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Synthesis of tetrazole- and imidazole-based compounds: prophetic molecules made real for biological studies

Valentina Pirota, Giovanni D'Acerno, and Paolo Quadrelli*

University of Pavia, Department of Chemistry, Viale Taramelli 12, 27100, Pavia, Italy
Email: paolo.quadrelli@unipv.it

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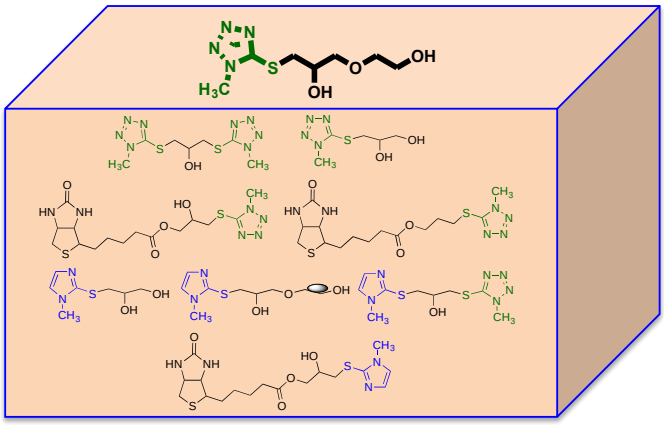
Abstract

The synthesis of tetrazole- and imidazole-based derivatives has been achieved via sulfur nucleophilic ring-opening of 2-oxiranyl-alcohols or chlorides. The derivatives obtained may represent interesting new chemical tools to investigate biological functions and in particular the mitochondrial molecular chaperone TRAP1. The results are discussed in the light of the availability of these molecules according to the proposed synthetic procedures.



Heterocyclic Box

Keywords: Tetrazole, imidazole, biotin, heterocyclic derivatives, prophetic molecules, epoxides

Introduction

Investigation of key biological mechanisms represents one of the modern applications of organic synthesis. Despite the central role of many biological systems, many details of the molecular mechanisms at the basis of specific functions are still unknown or at least uncertain. The reason for this is partially attributed to the lack of chemical tools able to interact specifically with a select portion of a cellular ambient and induce measurable functional perturbations. In this context, the use of small molecules as modulators of protein functions was proven to be a viable investigation methodology for a number of biomolecular systems.[1] Recently, we came in contact with the research focused on the HSP90 family of molecular chaperones, fundamental regulators of key biological mechanisms, such as cell survival, development, and metabolic adaptations to environmental changes, as targets for the discovery of new molecular entities that modulate their functions, interactions and signaling pathways. There are four isoforms of HSP90 in humans, namely HSP90alpha and beta in the cytosol, GRP94 in the ER and TRAP1 in mitochondria. The design and synthesis of small-molecules targeting the chaperone cycle of HSP90 and able to inhibit or stimulate the activity of the protein can open the way to finely dissect their biochemical activities; newly synthesized lead compounds can indeed become the core for mechanism-based drugs.[2]

In this context, we previously described a rational design of allosteric and selective inhibitors of the molecular chaperone TRAP1. Its activity as energy metabolism regulator has important implications in cancer, neurodegeneration, and ischemia. Selective inhibitors of TRAP1 could inform on its mechanisms of action and set the stage for further targeted drug development. In this framework, computational dynamics-based approaches helped identify a TRAP1 allosteric pocket distal to its active site that can host drug-like molecules.[3] Hence, small molecules with optimal stereoelectronic features to target the pocket were selected.

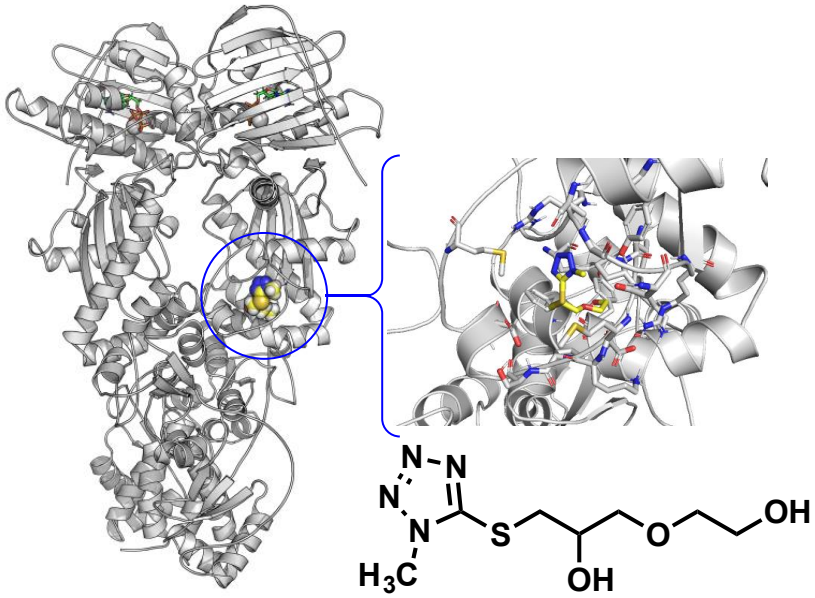


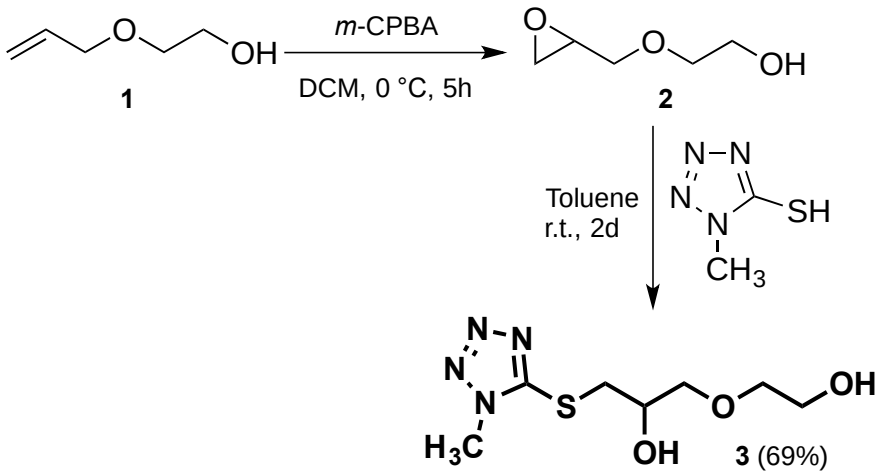
Figure 1. Structure of the 1-(2-hydroxyethoxy)-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]propan-2-ol and TRAP1 with the tetrazole derivative. TRAP1 monomers are shown in light gray; the ligand is depicted with C atoms in yellow. The inset shows the details of interactions in the complex.

Among the tested compounds, some tetrazole-based derivatives were also investigated for their activity in the inhibition of the formation of cancer foci and in particular 1-(2-hydroxyethoxy)-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]propan-2-ol (Figure 1, CAS 392707-61-8) was found to push the protein toward catalytically non-optimal sub-states.

These results define a link between the interference with TRAP1 enzymatic ATPase activity and the perturbation of its cell functions. On this basis, we launched a synthetic plan aiming to expand the diversity of tetrazole derivatives, generating new molecules, also containing a biotin moiety or different functionalizations for *in vivo* investigations of TRAP1 inhibition. Moreover, in view of potential structure-activity relationship (SAR) studies, imidazole derivatives were also prepared taking advantage of a commercially available drug scaffold.

Results and Discussion

In spite of the fact that 1-(2-hydroxyethoxy)-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]propan-2-ol (**3**) is a compound reported in chemical data bases, a CAS number has been attributed, and it has been made available for testing, to the best of our knowledge an easily accessible synthetic route has not been reported. It is a so-called "prophetic molecule". Its synthetic pathway is reported here (Scheme 1) and the experimental details given in the proper section of this paper to give a definitive synthetic frame to this molecule. Importantly, the chosen approach represents a key original strategy to a variety of tetrazole derivatives, as it will be demonstrated.



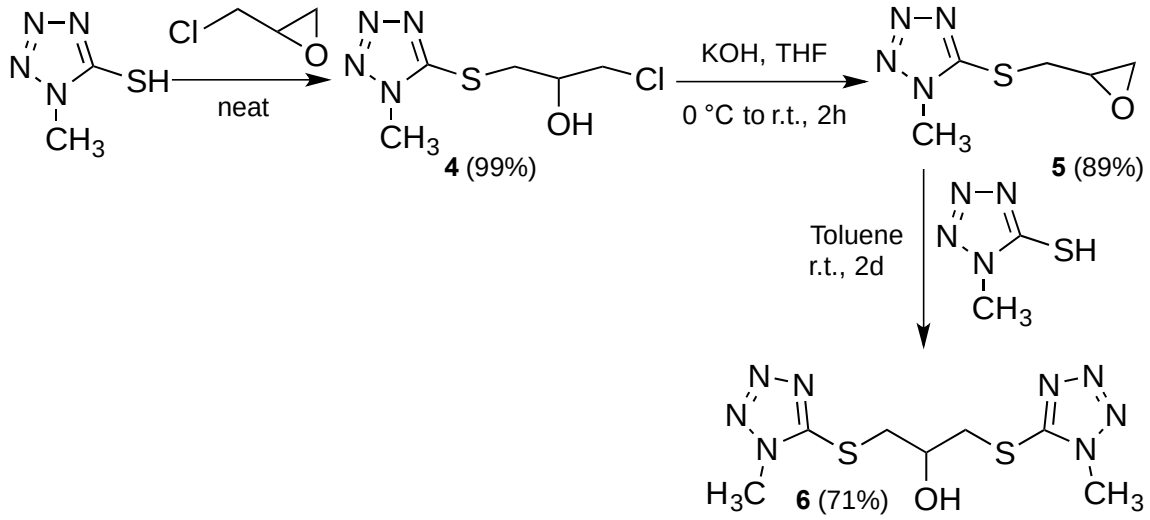
Scheme 1. Synthetic pathway to the 1-(2-hydroxyethoxy)-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]propan-2-ol (**3**).

2-Allyloxy-ethanol (**1**) was converted into the corresponding epoxide through typical *m*-CPBA epoxidation protocol[4] under standard conditions to afford the desired product **2** in good yields (*ca.* 50%). Careful purification by bicarbonate treatment in concentrated water solution was used to remove the *m*-chlorobenzoic acid. The epoxide **2** was then dissolved in toluene and the commercially available solid 1-methyl-1*H*-tetrazole-5-thiol was added and the solution left to stir at room temperature for 2 days. Toluene was then evaporated to dryness and the crude product taken with chloroform for a final washing with bicarbonate and brine. The organic phase was dried (Na_2SO_4) and the crude product **3**, obtained quantitatively

(in the crude) in the racemic form, was furtherly purified and fully characterized. In particular, in the ^{1}H NMR (DMSO-d_6) the methyl group at the tetrazole ring was found at δ_H 3.93 as a singlet while the methylene chain gave a group of signals centered at δ_H 3.46 as multiplets. The structure was also corroborated by ^{13}C NMR spectra with DEPT experiment, clarifying the type of carbon atoms contained in the structure.

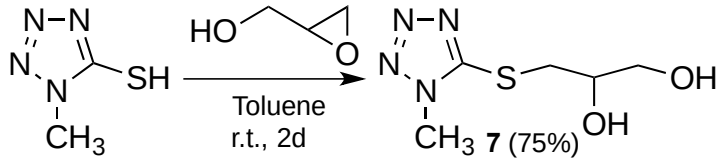
Compound **3** belong to the β-hydroxy sulfides family easily obtainable from sulfide-ring opening of epoxides. The β-hydroxy sulfides are an important class of organo sulfur compounds that have a key role in the synthesis of bioactive compounds. The thiolysis of epoxides is the most common and best route for the synthesis of β-hydroxy sulfides. During the last decade, the applications of this strategy, under diverse experimental conditions for the regioselective ring-opening reactions of epoxides with thiols, were studied by various research groups. A number of reviews focused on the important achievements reported in the literature for the thiolysis of epoxides.[5]

Given this background, a valuable confirmation of the synthetic method comes from the synthesis of the symmetric 1,3-bis[(1-methyl-1*H*-tetrazol-5-yl)thio]propan-2-ol (**6**) (Scheme 2). The 1-methyl-1*H*-tetrazole-5-thiol was derivatized with epichlorohydrin (neat) to afford quantitatively compound **4** as low-melting solid, fully characterized. Significantly, in the ^{1}H NMR (DMSO-d_6) spectrum, the AB system of one of the methylene groups is found at δ_H 3.56 while the second methylene clusters at δ_H 3.70. Standard treatment with KOH in THF as the solvent allowed to cyclize to the epoxide **5**; the ^{1}H NMR spectrum in fact clearly showed the presence of the epoxidic ring proton signals at δ_H 2.67 and 2.82. The epoxide **5** was then opened by further treatment with the same 1-methyl-1*H*-tetrazole-5-thiol under the previously set-up experimental conditions to afford quantitatively the expected symmetric bisheterocycles **6**. The product was fully characterized and the ^{1}H NMR spectrum (DMSO-d_6) showed half of the expected signals due to the symmetry of the molecule; in particular, the AB system is found at δ_H 3.53, coupled with the CH found at δ_H 3.94.



Scheme 2. Synthesis of 1,3-bis[(1-methyl-1*H*-tetrazol-5-yl)thio]propan-2-ol (**6**).

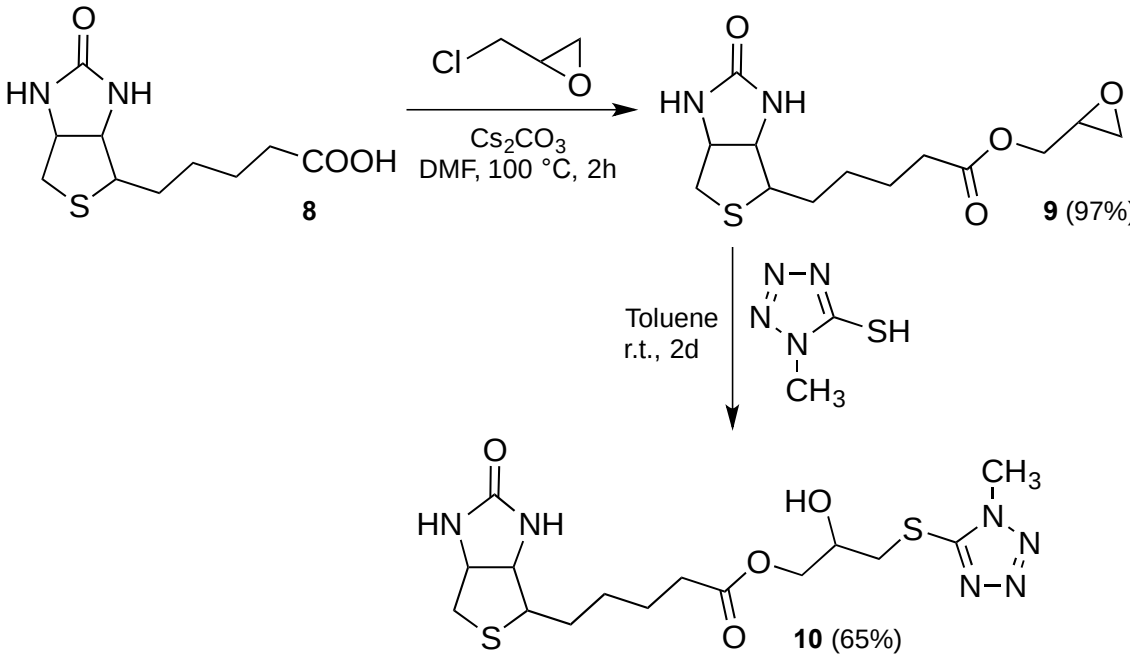
The same method used to prepare compounds **3**, **4** and **6**, find application with glycidol in the reaction with the 1-methyl-1*H*-tetrazole-5-thiol (Scheme 3) to afford compound **7** in quantitative yield. The characterization of **7** revealed the presence of the diol fragment linked to the heterocycle through a sulfur bridge.



Scheme 3. Synthesis of 3-[(1-methyl-1*H*-tetrazol-5-yl)thio]propane-1,2-diol (**7**).

The same synthetic strategy, standing on the mercapto-ring opening of the epoxide ring, was successfully applied to the synthesis of another tetrazole derivative containing a biotin residue (Scheme 4). Biotinylated compounds are indeed important in Co-Immunoprecipitation (Co-IP) studies, aimed at characterizing target engagement and at shedding light on the components of the TRAP1/HSP90 complexes the small molecule can attack.

Biotin **8** was esterified[6] with epichlorohydrin in the presence of Cs_2CO_3 in DMF at 100 °C for 2 h to afford the epoxide derivative **9** in 97% yield. The product was fully characterized and the ^{1}H NMR spectrum ($CDCl_3$) showed the presence of the epoxide moiety with the typical signals at δ_H 2.40 and 2.86 and the biotin moiety with the NH proton signals at δ_H 5.60 and 6.05. The biotine epoxide **9** was then dissolved in toluene and the solid 1-methyl-1*H*-tetrazole-5-thiol was added and the solution left to stir at room temperature for 2 days.



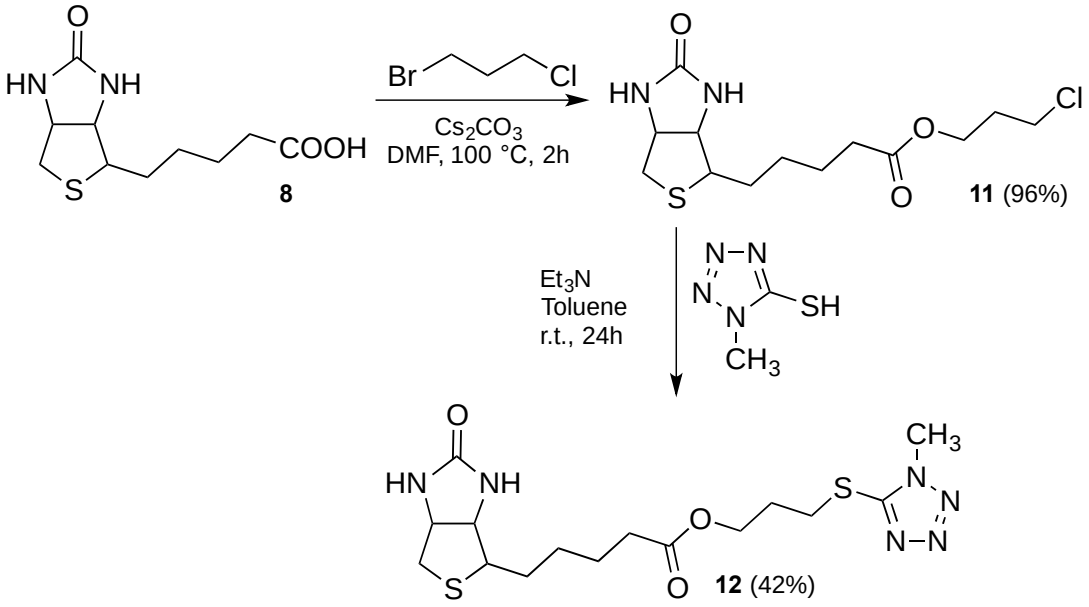
Scheme 4. Synthesis of 2-hydroxy-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]-propyl-5-(2-oxohexahydro-1*H*-thieno-[3,4-*d*]imidazol-4-yl)-pentanoate (**10**).

After toluene removal, the residue was taken up with DCM and the organic phase washed with bicarbonate and brine, to leave after drying over anhydrous Na_2SO_4 the crude product **10** in 65% yield. Compound **10** was fully characterized; in the ^{1}H NMR (DMSO-d_6) spectrum the main difference with the parent compound **9** is the presence of the diagnostic methyl group at δ_H 3.95 of the methyltetrazole. HRMS spectrum was also collected for confirmation and the mass of *m/z* 417.1390 corresponds to the MW+H.

Similarly, biotin **8** was esterified with the 1-bromo-3-chloropropane in the presence of Cs_2CO_3 in DMF at 100 °C to afford the ester **11** (Scheme 5). The relative MS spectrum is consistent with the reported structure. Coupling with tetrazole was conducted in the presence of triethylamine in equimolecular amount at room temperature in toluene as the solvent for 24 h under stirring. The desired product **12** differs from compound **10** by the absence of the hydroxyl group between the biotin and the tetrazole moieties.

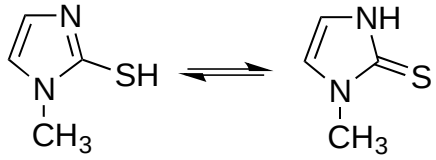
Compound **12** was obtained in 42% yield, in neat contrast with previous results due to the presence of unreacted starting material and degradation products. This indicated the minor efficiency of the nucleophilic substitution protocol with respect to the epoxide ring opening methodology that offer a reliable synthetic route to this type of compounds.

In the search of new molecules with related structures to those above described to be tested as allosteric inhibitors of TRAP1, we looked with interest to the 1-methyl-1*H*-imidazole-2-thiol (Thiamazole, Tapazole). This molecule belongs to the family of imidazole heterocycles having a pharmacological activity to contrast thyroid dysfunctions.



Scheme 5. Synthesis of 3-[(1-methyl-1*H*-tetrazol-5-yl)thio]propyl 5-(2-oxohexahydro-1*H*-thieno[3,4-*d*]-imidazol-4-yl)pentanoate (**12**).

Specifically, the imidazole is N-methylated with a thiol group in position 2. This last element, at the substituent level, allows the establishment of a tautomeric equilibrium that leads to the writing of the two formulas represented in Scheme 6.



1-methyl-1*H*-imidazole-2-thiol
(Thiamazole, Tapazole)

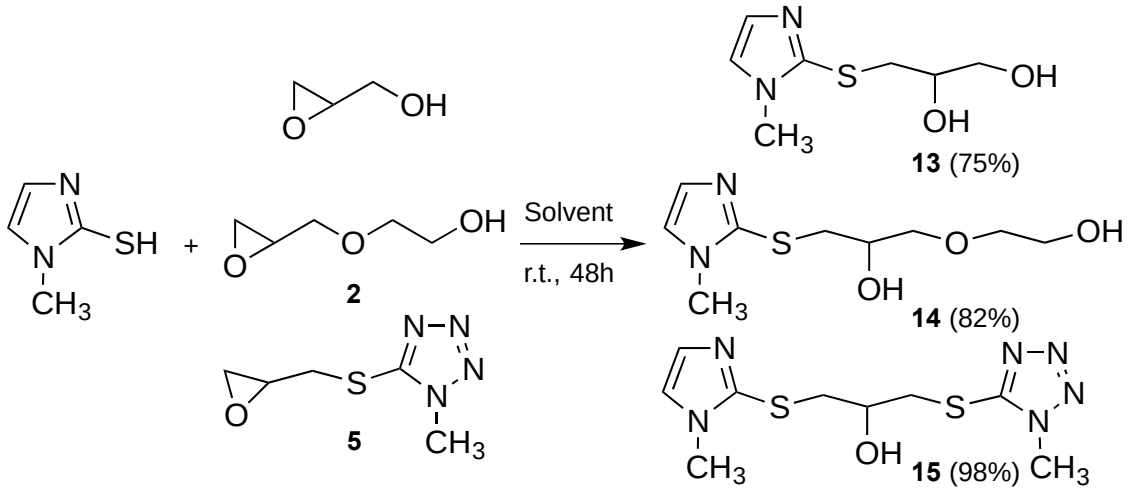
Scheme 6. 1-Methyl-1*H*-imidazole-2-thiol (Thiamazole, Tapazole), CAS No. 60-56-0 (mp 146-148 °C).

A classical synthetic approach to this drug is the reaction between CN triple bonds and aminoacetals in the presence of Cu(I) as the catalyst. The presence of appropriate carbon substituents of the nitrile group leads to obtaining the imidazole ring substituted in position 2. The final product is formed as a result of an acid-catalyzed eliminative cyclization.[7]

Thiamazole represents a viable heterocyclic partner of tetrazole for SAR studies and its structure nicely fits with the epoxide ring opening procedure to afford diol derivatives. Scheme 7 reports two application of such methodology in the reactions with glycidol and the epoxide **2**; after 2 days at room temperature in toluene solutions, the desired products **13**, **14** and **15** were isolated up to quantitative yields and good purity.

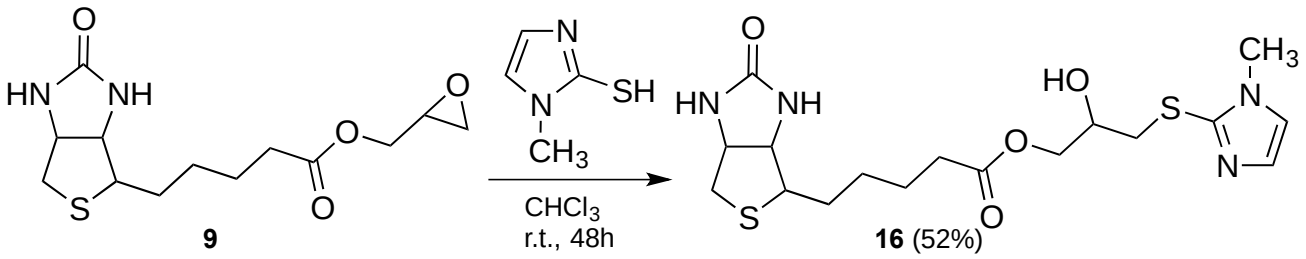
The structures of products **13**, **14** and **15** rely upon their analytical and spectroscopic data. In particular the ^{1}H NMR (DMSO-d_6) spectrum of **13** showed the AB system of one of the methylene groups at δ_H 3.00 and 3.21 while the second methylene cluster at δ_H 3.38.

Similarly, the spectrum of compound **14** showed the presence of the AB system of one of the methylene groups at δ_H 3.00 and 3.19 while the second methylene cluster at δ_H 3.43, along with the signals of the methylene chain at δ_H 3.41. Finally, the spectrum of compound **15** reports the AB system at δ_H 3.20 and the second methylene at δ_H 3.38. All these structures share the common signals of the CH=CH belonging to the imidazole moiety found at δ_H 6.91 and 7.21.



Scheme 7. Synthesis of 1-methyl-1*H*-imidazole-2-thiol derivatives **13**-**15**.

In a previous synthesis of the biotin derivative **10** we used the epoxide **9** that also serves in the case for the preparation of the Thiamazole derivative **16** (Scheme 8). The method is again the same although the solvent is different (chloroform) due to the need to dissolve both the reagent and make them to react properly. Compound **16** was obtained in 52% yield and its structure was confirmed by the analytical and spectroscopic data that are fully consistent for the assigned formula.



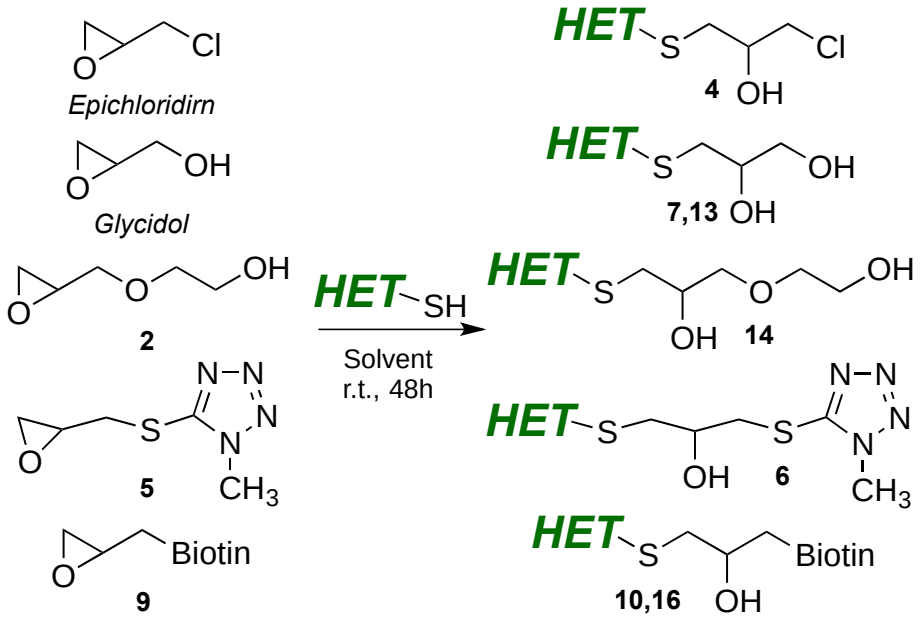
Scheme 8. Synthesis of 2-hydroxy-3-[(1-methyl-1*H*-imidazol-2-yl)thio]-propyl 5-(2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanoate (**16**).

The ^{1}H NMR (DMSO-d_6) spectrum showed the signals belonging to both the biotin and imidazole moieties in the expected spectral regions along the signals relative to the methylene chain.

Thiolysis of epoxides offers an efficient and simple synthetic approach to access β-hydroxy sulfides, valuable scaffolds in the synthesis of various important molecules in medicinal chemistry. A general overview in this topic recently suggests various methodologies to obtain the desired products from variable starting compounds under specific experimental conditions.[5,8]

In the present work, we have exploited the methodology relative to the epoxide ring-opening in epichlorohydrin or glycidol and other derivatives under mild and easily reproducible experimental conditions. Typically, the ring-opening of epoxides of type **2**, **5** and **9** along with epichlorohydrin and glycidol were conducted in medium polarity solvents or chloroform under high dilution conditions (0.014 M) to avoid duplicate reaction of the newly formed hydroxyl group over a second epoxide molecule (Scheme 9).

In fact, we have experienced this type of consecutive ring-opening process when concentrated solutions are allowed to react under the same conditions.



Scheme 9. Epoxide ring-opening plan with heterocyclic sulfur nucleophiles.

The optimized protocol was applied to several cases obtaining the desired products in very good yields and excellent robustness. A comparative example is reported in Scheme 5 where biotin **8** has been derivatized with 1-bromo-3-chloropropane to afford the intermediate **11** that is then transformed into the final compound **12** through nucleophilic substitution reaction of the 1-methyl-1*H*-tetrazole-5-thiol. This was the case where compound **12** was isolated with the lowest yield in comparison with all the other reactions performed.

The products were prepared in the racemic form for a rapid access to the compounds, available for a preliminary *in vitro* tests. With the support of docking studies and molecular dynamics investigations, the enantiomeric form to be used will be chosen. Further synthetic investigations are currently underway to design an enantiopure synthetic strategy, also to increase the molecular diversity of the derivatives and to include compounds either containing simple hydrophilic substituents or biotinylated derivatives.

Conclusions

In prior work, we reported the rational, structure-based, and dynamics-based discovery of ligands selective for TRAP1 targeting. The selected molecules were used to investigate the complex biology of the chaperone and further evolved into leads for the treatment of diseases in which TRAP1 plays a key role.[9] Our results demonstrated that the explicit consideration of the internal, functionally oriented dynamics of TRAP1 can unveil specific allosteric pockets, distal from the highly conserved ATP-binding site and characterized by structural and chemical features that are distinct from those of other paralogs of the HSP90 family.[3]

From the chemical point of view, some of the molecules and in particular compound **3** (Figure 2) were found of great interest for the studies at hand. Hence, the intense synthetic activity we report here aims to enlarge the chemical space available for TRAP1 modulators. With these new compounds at hand, SAR studies will be carried *in vitro* and *in vivo* in view of the possibility, offered by the results here reported, to use tetrazole- and imidazole-based derivatives sharing similar side chains as hits for further development towards isoform selective, administrable anticancer drugs.

In addition to this, the chemistry of tetrazole, suitably prepared from the 1,3-dipolar cycloaddition reaction of azides to nitriles, is currently under evaluation for implementation of the heterocyclic compound family.

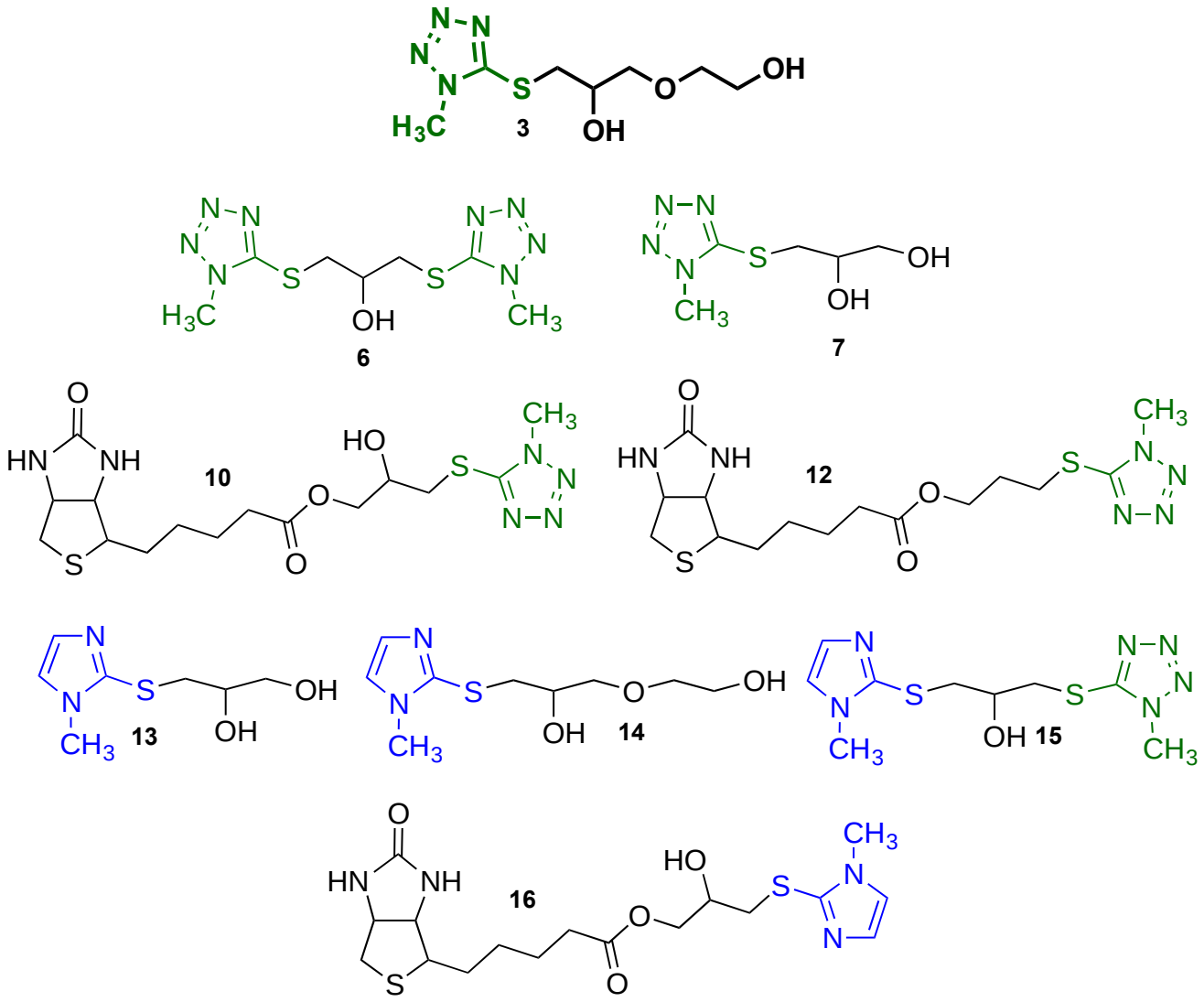


Figure 2. General view of the available tetrazole- and imidazole-based derivatives.

Experimental Section

General. All melting points are uncorrected. HRMS were done on a X500D QTOF system (ABSciex) available at the Centro Grandi Strumenti (CGS) of the University of Pavia. IR spectra (Nujol mulls for solids) were recorded on an FT-IR Bruker-Alpha. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 300 in the specified deuterated solvents at 25 °C. Chemical shifts are expressed in ppm from internal tetramethylsilane (*δ*). Column chromatography and tlc for monitoring the reactions: silica gel 60 (0.063-0.200 mm) (Merck); eluants: ethyl acetate/cyclohexane (90:10) to pure ethyl acetate. 2-(Allyloxy)ethan-1-ol, *m*-CPBA, epichlorohydrin, *N*-methyl-5-mercaptotetrazole, biotin, 1-bromo-3-chloropropane and all the required reagents for the syntheses were purchased from suitable chemical suppliers. We warmly thank Teofarma S.r.l. for generous amount of Thiamazole for chemical use. All other reagents and solvents were purchased from Sigma-Aldrich and Alfa-Aesar and used without any further purification.

2-(Oxiran-2-ylmethoxy)ethan-1-ol (2). To a cooled (0 °C, ice bath) stirred solution of 2-(allyloxy)ethan-1-ol (**1**) (9.6 g, 94 mmol) in DCM (300 mL) was added portion wise *m*-CPBA (24.2 g, 141 mmol). Once the addition was complete, the ice bath was removed and the reaction mixture was left to stir at room temperature for 5 h. The formed suspension was filtered and the organic phase washed three times with a saturated solution of $NaHCO_3$. The organic phase was then dried (Na_2SO_4) and, upon evaporation of the solvent, gave the title compound **2** as a straw yellow oily residue identical to that described in literature.[4] Compound **2** was used without further purification.

1-(2-Hydroxyethoxy)-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]propan-2-ol (3). To a stirred solution of 2-(oxiran-2-ylmethoxy)ethan-1-ol (**2**) (8.7 g, 74 mmol) in toluene (200 mL) at room temperature was added portion wise *N*-methyl-5-mercapto-tetrazole (8.6 g, 74 mmol). The reaction mixture was left to stir at room temperature for 48 h. An oily phase separates and was collected while the organic solvent was evaporated under reduced pressure. The collected oils are dissolved in chloroform and the organic phase is treated with a saturated solution of $NaHCO_3$ overnight under stirring. The organic phase is then separated and, after drying (Na_2SO_4), is concentrated to give the *title compound* **3** (11.95 g, 69%), as a straw yellow oil; IR: ν 3384 (OH), 1721 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ_H 3.20-3.27 (m, 1H, CH), 3.40-3.47 (m, 7H, CH and CH_2), 3.78 (s, 2H, OH), 3.93 (s, 4H, CH and CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 32.9, 33.1, 37.0, 59.8, 67.5, 72.2, 73.1; $C_7H_{14}N_4O_3S$ (234.28): HRMS: calcd. 257.0679 (MW+Na); found 257.0677.

1-Chloro-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]propan-2-ol (4). To excess neat stirred epichlorohydrin (32 mL) at room temperature was added portion wise *N*-methyl-5-mercaptotetrazole (5.0 g, 45 mmol). After 2.5 h, the excess epichlorohydrin was evaporated under reduced pressure to afford the *title compound* **4** (9.30 g, 99%), as a colorless solid; mp 68-70 °C (*i*-Pr_2O); IR: ν 3371 (OH), 1670 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ_H 3.56 (AB syst., 1H+1H, CH_2), 3.50-3.75 (m, 2H, CH_2), 3.96 (s, 3H, CH_3), 4.24-4.32 (m, 2H, CH and OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 33.6, 36.8, 47.2, 70.0, 154.3; $C_5H_9ClN_4OS$ (208.66): HRMS: calcd. 209.0258 (MW+H); found 209.0251.The product was used without further purification.

1-Methyl-5-[(oxiran-2-ylmethyl)thio]-1*H*-tetrazole (5). To a cooled (0 °C, ice bath) stirred solution of compound **4** (1.0 g, 4.8 mmol) in THF (100 mL) was added portion wise KOH (1.2 g, 21.4 mmol) is added portion wise. Once the addition was complete, the ice bath was removed and the reaction mixture was left to stir at room temperature. After 1.5 h, the suspension is filtered and the solvent is evaporated under reduced pressure and the residue is taken up in DCM and the organic phase washed twice with brine. The organic phase is dried (Na_2SO_4), then concentrated to give the *title compound* **5** (0.73 g, 89%), as a straw yellow oil; IR: ν 1726 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ_H 2.66-2.68 (m, 1H, H-C*H*), 2.81-2.83 (m, 1H, *H*-CH), 3.29-3.33 (m, 1H, CH), 3.27 and 3.63 (AB syst., 1H+1H, CH_2), 3.90 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 33.5, 35.5, 47.6, 50.0, 153.5; $C_5H_8N_4OS$ (172.21): HRMS: calcd. 173.0419 (MW+H); found 173.0420.

1,3-Bis[(1-methyl-1*H*-tetrazol-5-yl)thio]propan-2-ol (6). To a stirred solution of compound **5** (0.63 g, 3.7 mmol) in chloroform (200 mL) at room temperature was added in one portion *N*-methyl-5-mercaptotetrazole (0.43 g, 3.7 mmol). After 48 h, the solution was washed twice with brine, and the organic phase dried (Na_2SO_4), then concentrated to give the *title compound* **6** (0.75 g, 71%), as a straw yellow oil; IR: ν 3337 (OH), 1711 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ_H 3.53 (AB syst., 1H+1H, CH_2), 3.90-3.96 (m, 1H, CH), 3.97 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 33.2, 38.1, 69.0, 153.9; $C_7H_{12}N_8OS_2$ (288.35): HRMS: calcd. 289.0648 (MW+H); found 289.0645.

3-[(1-Methyl-1*H*-tetrazol-5-yl)thio]propane-1,2-diol (7). To a stirred solution of *N*-methyl-5-mercapto-tetrazole (0.39 g, 3.4 mmol) in toluene (150 mL) at room temperature was added in one portion glycidol (0.25 g, 3.4 mmol). After 48 h, the solvent is evaporated to reduced pressure and the residue is taken up with DCM and the organic phase washed twice with brine. The organic phase is dried (Na_2SO_4), then concentrated to give

the *title compound* **7** (0.49 g, 75%), as a straw yellow oil; IR: ν 3383 (OH), 1650 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ_H 3.41 (m, 4H, CH_2), 3.74 (m, 1H, CH), 3.94 (s, 3H, CH_3), 4.76 (t, 1H, *J* 6.0 Hz, OH), 5.17 (d, 1H, *J* 5.0 Hz, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 33.5, 37.4, 64.3, 69.8, 154.2; $C_5H_{10}N_4O_2S$ (190.22): HRMS: calcd. 191.0597 (MW+H); found 191.0595.

Oxiran-2-ylmethyl 5-(2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanoate (9). To a stirred solution of biotin **8** (2.00 g, 8.2 mmol) in DMF (50 mL) at 100 °C was added Cs_2CO_3 (2.67 g, 8.2 mmol) followed by epichlorohydrin (2.56 mL, 32.8 mmol). The reaction mixture was then heated to 100 °C for 1.5 h, and then allowed to cool to room temperature. The obtained suspension was filtrated and the organic phase diluted with chloroform and washed twice with brine. The organic phase was then dried (Na_2SO_4) and concentrated to give the *title compound* **9** (2.39 g, 97%), as a colorless solid; mp 73-78 °C ($CHCl_3$); IR: ν 3261 (NH), 1630 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ_H 1.45-1.48 (m, 2H, CH_2), 1.65-1.73 (m, 4H, CH and CH_2), 2.37-2.42 (m, 2H, CH_2), 2.84-2.88 (m, 2H, CH_2), 2.64-2.67 (m, 1H, CH), 3.12-3.18 (m, 2H, CH_2), 3.90-3.94 (m, 1H, CH), 4.25-4.60 (m, 4H, CH and CH_2), 5.60 (s, 1H, NH), 6.05 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 24.6, 28.1, 28.2, 33.5, 40.4, 44.6, 49.3, 55.4, 60.0, 61.8, 64.7, 163.7, 173.1; $C_{13}H_{20}N_2O_4S$ (300.37): HRMS: calcd. 301.1217 (MW+H); found 301.1219.

2-Hydroxy-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]propyl 5-(2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-pentanoate (10). To a stirred solution of compound **9** (0.80 g, 2.7 mmol) in toluene (80 mL) at room temperature was added *N*-methyl-5-mercapto-tetrazole (0.31 g, 2.7 mmol). After 48 h, toluene was removed, the residue taken up with DCM and the organic phase washed twice with brine. The organic phase was then dried (Na_2SO_4) and concentrated to give the *title compound* **10** (0.72 g, 65%), as a yellowish solid; mp 56-60 °C (Et_2O). IR: ν 3400 (OH, NH), 1699 (C=O), 1660 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ_H 1.20-1.50 (m, 2H, CH_2), 1.51-1.70 (m, 4H, CH and CH_2), 2.58 and 2.78 (AB syst., 2H, CH_2), 3.10-3.14 (m, 1H, CH), 3.29-3.33 (m, 1H, CH), 3.40-3.50 (m, 2H, CH_2), 3.70-3.73 (m, 2H, CH_2), 3.80-3.95 (m, 3H, CH_3), 4.01-4.03 (m, 2H, CH_2), 4.04-4.05 (m, 1H, CH), 4.30-4.34 (m, 1H, CH), 5.53 (d, 1H, OH), 6.26 (s, 1H, NH), 6.35 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 24.4, 28.0, 33.3, 33.6, 36.9, 40.3, 55.3, 59.2, 61.0, 63.2, 66.8, 162.7, 172.7; $C_{15}H_{24}N_6O_4S_2$ (416.52): HRMS: calcd. 417.1373 (MW+H); found 417.1390.

3-Chloropropyl 5-(2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanoate (11). To a stirred solution of biotin **8** (0.50 g, 2.1 mmol) in DMF (20 mL) at 100 °C was added Cs_2CO_3 (0.67 g, 2.1 mmol) followed by 1-bromo-3-chloropropane (0.82 mL, 8.3 mmol). The reaction mixture was then heated to 100 °C. After 2 h, the mixture was allowed to cool to room temperature and the suspension that formed was filtrated and the organic phase diluted with DCM. After washing twice with brine, the organic phase was dried (Na_2SO_4) and concentrated to give the *title compound* **11** (0.65 g, 96%), as a colorless solid; mp 100-105 °C (EtOH); IR: ν 3261 (NH), 1714 (C=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ_H 1.30-1.38 (m, 2H, CH_2), 1.60-1.64 (m, 4H, CH_2), 2.03 (qi, 2H, *J* 6.0 Hz, CH_2), 2.32 (t, 2H, *J* 7.0 Hz, CH_2), 2.57 and 2.82 (AB syst., 1H+1H, CH_2), 3.08-3.12 (m, 1H, CH), 3.69 (t, 2H, *J* 6.0 Hz, CH_2), 4.10-4.15 (m, 3H, CH and CH_2), 4.29-4.33 (m, 1H, CH), 6.35 (s, 1H, NH), 6.42 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 24.4, 28.0, 31.1, 33.2, 39.8, 41.8, 55.3, 59.2, 60.7, 61.0, 162.7, 172.8; $C_{13}H_{21}ClN_2O_3S$ (320.83): HRMS: calcd. 321.1034 (MW+H); found 321.1031.

3-[(1-Methyl-1*H*-tetrazol-5-yl)thio]propyl 5-(2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanoate (12). To a stirred solution of compound **11** (0.29 g, 0.9 mmol) in toluene (100 mL) at room temperature was added *N*-methyl-5-mercaptotetrazole (0.21 g, 1.8 mmol) followed by Et_3N (0.26 mL, 1.8 mmol). After 24 h, toluene was removed and the residue was taken up in chloroform and the organic phase washed twice with brine. The organic phase was then dried (Na_2SO_4) and concentrated to give the *title compound* **12** (0.15 g, 42%), as a brown solid; mp 51-55 °C (Et_2O); IR: ν 3249 (NH), 1695 (C=O, C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ_H 1.30-1.35 (m, 2H, CH_2), 1.50-1.68 (m, 4H, CH_2), 2.04 (qi, 2H, *J* 6.0 Hz, CH_2), 2.31 (t, 2H, *J* 7.0 Hz, CH_2) 2.57 and 2.83

(AB syst., 1H+1H, CH_2), 3.08-3.12 (m, 1H, CH), 3.32 (s, 3H, CH_3), 3.67 (t, 2H, *J* 6.0 Hz, CH_2), 3.93-4.13 (m, 3H, CH and CH_2), 4.29-4.33 (m, 1H, CH), 6.36 (s, 1H, NH), 6.42 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 24.4, 28.0, 31.1, 33.2, 33.6, 39.9, 41.9, 55.3, 59.2, 60.7, 61.0, 162.7, 172.8. $C_{15}H_{24}N_6O_3S_2$ (400.52): HRMS: calcd. 401.1424 (MW+H); found 401.1425.

Synthesis of compounds 13-15; general procedure: 1-Methyl-1*H*-imidazole-2-thiol (0.39 g, 3.4 mmol) is dissolved in 125 mL toluene and the epoxide derivatives (glycidol, **2** or **5**) (3.4 mmol) in 125 mL toluene to prepare compound **13**. To prepare compounds **14,15** $CHCl_3$ (125 mL) is needed instead of toluene. The epoxide solutions are added dropwise to the previous solutions and the reactions are left to stir at room temperature. After 48 h, solvent is evaporated and the residues are taken up with DCM and the solutions are washed twice with brine. The organic phases are dried (Na_2SO_4), then concentrated to leave straw yellow oils corresponding to the products **13-15** that were fully characterized.

Compound 13. (0.48 g, 75%), straw yellow oil; IR: ν 3263 (OH), 1667 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 3.00 and 3.21 (AB syst., 1H+1H, CH_2), 3.35-3.40 (m, 2H, CH_2), 3.57 (s, 3H, CH_3), 3.62-3.68 (m, 1H, CH), 4.84 (t, 1H, OH), 5.26 (d, 1H, OH), 6.91 (s, 1H, CH=), 7.21 (s, 1H, CH=); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 32.9, 37.4, 64.0, 70.7, 123.0, 127.9, 141.4; $C_7H_{12}N_2O_2S$ (188.25): HRMS: calcd. 189.0692 (MW+H); found 189.0688.

Compound 14. (0.65 g, 82%), straw yellow oil; IR: ν 3356 (OH), 1659 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 3.00 and 3.19 (AB syst., 1H+1H, CH_2), 3.38-3.42 (m, 4H, CH_2), 3.43-3.45 (m, 2H, CH_2), 3.58 (s, 3H, CH_3), 3.75-3.82 (m, 1H, CH), 4.49 (bs, 1H, OH), 5.32 (bs, 1H, OH), 6.91 (s, 1H, CH=), 7.20 (s, 1H, CH=); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 32.9, 37.6, 60.2, 68.8, 72.6, 73.7, 123.0, 128.1, 140.9; $C_9H_{16}N_2O_3S$ (232.30): HRMS: calcd. 233.0954 (MW+H); found 233.0948.

Compound 15. (0.96 g, 98%), colorless solid; mp 95-98 °C (EtOH/*i*-Pr_2O); IR: ν 3114 (OH), 1658 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 3.20 (AB syst., 2H, CH_2), 3.35-3.40 (m, 1H, CH_2), 3.53-3.55 (m, 1H, CH), 3.58 (s, 3H, CH_3), 3.93 (s, 3H, CH_3), 3.98-4.01 (m, 1H, CH), 5.94 (bs, 1H, OH), 6.91 (s, 1H, CH=), 7.22 (s, 1H, CH=); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 32.9, 33.6, 68.4, 123.2, 128.1, 140.4, 153.9; $C_9H_{14}N_6OS_2$ (286.37): HRMS: calcd. 287.0743 (MW+H); found 287.0741.

2-Hydroxy-3-[(1-methyl-1*H*-imidazol-2-yl)thio]propyl 5-(2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-pentanoate (16). To a stirred solution of compound **9** (0.30 g, 1.0 mmol) in chloroform (400 mL) at room temperature was added in one portion 1-methyl-1*H*-imidazole-2-thiol (0.12 g, 1.0 mmol). After 48 h, the solution was washed twice with brine. The organic phase was then dried (Na_2SO_4) and concentrated to give the *title compound* **16** (0.21 g, 52%), as a colorless oil: IR: ν 3227 (OH, NH), 1794 (C=O), 1681 (C=O, C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 1.32-1.36 (m, 2H, CH_2), 1.53-1.56 (m, 4H, CH_2), 2.27-2.32 (m, 2H, CH_2), 2.60 and 2.80 (AB syst., 1H+1H, CH_2), 3.05-3.13 (m, 2H, CH_2), 3.30-3.34 (m, 9H, CH, CH_2 and CH_3), 4-28-4.32 (m, 1H, CH), 6.35 (s, 1H, NH), 6.42 (s, 1H, NH), 6.86 (s, 1H, CH=), 6.97 (s, 1H, CH=); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 24.4, 28.0, 33.5, 39.8, 55.3, 59.2, 61.0, 114.0, 119.4, 123.1, 128.2, 161.2, 162.7, 172.7; $C_{17}H_{26}N_4O_4S_2$ (414.54): HRMS: calcd. 415.1468 (MW+H); found 415.1457.

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Supplementary Material

^{1}H and ^{13}C NMR spectra of synthetized compounds are available in the Supplementary Material.

References

1. Sanchez-Martin, C.; Serapian, S. A.; Colombo, G.; Rasola, A. *Front. Oncol.* **2020**, *10*, 1177 (article no.). https://doi.org/10.3389/fonc.2020.01177
2. Ferraro, M.; D'Annessa, I.; Moroni, E.; Morra, G.; Paladino, A.; Rinaldi, S.; Compostella, F.; Colombo, G. *J. Med. Chem*. **2019**, *62*, 60. https://doi.org/10.1021/acs.jmedchem.8b00825
3. Sanchez-Martin, C.; Moroni, E.; Ferraro, M.; Laquatra, C.; Cannino, G.; Masgras, I.; Negro, A.; Quadrelli, P.; Rasola, A.; Colombo, G. *Cell Rep.* **2020**, *31*, 107531 (article no.). https://doi.org/10.1016/j.celrep.2020.107531
4. Paraja, M.; Matile, S. *Angew. Chem. Int. Ed.* **2020**, *59*, 6273. https://doi.org/10.1002/anie.202000579
5. Chen, Z.; Mohammadi Nasr, S.; Kazemi, M.; Mohammadi, M. *Mini-Rev. Org. Chem.* **2020**, *17*, 352. https://doi.org/10.2174/1570193X16666190723111746
6. Faita, G.; Mella, M.; Mortoni, A.; Paio, A.; Quadrelli, P.; Seneci, P. *Eur. J. Org. Chem.* **2002**, 1175. https://doi.org/10.1002/1099-0690(200204)2002:7<1175::AID-EJOC1175>3.0.CO;2-Z
7. Eicher, T.; Hauptmann, S.; Speicher, A. "The Chemistry of Heterocycles" Wiley-VCH, Ed.; Weinheim: **2012**, 226.
8. Ahmad, S.; Fawad Zahoor, A.; Raza Naqvi, S. A.; Akash, M. *Mol. Divers.* **2018**, *22*, 191. https://doi.org/10.1007/s11030-017-9796-x
9. Aeluri, M.; Chamakuri, S.; Dasari, B.; Reddy Guduru, S. K.; Jimmidi, R.; Jogula, S.; Arya, P. *Chem. Rev.* **2014**, *114*, 4640. https://doi.org/10.1021/cr4004049