several mechanistic possibilities for the reaction with thiolate were considered. The first possibility is a direct displacement of the halide involving a transition state (**TS1**) like that shown for **6(2)** in Figure 1. The barrier for this process (Table 2) was computed to be at 39 kcal/mol or higher apart from for one example (entry 6) that corresponds to compound **2f** that is experimentally found to be unreactive. This strongly suggests that this direct mechanism is not responsible for the observed reactivity.

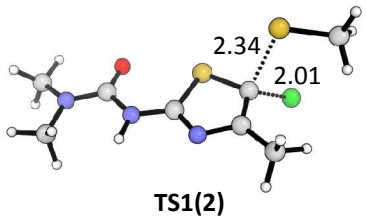
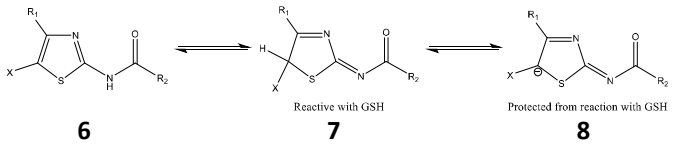


Figure 1. Transition state **TS1(2)** for direct S_N2 reaction of compound **6(2)** with MeS^-

One possibility, highlighted in the literature, is that the system might undergo a tautomerism to **7** (Scheme 2). [10] As detailed below, this was found to be the species that best explains the observed reactivity. However, as with many tautomerisms, the ability to deprotonate either of the tautomers would yield a common anion, which in this case would be unreactive with the nucleophilic GSH. Tautomer **7** is computed to be higher in energy than **6**. For all of the compounds investigated, the relative energy of anion **8** compared to **6** was computed and is also in Table 2. Examples in which the anion is particularly stable (entries 6 and 10) correspond to compounds that are found to be unreactive (**2f** and **4**). The reasons for the relative stability of the anion are revealed when the structures of the reactant in its two tautomers are placed alongside those of the anion in Figure 2. Three key bond lengths are picked out that reflect the general picture: for low energy **6(2)** and anion **8(2)**, the bond lengths suggest a more aromatic character to the ring than for higher energy tautomer **7(2)** in which alternating bond lengths are present. The anion is stabilised by overlap with the aromatic ring as reflected by the shorter exocyclic C-N bond length but this does not disrupt the aromaticity markedly. The strongly electron-withdrawing CF_3 group in entry 6 of Table 2 stabilises the anion **8(6)**.



Scheme 2. Tautomerism and deprotonation of **6**.

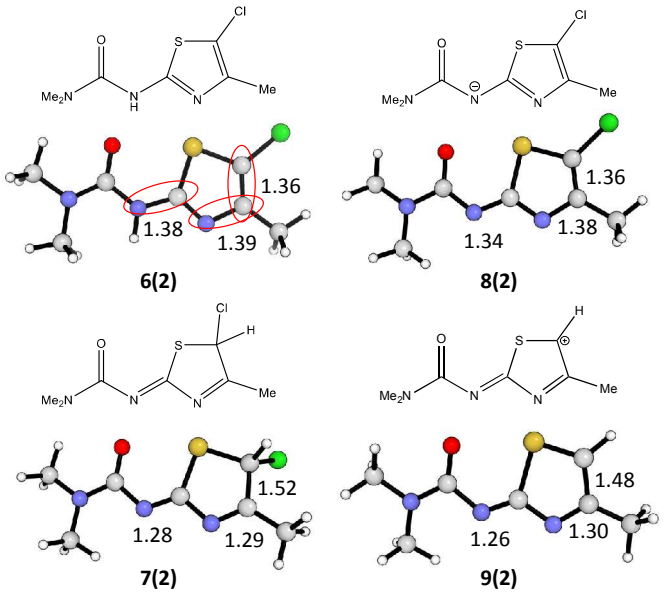
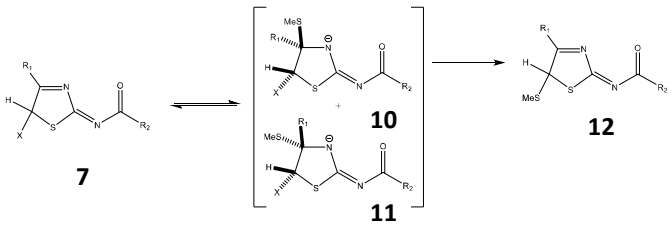


Figure 2. B3LYP/6-31+G* optimized structures of **6(2)**, **7(2)**,**8(2)** and **9(2)**.

The higher energy tautomeric structure **7** can undergo displacement in either an S_N1 or S_N2 fashion. The former requires access to the carbocation **9** and this was found and is shown in Figure 2 for **9(2)**. By contrast with the anion, the cation could be anti-aromatic so adopts a less delocalised structure, rather similar to that of the non-aromatic tautomer **7(2)**. Unsurprisingly, the electron withdrawing groups surrounding the carbocation ensure that, for all the cases examined (Table 2), these are too high in energy to contribute to the observed reactivity. Furthermore, the most stable of these species is **9(7)** for the reaction of the t-Bu substituted example (entry 7) which is inconsistent with the observed rates of reaction.

The S_N2 reaction was found to be a two-step process because the most electrophilic site of **7** is not the carbon attached to the halide but the imine-like carbon next to it. Thus, initial attack is at this position and can lead to one of two stereoisomeric possibilities (**10** or **11**) in which the thiol is attached either syn or anti to the halide (Scheme 3). The isomer **10**, in which the thiol is anti to the leaving group, can undergo a 1,2-migration of the thiol with loss of the halide to yield the tautomeric product, **12**.



Scheme 3. The two step process for the S_N2 reaction of **7**

The lowest energy path for the reaction goes through the intermediacy of the adduct **10**. The rate-limiting step was computed (in most cases) to be the initial addition step, **TS2** (Figure 3). In two cases (entries 7 and 8), the 1,2 shift via **TS3** was found to be the rate-limiting step. In general, the trans-substituted intermediate **10** is the most stable one but entry 7 also sees this reversed. The data in Table 2 combine to suggest that it is the combination of two considerations that govern the reactivity of these compounds with glutathione: the propensity of the imine tautomer **7** to deprotonate or else to undergo nucleophilic addition. This can be summarised as the energy difference between anion **8** and whichever of **TS2** and **TS3** is highest in energy, referred to as the computed barrier. These values are plotted against the corresponding half-lives in Figure 4 As can be seen in Figure 4, this combination of anion stability and ability to undergo nucleophilic attack conspire to cause **2l** and **2e** to be particularly reactive with glutathione. The reduced reactivity of **6(1)** and **6(10)** can be understood by considering the imine group in tautomer **7**, which resembles **TS2** and **TS3**. When the electron-donating alkyl groups that stabilise the imine are removed (as in entry 1), **TS2** and **TS3** are destabilised. When the strongly electron-withdrawing amide replaces the urea, as in entry 10, a similar effect occurs.

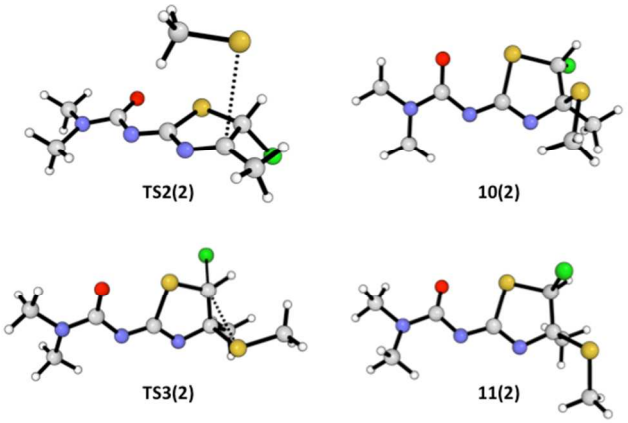


Figure 3. B3LYP/6-31+G* optimized structures of **TS2(2)**, **TS3(2)**, **10(2)** and **11(2)**.

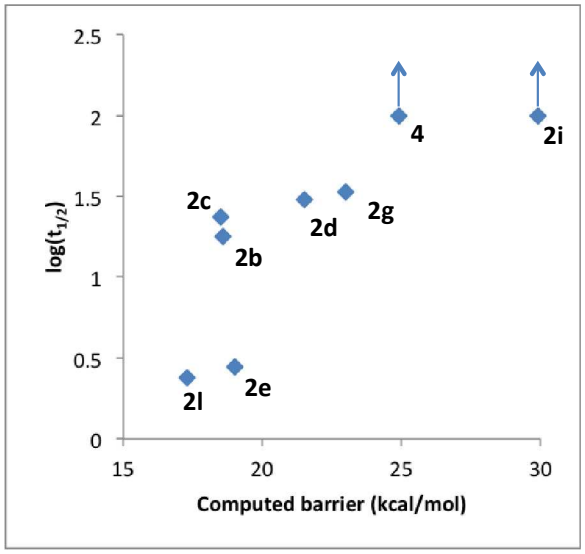
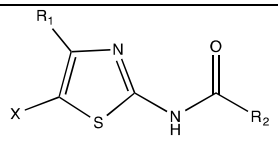


Figure 4. Measured half-lives in hours (as log(t1/2)) plotted against the difference in energy between the highest of **TS2** or **TS3** and anion **8** (computed barrier) in kcal/mol. Compounds **2i** and **4** half lives beyond the limit of the assay (100 hours).

Table 2. Computed energies of species involved in the reactivity of halothiazoles with methane thiolate. Energies are in kcal/mol relative to reactant **6**.

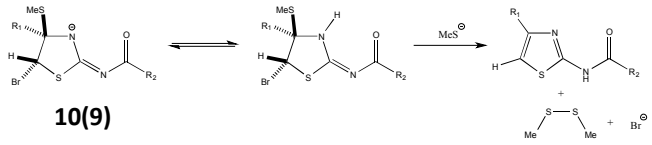


6

Entry	Compound being modelled	R_1	R_2	X	Direct S_N2 **TS1**	Tautomer **7**	Anion **8**	Cation **9**	Addition TS **TS2**	Trans intermediate **10**	Cis intermediate **11**	1,2-shift **TS3**
1	**2a**	H	NMe_2	Cl	+39.2	+8.7	-4.0	+31.6	-	+2.6	+5.4	-
2	**2b**	Me	NMe_2	Cl	+41.3	+5.2	-3.7	+26.2	+14.9	+8.9	+12.6	+12.7
3	**2c**	Et	NMe_2	Cl	+42.0	+5.3	-3.0	+26.8	+15.5	+13.3	+14.5	+14.1
4	**2d**	iPr	NMe_2	Cl	+43.4	+3.1	-3.4	+23.5	+18.1	+13.5	+14.9	+17.2
5	**2e**	cPr	NMe_2	Cl	+41.2	+3.3	-3.6	+24.0	+15.4	+11.5	+14.4	+14.6
6	**2f**	CF_3	NMe_2	Cl	+29.6	+13.2	-7.8	+39.1	-	+6.0	+6.8	-
7	**2g**	tBu	NMe_2	Cl	+41.4	+4.6	-2.9	+21.6	+17.7	+15.8	+15.3	+20.1
8	**2i**	tBu	NMe_2	F	+39.7	+2.6	-3.1	+41.6	+15.5	+10.1	+11.5	+26.8
9	**2l**	Me	NMe_2	Br	{+39.2}[a]	+3.3	-3.4	+20.7	+13.9	+9.0	+12.1	+8.1
10	**4**	Me	Me	Br	+38.7	+10.6	-5.1	+35.7	+19.8	+9.4	+12.1	+13.9

[a]Geometry optimisation repeatedly failed; this value is an estimate based on the lowest energy structure obtained.

The final observation requiring explanation is that compound **2l** does not yield an equivalent product to that seen with the other compounds. Our calculations suggest that this compound should react faster than any others, as is observed experimentally. Compound **2l** differs from **2b** by having bromine instead of chlorine such that any difference in reactivity must arise before the halide has left. Our investigations have found that an alternative reactivity for adduct **10(9)** is that rather than rearranging, it could instead pick up a proton from water. The pK_a describing this is challenging to compute but despite being delocalised, the anion in **10(9)** is likely very basic such that protonation is competitive even with the low barrier to rearrangement computed for **10(9)**. Subsequent to protonation, reaction with another molecule of glutathione is possible via a nucleophilic attack at sulphur, with the thiazole and bromide as leaving groups (Scheme 4). This step regenerates the aromaticity of the thiazole and generates disulphide but requires a good leaving group. Uncertainties in the calculation of pK_as makes the exact energy of the transition states difficult to be computed but this process has a barrier that is 2.4 kcal/mol lower in free energy for **10(9)** than for **10(2)** suggesting that it will be significantly faster for the bromine containing compound. The energies calculated are consistent with a barrier that favours this reaction for **10(9)** but which would favour the rearrangement leading to the thiol containing product for **10(2)**. The transition state for this process, **TS4(9),** features the expected antiperiplanar arrangement of each reaction component (Figure 5).



Scheme 4. The process leading to hydrogen substituted thiazole from **10(9)**.

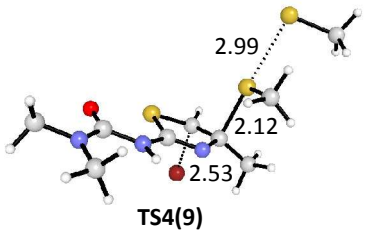


Figure 5. B3LYP/6-31+G* optimized structures of **TS4(9)**.

The most potent compound that did not show reactivity towards glutathione was **2f**. Further profiling of this compound showed it to be an extremely potent CCR2 antagonist with IC_{50}s in the human and rat calcium flux assays of 0.13 ± 0.07 (n = 8) and 1.3 ± 0.2 nM (n = 5) respectively. As well as very high rat CCR2 potency, **2f** (also known as AZ889) also has good pharmacokinetic properties including CNS penetration, making it suitable as a tool compound for the study of the role of CCR2 in disease models. Studies demonstrating the role of the CCR2/CCL-2 system in several rat models of neuropathic pain using AZ889 have been published. [16]

Conclusions

Certain aminohalothiazoles undergo a reaction with glutathione. The reaction is computed to proceed via a tautomer that undergoes a two step addition-1,2-migration process. Competition between deprotonation and nucleophilic addition explains the observed structure-reactivity relationship and products. Compound **2f** containing 4-trifluoromethyl-5-chloro-thiazole does not react with glutathione and is a highly potent antagonist of rat and human CCR2.

Acknowledgments

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Notes and references

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/c000000x/

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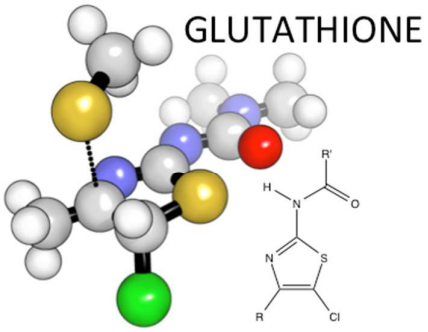
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