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Article

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Design of Potent and Drug Like Non Phenolic Inhibitors for Catechol *O*-Methyltransferase Derived from a Fragment Screening Approach Targeting the *S*-Adenosyl-L-Methionine Pocket

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ABSTRACT

A fragment screening approach designed to target specifically the *S*-adenosyl-L-methionine pocket of catechol *O*-methyl transferase, allowed the identification of structurally related fragments of high ligand efficiency and with activity on the described orthogonal assays. Using a reliable enzymatic assay together with X-ray crystallography as guidance, a series of fragment modifications revealed an SAR and, after several expansions, potent lead compounds could be obtained. For the first time non phenolic and small low nanomolar potent, SAM competitive COMT inhibitors are reported. These compounds represent a novel series of potent COMT

inhibitors that might be further optimized to new drugs useful for the treatment of Parkinson's disease, as adjuncts in levodopa based therapy, or for the treatment of schizophrenia.

INTRODUCTION

The enzyme catechol *O*-methyltransferase (COMT) catalyzes the Mg^{2+} dependent methyl transfer from its cofactor *S*-adenosyl-L-methionine (SAM), to one of the hydroxyl groups of endogenous catechols and neurotransmitters such as dopamine and noradrenaline, resulting in termination of their biological activity. In the brain, COMT-dependent dopamine degradation is of high relevance in regions with low expression of the presynaptic dopamine transporter (DAT), in particular in the prefrontal cortex. For this reason COMT inhibition offers a unique opportunity to influence pathologies characterized by low dopamine levels in the prefrontal area such as cognitive impairment associated with schizophrenia.[1]

Most known COMT inhibitors are nitro-catechol based compounds, such as tolcapone and entacapone (Figure 1) which are successfully used as adjuncts to the L-Dopa/based treatment of Parkinson's Disease.[2] They are highly metabolized and mainly peripherally acting, protecting L-Dopa from methylation and deactivation mostly in the liver.[3] While entacapone is almost exclusively peripherally acting, there is evidence for central effects with tolcapone, despite the limited brain penetration (brain to plasma ratio up to 0.01).[4]

Bisubstrate and hydroxy-pyridone based COMT inhibitors (Figure 1) have also been described.[5,6] They do not require the presence of a nitro group, however like the catechols, they have chelating motives of phenolic nature binding to the Mg^{2+} site. Such phenolic compounds are prone to high metabolic clearance, notably glucuronidation and sulfatation causing low oral bioavailability.[3,7] In addition, their acidity and high polarity make it challenging to develop them

as centrally acting drugs. Accordingly, to date the focus has been on peripheral COMT inhibition,[8] while evidence for an important role of COMT in schizophrenia is accumulating.

We want to report the identification of structurally differentiated COMT inhibitors targeting exclusively the SAM pocket instead of the Mg^{2+} site with its strict limitations for binding.[9] Ideally, the adenosine replacing motive should be of low molecular weight, with an acceptable polarity reflected in an adequate number of H-bond donors (typically HBD <3), with less groups able to function as HBA (<7) and with a suitable lipophilicity for CNS penetration (clogP, best between 1-4),[10] in contrast to previous examples targeting the SAM pocket.[11]

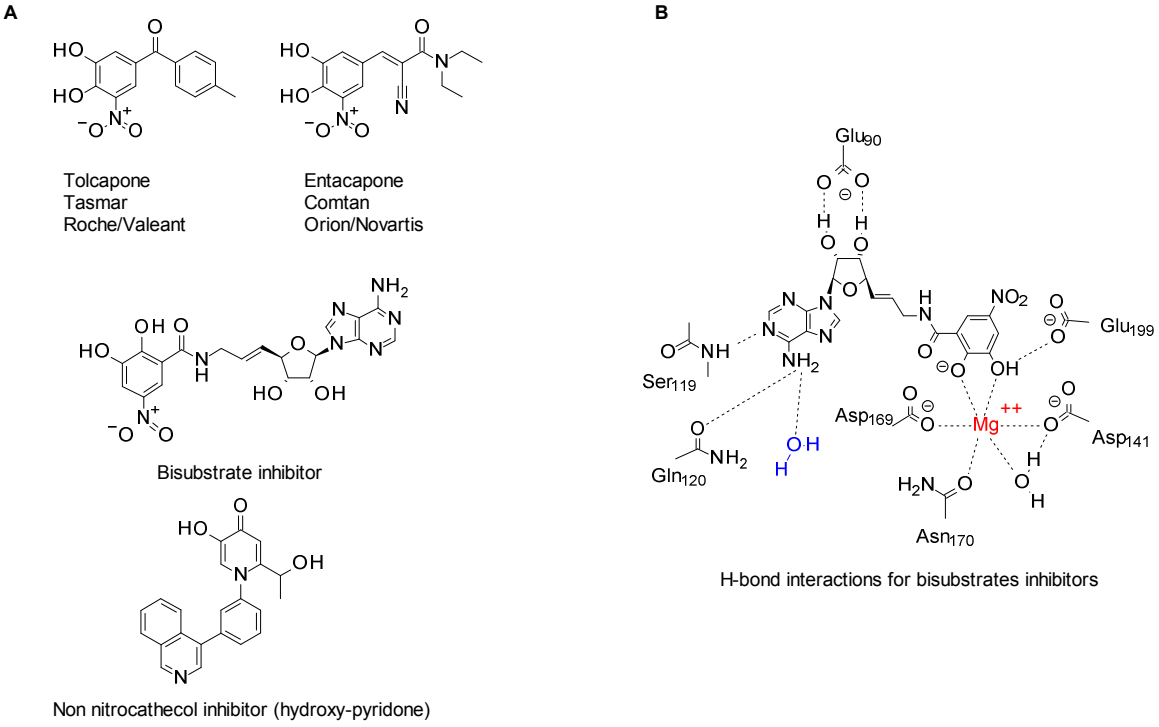


Figure 1. A) Reported COMT inhibitors. B) Interactions for bisubstrate inhibitors binding to SAM and Mg^{2+} pockets.[5a]

In the recent past, fragment-based drug discovery was successfully applied to the generation of hits or chemical tools for drug targets. A critical challenge is to further elaborate the fragment hits to potent leads with balanced properties[12] with particular examples such as Zelboraf, which could be further developed into a marketed drug.[13] Fragment hits typically show very low affinity to the targets, so sensitivity of the screening assay and confirmation of binding by orthogonal methods is required.[14]

RESULTS AND DISCUSSION

We performed a fragment screening for COMT targeting the SAM pocket. We used a library consisting of 6000 compounds with molecular weights below 250 Da, up to three hydrogen bond donors, up to three hydrogen bond acceptors, and calculated logP (clogP) of ≤3, in compliance with the rule of three.[14] Affinity screening was first based on surface plasmon resonance (SPR).[15] Binding of the fragments to wild type human COMT apoprotein was measured at a single concentration of 200 μM, in absence of SAM and Mg^{2+} to exclude chelators.

Results obtained with this protocol were compared with the binding to six enzyme mutants harboring variations in the SAM binding domain.[16] This counter screen ensured the specificity of the interaction with the desired co-factor pocket.

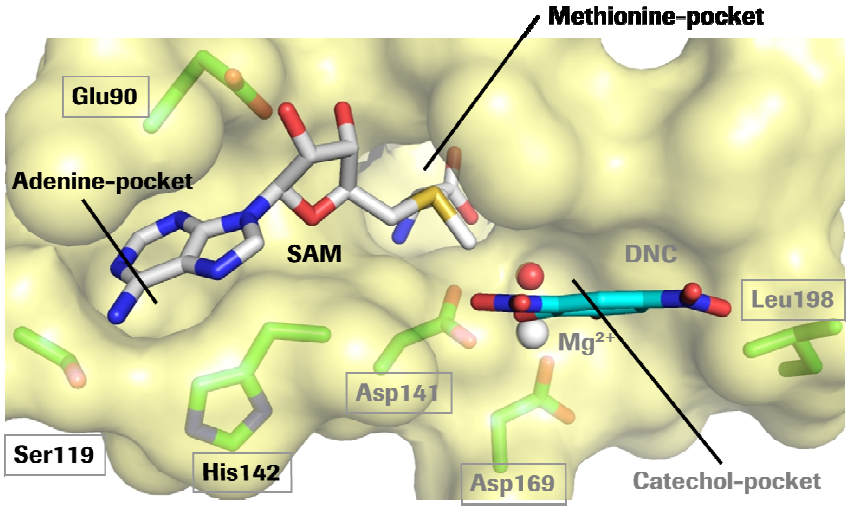
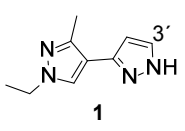


Figure 2. Selection of specific mutants on the adenosine binding pocket. Amino acids in bold (Glu90, Ser119 and His142) were mutated

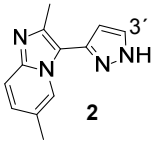
Upon selection of 600 fragments based on affinity and site specificity, hit confirmation was performed with a 10 point SPR dose response measurement.[15] For 200 confirmed binders identified, one dimensional (1D) ^{1}H NMR Spin Lock variation of Carr-Purcell-Meiboom Gill (CPMG)[14,17] ligand detected method was performed, by measuring the NMR spectra of the compound without/with hCOMT protein and observing line broadening due to fragment binding to the slow tumbling hCOMT and the differences in relaxation. Binding of fragments confirmed by 1D ^{1}H NMR, was further characterized by two-dimensional (2D) ^{1}H NMR target detect experiments method performed with ^{15}N labeled hCOMT protein by use of heteronuclear single-quantum coherence (^{1}H/^{15}N HSQC) NMR with chemical shifts mapping, where fragment-induced chemical shift differences of ^{1}H/^{15}N cross peaks of hCOMT amide backbones involved in binding, are observed.[14,17a,18] Additionally, all 600 fragments were tested in a functional enzymatic assay[19] to determine the IC_{50} values for COMT inhibition. Very few fragments showed significant binding in the co-factor pocket combined with a weak enzymatic inhibition and only four fragments were active in all assays. Following this screening/validation cascade we

identified three pyrazole derivatives as fragment hits (Table 1) with high ligand efficiencies (LE).[20] It is worth noting that we found similar starting points to the ones recently reported, in particular fragment **1.**[21] This supports our findings and it is not surprising since, unlike the high-throughput screening libraries, fragment libraries typically contain commercially available molecules selected with similar property criteria.[14]

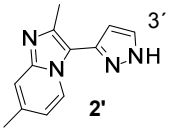
Table 1. Selected fragment screening hits. Binding, functional and ligand efficiency data.



1



2



2'

Fragment hit	**1**	**2**	**2´**
SPR KD (μM)	9	18	18
IC_{50} (μM)	69	80	85
LE	0.44	0.37	0.36

In order to avoid interspecies differences in our hit to lead optimization process, we performed co-crystallization efforts with humanized rat COMT as this surrogate crystallizes better that human COMT and yields data relevant to human.[5c] The crystal structure of humanized rat COMT in complex with fragment **2** (PDB code 5k03) confirmed its binding in the SAM pocket (Figure 3).

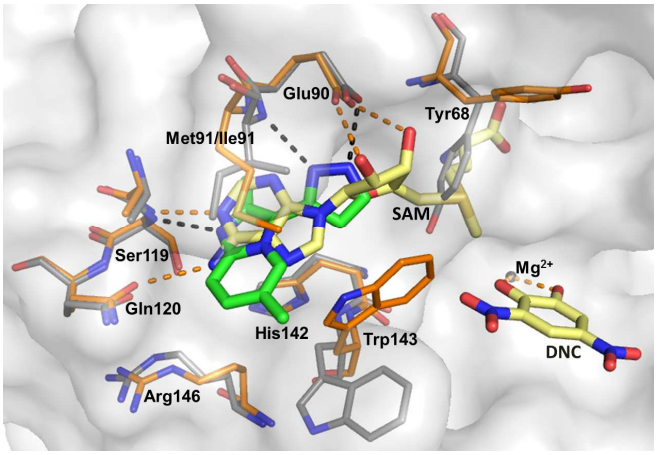


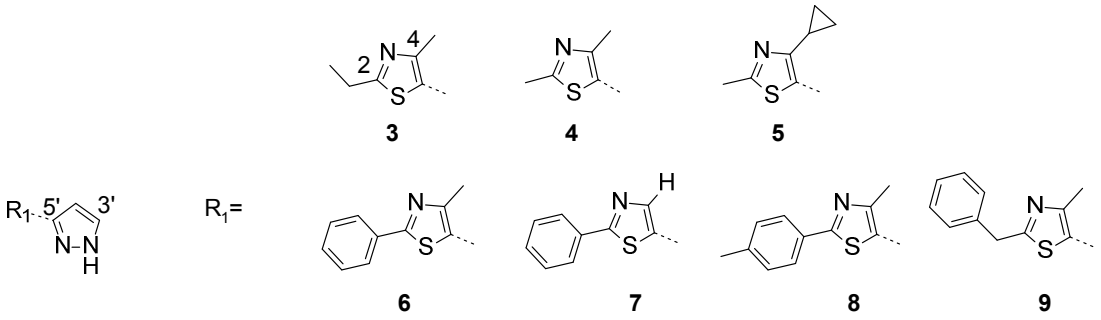
Figure 3. Superposition of the crystal structures of rat COMT[9] (orange) in complex with *S*-adenosylmethionine (SAM, yellow), dinitrocatechol (DNC, yellow), magnesium (Mg^{2+}) and humanized rat COMT (gray) bound to fragment **2** (green). Met91 of the rat COMT is mutated in the humanized protein to Ile91.

Most striking is the H-bond (290 pm) between the backbone NH of Ser119 and the nitrogen of the imidazopyridine from **2**. It corresponds to the H-bond interaction of Ser119 to the N-1 of adenine in the classical SAM binding mode. The pyrazole occupies the ribose binding region and the NH serves as H-bond donor (280 pm) to the carboxylate of Glu90, mimicking the interaction of the hydroxyl groups of the ribose in SAM which are normally the partners for the Glu90 carboxylate. There is a novel element in the binding of **2** to the adenosine pocket which is not present in the SAM bound structure, the H-bond (310 pm) between the backbone NH of Ile91 and the unprotonated N-1 of the pyrazole moiety. Additionally, the imidazopyridine ring forms a sandwich-like interaction with the aliphatic side chain of Ile91 on one side and with the benzylic CH_2 of Trp143 on the other side. The benzylic CH_2 of His142 interacts with both the pyrazole ring and the imidazopyridine. Finally, the pyrazole also interacts with the aromatic ring of Tyr68 in an edge-to-face interaction. Comparing the X-ray structures with SAM and fragment

2, the side chains of Trp143 and to a lesser extend of Arg146, move to offer space to the pyridine ring. Also, the previously mentioned Tyr68 flips over to close the methionine channel indicating significant flexibility.

Fragment expansion using interactive substructure searches and similarity searches within the Roche compound library, followed by chemical synthesis, led to the identification of a series of thiazole analogs (Table 2) with affinities in the micromolar range.

Table 2: IC_{50} values obtained from the enzymatic assay for representative first fragment modifications.



3 4 5 6 7 8 9

Compd	**3**	**4**	**5**	**6**	**7**	**8**	**9**
IC_{50}[a](μM)	21±3	16±2	>625	24±2	>626	11±2.5	>425

[a] IC_{50}: half maximal inhibitory concentration in enzymatic functional assay. IC_{50} values are the average of two to four independent measurements ±standard deviation.

A formal exchange of the corresponding pyrazole ring in the original fragment **1** (IC_{50} = 69 μM) by an identically substituted thiazole **3** (IC_{50} = 21 μM), led to a threefold increase in affinity. In general, the thiazolylpyrazoles were slightly more potent. Another group significantly affecting affinity is the 4-methyl substitution at the thiazole ring. 4-Methyl substituted **6** (IC_{50} = 24 μM) compares favorably with its unsubstituted analog **7** (>625 μM).

Indeed, 4-methyl seems to be optimal, illustrated by **4** (16 μM), and **5** (>625 μM). At position 2 of the thiazole ring, only a 2-tolyl substituted compound **8** was similarly potent as its 2-methyl analog **4**. Other alkyl- or aryl substituents with improved potency at this position, could not be identified (data not shown). The more sterically demanding benzylic derivative **9** was inactive.

To better understand the affinity differences, we examined the crystal structure of compound **8** in complex with humanized rat COMT (PDB code: 5k05) (Figure 4).

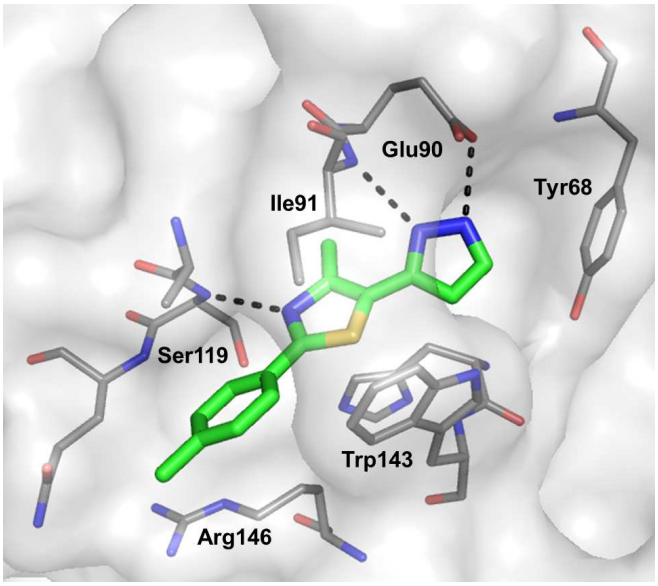


Figure 4. Crystal structure of humanized rat COMT with fragment **8**

The crystal structure shows that the nitrogen atom of the thiazole ring is involved in hydrogen bonding to the backbone NH (280 pm) of Ser119. The pyrazole ring, as described for compound **2**, occupies the ribose binding region and both nitrogen atoms form hydrogen bonds to Glu90 (270 pm) and the backbone NH of Ile-91 (310 pm).

For compound **8**, the flexible Trp143 is now in close contact to the thiazole, binding edge-to-face to its sulfur. Extensions at position 2 in the thiazole ring, are at the surface of the

enzyme pointing to the solvent. Larger substituents could even reach out, through the surface of the enzyme into the surrounding water. This may partially explain why no significant gain in affinity is observed with addition of aromatic rings comparing compound **4** with **6**, **8** and the lost of activity for compound **9** in Table 2.

A methyl group at position 4 in the thiazole (**4, 6, 8**) fits perfectly into a small pocket deep in the binding site (Figure 5). A larger substituent such as cyclopropyl (**5**) is already too big to accommodate, while a hydrogen (**7**) leaves an unoccupied cavity.

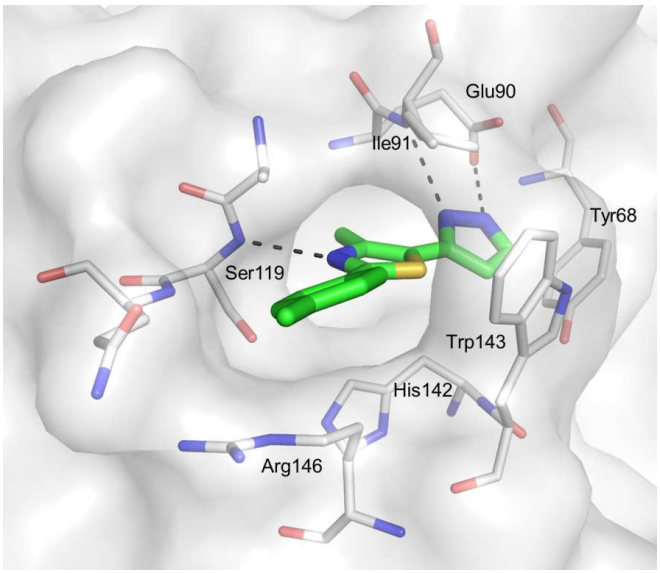
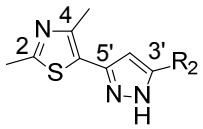


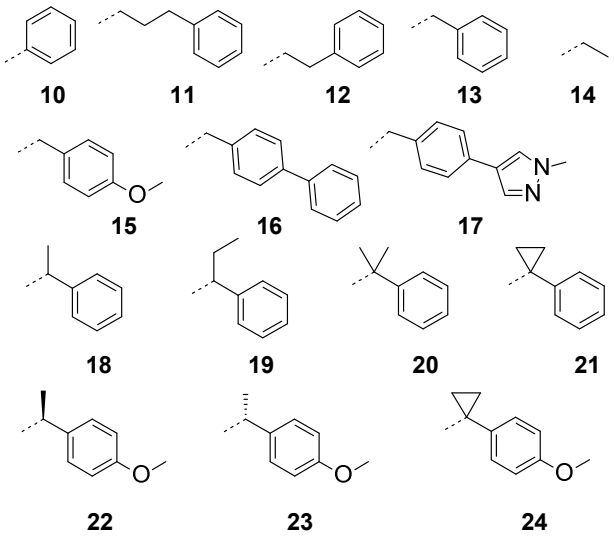
Figure 5. Crystal structure of humanized rat COMT with fragment **8**, oriented in order to show the small pocket at position 4 in the thiazole.

Although the co- crystal structures of **2** and **8** (Figures 3 and 4) indicate that there is limited space due to the flipped conformation of Tyr68 compared to the SAM-bound state, considering the observed flexibility, we decided to explore substituent effects at position 3' (R_2) of the pyrazole while keeping constant the 5-(1H-pyrazol-5-yl)-2,4-dimethyl-thiazole motif of compound **4** (Table 3 with key representatives).

Table 3: IC_{50} values and physicochemical parameters to assess brain penetration for 3´-substituted derivatives (R_2).



R_2 =



compd	IC_{50} (μM)	PSA[a]	clogP [b]	logD[c]	Solubility (μg/mL)
10	130±20	37.0	4.2	-	-
11	34±5	37.1	3.2	4.4	8
12	8±1	37.1	3.0	4.0	4
13	2±0.2	36.9	3.4	3.6	15
14	20±1	37.1	3.2	2.6	-
15	1.0±0.1	45.5	3.3	3.7	2
16	3.2±0.5	37.0	3.6	-	-
17	1.3±0.1	53.9	3.2	3.9	4
18	0.9±0.1	37.3	3.5	3.9	3
18S	1.1±0.001	37.3	3.5	-	-
18R	0.7±0.05	37.3	3.5	4.0	5
19	21±3	37.3	3.4	4.3	24
20	1.1±0.3	35.8	3.5	4.1	4

21	0.21±0.04	36.8	3.2	4.2	1
22	0.86±0.16	45.9	3.4	3.9	5
23	0.47±0.14	45.8	3.4	4.0	5
24	0.075±0.01	45.6	3.0	4.1	2

[a] PSA: Polar surface area, (for CNS penetration< 60-70Å^2), [b] clogP = calculated logarithm of the partition coefficient, [c] logD = logarithm of the octanol-water distribution coefficient @ pH 7.4. Note: compounds on table 3 have: MW<350 Da, Hydrogen bond donors (HBD):1 (NHs+OHs), and Hydrogen bond acceptors (HBA): 2-3 (Ns+Os).

Exploring substituent effects at position 3' (R_2) on Table 3, we observed that direct attachment of a sterically demanding phenyl ring at the pyrazole, compound **10**, led to a strong decrease in potency. Variation on the length of a slim linker revealed a key trend for potency improvement (compounds **11** to **13**), with 2 μM binding affinity for the shortest benzylic compound **13**. However, the lack of a phenyl ring attached to the linker (alkyl compound **14** compared with **13**) reduced potency.

Further modifications were performed to understand the binding requirements at the (R_2) side of the molecule for the benzylic compounds.

We observed a tendency for potency increase with higher electron densities at the phenyl ring and in particular with a small alkoxy group (**15**) in para position. In this regard, alternatives with lower electron density such as a pyridine or chloro substituted rings have IC_{50} values > 3 μM, (data not shown). Increase of size at the benzylic aromatic ring does not contribute to potency improvement (**16**), however heterocyclic biaryls such as a methyl pyrazole **17** are tolerated and

show similar potency to the alkoxy derivative **15**. Significant changes in potency were achieved with different substitutions at the benzylic position (**18**-**21**). Introducing a methyl substituent leads to submicromolar potency (**18**) while for example an ethyl group, is already too large (**19**). For the monosubstitution at the benzylic side, the *R* enantiomers are consistently more potent by about 2-fold (e.g **23** vs **22**).[22] Bridging of the dimethyl (**20**) to a cyclopropyl group (**21**) leads to a further gain in affinity. By combining the cyclopropyl at the benzylic position with an optimally substituted aromatic ring, potencies below 100 nM are obtained for the first time (**24**). Pyrazolo-pyrazole analogs follow a similar increase in potency as the thiazolo-pyrazoles with introduction of the benzyl and cyclopropyl functions and are less potent.[23] We were able to obtain a crystal structure of the most active compound (**24**) co-crystallized with humanized rat COMT (PDB code: 5k0l) to clarify key interactions (Figure 6).

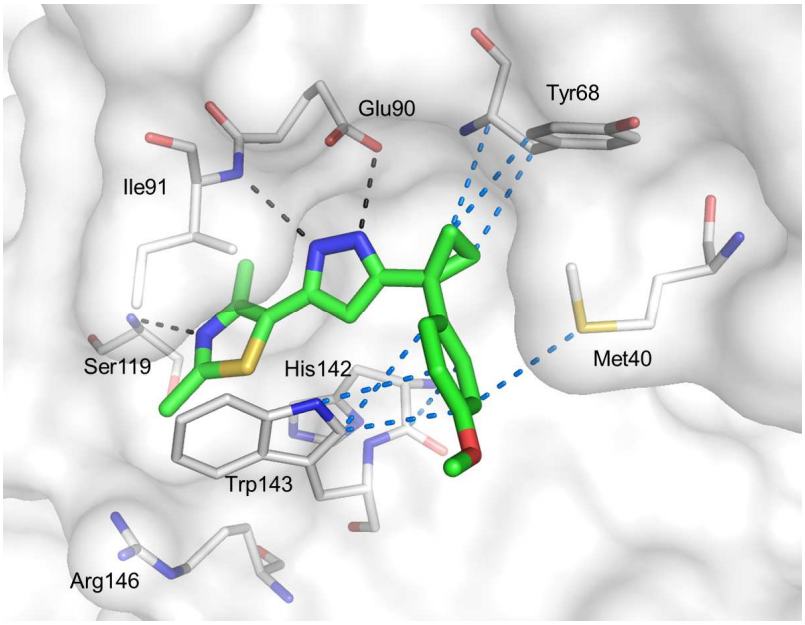


Figure 6. Crystal structure of humanized rat COMT with inhibitor **24:** hydrogen bonds (black) and hydrophobic interactions (blue) indicated.

The thiazolo-pyrazole H-bond pattern is confirmed to be as described for the previous compounds. Trp143 is in close contact to the thiazole sulfur atom (330 pm) and the methoxybenzene ring (350 pm). The methoxybenzene ring additionally interacts with the side chain of Met40 and is thus in a sandwich constellation.

These interactions rationalize the importance of the aromatic ring as observed in the comparison of compounds **13** with **14** (Table 3). Importantly, the side chain of Tyr68 moves out to create space for the binding of the cyclopropylene linker. The two cyclopropyl carbon atoms make 3 vdW interactions with the side chain of Tyr68.

Although the binding potencies for the monomethyl analogs are not of big difference, we observed that the *R* enantiomers show about 2 fold higher potencies than the *S* enantiomers. The difference in potency becomes larger when comparing the weakest *S*-methyl enantiomers (e.g **22** with IC_{50}: 0.86 μM) with the cyclopropyl derivative **24** (IC_{50}: 0.075 μM) or the **18** *S*-methyl enantiomer **18S** (IC_{50}: 1.1 μM) with the cyclopropyl **21** (0.21 μM). There is also a five fold reduction in potency from the cyclopropyl derivative **21** to the dimethyl analog **20** (IC_{50}: 1.1 μM) which binds with similar potency compared to the weakest **18S** enantiomer. Interestingly, superpositions for the most rigid cyclopropyl (**24** in green), the dimethyl (**20** in yellow), and the *R* and *S* monomethyl enantiomers (racemic **18** in blue and **22**+**23** racemic mixture in orange) reveals different conformational trends for the most differentiated compounds (Figure 7). The dimethyl substitution is sterically more demanding than cyclopropyl and seems to fit less well into the pocket. This dimethyl substitution could also affect the preferred torsional angle of the

aryl group leading to different and probably worse, interactions of the terminal phenyl. These less favorable interactions for the terminal aromatic ring are also observed for the weakest S enantiomers (see superpositions of the crystal structures on Figure 7). Furthermore, for the dimethyl and for the *R*/*S* monomethyl analogs at the benzylic side, the number of vdW interactions with the Tyr 68 (indicated on Figure 6) are reduced with respect to the best fitting two carbon interactions on the cyclopropyl analog.

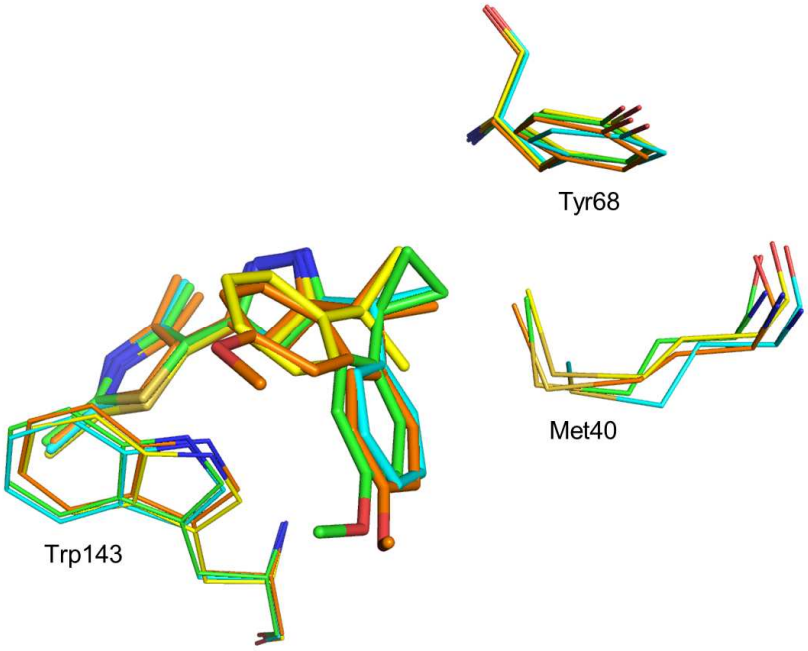


Figure 7. Superposition of the crystal structures of humanized rat COMT with inhibitors reported in Table 3: **22**/**23** (both in orange, co-crystallized as racemate PDB code 5k0f), **18** (blue, co-crystallized as racemate, however one enantiomer (**18R**) observed in the electron density, PDB code 5k0b), **20** (yellow, PDB code 5k0c) and **24** (green, PDB code 5k01).

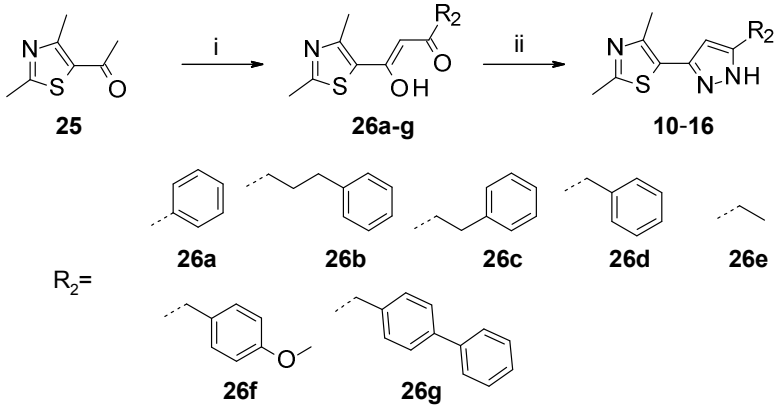
The role of key amino acids (in particular Ty68) in the enzymatic methyl transfer reaction catalyzed by COMT, has recently been analyzed in great detail.[24]

CHEMISTRY

The synthetic strategy to obtain compounds **10-16** is depicted in Scheme 1. Deprotonation of ketone **25** using NaH followed by addition of the corresponding ester gave the diketone intermediates **26a-g** as a mixture of tautomers. Attempts to use acid chlorides instead of esters in this transformation gave no product or very poor yields. Final compounds **10-16** were obtained by condensation of intermediates **26a-g** with hydrazine hydrate in ethanol with catalytic HCl.

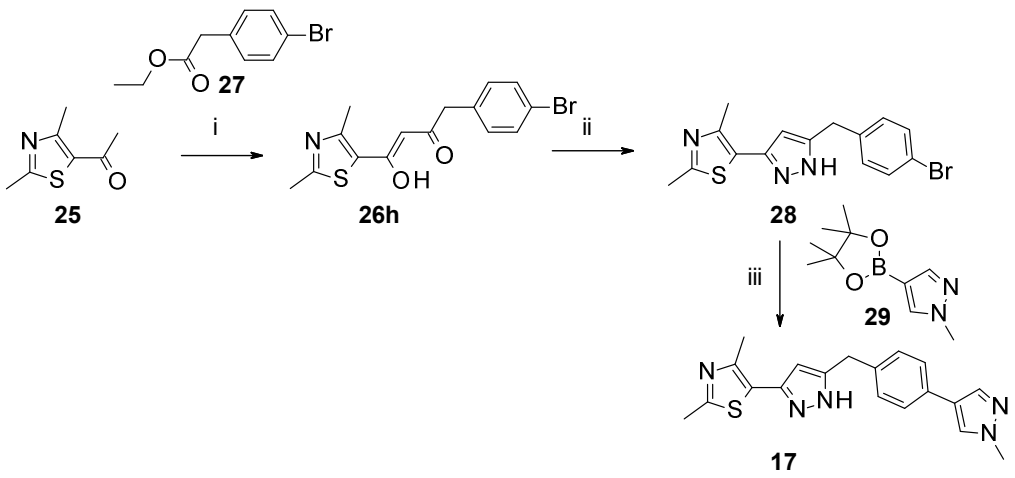
Suzuki coupling with a suitable bromo phenyl substituted intermediate **28** (Scheme 2) prepared similarly as compounds **10-16,** allowed the preparation of bicyclic analogs such as compound **17**.

Scheme 1. Synthesis of compounds 10-16 described in Table 3[a]



[a]Reagents and conditions: (i) NaH, 0 °C, 15 min then R_2CO_2Et, 0-100 °C, 1-3 h. 56-65%; (ii) $N_2H_4 \cdot H_2O$, EtOH/25% aq. HCl (80:1), 60 °C, overnight 60-90%.

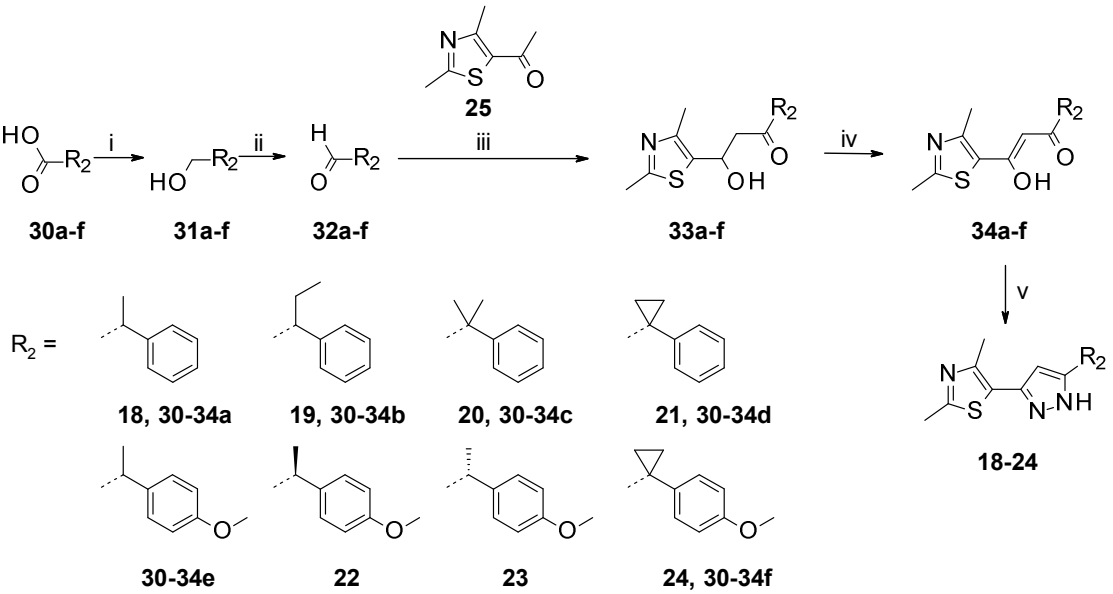
Scheme 2. Synthesis of compound 17 in Table 3[a]



[a]Reagents and conditions: (i) NaH, 0 °C, 30 min then **27**, 0-100 °C, 1 h, 63%; (ii) $N_2H_4 \cdot H_2O$, EtOH/25% aq. HCl (80:1), 60 °C, 3 h, 99%; (iii) **29**, Cs_2CO_3, $(PPh_3)_4Pd$, dioxane, H_2O (4:1), 165 °C, microwave, 20 min, 51%.

For the preparation of the substituted methylene bridged benzylic derivatives (**18**-**24**) (Scheme 3) the addition to the deprotonated methyl ketone **25,** required aldehydes **32a-f** and very low temperatures (-78 °C) in order to obtain acceptable yields. Deprotonation was best performed using a lithium base. The corresponding starting aldehydes **32a-f** (many of them commercially available) were obtained by reduction of available acids **30a-f** to alcohols **31a-f**, followed by oxidation using Dess-Martin periodinane. Oxidation of the β-hydroxy ketone intermediates **33a-f** to diketones **34a-f** was also performed with Dess-Martin periodinane. Reaction of intermediates **34a-f** with hydrazine gave the final compounds **18**-**24** with the central pyrazole ring in one unique tautomeric form in DMSO, as corroborated by NOESY-NMR with some observed NOEs between the NH of the imidazole and the benzylic CH or CH_2. Enantiomers such as **22** and **23,** were initially obtained from racemic precursor by chromatographic separation using a chiral column.

Scheme 3. Synthesis of compounds 18-24 described in Table 3[a]



[a]Reagents and conditions: (i) $LiAlH_4$, THF, 0-20 °C, 70-98%; (ii) Dess-Martin periodinane, CH_2Cl_2, 0-25 °C, 10 h, 70-83%; (iii) **25**, $LiN(SiMe_3)_2$, THF, -78 °C, 0.5 h; then **32**, 3 h, -78 °C, 60-99%; (iv) Dess-Martin periodinane, CH_2Cl_2, 0-25 °C, 5 h, 61-88%; (v) $N_2H_4 \cdot H_2O$, EtOH/25% aq. HCl (80:1), 60 °C, 4 h, 61-92%.

CONCLUSION

This study illustrates the successful application of a fragment-based approach followed by fragment optimization, to obtain for the first time potent and novel inhibitors that bind to the SAM pocket of COMT and do not require a catechol or phenol like motif for high potency. These compounds behave as competitive inhibitors of SAM.

In the reported lead optimization process, a challenging fragment derivatization was performed by observing key binding pocket characteristics, described in a stepwise manner with

the important use of X-ray crystallography. Key interactions are described such as hydrogen bonding between the Ser118 backbone NH and the thiazole or the pyrazole nitrogen for the thiazolo-pyrazole and pyrazolo-pyrazole sub-series, as well as interactions of the central pyrazole with Glu90 and with the backbone NH of Ile91. A small lipophilic pocket was identified occupied ideally with a 4-methyl substituent in the thiazole ring, that has an edge-to-face contact with Trp143.

Flexibility of the enzyme was confirmed, as previously described,[25] for several amino acids such as the Trp143 side chain movement. In addition, we provide insights for a relevant movement and interactions of Tyr68 that opens a novel pocket, offering best contacts with a cyclopropylene linker at a benzylic position. This allows the aromatic ring, to fit best into the pocket created by Trp143 and Met40 with some preferential substitution patterns.

The designed potent lead compounds have CNS drug like properties. Taken together, the reported study and observations represent an advance towards the rational design of CNS drug like and potent non phenolic COMT inhibitors.

EXPERIMENTAL SECTION

COMT Enzyme Inhibition Assay.

The principle of the assay relies on methylation of a 4-nitrocatechol-Alexa Fluor 488 substrate. After transfer of the methyl group from *S*-adenosylmethionine (SAM) to the substrate, an increase of fluorescence intensity is observed because the methylation disturbs the intramolecular static quenching of Alexa Fluor 488. This increase of fluorescence intensity can be followed in a kinetic measurement. An inhibitor compound of COMT decreases the slope.

In detail, 10 µl of recombinant hCOMT (expressed in E.coli.) diluted to 80 nM in assay buffer, was pipetted into a 384 well microtiter plate (black with flat clear bottom, non-binding surface treated polystyrene, Corning ref. 3655). 2 µl of compound dilution in 100% DMSO was then added to the enzyme solution and the plate was shaken with 1500 rpm for 1 min. After adding 20 µl of a mixture of 320 nM 4-nitrocatechol-Alexa488 (produced in house) and 800 nM SAM (Sigma-Aldrich A2804) in assay buffer the plate was shaken for 5 min at 1500 rpm. The plate was then transferred into a microtiter plate reader and the fluorescence was measured every 60s for 180 times at an excitation wavelength of 475 nm and an emission wavelength of 535 nm. The slope was then calculated from the linear range of the kinetic. The assay buffer used was 40 mM phosphate buffer at pH 7.6 containing 2.88 mM $MgCl_2$, 0.9 mM DTT and 0.25 mM $CaCl_2$.

The patented COMT Enzyme Inhibition Assay is a very sensitive homogenous assay, suitable for screening large numbers of compounds for their COMT modulating activity.[19] It provides a robust read out, with IC_{50} values determined by the average of two to four independent repeat measurements. The average coefficient of variation (%CV) of the IC_{50} values is 15%.

Enzyme Cloning, Expression and Purification.

The synthetically made gene for human, soluble COMT encoding the sequence from M51 to P271 (UniProt: P21964) was purchased from GenScript Corp. (NJ, USA) and subcloned in pET32a. The recombinant protein was expressed in BL21 (DE3) cells. The cDNA encoding for rat COMT was PCR cloned into the E. coli expression vector pDS56/RBSII. The construct encoded for the soluble part of rat COMT from M44 to S264 (UniProt: 22734). Recombinant rCOMT was produced in E. coli W3110[pREP4]. In our numbering, M51 for human and M44

for rat of the soluble COMT enzymes were set as first amino acids in the alignment of the sequences. Cell lysis was made in 50 mM Tris/HCL pH 7.6 containing 10 mM 2-mercaptoethanol, inhibitor complete (Roche Applied Science), lysozyme, DNAse and RNAse. After one passage through the cell disruptor at 700 bar the solution was centrifuged (20 min, 30'000 x g, 4°C). The supernatant was collected and ammonium sulfate added to 55% saturation. The protein pellet obtained after centrifugation at 30'000 x g was dissolved in 20 mM Bis-Tris/HCl pH 6.2 containing 10 mM DTT and 1 mM $MgCl_2$, dialyzed against the same buffer and then again centrifuged. The clear supernatant was applied on a Superdex S-75 column (100 x 2.6 cm). Enzyme containing fractions were pooled and further purified on a HiTrap Q FF (5 ml) column in the same Bis-Tris buffer. The protein was eluted in the course of a linear NaCl gradient. Finally, the protein was again applied on a Superdex S-75 column (60 x 1.6 cm) in 50 mM Tris/HCl pH 7.5, 50 mM NaCl, 10 mM DTT, 2 mM $MgCl_2$. As shown by SDS-PAGE and HPLC, >98% pure protein was obtained. The purified protein was monomeric as detected by analytical ultracentrifugation and active in a specific enzyme assay.

Protein engineering by site directed mutagenesis, according to the Statagene Quik Change Mutagenesis Method, was applied to mutate specific amino acids in the active site of the enzyme. In order to block the *S*-adenosyl-methionine pocket, bulky amino acids like tryptophane, arginine and lysine were chosen. In fact the mutations E90R, E90W, S119W, H142R, H142K, H142W were made. Using the same mutagenesis strategy a "humanized" rat protein was made. Thereby the methionine M91 in the so called methionine pocket of the wild type rat enzyme was mutated to isoleucin as it is found in the wild type human enzyme. All mutant proteins could be expressed and purified as described above for the wild type enzymes.

Compound Synthesis and Characterization. Chemistry.

General. All compounds from Table 3 are described below. Reactions were carried out under argon atmosphere. Unless otherwise stated, the reactions were stopped when the starting material was fully consumed according to thin layer chromatography (TLC). The solvent was then removed and the residue was purified by column chromatography. Unless otherwise mentioned, all reagents and chemicals were obtained from commercial suppliers and used without further purification. The purity of final compounds as measured by HPLC was at least above 95%. Column chromatography was carried out either using cartridges packed with silica gel (Isolute Columns, Telos Flash Columns) or on glass columns on silica gel 60 (32-60 mesh, 60Å) with the eluent mixtures given for the corresponding procedures. LC high resolution spectra were recorded with a Agilent LC-system consisting of Agilent 1290 high pressure system, a CTC PAL auto sampler and a Agilent 6520 QTOF. The separation was achieved on a Zorbax Eclipse Plus C18 1,7 µm 2.1*50mm column at 55°C; A=0.01% formic acid in Water; B= 0.01% formic acid in acetonitrile at flow 1 mL/min. gradient: 0 min 5%B, 0.3 min 5%B, 4.5 min 99 %B 5 min 99%B. The NMR spectras were measured on a Bruker 600 MHz machine in a 5 mm TCI cryoprobe at 298 K. TMS was used for referencing. Chemical shifts are reported relative to the solvent in ppm as reference. Coupling constants (J) are given in Hz. The resonance multiplicity is described as s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet), and br. (broad). ^{1}H NMR is reported for the major tautomer.

1-(2,4-Dimethylthiazol-5-yl)-3-phenyl-propane-1,3-dione (10, intermediate 26a). Sodium hydride (60 %, 186 mg, 7.73 mmol) was suspended in THF (20 mL). At 0 °C a solution of 1-(2,4-dimethylthiazol-5-yl)ethanone **25** (CAS 38205-60-6, 600 mg, 3.87 mmol) in THF (4 mL) was added dropwise. The reaction mixture was allowed to warm to room temperature and

stirred for 15 minutes. A solution of ethyl benzoate (1.16 g, 1.11 mL, 7.73 mmol) in THF (4 mL) was added dropwise at 0 °C. After 5 minutes at 0 °C the mixture was stirred at 100 °C for 2 hrs and then cooled to 0 °C. A saturated aqueous solution of ammonium chloride (25 mL) was added. Extraction with water/ethyl acetate and chromatography (silica, 0-50% EtOAc in heptane) yielded 602 mg (60% yield) of **26a** (CAS1467020-71-8) as a yellow solid. R_f: 0.46 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 16.53 (s, 1H), 7.91 (dd, *J*=8.2, 1.1 Hz, 2H), 7.53 - 7.57 (m, 1H), 7.47 - 7.50 (m, 2H), 6.47 (s, 1H), 2.78 (s, 3H), 2.72 (s, 3H) (compound present only as enol-form); LC-HRMS: m/z = 260.0746 [(M+H)+ calculated for $C_{14}H_{13}NO_2S$ = 260.074; Diff = 0.60mD].

2,4-Dimethyl-5-(5-phenyl-1H-pyrazol-3-yl)thiazole (10). 1-(2,4-Dimethylthiazol-5-yl)-3-phenyl-propane-1,3-dione (**26a**) (300 mg, 1.16 mmol) was combined with ethanol (15 ml). Hydrazine monohydrate (99.5 mg, 98 µl, 1.27 mmol) and hydrochloric acid (25%, 75.0 µl) were added. The solution was stirred at 60 °C overnight. For compounds **11-17** with sterically less demanding linkers, the reaction was typically finished after 2-3 hrs. The reaction mixture was basified with saturated aqueous sodium bicarbonate and concentrated. Chromatography (silica, 0-5% MeOH in dichloromethane) and trituration with diethyl ether/pentane gave compound **10** as off-white solid (105 mg, 36% yield). R_f: 0.43 (dichloromethane/methanol 9:1); ^{1}H NMR (600 MHz, DMSO-d6) δ ppm 13.46 (br s, 1H), 7.82 (br d, *J*=6.6 Hz, 2H), 7.47 (br d, *J*=6.3 Hz, 2H), 7.38 (br d, *J*=6.5 Hz, 1H), 6.94 (s, 1H), 2.60 (br s, 3H), 2.53 (br s, 3H); LC-HRMS: m/z = 256.0909 [$(M+H)^+$ calculated for $C_{14}H_{13}N_3S$ = 256.0903 ; Diff = 0.60mD]

1-(2,4-Dimethylthiazol-5-yl)-6-phenyl-hexane-1,3-dione (11, intermediate 26b). 1-(2,4-Dimethylthiazol-5-yl)ethanone **25** (150 mg, 966 µmol), NaH (60% in mineral oil, 46.4 mg, 1.16 mmol) and ethyl 4-phenylbutanoate (372 mg, 1.93 mmol) were used to synthesize **26b** following

the procedure described for the preparation of **26a.** Compound **26b** (40.0 mg, 13% yield) was obtained as a yellow oil. R_f: 0.54 (EtOAc/heptane 1:1); (600 MHz, $CDCl_3$) δ ppm 15.95 (s, 1H), 7.31 - 7.27 (m, 2H), 7.23 - 7.15 (m, 1H), 7.21 - 7.17 (m, 2H), 5.77 (s, 1H), 2.70 (s, 3H), 2.69 (s, 3 H), 2.71 – 2.68 (m, 2H), 2.37 (t, *J*=7.6 Hz, 2H), 2.00 (quin, *J*=7.6 Hz, 2 H) (9:1 tautomeric mixture of enol and di-ketone); LC-HRMS: m/z = 302.1206 [$(M+H)^+$ calculated for $C_{17}H_{19}NO_2S$ = 302.1209 ; Diff = -0.30mD].

2,4-Dimethyl-5-[5-(3-phenylpropyl)-1H-pyrazol-3-yl]thiazole (11). 1-(2,4-Dimethylthiazol-5-yl)-6-phenyl-hexane-1,3-dione **26b** (170 mg, 564 μmol), hydrazine monohydrate (48.5 mg, 47 μL, 620 μmol) and hydrochloric acid (25%, 10 μL) were used to synthesize **11** following the procedure described for the preparation of **10**. After chromatography, compound **11** (115 mg, 68% yield) was obtained as an off-white solid. R_f: 0.21 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 11.46-7.9 (br s, 1H), 7.32 - 7.28 (m, 2H), 7.23 - 7.19 (m, 1H), 7.20 - 7.17 (m, 2H), 6.23 (s, 1H), 2.73 - 2.69 (m, 2H), 2.72 - 2.68 (m, 2H), 2.67 (s, 3H), 2.55 (s, 3H), 2.03 (quin, *J*=7.6 Hz, 2H); LC-HRMS: m/z = 298.138 [$(M+H)^+$ calculated for $C_{17}H_{19}N_3S$ = 298.1373 ; Diff = 0.70mD].

1-(2,4-Dimethyl-thiazol-5-yl)-5-phenyl-pentane-1,3-dione (12, intermediate 26c). 1-(2,4-Dimethylthiazol-5-yl)ethanone **25** (100 mg, 0.644 mmol), NaH (60% in mineral oil, 31 mg, 0.77 mmol) and ethyl 3-phenylpropanoate (230 mg, 0.774 mmol) were used to synthesize **26c** following a similar procedure as described for the preparation of **26a.** In this case 1-(2,4-dimethylthiazol-5-yl)ethanone and NaH were stirred at 0 °C for 30 minutes in THF (0.5 mL) before adding the ester. After chromatography (silica gel, 0-20% EtOAc/heptane), compound **26c** (185 mg, 57% yield) was obtained as a light yellow oil. R_f: 0.39 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 15.93 (br. s., 1H), 7.32 - 7.28 (m, 2H), 7.27 - 7.17 (m, 3H), 5.73

(s, 1H), 3.01 - 2.97 (m, 2H), 2.68 (s, 3H), 2.66 (s, 3H), 2.69 - 2.65 (m, 2H); LC-HRMS: m/z = 288.1066 [$(M+H)^+$ calculated for $C_{16}H_{17}NO_2S$ = 288.1058 ; Diff = 1.30 mD].

2,4-Dimethyl-5-(5-phenethyl-2H-pyrazol-3-yl)-thiazole (12). 1-(2,4-Dimethyl-thiazol-5-yl)-5-phenyl-pentane-1,3-dione **26c** (40 mg, 0.14 mmol), hydrazine monohydrate (7.7 mg, 7.6 µL, 0.150 mmol) and hydrochloric acid (25%, 10 µL) were used to synthesize **12** following the procedure described for the preparation of **10**. After chromatography, compound **12** (36 mg, 91% yield) was obtained as a light brown solid. R_f: 0.13 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 7.34 - 7.28 (m, 2H), 7.25 - 7.22 (m, 1H), 7.21 - 7.17 (m, 2H), 6.20 (s, 1H), 3.07-2.90 (m, 4H), 2.66 (s, 3H), 2.53 (s, 3H); LC-HRMS: m/z = 284.1233 [$(M+H)^+$ calculated for $C_{16}H_{17}N_3S$ = 284.1216 ; Diff = 1.70 mD].

1-(2,4-Dimethyl-thiazol-5-yl)-4-phenyl-butane-1, 3-dione (13, intermediate 26d). 1-(2,4-Dimethylthiazol-5-yl)ethanone **25** (50 mg, 0.32 mmol), NaH (60% in mineral oil, 26 mg, 0.64 mmol) and ethyl 2-phenylacetate (10.6 mg, 0.64 mmol) were used to synthesize **26d** following the procedure described for the preparation of **26c.** Compound **26d** (49 mg, 56% yield) was obtained as a white powder. R_f: 0.49 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 7.36 - 7.34 (m, 2H), 7.31 - 7.27 (m, 3H), 5.72 (s, 1H), 3.66 (s, 2H), 2.67 (s, 3H), 2.61 (s, 3H); LC-HRMS: m/z = 274.0893 [$(M+H)^+$ calculated for $C_{15}H_{15}NO_2S$ = 274.0896 ; Diff = -0.30 mD].

2,4-Dimethyl-5-(5-benzyl-2H-pyrazol-3-yl)-thiazole (13). 1-(2,4-Dimethyl-thiazol-5-yl)-4-phenyl-butane-1,3-dione **26d** (100 mg, 0.37 mmol), hydrazine monohydrate (20 mg, 20 µL, 407 µmol) and hydrochloric acid (25%, 10 µL) were used to synthesize **13** following the procedure described for the preparation of **10**. The crude product was purified by trituration with

diethyl ether to afford **13** as an off white solid (50 mg, 51% yield). R_f: 0.19 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 7.36 - 7.32 (m, 2H), 7.28 (d, *J* = 7.3 Hz, 1H), 7.26 - 7.23 (m, 2H), 6.25 (s, 1H), 4.05 (s, 2H), 2.66 (s, 3H), 2.53 (s, 3H); LC-HRMS: m/z = 270.1070 $[(M+H)^+$ calculated for $C_{15}H_{15}N_3S$ = 270.1060 ; Diff = 1.00 mD].

1-(2,4-Dimethyl-thiazol-5-yl)-pentane-1,3-dione (14, intermediate 26e). 1-(2,4-Dimethylthiazol-5-yl)ethanone **25** (100 mg, 0.32 mmol), NaH (60% in mineral oil, 52 mg, 1.3 mmol) and ethyl proprionate (132 mg, 1.29 mmol) were used to synthesize **26e** following the procedure described for the preparation of **26c.** Compound **26e** (80 mg, 59% yield) was obtained as a light yellow oil. R_f: 0.51 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 5.79 (s, 1H), 2.70 (s, 3H), 2.69 (s, 3H), 2.39 (q, *J*=7.6 Hz, 2H), 1.19 (t, *J*=7.6 Hz, 3H); LC-HRMS: m/z = 212.0745 $[(M+H)^+$ calculated for $C_{10}H_{13}NO_2S$ = 212.074 ; Diff = 0.50mD].

2,4-Dimethyl-5-(5-ethyl-2H-pyrazol-3-yl)-thiazole (14). 1-(2,4-Dimethyl-thiazol-5-yl)-pentane-1,3-dione **26e** (30 mg, 0.38 mmol), hydrazine monohydrate (21 mg, 21 µL, 408 µmol) and hydrochloric acid (25%, 10 µL) were used to synthesize **14** following the procedure described for the preparation of **10**. The crude product was purified by trituration with diethyl ether to afford **14** as an off white solid (27 mg, 92% yield). R_f: 0.16 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 6.23 (s, 1H), 2.72 (q, *J* = 7.7 Hz, 2H), 2.67 (s, 3H), 2.56 (s, 3H), 1.32 (t, *J*=7.7 Hz, 3H); LC-HRMS: m/z = 208.0924 $[(M+H)^+$ calculated for $C_{10}H_{13}N_3S$ = 208.0903 ; Diff = 2.10 mD].

1-(2,4-Dimethyl-1,3-thiazol-5-yl)-4-(4-methoxyphenyl)butane-1,3-dione (15, intermediate 26f). 1-(2,4-Dimethylthiazol-5-yl)ethanone **25** (200 mg, 1.29 mmol), NaH (60% in mineral oil, 103 mg, 2.58 mmol) and ethyl 2-(4-methoxyphenyl)acetate (501 mg, 0.456 mL)

were used to synthesize **26f** following the procedure described for the preparation of **26c.** Compound **26f** (202 mg, 52% yield) was obtained as a white solid. R_f: 0.51 (EtOAc); ^{1}H-NMR (600 MHz, $CDCl_3$) (enol tautomer) δ ppm 15.84 (br s, 1H), 7.19 (br d, *J*=8.7 Hz, 2H), 6.88 (d, *J*=8.7 Hz, 2H), 5.71 (s, 1H), 3.81 (s, 2H), 3.60 (s, 2H), 2.67 (s, 4H), 2.62 (s, 3H); LC-HRMS: m/z = 304.1019 [$(M+H)^+$ calculated for $C_{16}H_{17}NO_3S$ = 304.1002 ; Diff = 1.70mD].

5-[5-[(4-Methoxyphenyl)methyl]-1H-pyrazol-3-yl]-2,4-dimethyl-thiazole (15). 1-(2,4-Dimethyl-thiazol-5-yl)-4-(4-methoxy-phenyl)-butane-1,3-dione (200 mg, 659 μmol) **26f**, hydrazine monohydrate (36.3 mg, 35 μL, 725 μmol) and HCl 25% (26 μL) were used to synthesize **15** following the procedure described for the preparation of **10**. After chromatography, compound **15** (159 mg, 81% yield) was obtained as a white solid. R_f: 0.343 (EtOAc); ^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm 7.17-7.16 (m, 2H); 6.88 - 6.87 (m, 2H), 6.23 (s, 1H), 3.99 (s, 2H), 3.81 (s, 3H), 2.66 (s, 3H), 2.53 (s, 3H); ^{1}H-NMR (600 MHz, DMSO-d6) δ 13.39 (br s, 1H, NH imidazole); 7.19 (d, *J*=8.66, 2H), 6.87 (d, *J*=8.66, 2H), 6.22 (s, 1H), 3.91 (s, 2H), 3.72 (s, 3H), 2.56 (s, 3H), 2.42 (s, 3H); NOESY (600 MHz, DMSO-d6): Interaction between δ 13.39 (br s, 1H, NH), and 3.91 (s, 2H, CH_2) indicating the imidazole tautomer is the one indicated. LC-HRMS: m/z = 300.1176 [$(M+H)^+$ calculated for $C_{16}H_{17}N_3OS$ = 300.1165 ; Diff = 1.10mD].

4-(Biphenyl-4-yl)-1-(2,4-dimethyl-1,3-thiazol-5-yl)butane-1,3-dione (16, intermediate 26g). 1-(2,4-Dimethylthiazol-5-yl)ethanone 25,(100 mg, 0.644 mmol, 87 μL), NaH (60% in mineral oil, 51.5 mg, 1.29 mmol) and ethyl biphenyl-4-ylacetate (CAS number 14062-23-8) (310 mg, 1.29 mmol) were used to synthesize **26g** following the procedure described for the preparation of **26c.** Compound **26g** (137 mg, 61% yield) was obtained as a white solid. R_f: 0.61

(EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$) δ 15.86 (br s, 1H, OH enol), 7.52 - 7.63 (m, 5H), 7.40 - 7.49 (m, 2H), 7.36 (br d, *J*=8.1 Hz, 2H), 5.78 (s, 1H), 3.70 (s, 2H), 2.67 (s, 3H), 2.63 (s, 3H); LC-HRMS: m/z = 350.121 [$(M+H)^+$ calculated for $C_{21}H_{19}NO_2S$ = 350.1209 ; Diff = 0.10mD].

5-[5-(Biphenyl-4-ylmethyl)-1H-pyrazol-3-yl]-2,4-dimethyl-1,3-thiazole (16).

4-(Biphenyl-4-yl)-1-(2,4-dimethyl-1,3-thiazol-5-yl)butane-1,3-dione **26g** (116 mg, 332 µmol), hydrazine monohydrate (18.3 mg, 18 µL, 365 µmol) in ethanol (1.3 mL) and HCl 25% (13 µL) were used to synthesize **16** following the procedure described for the preparation of **10**. After chromatography, compound **16** (81.4 mg, 71% yield) was obtained as a light yellow solid. R_f: 0.16 (pentane/EtOAc 1:1); ^{1}H NMR (600 MHz, $CDCl_3$): 7.61 - 7.55 (m, 4H). 7.46 - 7.41 (m, 2H), 7.37 - 7.34 (m, 1H), 7.32 (br d, *J*=8.4 Hz, 2H), 6.29 (s, 1H), 4.10 (br s, 2H), 2.67 (s, 3H), 2.55 (s, 3H); LC-HRMS: m/z = 346.1378 [$(M+H)^+$ calculated for $C_{21}H_{19}N_3S$ = 346.1373 ; Diff = 0.50mD].

4-(4-Bromophenyl)-1-(2,4-dimethyl-1,3-thiazol-5-yl)butane-1,3-dione (17, intermediate 26h). 1-(2,4-Dimethylthiazol-5-yl)ethanone **25** (200 mg, 1.29 mmol, 174 µL), sodium hydride (60% in mineral oil, 103 mg, 2.58 mmol) and ethyl (4-bromophenyl)acetate (626 mg, 2.58 mmol) were used to synthesize **26h** following the procedure described for the preparation of **26c.** Compound **26h** was obtained as a light yellow solid (283.8 mg, 63% yield). R_f: 0.54 (EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$): δ 15.82 (br s, 1H, OH enol), 7.43 - 7.51 (m, 2H), 7.16 (br d, *J*=8.5 Hz, 2H), 5.71 (s, 1H), 3.61 (s, 2H), 2.68 (s, 3H), 2.64 (s, 3H); GC-EI-MS: m/z = 351 ($M^{\cdot+}$; calculated: for $C_{15}H_{14}BrNO_2S$ m/z = 351)

5-[5-(4-Bromobenzyl)-1H-pyrazol-3-yl]-2,4-dimethyl-1,3-thiazole (17, intermediate 28). 4-(4-Bromophenyl)-1-(2,4-dimethyl-1,3-thiazol-5-yl)butane-1,3-dione **26h** (200 mg, 568 µmol), hydrazine monohydrate (31.3 mg, 31 µL, 625 µmol) in ethanol (2.3 mL) and HCl 25% (23 µL) were used to synthesize **28** following the procedure described for the preparation of **10**. After chromatography, compound **28** (195.2 mg, 99% yield) was obtained as a light yellow solid. *Rf*: 0.12 (EtOAc/heptane 1:1), R_f: 0.385 (EtOAc); ^{1}H NMR (600 MHz, DMSO-d6): δ 12.86 (br s, 1H, NH), 7.51 (d, *J*=8.26 Hz, 2H), 7.24 (br d, *J*=8.4 Hz, 2H), 6.27 (br s, 1H), 3.97 (s, 2H, CH_2), 2.56 (s, 3H), 2.43 (s, 3H); NOESY (600 MHz, DMSO-d6): Interaction between δ 12.86 (br s, 1H, NH), and 3.97 (s, 2H, CH_2) indicating the imidazole tautomer is the one indicated. LC-HRMS: m/z = 348.0168 [$(M+H)^+$ calculated for $C_{15}H_{14}BrN_3S$ = 348.0165 ; Diff = 0.30mD].

2,4-Dimethyl-5-{5-[4-(1-methyl-1H-pyrazol-4-yl)benzyl]-1H-pyrazol-3-yl}-1,3-thiazole (17). 5-[5-(4-Bromobenzyl)-1H-pyrazol-3-yl]-2,4-dimethyl-1,3-thiazole **28** (180 mg, 517 µmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (129 mg, 620 µmol), Cs_2CO_3 (674 mg, 2.07 mmol, Eq: 4) and tetrakis (triphenylphosphine) palladium (0) (59.7 mg, 51.7 µmol) were combined in dioxane (2.2 mL) and water (550 µL). The vial was capped and the mixture was heated in a microwave oven at 165 °C. After 20 min reaction was poured into a saturated sodium bicarbonate solution (50 mL) and extracted with dichloromethane (3x 50 mL). The organic layers were dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (silica gel, 0% to 100% EtOAc in heptane) to afford 92.3 mg of **17** (51% yield) as a light yellow solid. R_f: 0.17 (EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.74 (s, 1H), 7.60 (s, 1H), 7.45-7.42 (m, 2H), 7.26 - 7.22 (m, 2H), 6.26 (s, 1H), 4.05 (s, 2H), 3.95

(s, 3H), 2.66 (s, 3H), 2.54 (s, 3H); LC-HRMS: m/z = 350.1442 [$(M+H)^+$ calculated for $C_{19}H_{19}N_5S$ = 350.1434 ; Diff = 0.80mD].

1-(2,4-Dimethyl-1,3-thiazol-5-yl)-3-hydroxy-4-phenylpentan-1-one (18, intermediate 33a). 1-(2,4-dimethylthiazol-5-yl)ethanone **25** (500 mg, 3.22 mmol) was dissolved in THF (3 mL). The reaction mixture was cooled down to -78 °C and lithium bis(trimethylsilyl)amide in THF (3.54 mL, 3.54 mmol) was added. After stirring 30 min at 0 °C, the reaction was cooled again to -78 °C and 2-phenylpropanal **32a** (CAS Registry Number 93-53-8) (562 mg, 561 µL, 4.19 mmol), dissolved in THF (3 mL), was slowly added. The mixture was stirred for 3 h at -78 °C until TLC indicated the reaction was finished. The reaction was stopped by adding 15 mL H_2O at -78 °C and extracted with DCM at room temp. The organic layers were dried over $MgSO_4$ and concentrated. The crude material was purified by column chromatography (silica gel, 0% to 80% EtOAc in heptane) to afford 571 mg (61% yield) of desired intermediate **33a** as an oil. R_f: 0.28 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, DMSO-d6): δ 7.31-7.26 (m, 3H), 7.26-7.21 (m, 2H), 7.21-7.16 (m, 1H), 4.07-4.04 (m, 1H), 2.75-2.70 (m, 2H), 2.61 (s, 3H), 2.54 (s, 3H), 1.25 (s, 3H); LC-HRMS: m/z = 290.1224 [$(M+H)^+$ calculated for $C_{16}H_{19}NO_2S$ = 290.1209 ; Diff = 1.50mD].

1-(2,4-Dimethyl-1,3-thiazol-5-yl)-4-phenylpentane-1,3-dione (18, intermediate 34a). 1-(2,4-dimethylthiazol-5-yl)-3-hydroxy-4-phenylpentan-1-one **33a** (212 mg, 0.733 mmol) and Dess-Martin periodinane (15% in DCM, 2.07 g, 0.733 mmol) were combined at 0 °C with DCM (8 mL). The reaction mixture was stirred for 5 hrs at r.t., diluted with 25 mL of saturated solution of $NaHCO_3$ and extracted with DCM (3 x 30 mL). The organic layers were dried over $MgSO_4$ and concentrated. The crude material was purified by column chromatography (silica gel, 0% to

100% EtOAc in heptane) to afford 186.3 mg (88% yield) of **34a** as a light yellow viscous liquid. R_f: 0.40 (EtOAc/heptane 1:1); R_f: 0.59 (EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$): δ 15.83 (br s, 1H), 7.38-7.27 (m, 5H), 5.74 (s, 1H), 3.72 (q, *J*= 7.2 Hz, 1H), 2.66 (s, 3H), 2.59 (s, 3H), 1.55 (d, *J*=7.2 Hz, 3H); ^{1}H NMR (600 MHz, DMSO-d6): δ 16.00 (br s, 1H, OH), 7.37- 7.34 (m, 3H), 7.31-7.24 (m, 2H), 6.00 (s, 1H), 3.9-3.95 (m, 1H), 2.64 (s, 3H), 2.54 (s, 3H), 1.46 (d, *J*=7.2 Hz, 3H); GC-EI-MS: m/z = 287.1 (M·+; calculated for $C_{16}H_{17}NO_2S$ m/z = 287.1).

2,4-Dimethyl-5-[5-(1-phenylethyl)-1H-pyrazol-3-yl]-1,3-thiazole (18). 1-(2,4-Dimethyl-1,3-thiazol-5-yl)-4-phenylpentane-1,3-dione **34a** (162 mg, 564 μmol), hydrazine monohydrate (31 mg, 31 μL, 620 μmol) in ethanol (2.2 mL) and HCl 25% (23 μL) were used to synthesize **18** following the procedure described for the preparation of **10**. Compound **18** (100.2 mg, 63% yield) was obtained as an off-white solid. R_f: 0.41 (EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.38-7.32 (m, 2H), 7.28-7.24 (m, 3H), 6.30 (s, 1H), 4.19 (q, *J*=7.2 Hz, 1H), 2.67 (s, 3H), 2.56 (s, 3H), 1.68 (d, *J*=7.2 Hz, 3H); ^{1}H NMR (600 MHz, DMSO-d6): δ 12.82 (br s, 1H, NH), 7.33-7.28 (m, 4H), 7.22-7.20 (m, 1H), 6.30 (d, *J*=1.9 Hz, 1H), 4.22 (q, *J*=7.2 Hz, 1H), 2.56 (s, 3H), 2.45 (s, 3H), 1.60 (d, *J*=7.2 Hz, 3H); NOESY (600 MHz, DMSO-d6); NOE between 12.82 (br s, 1H, NH) and 4.22 (q, *J*=7.2 Hz, 1H); LC-HRMS: m/z = 284.1216 [$(M+H)^+$ calculated for $C_{16}H_{17}N_3S$ = 284.1216 ; Diff = 0.00mD]. The racemic mixture **18R/S** was separated using a chiral column (Chiralpack AD, 10% EtOH/ Heptane); the retention time for enantiomer A with $[\alpha]^{20}_D$ (*c* 1 $CHCl_3$) = + 71.347 (100% ee) was 8.58 minutes; for enantiomer B with $[\alpha]^{20}_D$ (*c* 1 $CHCl_3$) = -69.747 (99% ee), the retention time was 9.7 minutes. On the enzymatic assay, IC_{50}= 1.12 μM for enantiomer A (**18S**) and IC_{50}= 0.70 μM for enantiomer B (**18R**). The absolute configuration was determined by enantioselective synthesis, preparing enantiomer A using chiral *S* (+)2-phenylpropanal and comparing optical rotations from final

compounds ($[\alpha]^{20}_D$ (*c* 1 $CHCl_3$) = +73.79 for the prepared chiral *S* enantiomer). Also by co-injections in chiral HPLC.

2-Phenylbutan-1-ol (19, intermediate 31b) was prepared by reduction of 2-phenylbutanoic acid **30b** (500 mg, 3.05 mmol) in THF (5.5 mL) using $LiAlH_4$ 1M in THF (6.09 mL, 6.09 mmol) and stirring 1 h at 0 °C. Quenching the reaction with a saturated NH_4Cl aqueous solution and extraction using EtOAc afforded, after concentration of the organic phase, 457 mg of alcohol **31b** (98% yield) that was used as a crude on the next reaction.

2-Phenylbutanal (19, intermediate 32b) (CAS Registry Number 2439-43-2) was prepared from 2-phenylbutan-1-ol **31b** (445 mg, 2.96 mmol) using Dess-Martin periodinane (15% in DCM, 8.3 g, 2.96 mmol) in dichloromethane (15.5 mL) and stirring 6 h to room temperature. The reaction was poured into 50 mL of saturated $NaHCO_3$ and extracted with dichloromethane. The organic layers were dried over $MgSO_4$ and concentrated. The crude material was purified by column chromatography (silica gel, 0% to 50% EtOAc in heptane) to afford 202 mg (50% yield) of the desired aldehyde **32b**. R_f: 0.53 (EtOAc/heptane 1:2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.7 (s, 1H), 7.4-7.2 (m, 5H), 3.44-3.38 (m, 1H), 2.19-2.05 (dt, *J*=14.3, 7.0 Hz, 1H), 1.84-1.69 (dt, *J*=14.0, 7.1 Hz, 1H), 0.91 (br t, *J*=7.1 Hz, 3H).

1-(2,4-Dimethyl-1,3-thiazol-5-yl)-3-hydroxy-4-phenylhexan-1-one (19, intermediate 33b). 1-(2,4-Dimethylthiazol-5-yl)ethanone **25** (153 mg, 133 μL, 986 μmol) lithium bis(trimethylsilyl)amide in THF (1.08 mL, 1.08 mmol) and 2-phenylbutanal (190 mg, 1.28 mmol) **32b** were used to synthesize **33b** following the procedure described for the preparation of **33a**. Compound **33b** (239 mg, 60 % yield) was obtained as an oil. R_f: 0.24 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.32-7.29 (m, 2H), 7.24-7.14 (m, 3H), 4.32-4.2 (m, 1H), 3.34 (d,

J=4.1 Hz, 1H), 2.67 (m, 2H), 2.64 (s, 3H), 2,57 (s, 3H), 2.57-2.64 (m, 1H), 2.22-2.20 (m, 1H), 1.63-1.61 (m, 1H), 0.76 (t, *J*=7.4 Hz, 3H); LC-HRMS: m/z = 304.1378 [$(M+H)^+$ calculated for $C_{17}H_{21}NO_2S$ = 304.1366 ; Diff = 1.20mD].

1-(2,4-Dimethyl-1,3-thiazol-5-yl)-4-phenylhexane-1,3-dione (19, intermediate 34b). 1-(2,4-Dimethyl-1,3-thiazol-5-yl)-3-hydroxy-4-phenylhexan-1-one **33b** (100 mg, 0.330 mmol) and Dess-Martin periodinane (15% weight in DCM) (1.03 g, 0.363 mmol) were used to synthesize **34b** following the procedure described for the preparation of **34a**. Compound **34b** (76 mg, 76% yield) was obtained as a viscous liquid. R_f: 0.53 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) (major tautomer): δ 15.98 (s, 1H), 7.35-7.30 (m, 5H), 5.79 (s, 1H), 3.40 (t, *J*=7.7 Hz, 1H), 2.67 (s, 3H), 2.62 (s, 3H), 2.17-2.13 (m, 1H), 1.87-1.89 (m, 1H), 0.93 (t, *J*=7.4 Hz, 3H); LC-HRMS: m/z = 302.1223 [$(M+H)^+$ calculated for $C_{17}H_{19}NO_2S$ = 302.1209; Diff = 1.40mD].

1-(2,4-Dimethyl-5-[5-(1-phenylpropyl)-1H-pyrazol-3-yl]-1,3-thiazole (19). 1-(2,4-Dimethyl-1,3-thiazol-5-yl)-4-phenylhexane-1,3-dione **34b** (33 mg, 109 μmol), hydrazine monohydrate (6 mg, 36 μL, 120 μmol) and HCl 25% (5 μL) were used to synthesize **19** following the procedure described for the preparation of **10**. After chromatography, compound **19** (28.3 mg, 87% yield) was obtained as an off-white solid. R_f: 0.39 (EtOAc); ^{1}H NMR (600 MHz, DMSO-d6): δ 12.83 (br s, 1H), 7.31 (m, 4H), 7.22-7.19 (m, 1H), 6.35 (s, 1H), 3.91 (br t, *J*=7.51 Hz, 1H), 2.56 (s, 3H), 2.45 (s, 3H), 2.12-2.05 (m, 1H), 1.99-1.93 (m, 1H), 0.83 (t, *J*=7.30 Hz, 3H); LC-HRMS: m/z = 298.1385 [$(M+H)^+$ calculated for $C_{17}H_{19}N_3S$ = 298.1383 ; Diff = 0.20mD]. The racemic mixture **19R/S** was separated using a chiral column (Chiralpak AD, 15% EtOH/heptane). The retention time for enantiomer A (**19S**) was 8.22 minutes with $[\alpha]^{20}_D$ (*c* 0.99 $CHCl_3$) = +74.69 (100% ee); for enantiomer B (**19R** enantiomer) with $[\alpha]^{20}_D$ (*c* 1 $CHCl_3$) = -69.6

(96% ee), the retention time was 9.07 minutes. On the enzymatic assay, IC_{50}= 28 μM for the **19S** enantiomer and IC_{50}=16 μM for the **19R** enantiomer. The absolute configuration was determined by enantioselective synthesis using *S* (+)2-phenylbutanal.

2-Methyl-2-phenylpropanal (**20**, intermediate **32c).** 2-Methyl-2-phenylpropan-ol **31c** (300 mg, 1.99 mmol) and Dess-Martin periodinane (15% in DCM) (5.62 g, 1.99 mmol) were used to synthesize **32c** following the procedure described for the preparation of **32b**. Compound **32c** (296 mg, 45% yield) was obtained as a light yellow oil. NMR was the same as described in literature (CAS Registry Number 3805-10-5).

1-(2,4-Dimethyl-1,3-thiazol-5-yl)-3-hydroxy-4-methyl-4-phenylpentan-1-one (20, intermediate 33c). 1-(2,4-Dimethylthiazol-5-yl)ethanone **25** (91.8 mg, 79.8 μL, 592 μmol), lithium bis(trimethylsilyl) amide in THF (0.651 mL, 651μmol) and 2-methyl-2-phenylpropanal **32c** (114 mg, 769 μmol) were used to synthesize **33c** following the procedure described for the preparation of **33a**. Compound **33c** (103 mg, 57% yield) was obtained as a colorless oil. R_f: 0.25 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.42-7.39 (br d, *J*=7.9 Hz, 2H), 7.34 (br t, *J*=7.9 Hz, 2H), 7.24-7.20 (m, 1H), 4.31-4.33 (br dd, *J*=5.8, 3 Hz, 1H), 2.87 (d, *J*=3.3 Hz, 1H), 2.64-2.65 (m, 1H) inside 2.65 (s, 3H), 2,60 (s, 3H), 1.42 (s, 3H), 1.39 (s, 3H) ; ^{1}H NMR (600 MHz, DMSO-d6): δ 7.39-7.38 (br d, *J*=7.6 Hz, 2H), 7.32-7-29 (br t, *J*=7.6 Hz, 2H), 7.20-7.17 (m, 1H), 5.03 (d, *J*=5.8Hz, 1 H, O<u>H</u>), 4.08-4.03 (m, 1H), 2.63-2.59 (m, 1H) inside 2.60 (s, 3H), 2,50 (s, 3H), 2.36-2.39 (dd, *J*=15,2, 2.1 Hz 1H), 1.27 (s, 6H); LC-HRMS: m/z = 304.1369 [$(M+H)^+$ calculated for $C_{17}H_{21}NO_2S$ = 304.1366 ; Diff = 0.30mD].

1-(2,4-Dimethyl-1,3-thiazol-5-yl)-4-methyl-4-phenylpentane-1,3-dione (20, intermediate 34c). 1-(2,4-Dimethyl-1,3-thiazol-5-yl)-3-hydroxy-4-methyl-4-phenylpentan-1-

one **33c** (84 mg, 0.277 mmol) and Dess-Martin periodinane (15% in DCM) (861 mg, 0.305 mmol) were used to synthesize **34c** following the procedure described for the preparation of **34a.** Compound **34c** (65 mg, 78% yield) was obtained as a viscous liquid. R_f: 0.44 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) (major tautomer): δ 15.93 (s, 1H, OH), 7.36-7.26 (m, 5H), 5.65 (s, 1H), 2.65 (s, 3H), 2.52 (s, 3H), 1.59 (s, 6H); LC-HRMS: m/z = 302.1198 [$(M+H)^+$ calculated for $C_{17}H_{19}NO_2S$ = 302.1209 ; Diff = -1.10mD].

2,4-Dimethyl-5-[5-(2-phenylpropan-2-yl)-1H-pyrazol-3-yl]-1,3-thiazole (20). 1-(2,4-Dimethyl-1,3-thiazol-5-yl)-4-methyl-4-phenylpentane-1,3-dione **34c** (62 mg, 205 μmol), hydrazine hydrate (13 mg, 266 μmol) and HCl 25% (5 μL) were used to synthesize **20** following the procedure described for the preparation of **10**. After chromatography, compound **20** (56 mg, 92% yield) was obtained as an off white solid. R_f: 0.35 (EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.33-7.29 (m, 4H), 7.22-7.19 (m, 1H), 6.32 (s, 1H), 2.67 (s, 3H), 2.57 (s, 3H), 1.74 (s, 6H); LC-HRMS: m/z = 298.1385 [$(M+H)^+$ calculated for $C_{17}H_{19}N_3S$ = 298.1373 ; Diff = 1.00mD].

1-(2,4-Dimethyl-1,3-thiazol-5-yl)-3-hydroxy-3-(1-phenylcyclopropyl)propan-1-one (21, intermediate 33d). 1-(2,4-Dimethylthiazol-5-yl)ethanone **25** (100 mg, 87 μL, 644 μmol), lithium bis(trimethylsilyl)amide in THF (0.709 mL, 709 μmol), and 1-phenylcyclopropanecarbaldehyde (188 mg, 1.29 mmol) **32d** (CAS Registry Number 21744-88-7) were used to synthesize **33d** following the procedure described for the preparation of **33a**. Compound **33d** (130 mg, 67% yield) was obtained as a colorless oil. R_f: 0.54 (EtOAc), 0.31 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.40-7.38 (br d, J=8.2 Hz, 2H), 7.30-7.33 (br t, J=7.66 Hz, 2H), 7.29-7.25 (m, 1H), 3.87 (dd, J= 9.6, 2.2 Hz, 1H), 3.01-3.09 (br. s, 1H), 2.99 (dd, J=17, 2.4 Hz, 1H), 2.74 (dd, J=16.9, 9.6 Hz, 1H), 2.66 (s, 3H), 2.62 (s, 3H), 0.91-0.82

(m, 4H); LC-HRMS: m/z = 302.1218 [$(M+H)^+$ calculated for $C_{17}H_{19}NO_2S$ = 302.1209 ; Diff = 0.90mD].

1-(2,4-Dimethyl-1,3-thiazol-5-yl)-3-(1-phenylcyclopropyl)propane-1,3-dione (21, intermediate 34d). 1-(2,4-Dimethyl-1,3-thiazol-5-yl)-3-hydroxy-3-(1-phenylcyclopropyl)propan-1-one **33d** (81 mg, 0.269 mmol) and Dess-Martin periodinane (15% in DCM) (760 mg, 0.269 mmol) were used to synthesize **34d** following the procedure described for the preparation of **34a**. Compound **34d** (81 mg, 70.5% yield) was obtained as a viscous liquid. R_f: 0.54 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) (major tautomer in enol form): δ 16.38 (br. s, 1H), 7.40-7.34 (m, 5H), 5.44 (s, 1H), 2.61 (s, 3H), 2.37 (s, 3H), 1.78 (q, *J*=3.7 Hz, 2H), 1.31 (q, *J*=3.7 Hz, 2H) ; LC-HRMS: m/z = 300.1054 [$(M+H)^+$ calculated for $C_{17}H_{17}NO_2S$ = 300.1053 ; Diff = 0.10mD].

2,4-Dimethyl-5-[5-(1-phenylcyclopropyl)-1H-pyrazol-3-yl]-1,3-thiazole (21). 1-(2,4-Dimethyl-1,3-thiazol-5-yl)-3-(1-phenylcyclopropyl)propane-1,3-dione **34d** (61.3 mg, 205 μmol), hydrazine hydrate (13 mg, 266 μmol) and HCl 25% (5 μL) were used to synthesize **21** following the procedure described for the preparation of **10**. After chromatography, compound **21** (50 mg, 83% yield) was obtained as an off white solid. R_f: 0.33 (EtOAc); ^{1}H NMR (600 MHz, DMSO-d6): δ 12.85 (s, 1H), 7.32-7.20 (m, 5H), 6.21 (s, 1H), 2.56 (s, 3H), 2.42 (s, 3H), 1.36-1.28 (m, 4H) ; LC-HRMS: m/z = 296.1226 [$(M+H)^+$ calculated for $C_{17}H_{17}N_3S$ = 296.1216 ; Diff = 1.00mD].

(rac) 1-(2,4-Dimethyl-1,3-thiazol-5-yl)-3-hydroxy-4-(4-methoxyphenyl)pentan-1-one (22 and 23, intermediate 33e). 1-(2,4-Dimethylthiazol-5-yl)ethanone (100 mg, 644 μmol), lithium bis(trimethylsilyl)amide and 2-(4-methoxyphenyl)propanal **32e** (CAS Registry Number

5405-83-4) (111 mg, 676 μmol) were used to synthesize **33e** following the procedure described for the preparation of **33a**. Compound **33e** (146 mg, 71% yield) was obtained as a liquid. R_f: 0.18 (EtOAc/heptane 1:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.12 (br d, *J*=9 Hz, 2H), 6.85 (br d, *J*=8.8 Hz, 2H), 4.17 (dt, *J*=8.4, 2.7 Hz, 1H), 3.79 (s, 3H), 3.19 (br. s, 1H), 2.79-2.71 (m, 3H), 2.65 (s, 3H), 2.61 (s, 3H), 1.38 (d, *J*=7 Hz, 3H); LC-HRMS: m/z = 320.1317 [$(M+H)^+$ calculated for $C_{17}H_{21}NO_3S$ = 320.1315 ; Diff = 0.20mD].

(rac) 1-(2,4-Dimethyl-1,3-thiazol-5-yl)-4-(4-methoxyphenyl)pentane-1,3-dione (22 and 23, intermediate 34e). 1-(2,4-Dimethyl-1,3-thiazol-5-yl)-3-hydroxy-4-(4-methoxyphenyl)pentan-1-one **33e** (140 mg, 0.438 mmol) and Dess-Martin periodinane (15% in DCM) (1.28 g, 0.460 mmol) were used to synthesize **34e** following the procedure described for the preparation of **34a**. Compound **34e** (85 mg, 61% yield) was obtained as a light yellow viscous liquid. R_f: 0.36 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$) (major tautomer in enol form): δ 15.9 (br s, 1H, OH), 7.22 (br d, *J*=9 Hz, 2H), 6.88 (br d, *J*=8.8 Hz, 2H), 5.73 (s, 1H), 3.8 (s, 3H), 3.67 (q, *J*=7.2, 1H), 2.66 (s, 3H), 2.61 (s, 3H), 1.52 (d, *J*=7.2 Hz, 3H); LC-HRMS: m/z = 318.1159 [$(M+H)^+$ calculated for $C_{17}H_{19}NO_3S$ = 318.1159 ; Diff = 0.00mD].

(rac) 5-{5-[1-(4-Methoxyphenyl)ethyl]-1H-pyrazol-3-yl}-2,4-dimethyl-1,3-thiazole (22+23) . 1-(2,4-Dimethyl-1,3-thiazol-5-yl)-4-(4-methoxyphenyl)pentane-1,3-dione **34e** (79 mg, 249 μmol), hydrazine monohydrate (13.7 mg, 13.3 μL, 274 μmol) and HCl 25% (10 μL) were used to synthesize the racemic mixture of **22** and **23** following the procedure described for the preparation of **10**. After chromatography, the racemic compound **22+23** (78 mg, 74% yield) was obtained as an off-white solid. R_f: 0.1 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.16-7.14 (br d, *J*=8.4 Hz, 2H), 6.89-6.87 (br d, *J*=8.4 Hz, 2H), 6.28 (s, 1H), 4.14 (q, *J*= 7.2 Hz,

1H), 3.8 (s, 3H), 2.66 (s, 3H), 2.55 (s, 3H), 1.66 (d, *J*= 7.2 Hz, 3H); LC-HRMS: m/z = 314.134 [$(M+H)^+$ calculated for $C_{17}H_{19}N_3OS$ = 314.1322 ; Diff = 1.80mD].

5-{5-[(1S)-1-(4-Methoxyphenyl)ethyl]-1H-pyrazol-3-yl}-2,4-dimethyl-1,3-thiazole (22) and **5-{5-[(1R)-1-(4-Methoxyphenyl)ethyl]-1H-pyrazol-3-yl}-2,4-dimethyl-1,3-thiazole (23)**. The racemic compound (**22**+**23**) was separated using a chiral column (Reprosil Chiral NR, 10% EtOH in heptane). The retention time for enantiomer A (compound **23** on Table 3) was 14.37 minutes with IC_{50}= 0.47 μM and assigned as 5-{5-[(1R)-1-(4-methoxyphenyl)ethyl]-1H-pyrazol-3-yl}-2,4-dimethyl-1,3-thiazole) with $[\alpha]^{20}_D$ (*c* 1 $CHCl_3$) = -66.7 (100% ee). The retention time for enantiomer B (compound **22** on Table 3) was 15.65 minutes with IC_{50}= 0.86 μM and assigned as 5-{5-[(1S)-1-(4-methoxyphenyl)ethyl]-1H-pyrazol-3-yl}-2,4-dimethyl-1,3-thiazole) with $[\alpha]^{20}_D$ (*c* 1 $CHCl_3$) = +60.41 (93% ee). The absolute configuration of the enantiomers was assigned via X-ray crystallography and by enantioselective synthesis using chiral aldehyde.

1-(4-Methoxyphenyl) cyclopropanecarbaldehyde (24, intermediate 32f). (1-(4-Methoxyphenyl) cyclopropyl)methanol **31f** (500mg, 2.80 mmol) and Dess-Martin periodinane (15% in DCM) (1.25g, 2.04 mmol) were used to synthesize **32f** following the procedure described for the preparation of **32b**. Compound **32f** (494 mg, 83% yield) was obtained as a light yellow oil. NMR was the same as described in literature (CAS Registry Number 34603-55-9).

1-(2,4-Dimethyl-1,3-thiazol-5-yl)-3-hydroxy-3-[1-(4-methoxyphenyl)cyclopropyl] propan-1-one (24, intermediate 33f). 1-(2,4-Dimethylthiazol-5-yl)ethanone **25** (150 mg, 966 μmol), lithium bis(trimethylsilyl)amide in THF (1.06 mL, 1.06 mmol) and 1-(4-methoxyphenyl) cyclopropanecarbaldehyde **32f** were used to synthesize **33f** following the procedure described

for the preparation of **33a**. Compound **33f** (319 mg, 99% yield) was obtained as a yellowish oil. R_f: 0.19 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.32 (m, 2H), 6.85 (d, *J*= 8.8 Hz, 2H), 3.82-3.77 (m, 4H), 3.01-2.96 (m, 1H), 2.97 (br. s, 1H), 2.73 (dd, 16.9, 9.6 Hz, 1H), 2.67 (s, 3H), 2.63 (s, 3H), 1.00-0.96 (m, 1H), 0.86-0.82 (m, 2H), 0.82-0.79 (m, 1H); LC-HRMS: m/z = 332.131 [$(M+H)^+$ calculated for $C_{18}H_{21}NO_3S$ = 332.1313 ; Diff = -0.30mD].

1-(2,4-Dimethyl-1,3-thiazol-5-yl)-3-[1-(4-methoxyphenyl)cyclopropyl]propane-1,3-dione (24, intermediate 34f). 1-(2,4-Dimethyl-1,3-thiazol-5-yl)-3-hydroxy-3-[1-(4-methoxyphenyl)cyclopropyl] propan-1-one **33f** (150 mg, 0.45 mmol) and Dess-Martin periodinane (15% weight in DCM) (1.27 g, 0.45 mmol) were used to synthesize **34f** following the procedure described for the preparation of **34a**. Compound **34f** (91 mg, 61% yield) was obtained as an oil. R_f: 0.38 (EtOAc/heptane 1:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ 16.43 (br. s, 1H, OH), 7.31 (br. d , *J*= 9 Hz, 2H), 6.90 (br. d, *J*= 8.8 Hz, 2H), 5.48 (s, 1H), 3.84 (s, 3H), 2.62 (s, 3H), 2.42 (s, 3H), 1.76-1.74 (m, 2H), 1.27-1.25 (m, 2H); LC-HRMS: m/z = 330.1161 [$(M+H)^+$ calculated for $C_{18}H_{19}NO_3S$ = 330.1159 ; Diff = 0.20mD].

5-{5-[1-(4-Methoxyphenyl)cyclopropyl]-1H-pyrazol-3-yl}-2,4-dimethyl-1,3-thiazole (24). 1-(2,4-Dimethyl-1,3-thiazol-5-yl)-3-[1-(4-methoxyphenyl)cyclopropyl]propane-1,3-dione (90 mg, 273 µmol) **34f**, hydrazine monohydrate (15 mg, 15 µL, 301 µmol) and HCl 25% (10 µL) were used to synthesize **24** following the procedure described for the preparation of **10**. After chromatography, compound **24** (45 mg, 51% yield) was obtained as an off-white solid. R_f: 0.15 (EtOAc/heptane 1:1) ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.33 (d , *J*= 8.5 Hz, 2H), 6.83 (d, *J*= 8.5 Hz, 2H), 6.03 (s, 1H), 3.82 (s, 3H), 2.65 (s, 3H), 2.53 (s, 3H), 1.35-1.32 (m, 4H); LC-HRMS: m/z = 326.1334 [$(M+H)^+$ calculated for $C_{18}H_{19}N_3OS$ = 326.1322 ; Diff = 1.20mD].

ASSOCIATE CONTENT

Supporting Information

Experimental procedures for non commercial available compounds on Table 2, pyrazolo-pyrazole analogs and 4-Ethyl analogs. Crystallization conditions, X-ray data collection and experimental details on methods used for fragment screening (SPR, 1D/2D NMR). This material is free of charge via the Internet at http://pubs.acs.org.

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Note

The authors declare the following competing financial interest(s): The authors are employees of F. Hoffmann-La Roche AG.

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PDB ID codes for compounds bound to humanized rat COMT:

5k01 (compound **2'**), 5k03 (compound **2**), 5k05 (compound **8**), 5k09 (compound **15**), 5k0b (compound **18**), 5k0c (compound **20** in Figure 7), 5k0e (alternative X-ray of compound **20,** not shown), 5k0f (**22**+**23,** co-crystallized as racemate), 5k0l (compound **24** in Figure 7), 5k0j (alternative X-ray of compound **24**, not shown) 5k0n (**24´**, pyrazolo-pyrazole analog of **24** in supporting information). Authors will release the atomic coordinates and experimental data upon article publication.

ABBREVIATIONS USED:

COMT catechol *O*-methyl transferase; SAM *S*-adenosyl L-methionine; DAT dopamine transporter; SPR surface plasmon resonance; CPMG Carr-Purcel-Meiboom Gill; HSQC-NMR heteronuclear single quantum coherence; LE ligand efficiency; DNC dinitrocatechol.

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