

CrossMark
click for updatesCite this: *RSC Adv.*, 2017, 7, 17427

Laccase-catalyzed green synthesis and cytotoxic activity of novel pyrimidobenzothiazoles and catechol thioethers†‡

H. T. Abdel-Mohsen,^a J. Conrad,^b K. Harms,^c D. Nohr^d and U. Beifuss^{*b}

The laccase-catalyzed reaction between unsubstituted catechol and 2-thioxypyrimidin-4(1*H*)-ones using aerial O₂ as the oxidant delivers novel pyrimidobenzothiazoles with high yields in an aqueous solvent system under mild reaction conditions. With 4-substituted catechols, catechol thioethers are formed exclusively. The synthetic protocols developed provide a sustainable approach for these compound classes. In addition, the cytotoxicity of the products against HepG2 cell line is reported. Most compounds exhibit antiproliferative activities with IC₅₀ values at the micromolar level. A structure–activity relationship study will facilitate the further development of these compounds as cytotoxic agents.

Received 12th December 2016

Accepted 15th February 2017

DOI: 10.1039/c6ra28102h

rsc.li/rsc-advances

Introduction

Pyrimidines are the building blocks of pyrimidine nucleosides and thiamine (vitamin B1). Therefore, the pyrimidine skeleton is regarded as an interesting scaffold for the development of molecules with potential applications in medicine.¹ Over the years, a wide range of pharmaceutical agents with a pyrimidine moiety have been developed.^{1–3} Zidovudine, stavudine and other potent anti-HIV agents are pyrimidines.^{2,3a} Other important drugs with a pyrimidine ring are the antitumor agents fluorouracil (I), tegafur (II), nimustine (III), monastrol (IV) and pazopanib (V) (Fig. 1).^{2,3b,c}

Recently, the development of more environmentally friendly approaches for the synthesis of pharmaceutical active ingredients has increasingly come into focus of the pharmaceutical chemistry.⁴ Green chemistry is a valuable concept for the development of new, more effective, less toxic and cost efficient methods for the synthesis of bioactive molecules. This can be achieved, for example, by developing highly atom economic transformations,⁵ using nontoxic reagents and catalysts of

natural origin (*e.g.* enzymes), the development of one-pot multi-step reactions, which reduce the amount of waste formed during reaction and work up as well as the development of environmentally benign reaction conditions.^{4,6}

Hepatocellular carcinoma (HCC) is regarded as one of the leading causes of cancer related mortality in the world. Every year more than half a million new cases with HCC are diagnosed worldwide. Hepatitis B, hepatitis C viruses and non-alcoholic fatty liver disease are the main risk factors for the development of chronic liver disease and subsequent development of HCC.^{7a,b} Despite the tremendous progress that has been achieved in cancer therapy over the last decades, the currently available drugs suffer from serious disadvantages, such as lack

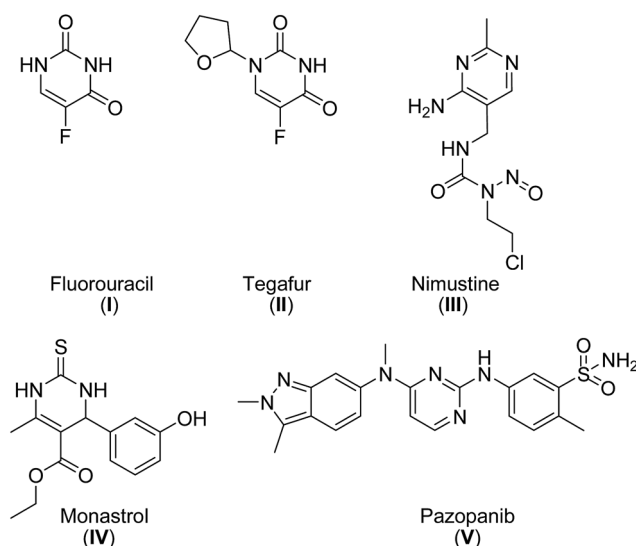


Fig. 1 Antitumor agents with a pyrimidine moiety.

^aChemistry of Natural and Microbial Products Department, Pharmaceutical Industries Research Division, National Research Centre, Cairo, Egypt

^bBioorganische Chemie, Institut für Chemie, Universität Hohenheim, Garbenstr. 30, Stuttgart, D-70599, Germany. E-mail: ubeifuss@uni-hohenheim.de; Fax: +49 711 459 22951; Tel: +49 711 459 22171

^cFachbereich Chemie, Universität Marburg, Hans-Meerwein-Str. 4, D-35032 Marburg, Germany

^dInstitut für Biologische Chemie und Ernährungswissenschaft, Universität Hohenheim, Garbenstr. 30, Stuttgart, D-70599, Germany

† This paper is dedicated to Professor Dr Dr hc Wolfgang Haubold on the occasion of his 80th birthday.

‡ Electronic supplementary information (ESI) available: Copies of the ¹H NMR and ¹³C NMR spectra. CCDC 1514742 and 1514743. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c6ra28102h

of selectivity, toxicity and resistance that limit their use.^{3b,7c} For these reasons, the development of novel anti-tumor agents remains a challenge.

Against this background, the importance of enzyme-catalyzed transformations in organic synthesis is steadily growing.⁸ Laccases (benzenediol: O₂ oxidoreductase E.C. 1.10.3.2.), which are multicopper oxidases, are among the most attractive enzymes in this respect.⁹ Over the last few years, laccases have proven their ability to catalyze a number of important oxidative transformations in aqueous solvent systems under mild reaction conditions (temperature, pH, pressure) using aerial oxygen as an oxidant. The oxidation of the substrates is linked to the reduction of oxygen to water which is the only byproduct of laccase-catalyzed reactions. Moreover, the application range of laccases can be broadened by employing mediators. Using laccase-mediator systems, the oxidation of substrates with higher oxidation potentials can also be achieved.¹⁰ Laccases have been successfully used to catalyze the oxidation of a number of functional groups,¹¹ the dimerization of phenolic compounds by oxidative coupling¹² and the oxidation of catechols and hydroquinones to the corresponding highly reactive quinoid systems, followed by reaction with different nucleophiles.^{13–16} With the latter approach, a range of simple 1,4-adducts^{13–15} as well as heterocyclic systems¹⁶ has been made available.

In contrast to the additions of C and N nucleophiles, only little is known about laccase-initiated 1,4-additions of S nucleophiles.^{15,16b,d} Recently, we have studied the laccase-catalyzed generation of *o*-quinones and their reaction with different S nucleophiles for the synthesis of thioethers as well as pyrimidobenzothiazoles.^{15b,16d} Ragauskas and coworkers have shown the successful application of laccases for the synthesis of 2,3-ethylenedithio-1,4-quinones.^{16b} In 2016, Schauer *et al.* reported on the multiple C–S bond formation between *p*-hydroquinones and aromatic thiols using laccase as a catalyst.^{15a}

Here, we report on the laccase-catalyzed reaction between catechols **1** and 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2** for the green synthesis of novel biologically active compounds. Depending on the substitution pattern of the catechols **1**, either pyrimidobenzothiazoles **3**, **4** or catechol thioethers **5** are formed (Fig. 2). Moreover, the cytotoxic activity of selected reaction products against HepG2 cell line is reported.

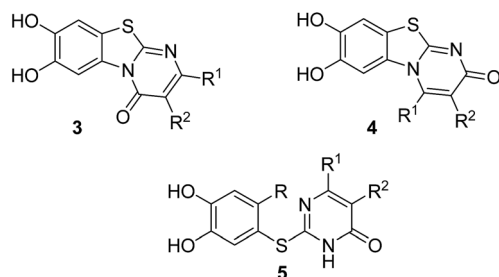


Fig. 2 Structures of the regioisomeric pyrimidobenzothiazoles **3**, **4** and the catechol thioethers **5**.

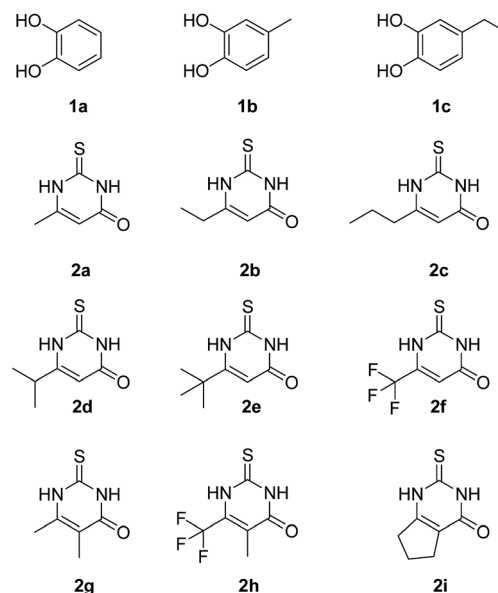


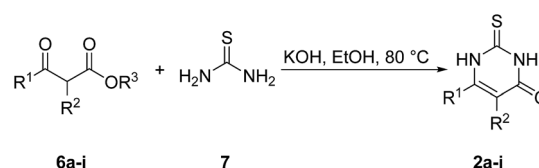
Fig. 3 Substrates for the laccase-catalyzed transformations.

Results and discussion

Fig. 3 depicts the structures of the catechols **1** and the 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2** that were chosen as substrates for the enzyme-catalyzed oxidative transformations.

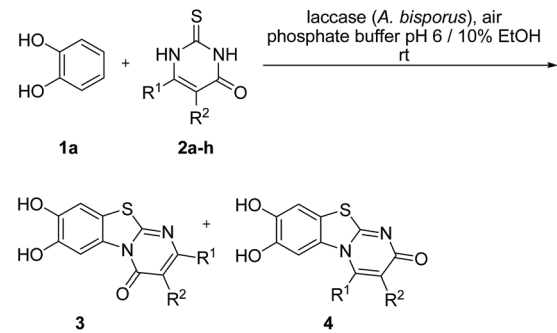
The catechols **1a–c** are commercially available, while the 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2a–h** were synthesized by reaction of different acyclic β -ketoesters **6a–h** with thiourea (**7**) under basic conditions in ethanol at 80 °C (general procedure I).¹⁷ 2,3,6,7-Tetrahydro-2-thioxo-1*H*-cyclopenta[*d*]pyrimidin-4(5*H*)-one (**2i**) was obtained from cyclic β -ketoester **6i** as substrate (Scheme 1).

First, the laccase-catalyzed reactions with the unsubstituted catechol (**1a**) were studied. In an initial experiment, 0.58 mmol (1.16 equiv.) catechol (**1a**) were reacted with 0.50 mmol (1 equiv.) 2,3-dihydro-6-methyl-2-thioxopyrimidin-4(1*H*)-one (**2a**) in the presence of 12 U laccase from *Agaricus bisporus* (1.2 U mg^{−1})¹⁸ as a catalyst in 20 mL of a 9 : 1 mixture of phosphate buffer (pH 6) and ethanol under air (general procedure II). The reaction proceeded smoothly at room temperature and delivered 97% of a crude product (purity > 95%; ¹H NMR) of a 37 : 63 mixture of the two regioisomers 7,8-dihydroxy-4*H*-2-methyl-pyrimido[2,1-*b*]benzothiazol-4-one (**3a**) and 7,8-dihydroxy-2*H*-4-methyl-pyrimido[2,1-*b*]benzothiazol-2-one (**4a**) after 12 h (Table 1, entry 1). A control experiment was conducted to show that the



Scheme 1 Synthesis of 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2a–i**.



Table 1 Laccase-catalyzed reaction between catechol (**1a**) and 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2a–h**


Entry	2	R ¹	R ²	t (h)	3 + 4	3 : 4 ^d	Yield of 3 + 4 ^e (%)
1 ^a	a	CH ₃	H	12	a	37 : 63	97
2 ^a	b	C ₂ H ₅	H	12	b	54 : 46	95
3 ^a	c	C ₃ H ₇	H	18	c	44 : 56	94
4 ^a	d	CH(CH ₃) ₂	H	13	d	76 : 24	95
5 ^a	e	C(CH ₃) ₃	H	16	e	100 : 0	97
6 ^a	f	CF ₃	H	16	f	100 : 0	78
7 ^b	g	CH ₃	CH ₃	28	g	36 : 64	89
8 ^b	h	CF ₃	CH ₃	17	h	100 : 0	95 ^f
9 ^c	a	CH ₃	H	72	—	—	— ^g

^a General procedure II: reactions were carried out using 0.58 mmol catechol (**1a**), 0.50 mmol 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2a–f**, laccase (*A. bisporus*, 10 mg, 12 U), phosphate buffer pH 6 (18 mL) and EtOH (2 mL). ^b General procedure III: reactions were carried out using 0.29 mmol catechol (**1a**), 0.25 mmol 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2g, h**, laccase (*A. bisporus*, 10 mg, 12 U), phosphate buffer pH 6 (18 mL) and EtOH (2 mL). ^c Reaction was carried out using 0.58 mmol **1a**, 0.50 mmol **2a**, phosphate buffer pH 6 (18 mL) and EtOH (2 mL). ^d Ratios of regioisomers were determined by ¹H NMR of the crude products. ^e Yields refer to crude products (purity > 95%, as determined by ¹H NMR). ^f Crude product contain traces of unknown impurities. ^g No reaction.

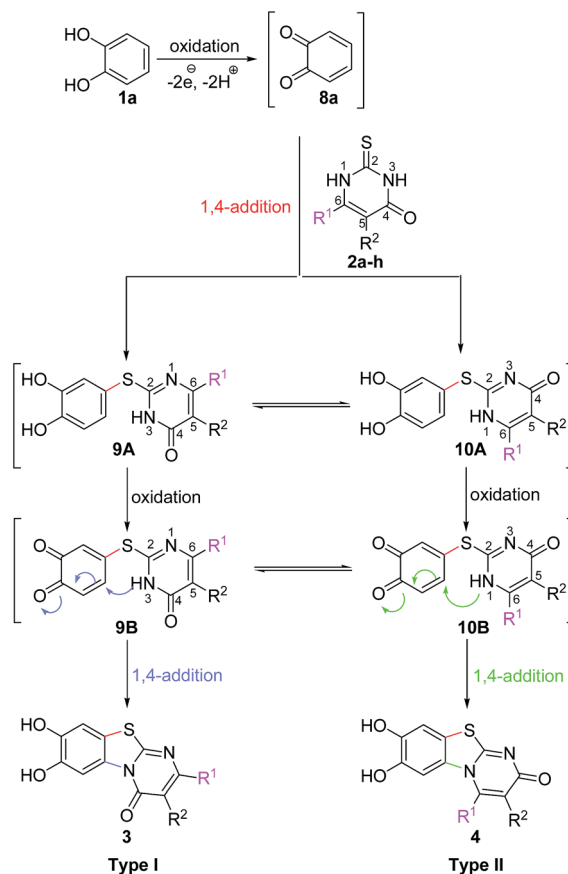
formation of the two regioisomers **3a** and **4a** does not proceed in the absence of the laccase (Table 1, entry 9). Subsequently, catechol (**1a**) was reacted with the other monosubstituted 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2b–f** under similar conditions (Table 1, entries 2–6). With **2b–d**, mixtures of the corresponding regioisomers **3b–d** and **4b–d** were obtained in excellent yields (purity of crude products >95%; ¹H NMR) (Table 1, entries 2–4). When the 6-*tert*-butyl- and the 6-trifluoromethyl substituted 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2e, f** were used as substrates, the 7,8-dihydroxy-4*H*-pyrimido[2,1-*b*]benzothiazol-4-ones **3e** and **3f** were isolated exclusively (Table 1, entries 5, 6). It seems that with increasing space demand of the R¹ group in the 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2**, the formation of regioisomers **3** is favoured over the formation of **4**.

When the disubstituted 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2g, h** which carry an additional methyl group in 5-position were employed as substrates, the reactions were not complete even after 72 h. In addition to the cyclized products **3g/4g** and **3h**, uncyclized material could be detected by ¹H NMR.

Optimization experiments revealed that the reactions can be driven to completion by simply increasing the amount of laccase and solvent by a factor of 2. When 0.29 mmol catechol (**1a**) were reacted with 0.25 mmol of the 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2g, h** in the presence of 12 U laccase from *A. bisporus* in 20 mL of a 9 : 1 mixture of phosphate buffer (pH 6) and ethanol under air (general procedure III) a mixture of regioisomers **3g** and **4g** in 89% yield (purity of crude product >95%; ¹H NMR) and the regioisomer **3h** in 95% yield were isolated (purity of crude product >95%; ¹H NMR) (Table 1, entries 7, 8).

It can be expected that the reactions proceed *via* the laccase-catalyzed *in situ* oxidation of catechol (**1a**) to the highly reactive *o*-quinone (**8a**) (Scheme 2). The latter one is then attacked at C-4 by the S-atom of the 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2a–h** to give the tautomers **9A** and **10A**. The intermolecular 1,4-addition is followed by a second laccase-catalyzed oxidation, namely the oxidation of the intermediates **9A** and **10A** to **9B** and **10B**, respectively. The final intramolecular 1,4-addition proceeds either by nucleophilic attack of N-3 or N-1 and delivers the corresponding regioisomeric cyclization products **3** and **4**, respectively.

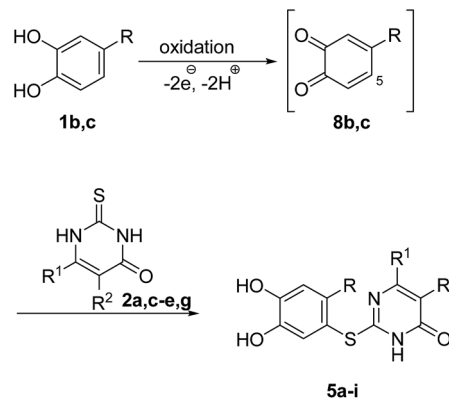
It is interesting to note that the substituent R¹ at C-6 of the 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2a–h** exerts a tremendous influence on the ratio of the regioisomeric 7,8-dihydroxy-4*H*-pyrimido[2,1-*b*]benzothiazol-4-ones **3** and 7,8-dihydroxy-2*H*-

**Scheme 2** Mechanistic proposal for the laccase-catalyzed synthesis of pyrimidobenzothiazoles **3**, **4**.

pyrimido[2,1-*b*]benzothiazol-2-ones **4**. With unbranched alkyl groups at C-6, the formation of 7,8-dihydroxy-2*H*-pyrimido[2,1-*b*]benzothiazol-2-ones **4** is either favoured or roughly equal amounts of the two isomers **3** and **4** are observed (Table 1, entries 1–3, 7). With branched alkyl groups (i-propyl- or *tert*-butyl group) or a trifluoromethyl group at C-6, the 7,8-dihydroxy-4*H*-pyrimido[2,1-*b*]benzothiazol-4-ones **3** are strongly favoured (Table 1, entries 4–6, 8). These findings are in good agreement with the assumption that the intramolecular 1,4-addition *via* N-1 in **10B** to regioisomers **4** (Scheme 2) is hindered by sterically demanding as well as strongly electronegative substituents.

The reactions between **1a** and **2a–g** delivered only either one major product (**3e**, **3f**) or two major products, consisting of exclusively mixtures of the regioisomeric products **3a–d,g** and **4a–d,g** in remarkably high yields. No other products could be detected by ¹H NMR. Only the reaction between **1a** and **2h** delivered in addition to **3h** traces of an additional product of unknown structure (Table 1, entry 8).

Subsequently, the laccase-catalyzed reactions of 4-substituted catechols **1b, c** with 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2a, c–e, g** were studied. For this purpose, 4-methylcatechol (**1b**) and 4-ethylcatechol (**1c**) were reacted with selected 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2a, c–e, g** under the conditions developed for the reactions of unsubstituted catechol (**1a**) (general procedure II). In all cases, the corresponding thioethers were formed exclusively with yields ranging between



Scheme 3 Mechanistic proposal for the laccase-catalyzed synthesis of catechol thioethers **5a–i**.

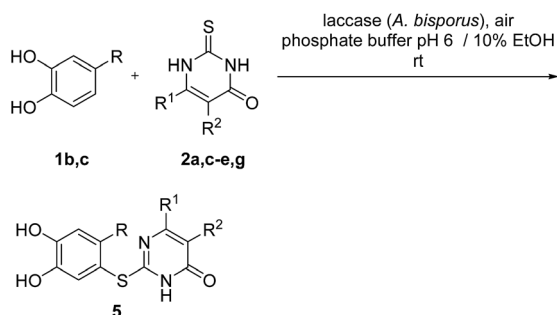
76 and 92% (purity of crude products >95%; ¹H NMR) (Table 2). Obviously, only the S atoms of the 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2** act as nucleophiles in the intermolecular 1,4-additions. This can be attributed to the higher nucleophilicity of 2-S in comparison to N-1 and N-3 (Scheme 3).

Finally, the laccase-catalyzed reactions between the 4-substituted catechols **1b, c** and 2,3,6,7-tetrahydro-2-thioxo-1*H*-cyclopenta[*d*]pyrimidin-4(5*H*)-one (**2i**) were studied (general procedure II) to give the corresponding catechol thioethers **5j, k** in high yields (Table 3).

It can be taken for granted that the reactions start with the laccase-catalyzed oxidation of the 4-substituted catechols **1b, c** to the corresponding *o*-benzoquinones **8b, c**. This is followed by intermolecular 1,4-addition of the 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2** *via* the S atom at C-5 to afford the corresponding catechol thioethers **5a–i** as sole products. Due to the presence of a methyl or an ethyl substituent at C-4 of the catechol, the intramolecular 1,4-addition occurs exclusively at C-5 (Scheme 3).

It should be highlighted that the reactions presented are highly efficient and deliver the products **3, 4** and **5** in remarkably

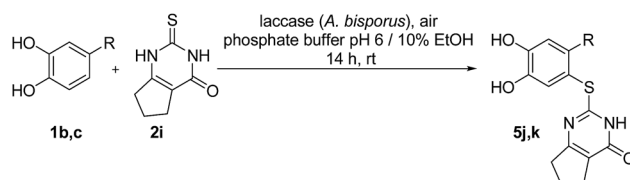
Table 2 Laccase-catalyzed synthesis of catechol thioethers **5**



Entry ^a	1	R	2	R ¹	R ²	Time (h)	5	Yield ^b (%)
1	b	CH ₃	a	CH ₃	H	12	a	82
2	b	CH ₃	c	C ₃ H ₇	H	13	b	88
3	b	CH ₃	d	CH(CH ₃) ₂	H	16	c	92
4	b	CH ₃	e	C(CH ₃) ₃	H	17	d	90
5	b	CH ₃	g	CH ₃	CH ₃	20	e	86
6	c	C ₂ H ₅	a	CH ₃	H	14	f	90
7	c	C ₂ H ₅	d	CH(CH ₃) ₂	H	12	g	76
8	c	C ₂ H ₅	e	C(CH ₃) ₃	H	16	h	81
9	c	C ₂ H ₅	g	CH ₃	CH ₃	16	i	91

^a General procedure II: reactions were carried out using 0.58 mmol 4-substituted catechols **1b, c**, 0.50 mmol 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2a, c–e, g**, laccase (*A. bisporus*, 10 mg, 12 U), phosphate buffer pH 6 (18 mL) and EtOH (2 mL). ^b Yields refer to crude products (purity > 95%, as determined by ¹H NMR).

Table 3 Synthesis of dihydroxyphenylthio-6,7-dihydro-3*H*-cyclopenta[*d*]pyrimidin-4(5*H*)-ones **5j, k** by laccase-catalyzed transformations under aerobic conditions



Entry ^a	1	R	5	Yield ^b (%)
1	b	CH ₃	j	90
2	c	C ₂ H ₅	k	76

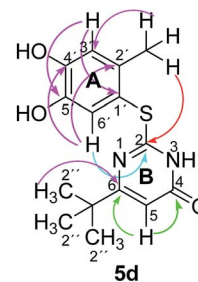
^a General procedure II: reactions were carried out using 0.58 mmol 4-substituted catechols **1b, c**, 0.50 mmol 2,3,6,7-tetrahydro-2-thioxo-1*H*-cyclopenta[*d*]pyrimidin-4(5*H*)-one (**2i**), laccase (*A. bisporus*, 10 mg, 12 U), phosphate buffer pH 6 (18 mL) and EtOH (2 mL). ^b Yields refer to crude products (purity > 95%, as determined by ¹H NMR).



high yields and in a highly chemoselective manner. The reactions proceed without the formation of any relevant amounts of byproducts arising from competing reactions, such as (a) the homocoupling of the catechols **1**; *i.e.*, the reaction of the *o*-quinone intermediates **8** with the corresponding parent catechols **1**, which is followed by formation of the benzofurans or (b) the formation of disulfides. We assume that this favourable outcome is the result of a combination of several factors. Among them are (a) the high nucleophilicity of the S-nucleophilic substrates **2**, (b) the use of a laccase which is particularly suitable for this type of reactions and (c) the careful choice of reaction conditions, such as pH, reaction temperature and substrate concentrations. This view is supported by previous work done in our laboratory.^{16k} The reactions between catechols and 1,3-dicarbonyls prove that the selection of laccase from *A. bisporus* as the catalyst as well as the choice of suitable reaction conditions exert a tremendous impact on product yields.

Structure elucidation

Structures of all pyrimidobenzothiazole regioisomers **3**, **4** as well as catechol thioethers **5** were unambiguously elucidated by mass spectrometry and NMR spectroscopy including 2D NMR for the full assignment of the ¹H and ¹³C chemical shifts. Analysis of the ¹H NMR spectrum of the crude products obtained from the reaction between catechol (**1a**) and 2-thioxopyrimidin-4-ones **2a–h** showed the appearance of either a mixture of 2 regioisomers **3** (type I) and **4** (type II) or a single regioisomer **3**. 2D ROESY as well as super long range gHMBC experiments were carried out to differentiate between the 2 regioisomers. Taking the mixture of **3a** and **4a** as an example, in **4a** a strong ROESY correlation between the methyl group at C-4 (δ_{H} 2.73 ppm) to both the aromatic proton 6-H (δ_{H} 7.52 ppm) and the aromatic proton 3-H (δ_{H} 6.07 ppm) confirms the type II regioisomer. However, in **3a** only a single ROESY correlation between the methyl group at C-2 (δ_{H} 2.26 ppm) and the aromatic proton at 3-H (δ_{H} 6.17 ppm) can be observed (Fig. 4). ⁴J HMBC correlations (as observed in the super long range gHMBC spectrum) between 6-H (δ_{H} 8.44 ppm) and C-4 (δ_{C} 159.97 ppm) along with 9-H (δ_{H} 7.30 ppm) and C-10a (δ_{C} 161.71 ppm) established a type I regioisomer. Evaluation of the experimental ¹H–¹³C long-range coupling constants (PIP-HSQMBC), for example in **4a**, between 9-H (δ_{H} 7.25 ppm) to C-5a (³J = 9.5 Hz)



→ ²J HMBC correlations → ³J HMBC correlations
→ ⁴J HMBC correlations → ⁵J HMBC correlations

Fig. 5 Important ²J, ³J, ⁴J and ⁵J HMBC correlations of **5d**.

and C-7 (³J = 7.6 Hz) allows the successful assignment of the quaternary carbons C-5a at δ_{C} 128.82 ppm and C-7 at δ_{C} 145.25 ppm. Similarly, two strong correlations can be seen from H-6 (δ_{H} 7.52 ppm) to C-9a (³J = 8.4 Hz) and C-8 (³J = 6.2 Hz), to be assigned at δ_{C} 112.65 ppm and δ_{C} 144.68 ppm, respectively. The quaternary carbons C-2, C-4, C-10a of ring C were assigned by standard gHMBC and super long range gHMBC at δ_{C} 166.32, 148.31, 164.60 ppm, respectively (Fig. 4).

In the reactions between 4-substituted catechols **1b, c** and 2-thioxopyrimidin-4-ones **2a, c–e, g, i** single products were formed exclusively. The products **5a–i** consist of the 2 ring systems A and B. Taking **5d** as an example, the complete assignment of ring A was carried out by PIP-HSQMBC ¹H–¹³C correlations. The difficulty in assigning C-2, C-4, and C-6 was solved by ¹H–¹³C super long gHMBC along with standard gHMBC correlations between 6'-H at δ_{H} 7.66 ppm and C-2 at δ_{C} 165.32 ppm (⁴J) as well as between 2'-CH₃ at δ_{H} 2.42 ppm and C-2 at δ_{C} 165.32 ppm (⁵J), between 2''-H at δ_{H} 1.14 and C-6 at δ_{C} 176.73 ppm (³J) and finally between 5-H at δ_{H} 6.43 ppm and C-4 at δ_{C} 167.61 (²J) as well as C-6 at δ_{C} 176.73 ppm (²J) (Fig. 5).

Unequivocal evidence for the structures of **3f** and **5a** was provided by X-ray crystal structure analysis.¹⁹ The molecular structures of **3f** and **5a** are depicted in Fig. 6 and 7.

Greenness of the reactions

The newly developed methods for the synthesis of pyrimidobenzothiazoles **3**, **4** and catechol thioethers **5** address many of the principles of green chemistry.^{6,20} The reactions are enzyme-

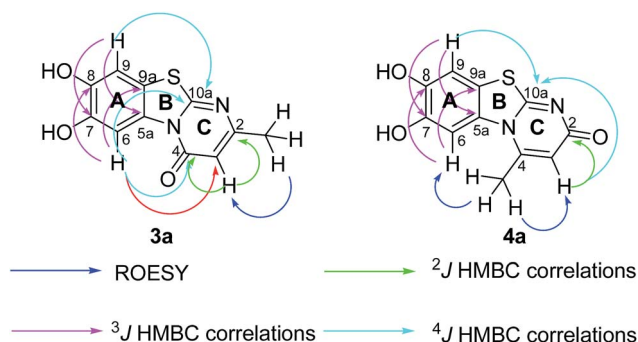


Fig. 4 Important ROESY, ²J, ³J, and ⁴J HMBC correlations of **3a** and **4a**.

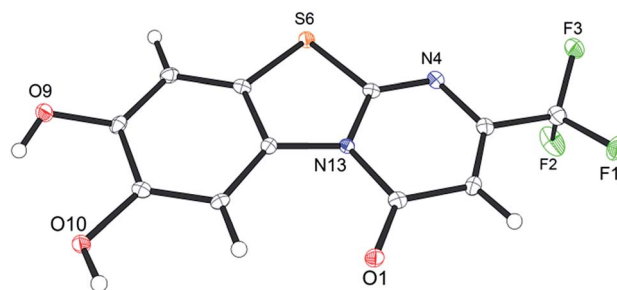


Fig. 6 Molecular structure of 7,8-dihydroxy-4H-2-trifluoromethylpyrimido[2,1-b]benzothiazol-4-one (**3f**), derived from X-ray crystal structure analysis.



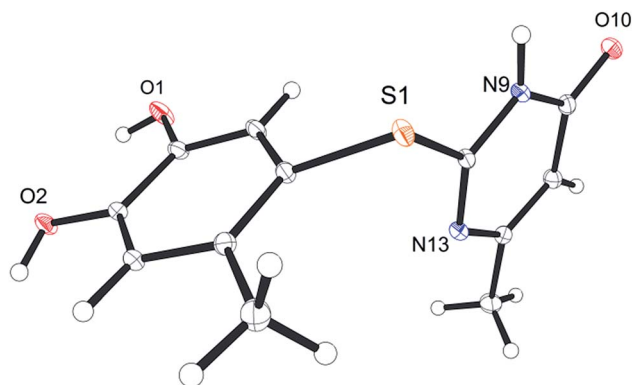


Fig. 7 Molecular structure of 2-(4,5-dihydroxy-2-methylphenylthio)-6-methylpyrimidin-4(3H)-one (**5a**), derived from X-ray crystal structure analysis.

catalyzed transformations using completely safe and non-toxic aerial oxygen as the sole oxidant. The transformations deliver the pyrimidobenzothiazoles **3**, **4** and the catechol thioethers **5** exclusively and with high yields. The only byproduct formed is water which stems from the reduction of oxygen. The laccase-catalyzed domino reactions presented combine several individual reactions in multistep processes. This allows the reduction of the amount of waste formed during reaction and work up and complies well with the first principle of green chemistry. In addition, environmentally benign reaction conditions have been developed. Taking the preparation of **3e** as a typical example for the synthesis of pyrimidobenzothiazoles **3**, the E-factor (kg waste per kg product)²¹ of the process is 4.57 kg kg⁻¹. The atom economy⁵ of this transformation amounts to 94%. The reaction was carried out in a mixture of phosphate buffer (pH 6.0) and 10 vol% ethanol, which are completely safe and environmentally acceptable solvents.²² The method highlights the use of enzymes in catalytic rather than stoichiometric amounts. The turnover number of the laccase in this process is high, for the synthesis of **3e** TON amounts to 4656; the turnover frequency of this transformation is good; in the example discussed TOF amounts to 291 h⁻¹. Taking the preparation of **5j** as a representative example for the synthesis of thioethers **5**, the E-factor of this process was calculated to be 5.00 kg kg⁻¹. The atom economy of this process, calculated as 94%, is also very good. The turnover number and the turnover frequency of the laccase for the synthesis of **5j** are high. They amount to 4320 and 308.57 h⁻¹, respectively.

In vitro cytotoxic activity

Selected compounds and doxorubicin (positive control) were evaluated for their *in vitro* cytotoxic activity against HepG2 cancer cell line using SRB assay.²³ The IC₅₀ values of the tested compounds are summarized in Table 4. All compounds tested exhibited cytotoxic activities, but all of them were less potent than doxorubicin.

In the catechol thioether series **5a–k**, the substituents on C-5, C-6 and C-2' have a great influence on the cytotoxic activity. In compounds **5a–e**, it is assumed that increasing the chain length from methyl in **5a** (IC₅₀ > 40 μM, Table 4, entry 4) to *n*-propyl in

Table 4 IC₅₀ of selected compounds **3**, **5** against HepG2 cell line

Entry	Product	IC ₅₀ ^{a,b} (μM)
1	3d	23.28 ± 2.16
2	3e	12.70 ± 0.53
3	3f	12.01 ± 1.46
4	5a	>40
5	5b	21.25 ± 0.46
6	5c	7.77 ± 0.30
7	5d	14.30 ± 0.11
8	5e	30.57 ± 1.05
9	5f	27.82 ± 2.36
10	5g	2.74 ± 0.29
11	5h	14.92 ± 1.16
12	5i	31.98 ± 1.43
13	5j	40.48 ± 3.52
14	5k	31.34 ± 3.57
15	Doxorubicin	0.28 ± 0.04

^a IC₅₀ are the mean of 2–5 independent experiments ± SE. ^b DMSO alone (2% final concentration) had no effect on the cell viability.

5b (IC₅₀ = 21.25 μM, Table 4, entry 5) or introduction of a methyl group in the 5-position in **5e** (IC₅₀ = 30.57 μM, Table 4, entry 8) increases the potency. Moreover, the presence of a branched isopropyl group at C-6 in **5c** results in a great increase in potency (IC₅₀ = 7.77 μM, Table 4, entry 6) compared to **5a** (IC₅₀ > 40 μM, Table 4, entry 4). However, the presence of a bulky *tert*-butyl group in **5d** results in a decrease of activity (IC₅₀ = 14.30 μM, Table 4, entry 7) in comparison to **5c**. In the **5f–i** series, where an ethyl group is present at C-2', the introduction of an isopropyl group in **5g** increases the activity (IC₅₀ = 2.74 μM, Table 4, entry 10) and the presence of a *tert*-butyl group in **5h** decreases the potency (IC₅₀ = 14.92 μM, Table 4, entry 11) in comparison to **5g**. Introduction of a methyl group at C-5 of **5i** results in a slight decrease of the cytotoxic activity (IC₅₀ = 31.98 μM, Table 4, entry 12) in comparison to **5f** (IC₅₀ = 27.82 μM, Table 4, entry 9). The presence of a bicyclic system in **5j** and **5k** has no favourable effect on the cytotoxic activity compared to **5a** and **5f**. Comparison of the IC₅₀ values of the **5a–e**, **j** series with the **5f–i**, **k** series reveals that the most potent compounds are **5c** and **5g** which have an isopropyl group at C-6. The presence of an ethyl group at C-2' of **5g** increases the cytotoxic potency in comparison to **5c**. In the pyrimidobenzothiazole derivatives **3d–f**, it was found that replacing the isopropyl group at C-2 of **3d** (IC₅₀ = 23.28 μM, Table 4, entry 1) with a *tert*-butyl group in **3e** (IC₅₀ = 12.70 μM, Table 4, entry 2) or a trifluoromethyl group in **3f** (IC₅₀ = 12.01 μM, Table 4, entry 3) results in an increase of the antiproliferative activity. The presented study provides a novel class of compounds, which will be further optimized to increase their potency. The mechanism of their cytotoxic activity is still under study.

Experimental

Chemistry

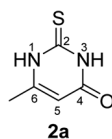
General remarks. All chemicals were purchased from commercial suppliers. Laccase from *Agaricus bisporus* (1.2 U



mg⁻¹)¹⁸ was purchased from ASA Spezialenzyme. Solvents used in extraction and purification were distilled prior to use. The pH of the buffer was adjusted using a pH 330/SET-1 pH-meter. Analytical thin layer chromatography (TLC) was performed on precoated silica gel 60 F₂₄₅ aluminium plates (Merck) with visualization under UV light. Flash chromatography was carried out on silica gel MN 60, 0.04–0.053 mm (Macherey & Nagel). Melting points were determined on a Büchi melting point apparatus B-545 with open capillary tubes and are uncorrected. IR spectra were measured on a Perkin-Elmer Spectrum One (FT-IR-spectrometer). ¹H and ¹³C NMR spectra were recorded at 300 (75) MHz and at 500 (125) MHz on Varian Unity Inova instruments using DMSO-*d*₆ and pyridine-*d*₅ as solvents. The chemical shifts were referenced to the solvent signals at $\delta_{\text{H/C}}$ 2.49/39.50 ppm (DMSO-*d*₆) and 8.71/149.80 ppm (pyridine-*d*₅) relative to TMS as internal standards. 2-D ROESY, gHSQC, ASAPHMQC, standard gHMBCAD, super long range gHMBCAD as well as band selective gHMBC, PIP-HSQMBC and gCOSY spectra were recorded on a Varian Unity Inova spectrometer (500 MHz). Coupling constants *J* [Hz] were directly taken from the spectra and are not averaged. Low resolution electron impact mass spectra (EI-LRMS) and exact electron impact mass spectra (HRMS) were recorded at 70 eV on a Finnigan MAT 95 instrument. Low resolution electron spray ionisation mass spectra (ESI-LRMS) and exact electron spray ionisation mass spectra (HRMS) were recorded on a Bruker Daltonics (micro TOFQ) instrument. The intensities are reported as percentages relative to the base peak (*I* = 100%).

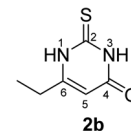
General procedure I for the synthesis of 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones 2a–h and 2,3,6,7-tetrahydro-2-thioxo-1*H*-cyclopenta[*d*]pyrimidin-4(5*H*)-one (2i).¹⁷ A 50 or 150 mL round bottomed flask with a magnetic stir bar was charged with a suspension of β -ketoester 6, thiourea (7) and KOH in ethanol. The mixture was stirred at 80 °C for 5 h. Ethanol was evaporated under reduced pressure to one third of its original volume, the reaction mixture was poured into water and neutralized with 2 N HCl. The resulting mixture was left stirring overnight at rt. Filtration gave the crude product which was purified by crystallization from methanol.

Synthesis and analytical data of 2,3-dihydro-6-methyl-2-thioxopyrimidin-4(1*H*)-one (2a).



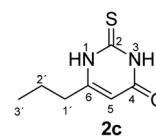
According to general procedure I, ethyl acetoacetate (6a) (6.50 g, 50 mmol), thiourea (7) (3.81 g, 50 mmol), KOH (2.81 g, 50 mmol), ethanol (80 mL) were reacted. Work up gave 2,3-dihydro-6-methyl-2-thioxopyrimidin-4(1*H*)-one (2a) as a white powder (5.00 g, 70%), mp > 300 °C (lit.^{24a} > 300 °C); *R*_f = 0.54 (CH₂Cl₂/EtOAc = 2 : 1); δ_{H} (300 MHz; DMSO-*d*₆) 2.05 (3H, s, 6-CH₃), 5.66 (1H, s, 5-H), 12.22 (2H, br, 1-H and 3-H).

Synthesis and analytical data of 6-ethyl-2,3-dihydro-2-thioxopyrimidin-4(1*H*)-one (2b).



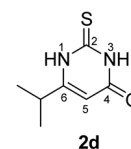
According to general procedure I, ethyl propionylacetate (6b) (1.44 g, 10 mmol), thiourea (7) (0.76 g, 10 mmol), KOH (0.56 g, 10 mmol), ethanol (20 mL) were reacted. Work up gave 6-ethyl-2,3-dihydro-2-thioxopyrimidin-4(1*H*)-one (2b) as a white powder (0.78 g, 50%), mp 227–229 °C (lit.^{24a} 228.5–230.5 °C); *R*_f = 0.44 (CH₂Cl₂/EtOAc = 2 : 1); δ_{H} (300 MHz; DMSO-*d*₆) 1.08 (3H, t, ³*J* = 7.5 Hz, CH₃), 2.35 (2H, q, ³*J* = 7.5 Hz, CH₂), 5.66 (1H, s, 5-H) and 12.24 (2H, s, 1-H and 3-H); δ_{C} (75 MHz; DMSO-*d*₆) 11.60, 24.73, 102.04, 158.21, 161.19, 175.97.

Synthesis and analytical data of 2,3-dihydro-6-propyl-2-thioxopyrimidin-4(1*H*)-one (2c).



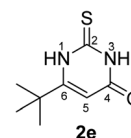
According to general procedure I, ethyl butyrylacetate (6c) (3.16 g, 20 mmol), thiourea (7) (1.52 g, 20 mmol), KOH (1.12 g, 20 mmol), ethanol (20 mL) were reacted. Work up gave 2,3-dihydro-6-propyl-2-thioxopyrimidin-4(1*H*)-one (2c) as a white powder (1.40 g, 41%), mp 216–218 °C (lit.^{24a} 218–220 °C); *R*_f = 0.52 (CH₂Cl₂/EtOAc = 2 : 1); δ_{H} (300 MHz; DMSO-*d*₆) 0.86 (3H, t, ³*J* = 7.4 Hz, 3'-H), 1.53 (2H, sex, ³*J* = 7.4 Hz, 2'-H), 2.30 (2H, t, ³*J* = 7.7 Hz, 1'-H), 5.65 (1H, s, 5-H) and 12.20 (2H, s, 1-H and 3-H); δ_{C} (75 MHz; DMSO-*d*₆) 13.20, 20.50, 33.35, 102.90, 156.97, 161.17, 176.08.

Synthesis and analytical data of 2,3-dihydro-6-isopropyl-2-thioxopyrimidin-4(1*H*)-one (2d).



According to general procedure I, ethyl isobutyrylacetate (6d) (1.58 g, 10 mmol), thiourea (7) (0.76 g, 10 mmol), KOH (0.56 g, 10 mmol), ethanol (20 mL) were reacted. Work up gave 2,3-dihydro-6-isopropyl-2-thioxopyrimidin-4(1*H*)-one (2d) as a white powder (0.60 g, 35%), mp 176–178 °C (lit.^{24a} 179–180 °C); *R*_f = 0.50 (CH₂Cl₂/EtOAc = 2 : 1); δ_{H} (300 MHz; DMSO-*d*₆) 1.11 (6H, d, ³*J* = 6.9 Hz, CH(CH₃)₂), 2.65 (1H, sep, ³*J* = 6.9 Hz, CH(CH₃)₂), 5.65 (1H, s, 5-H) and 12.20 (2H, br s, 1-H and 3-H); δ_{C} (75 MHz; DMSO-*d*₆) 20.39, 30.33, 100.33, 161.33, 162.22, 176.08.

Synthesis and analytical data of 6-tert-butyl-2,3-dihydro-2-thioxopyrimidin-4(1*H*)-one (2e).



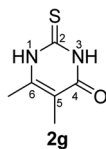
According to general procedure I, ethyl 4,4-dimethyl-3-oxovalerate (**6e**) (1.72 g, 10 mmol), thiourea (7) (0.76 g, 10 mmol), KOH (0.56 g, 10 mmol), ethanol (20 mL) were reacted. Work up gave 6-*tert*-butyl-2,3-dihydro-2-thioxopyrimidin-4(1*H*)-one (**2e**) as a white powder (0.50 g, 27%), mp 179–181 °C (lit.^{24a} 178–180 °C); R_f = 0.63 (CH₂Cl₂/EtOAc = 2 : 1); δ_H (300 MHz; DMSO-*d*₆) 1.21 (9H, s, C(CH₃)₃), 5.63 (1H, s, 5-H), 11.79 and 12.34 (2H, s, 1-H and 3-H); δ_C (75 MHz; DMSO-*d*₆) 27.30, 34.49, 100.50, 161.16, 163.36, 176.49.

Synthesis and analytical data of 6-trifluoromethyl-2,3-dihydro-2-thioxopyrimidin-4(1*H*)-one (2f).



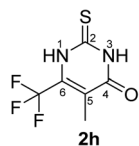
According to general procedure I, ethyl-4,4,4-trifluoroacetoacetate (**6f**) (1.84 g, 10 mmol), thiourea (7) (0.76 g, 10 mmol), KOH (0.56 g, 10 mmol), ethanol (20 mL) were reacted. Work up gave 6-trifluoromethyl-2,3-dihydro-2-thioxopyrimidin-4(1*H*)-one (**2f**) as a white powder (0.69 g, 35%), mp 246–248 °C (lit.^{24b} 247–249 °C); R_f = 0.50 (CH₂Cl₂/EtOAc = 2 : 1); δ_H (300 MHz; DMSO-*d*₆) 6.40 (1H, s, 5-H), 12.83 (1H, s, 1-H or 3-H) and 13.50 (1H, br, 1-H or 3-H); δ_C (75 MHz; DMSO-*d*₆) 105.14 (q, $^3J_{C,F}$ = 3.9 Hz, C-5), 118.83 (q, $^1J_{C,F}$ = 273.7 Hz, CF₃), 140.56 (q, $^2J_{C,F}$ = 36.1 Hz, C-6), 159.84, 176.82.

Synthesis and analytical data of 2,3-dihydro-5,6-dimethyl-2-thioxopyrimidin-4(1*H*)-one (2g).



According to general procedure I, ethyl 2-methylacetoacetate (**6g**) (7.21 g, 50 mmol), thiourea (7) (3.81 g, 50 mmol), KOH (2.81 g, 50 mmol), ethanol (60 mL) were reacted. Work up gave 2,3-dihydro-5,6-dimethyl-2-thioxopyrimidin-4(1*H*)-one (**2g**) as a white powder (3.80 g, 49%), mp 283–285 °C (lit.^{24a} 283–285 °C); R_f = 0.48 (CH₂Cl₂/EtOAc = 2 : 1); δ_H (300 MHz; DMSO-*d*₆) 1.74 (3H, s, CH₃), 2.09 (3H, s, CH₃) and 12.19 (2H, br, 1-H and 3-H).

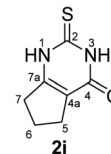
Synthesis and analytical data of 6-trifluoromethyl-2,3-dihydro-5-methyl-2-thioxopyrimidin-4(1*H*)-one (2h).



According to general procedure I, ethyl-4,4,4-trifluoro-2-methylacetoacetate (**6h**) (1.98 g, 10 mmol), thiourea (7) (0.76 g, 10 mmol), KOH (0.56 g, 10 mmol), ethanol (15 mL) were reacted. Work up gave 6-trifluoromethyl-2,3-dihydro-5-methyl-2-thioxopyrimidin-4(1*H*)-one (**2h**) as a white powder (0.60 g, 29%), mp 244–246 °C; R_f = 0.66 (CH₂Cl₂/EtOAc = 2 : 1); $\tilde{\nu}_{max}$

(atr)/cm^{−1} 3150 (NH), 2874 (CH), 1664 (C=O), 1570 and 1220; δ_H (300 MHz; DMSO-*d*₆) 1.93 (3H, q, $^5J_{H,F}$ = 3.3 Hz, 5-CH₃) and 12.85 (2H, br, 1-H and 3-H); δ_C (75 MHz; DMSO-*d*₆) 9.48 (q, $^4J_{C,F}$ = 2.5 Hz, 5-CH₃), 115.57 (br, C-5), 119.89 (q, $^1J_{C,F}$ = 275.7 Hz, CF₃), 135.16 (q, $^2J_{C,F}$ = 34.6 Hz, C-6), 161.18, 174.55.

Synthesis and analytical data of 2,3,6,7-tetrahydro-2-thioxo-1*H*-cyclopenta[d]pyrimidin-4(5*H*)-one (2i).

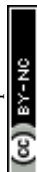


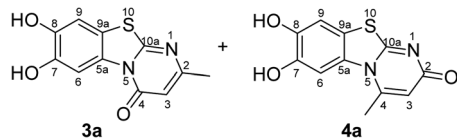
According to general procedure I, ethyl 2-oxocyclopentane-carboxylate (**6i**) (4.69 g, 30 mmol), thiourea (7) (2.28 g, 30 mmol), KOH (1.68 g, 30 mmol), ethanol (30 mL) were reacted. Work up gave 2,3,6,7-tetrahydro-2-thioxo-1*H*-cyclopenta[d]pyrimidin-4(5*H*)-one (**2i**) as a white powder (1.20 g, 24%), mp > 300 °C (lit.^{24a} 336–337 °C); R_f = 0.38 (CH₂Cl₂/EtOAc = 2 : 1); δ_H (300 MHz; DMSO-*d*₆) 1.94 (2H, quin, 3J = 7.3 Hz, 6-H), 2.47 (2H, ov. t like, 3J = 7.3 Hz, 7-H), 2.67 (2H, t, 3J = 7.6 Hz, 5-H), 12.17 and 12.18 (2H, br, 1-H and 3-H); δ_C (75 MHz; DMSO-*d*₆) 20.78, 26.60, 31.00, 115.52, 156.51, 159.52, 175.57.

General procedure II for the laccase-catalyzed domino reaction between catechols 1 and 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones 2. A 100 mL round bottomed flask with a magnetic stir bar was charged with a solution or suspension of a catechol **1** (0.58 mmol) and a 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2** (0.50 mmol) in ethanol (2 mL). Phosphate buffer (0.2 M, pH 6, 18 mL) and laccase from *A. bisporus* (10 mg, 12 U, 1.2 U mg^{−1}) were added and the mixture was stirred under air at rt for the time given. The reaction mixture was acidified with 2 N HCl to pH ~ 4. The precipitated product was filtered with suction using a Buchner funnel. The filter cake was washed with water. The crude products obtained after drying exhibit a purity of >95% (¹H NMR). Analytically pure products were obtained by column filtration (CH₂Cl₂/EtOAc = 1 : 1 to CH₂Cl₂/EtOAc/MeOH = 1 : 1 : 0.1) of the crude products.

General procedure III for the laccase-catalyzed domino reaction between catechol (1a) and 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones 2g, h. A 100 mL round bottomed flask with a magnetic stir bar was charged with a solution of catechol (**1a**) (0.29 mmol) and 2,3-dihydro-2-thioxopyrimidin-4(1*H*)-ones **2g**, **h** (0.25 mmol) in ethanol (2 mL). Phosphate buffer (0.2 M, pH 6, 18 mL) and laccase from *A. bisporus* (10 mg, 12 U, 1.2 U mg^{−1}) were added and the mixture was stirred under air at rt for the time given. The reaction mixture was acidified with 2 N HCl to pH ~ 4. The precipitated product was filtered with suction using a Buchner funnel. The filter cake was washed with water. The crude products obtained after drying exhibit a purity of >95% (¹H NMR). Analytically pure products were obtained either by acetylation or by column filtration (CH₂Cl₂/EtOAc = 1 : 1 to CH₂Cl₂/EtOAc/MeOH = 1 : 1 : 0.1) of the crude products.

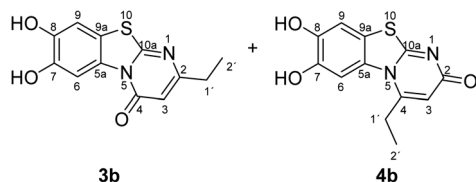
Synthesis and analytical data of 7,8-dihydroxy-4*H*-2-methyl-pyrimido[2,1-*b*]benzothiazol-4-one (3a) and 7,8-dihydroxy-2*H*-4-methyl-pyrimido[2,1-*b*]benzothiazol-2-one (4a).





According to general procedure II, catechol (**1a**) (64 mg, 0.58 mmol), 2,3-dihydro-6-methyl-2-thioxopyrimidin-4(1H)-one (**2a**) (71 mg, 0.50 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U, 10 mg, *A. bisporus*) were reacted for 12 h. Workup gave a mixture of 7,8-dihydroxy-4H-2-methyl-pyrimido[2,1-*b*]benzothiazol-4-one (**3a**) and 7,8-dihydroxy-2H-4-methyl-pyrimido[2,1-*b*]benzothiazol-2-one (**4a**) as a brown powder (120 mg, 97%), mp > 300 °C; $R_f = 0.27$ (CH₂Cl₂/EtOAc/MeOH = 2 : 2 : 0.1); $\tilde{\nu}_{\max}$ (atr)/cm⁻¹ 3406 (OH), 3023 (CH), 1628 (C=O), 1510 (C=N) and 1185; δ_H (500 MHz; DMSO-*d*₆) of **3a** 2.26 (3H, s, 2-CH₃), 6.17 (1H, s, 3-H), 7.30 (1H, s, 9-H), 8.44 (1H, s, 6-H) and 9.66 (2H, ov. br, 7,8-OH); δ_H (500 MHz; DMSO-*d*₆) of **4a** 2.73 (3H, s, 4-CH₃), 6.07 (1H, s, 3-H), 7.25 (1H, s, 9-H), 7.52 (1H, s, 6-H) and 9.66 (2H, ov. br, 7,8-OH); δ_C (125 MHz; DMSO-*d*₆) of **3a** 23.10 (2-CH₃), 105.68 (C-3), 106.38 (C-6), 108.29 (C-9), 113.21 (C-9a), 128.12 (C-5a), 144.96 (C-7), 145.65 (C-8), 159.97 (C-4), 161.71 (C-10a) and 161.99 (C-2); δ_C (125 MHz; DMSO-*d*₆) of **4a** 21.15 (4-CH₃), 104.76 (C-6), 108.97 (C-9), 110.80 (C-3), 112.65 (C-9a), 128.82 (C-5a), 144.68 (C-8), 145.25 (C-7), 148.31 (C-4), 164.60 (C-10a) and 166.32 (C-2); MS (EI-70 eV) m/z 248 (M^+ , 100%), 220 (35) and 181 (15); HRMS calcd for C₁₁H₈N₂O₃S (248.0256), found 248.0253.

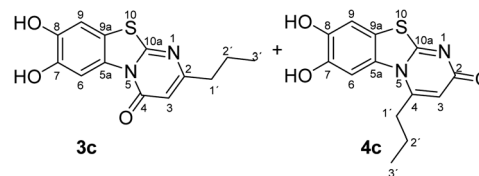
*Synthesis and analytical data of 7,8-dihydroxy-4H-2-ethyl-pyrimido[2,1-*b*]benzothiazol-4-one (3b) and 7,8-dihydroxy-2H-4-ethyl-pyrimido[2,1-*b*]benzothiazol-2-one (4b).*



According to general procedure II, catechol (**1a**) (64 mg, 0.58 mmol), 6-ethyl-2,3-dihydro-2-thioxopyrimidin-4(1H)-one (**2b**) (78 mg, 0.50 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U, 10 mg, *A. bisporus*) were reacted for 12 h. Workup gave a mixture of 7,8-dihydroxy-4H-2-ethyl-pyrimido[2,1-*b*]benzothiazol-4-one (**3b**) and 7,8-dihydroxy-2H-4-ethyl-pyrimido[2,1-*b*]benzothiazol-2-one (**4b**) as a brown powder (124 mg, 95%), mp 298–300 °C; $R_f = 0.27$ (CH₂Cl₂/EtOAc = 2 : 1); $\tilde{\nu}_{\max}$ (atr)/cm⁻¹ 3400 (OH), 2979 (CH), 1625 (C=O), 1512 and 1299; δ_H (500 MHz; DMSO-*d*₆) of **3b** 1.17 (3H, t, $^3J = 7.5$ Hz, 2'-H), 2.54 (2H, q, $^3J = 7.5$ Hz, 1'-H), 6.16 (1H, s, 3-H), 7.30 (1H, s, 9-H), 8.45 (1H, s, 6-H) and 9.65 (2H, ov. br, 7,8-OH); δ_H (500 MHz; DMSO-*d*₆) of **4b** 1.27 (3H, t, $^3J = 7.1$ Hz, 2'-H), 3.09 (2H, q, $^3J = 7.1$ Hz, 1'-H), 6.02 (1H, s, 3-H), 7.25 (1H, s, 9-H), 7.49 (1H, s, 6-H) and 9.65 (2H, br, 7,8-OH); δ_C (125 MHz; DMSO-*d*₆) of **3b** 12.25 (C-2'), 29.56 (C-1'), 104.36 (C-3), 106.37 (C-6), 108.30 (C-9), 113.24 (C-9a), 128.09 (C-5a), 144.94 (C-7), 145.65 (C-8), 160.25 (C-4), 161.82 (C-10a), 166.68 (C-2); δ_C (125 MHz; DMSO-*d*₆) of **4b** 11.57 (C-2'), 25.84 (C-1'), 104.93 (C-6), 108.52 (C-3), 108.96 (C-9),

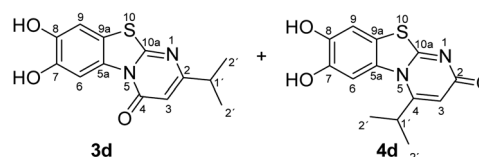
112.73 (C-9a), 128.40 (C-5a), 144.63 (C-8), 145.33 (C-7), 153.26 (C-4), 164.91 (C-10a) and 166.35 (C-2); MS (EI-70 eV) m/z 262 (M^+ , 100%), 263 ($[M + H]^+$, 37) and 219 (19); HRMS calcd for C₁₂H₁₀N₂O₃S (262.0412), found 262.0413.

*Synthesis and analytical data of 7,8-dihydroxy-4H-2-propyl-pyrimido[2,1-*b*]benzothiazol-4-one (3c) and 7,8-dihydroxy-2H-4-propyl-pyrimido[2,1-*b*]benzothiazol-2-one (4c).*



According to general procedure II, catechol (**1a**) (64 mg, 0.58 mmol), 2,3-dihydro-6-propyl-2-thioxopyrimidin-4(1H)-one (**2c**) (85 mg, 0.50 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U, 10 mg, *A. bisporus*) were reacted for 18 h. Workup gave a mixture of 7,8-dihydroxy-4H-2-propyl-pyrimido[2,1-*b*]benzothiazol-4-one (**3c**) and 7,8-dihydroxy-2H-4-propyl-pyrimido[2,1-*b*]benzothiazol-2-one (**4c**) as a brown powder (130 mg, 94%), mp 258–260 °C; $R_f = 0.27$ (CH₂Cl₂/EtOAc = 2 : 1); $\tilde{\nu}_{\max}$ (atr)/cm⁻¹ 3310 (OH), 2960 (CH), 1625 (C=O), 1453 and 1307; δ_H (500 MHz; DMSO-*d*₆) of **3c** 0.89 (3H, t, $^3J = 7.3$ Hz, 3'-H), 1.65 (2H, ov., 2'-H), 2.49 (2H, ov., 1'-H), 6.15 (1H, br s, 3-H), 7.30 (1H, s, 9-H) and 8.45 (1H, s, 6-H); δ_H (500 MHz; DMSO-*d*₆) of **4c** 1.04 (3H, t, $^3J = 7.1$ Hz, 3'-H), 1.65 (2H, ov. sex, $^3J = 7.7$ Hz, 2'-H), 3.00 (2H, t, $^3J = 7.5$ Hz, 1'-H), 6.03 (1H, br s, 3-H), 7.25 (1H, s, 9-H) and 7.41 (1H, s, 6-H); δ_C (125 MHz; DMSO-*d*₆) of **3c** 13.50 (C-3'), 20.87 (C-2'), 38.26 (C-1'), 105.26 (C-3), 106.39 (C-6), 108.29 (C-9), 113.24 (C-9a), 128.09 (C-5a), 144.95 (C-7), 145.66 (C-8), 160.10 (C-4), 161.84 (C-10a), 165.30 (C-2); δ_C (125 MHz; DMSO-*d*₆) of **4c** 13.14 (C-3'), 20.09 (C-2'), 34.20 (C-1'), 104.63 (C-6), 109.00 (C-9), 109.73 (C-3), 112.73 (C-9a), 128.28 (C-5a), 144.72 (C-8), 145.38 (C-7), 151.48 (C-4), 164.98 (C-10a), 166.24 (C-2); MS (ESI) m/z 299 ($[M + Na]^+$, 100%) and 277 ($[M + H]^+$, 35); HRMS calcd for C₁₃H₁₂N₂O₃S + Na (299.0461), found 299.0457.

*Synthesis and analytical data of 7,8-dihydroxy-4H-2-isopropyl-pyrimido[2,1-*b*]benzothiazol-4-one (3d) and 7,8-dihydroxy-2H-4-isopropyl-pyrimido[2,1-*b*]benzothiazol-2-one (4d).*

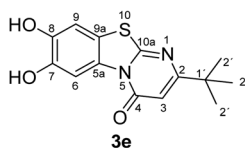


According to general procedure II, catechol (**1a**) (64 mg, 0.58 mmol), 2,3-dihydro-6-isopropyl-2-thioxopyrimidin-4(1H)-one (**2d**) (85 mg, 0.50 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U, 10 mg, *A. bisporus*) were reacted for 13 h. Workup gave a mixture of 7,8-dihydroxy-4H-2-isopropyl-pyrimido[2,1-*b*]benzothiazol-4-one (**3d**) and 7,8-dihydroxy-2H-4-isopropyl-pyrimido[2,1-*b*]benzothiazol-2-one (**4d**) as a brown powder (131 mg, 95%); mp 250–252 °C; $R_f = 0.36$ (CH₂Cl₂/EtOAc = 0.1 : 1); $\tilde{\nu}_{\max}$ (atr)/cm⁻¹ 3350 (OH), 2962 (CH),



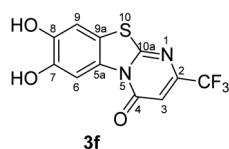
1636 (C=O), 1517 and 1234; δ_{H} (500 MHz; DMSO- d_6) of **3d** 1.17 (6H, d, $^3J = 6.9$ Hz, 2'-H), 2.77 (1H, sep, $^3J = 6.8$ Hz, 1'-H), 6.15 (1H, s, 3-H), 7.35 (1H, s, 9-H), 8.44 (1H, s, 6-H) and 9.77 (2H, ov. br, 7,8-OH); δ_{H} (500 MHz; DMSO- d_6) of **4d** 1.32 (6H, d, $^3J = 6.3$ Hz, 2'-H), 3.64 (1H, ov., 1'-H), 6.08 (1H, s, 3-H), 7.30 (1H, s, 9-H), 7.68 (1H, s, 6-H) and 9.77 (2H, ov. br, 7,8-OH); δ_{C} (125 MHz; DMSO- d_6) of **3d** 21.19 (C-2'), 34.65 (C-1'), 103.22 (C-3), 106.53 (C-6), 108.34 (C-9), 113.20 (C-9a), 128.02 (C-5a), 144.98 (C-7), 145.77 (C-8), 160.42 (C-4), 161.90 (C-10a) and 170.33 (C-2); δ_{C} (125 MHz; DMSO- d_6) of **4d** 21.48 (C-2'), 28.59 (C-1'), 105.33 (C-6), 106.43 (C-3), 109.02 (C-9), 112.65 (C-9a), 128.00 (C-5a), 144.81 (C-8), 145.64 (C-7), 158.10 (C-4), 165.25 (C-10a), 166.53 (C-2); MS (ESI) m/z 315 ($[\text{M} + \text{K}]^+$, 20%), 277 ($[\text{M} + \text{H}]^+$, 100), 249 (8); HRMS calcd for $\text{C}_{13}\text{H}_{12}\text{N}_2\text{O}_3\text{S} + \text{H}$ (277.0641), found 277.0615. Column filtration using the eluent $\text{CH}_2\text{Cl}_2/\text{EtOAc} = 1 : 1$ gave **3d** in pure form; mp 259–261 °C.

Synthesis and analytical data of 7,8-dihydroxy-4H-2-tert-butyl-pyrimido[2,1-*b*]benzothiazol-4-one (3e).



According to general procedure II, catechol (**1a**) (64 mg, 0.58 mmol), 6-tert-butyl-2,3-dihydro-2-thioxopyrimidin-4(1H)-one (**2e**) (92 mg, 0.50 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U, 10 mg, *A. bisporus*) were reacted for 16 h. Workup gave 7,8-dihydroxy-4H-2-tert-butyl-pyrimido[2,1-*b*]benzothiazol-4-one (**3e**) as a brown powder (140 mg, 97%), mp > 300 °C; $R_f = 0.38$ ($\text{CH}_2\text{Cl}_2/\text{EtOAc} = 0.1 : 1$); $\tilde{\nu}_{\text{max}}$ (atr)/ cm^{-1} 3295 (OH), 2954 (CH), 1638 (C=O), 1546 and 1185; δ_{H} (500 MHz; DMSO- d_6) 1.23 (9H, s, 2'-H), 6.21 (1H, s, 3-H), 7.31 (1H, s, 9-H), 8.44 (1H, s, 6-H) and 9.75 (2H, br, 7,8-OH); δ_{C} (125 MHz; DMSO- d_6) 28.67 (C-2'), 36.78 (C-1'), 102.08 (C-3), 106.42 (C-6), 108.32 (C-9), 113.22 (C-9a), 127.90 (C-5a), 144.94 (C-7), 145.73 (C-8), 160.52 (C-4), 161.36 (C-10a) and 172.33 (C-2); MS (ESI) m/z 329 ($[\text{M} + \text{K}]^+$, 31%) and 291 ($[\text{M} + \text{H}]^+$, 100); HRMS calcd for $\text{C}_{14}\text{H}_{14}\text{N}_2\text{O}_3\text{S} + \text{H}$ (291.0798), found 291.0802.

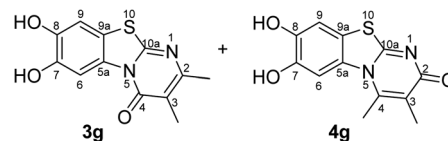
Synthesis and analytical data of 7,8-dihydroxy-4H-2-trifluoromethyl-pyrimido[2,1-*b*]benzothiazol-4-one (3f).



According to general procedure II, catechol (**1a**) (64 mg, 0.58 mmol), 6-trifluoromethyl-2,3-dihydro-2-thioxopyrimidin-4(1H)-one (**2g**) (98 mg, 0.50 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U, 10 mg, *A. bisporus*) were reacted for 16 h. Workup gave 7,8-dihydroxy-4H-2-trifluoromethyl-pyrimido[2,1-*b*]benzothiazol-4-one (**3f**) as a brown powder (118 mg, 78%), mp > 300 °C; $R_f = 0.31$ (cyclohexane/EtOAc = 1 : 1); $\tilde{\nu}_{\text{max}}$ (atr)/ cm^{-1} 3408 (OH), 1654 (C=O), 1526 (C=N) and 1151; δ_{H} (500 MHz; DMSO- d_6) 6.79 (1H, s, 3-H), 7.42

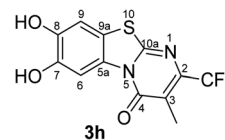
(1H, s, 9-H), 8.47 (1H, s, 6-H), 9.82 and 9.90 (2H, 2s, 7,8-OH); δ_{C} (125 MHz; DMSO- d_6) 105.31 (q, $^3J_{\text{C,F}} = 3.1$ Hz, C-3), 106.33 (C-6), 108.35 (C-9), 114.19 (C-9a), 120.78 (q, $^1J_{\text{C,F}} = 273.0$ Hz, CF_3), 127.82 (C-5a), 145.42 (C-7), 146.41 (C-8), 148.25 (q, $^2J_{\text{C,F}} = 34.4$ Hz, C-2), 159.47 (C-4), 164.29 (C-10a); MS (ESI) m/z 341 ($[\text{M} + \text{K}]^+$, 49%), 303 ($[\text{M} + \text{H}]^+$, 100) and 283 (73); HRMS calcd for $\text{C}_{11}\text{H}_5\text{F}_3\text{N}_2\text{O}_3\text{S} + \text{H}$ (303.0046), found 303.0046.

Synthesis and analytical data of 7,8-dihydroxy-4H-2,3-dimethyl-pyrimido[2,1-*b*]benzothiazol-4-one (3g) and 7,8-dihydroxy-2H-3,4-dimethyl-pyrimido[2,1-*b*]benzothiazol-2-one (4g).



According to general procedure III, catechol (**1a**) (32 mg, 0.29 mmol), 2,3-dihydro-5,6-dimethyl-2-thioxopyrimidin-4(1H)-one (**2g**) (39 mg, 0.25 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U, 10 mg, *A. bisporus*) were reacted for 28 h. Workup gave a mixture of 7,8-dihydroxy-4H-2,3-dimethyl-pyrimido[2,1-*b*]benzothiazol-4-one (**3g**) and 7,8-dihydroxy-2H-3,4-dimethyl-pyrimido[2,1-*b*]benzothiazol-2-one (**4g**) as a brown powder (58 mg, 89%), mp > 300 °C; $R_f = 0.46$ ($\text{CH}_2\text{Cl}_2/\text{EtOAc} = 1 : 1$); $\tilde{\nu}_{\text{max}}$ (atr)/ cm^{-1} 3352 (OH), 3023 (CH), 1631 (C=O), 1488 and 1185; δ_{H} (500 MHz; DMSO- d_6) of **3g** 2.03 (3H, s, 3-CH₃), 2.28 (3H, s, 2-CH₃), 7.28 (1H, s, 9-H), 8.48 (1H, s, 6-H) and 9.61 (2H, ov. br, 7,8-OH); δ_{H} (500 MHz; DMSO- d_6) of **4g** 2.00 (3H, s, 3-CH₃), 2.73 (3H, s, 4-CH₃), 7.25 (1H, s, 9-H), 7.58 (1H, s, 6-H) and 9.61 (2H, ov. br, 7,8-OH); δ_{C} (125 MHz; DMSO- d_6) of **3g** 10.98 (3-CH₃), 21.71 (2-CH₃), 106.46 (C-6), 108.29 (C-9), 112.82 (C-3), 113.33 (C-9a), 128.12 (C-5a), 144.77 (C-7), 145.52 (C-8), 157.37 (C-2), 157.83 (C-10a) and 160.46 (C-4); δ_{C} (125 MHz; DMSO- d_6) of **4g** 12.01 (3-CH₃), 17.97 (4-CH₃), 105.06 (C-6), 108.99 (C-9), 112.76 (C-9a), 116.70 (C-3), 129.21 (C-5a), 144.00 (C-4), 144.49 (C-8), 145.02 (C-7), 162.86 (C-10a) and 166.21 (C-2); analytically pure product was obtained by acetylation; MS (ESI) m/z 369 ($[\text{M} + \text{Na}]^+$, 93%), 347 ($[\text{M} + \text{H}]^+$, 49), 305 (53) and 263 (100); HRMS calcd for $\text{C}_{16}\text{H}_{14}\text{N}_2\text{O}_5\text{S} + \text{Na}$ (369.0516), found 369.0522.

Synthesis and analytical data of 7,8-dihydroxy-4H-3-methyl-2-trifluoromethyl-pyrimido[2,1-*b*]benzothiazol-4-one (3h).

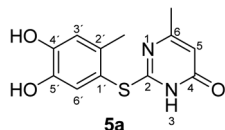


According to general procedure III, catechol (**1a**) (32 mg, 0.29 mmol), 6-trifluoromethyl-2,3-dihydro-5-methyl-2-thioxopyrimidin-4(1H)-one (**2h**) (52.5 mg, 0.25 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U, 10 mg, *A. bisporus*) were reacted for 17 h. Workup gave 7,8-dihydroxy-4H-2-trifluoromethyl-pyrimido[2,1-*b*]benzothiazol-4-one (**3h**) as a brown powder (75 mg, 95%), mp > 300 °C; $R_f = 0.49$ ($\text{CH}_2\text{Cl}_2/\text{EtOAc} = 2 : 1$); $\tilde{\nu}_{\text{max}}$ (atr)/ cm^{-1} 3533 (OH), 3120 (CH), 1632 (C=O), 1531 (C=N) and 1224; δ_{H} (500 MHz; DMSO- d_6)



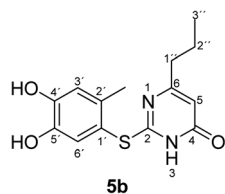
2.20 (3H, q, $^5J_{\text{H,F}} = 2.2$ Hz, 3-CH₃), 7.39 (1H, s, 9-H), 8.49 (1H, s, 6-H) and 9.85 (2H, br, 7,8-OH); δ_{C} (125 MHz; DMSO-*d*₆) 10.18 (q, $^4J_{\text{C,F}} = 2.2$ Hz, 3-CH₃), 106.40 (C-6), 108.36 (C-9), 114.29 (C-9a), 116.29 (br s, C-3), 121.78 (q, $^1J_{\text{C,F}} = 274.6$ Hz, CF₃), 127.59 (C-5a), 143.73 (q, $^2J_{\text{C,F}} = 33.0$ Hz, C-2), 145.22 (C-7), 146.27 (C-8), 159.79 (C-10a) and 160.82 (C-4); MS (ESI) *m/z* 355 ([M + K]⁺, 72%), 317 ([M + H]⁺, 74), 297 (100) and 249 (60); HRMS calcd for C₁₂H₇F₃N₂O₃S + H (317.0202), found 317.0193.

Synthesis and analytical data of 2-(4,5-dihydroxy-2-methylphenylthio)-6-methylpyrimidin-4(3H)-one (5a).



According to general procedure II, 4-methylcatechol (**1b**) (72 mg, 0.58 mmol), 2,3-dihydro-6-methyl-2-thioxopyrimidin-4(1H)-one (**2a**) (71 mg, 0.50 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U, 10 mg, *A. bisporus*) were reacted for 12 h. Workup gave 2-(4,5-dihydroxy-2-methylphenylthio)-6-methylpyrimidin-4(3H)-one (**5a**) as a pale yellow powder (108 mg, 82%), mp 213–215 °C; *R*_f = 0.27 (CH₂Cl₂/EtOAc/MeOH = 3 : 3 : 0.1); $\tilde{\nu}_{\text{max}}$ (atr)/cm^{−1} 3406 (OH), 2916 (CH), 1644 (C=O), 1508 and 1264; δ_{H} (500 MHz; pyridine-*d*₅) 2.13 (3H, s, 6-CH₃), 2.43 (3H, s, 2'-CH₃), 6.29 (1H, s, 5-H), 7.23 (1H, s, 3'-H) and 7.69 (1H, s, 6'-H); δ_{C} (125 MHz; pyridine-*d*₅) 20.40 (2'-CH₃), 23.67 (6-CH₃), 105.59 (C-5), 116.82 (C-1'), 118.95 (C-3'), 125.04 (C-6'), 135.40 (ov., C-2'), 145.63 (C-5'), 149.64 (C-4'), 166.69 (C-6), 167.13 (C-2) and 167.87 (C-4); MS (ESI) *m/z* 287 ([M + Na]⁺, 100%), 265 ([M + H]⁺, 75) and 143 (60); HRMS calcd for C₁₂H₁₂N₂O₃S + H (265.0641), found 265.0643.

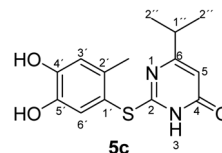
Synthesis and analytical data of 2-(4,5-dihydroxy-2-methylphenylthio)-6-propylpyrimidin-4(3H)-one (5b).



According to general procedure II, 4-methylcatechol (**1b**) (72 mg, 0.58 mmol), 2,3-dihydro-6-propyl-2-thioxopyrimidin-4(1H)-one (**2c**) (85 mg, 0.50 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U, 10 mg, *A. bisporus*) were reacted for 13 h. Workup gave 2-(4,5-dihydroxy-2-methylphenylthio)-6-propylpyrimidin-4(3H)-one (**5b**) as a pale yellow powder (128 mg, 88%), mp 189–191 °C; *R*_f = 0.40 (CH₂Cl₂/EtOAc/MeOH = 3 : 3 : 0.1); $\tilde{\nu}_{\text{max}}$ (atr)/cm^{−1} 3439 (OH), 3179 (NH), 2957 (CH), 1634 (C=O), 1508 (C=N) and 1227; δ_{H} (300 MHz; pyridine-*d*₅) 0.75 (3H, t, $^3J = 7.2$ Hz, 3''-H), 1.58 (2H, sex like, $^3J = 7.5$ Hz, 2''-H), 2.39 (2H, t, $^3J = 7.6$ Hz, 1''-H), 2.43 (3H, s, 2'-CH₃), 6.31 (1H, s, 5-H), 7.25 (1H, s, 3'-H) and 7.67 (1H, s, 6'-H); δ_{C} (75 MHz; pyridine-*d*₅) 13.64 (C-3''), 20.34 (2'-CH₃), 21.44 (C-2''), 39.32 (C-1''), 105.31 (C-5), 116.79 (C-1'),

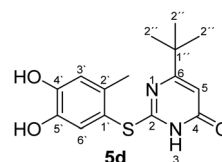
118.85 (C-3'), 124.96 (C-6'), 135.39 (C-2'), 145.57 (C-5'), 149.60 (C-4'), 166.79 (C-2), 167.68 (C-4) and 170.09 (C-6); MS (ESI) *m/z* 331 ([M + K]⁺, 47%), 315 ([M + Na]⁺, 100), 293 ([M + H]⁺, 83) and 171 (53); HRMS calcd for C₁₄H₁₆N₂O₃S + Na (315.0774), found 315.0767.

Synthesis and analytical data of 2-(4,5-dihydroxy-2-methylphenylthio)-6-isopropylpyrimidin-4(3H)-one (5c).



According to general procedure II, 4-methylcatechol (**1b**) (72 mg, 0.58 mmol), 2,3-dihydro-6-isopropyl-2-thioxopyrimidin-4(1H)-one (**2d**) (85 mg, 0.50 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U, 10 mg, *A. bisporus*) were reacted for 16 h. Workup gave 2-(4,5-dihydroxy-2-methylphenylthio)-6-isopropylpyrimidin-4(3H)-one (**5c**) as a pale yellow powder (134 mg, 92%), mp 204–206 °C; *R*_f = 0.27 (CH₂Cl₂/EtOAc/MeOH = 3 : 3 : 0.1); $\tilde{\nu}_{\text{max}}$ (atr)/cm^{−1} 3240 (OH), 2965 (CH), 1644 (C=O), 1509 and 1281; δ_{H} (300 MHz; pyridine-*d*₅) 1.09 (6H, d, $^3J = 6.9$ Hz, 2''-H), 2.42 (3H, s, 2'-CH₃), 2.65 (1H, sep, $^3J = 6.9$ Hz, 1''-H), 6.32 (1H, s, 5-H), 7.26 (1H, s, 3'-H) and 7.67 (1H, s, 6'-H); δ_{C} (75 MHz; pyridine-*d*₅) 20.32 (2'-CH₃), 21.20 (C-2''), 35.60 (C-1''), 103.23 (C-5), 116.82 (C-1'), 118.77 (C-3'), 124.94 (C-6'), 135.40 (ov., C-2'), 145.51 (C-5'), 149.56 (C-4'), 166.51 (C-2), 167.84 (C-4) and 174.91 (C-6); MS (ESI) *m/z* 315 ([M + Na]⁺, 100%), 293 ([M + H]⁺, 44) and 171 (27); HRMS calcd for C₁₄H₁₆N₂O₃S + H (293.0954), found 293.0950.

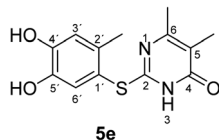
Synthesis and analytical data of 2-(4,5-dihydroxy-2-methylphenylthio)-6-tert-butylpyrimidin-4(3H)-one (5d).



According to general procedure II, 4-methylcatechol (**1b**) (72 mg, 0.58 mmol), 6-tert-butyl-2,3-dihydro-2-thioxopyrimidin-4(1H)-one (**2e**) (92 mg, 0.50 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U, 10 mg, *A. bisporus*) were reacted for 17 h. Workup gave 2-(4,5-dihydroxy-2-methylphenylthio)-6-tert-butylpyrimidin-4(3H)-one (**5d**) as a pale yellow powder (138 mg, 90%), mp 205–207 °C; *R*_f = 0.45 (CH₂Cl₂/EtOAc/MeOH = 3 : 3 : 0.1); $\tilde{\nu}_{\text{max}}$ (atr)/cm^{−1} 3450 (OH), 2957 (CH), 1638 (C=O), 1577 (C=N) and 1279; δ_{H} (500 MHz; pyridine-*d*₅) 1.14 (9H, s, 2''-H), 2.42 (3H, s, 2'-CH₃), 6.43 (1H, s, 5-H), 7.28 (1H, s, 3'-H) and 7.66 (1H, s, 6'-H); δ_{C} (125 MHz; pyridine-*d*₅) 20.34 (2'-CH₃), 28.73 (C-2''), 37.20 (C-1''), 102.37 (C-5), 116.75 (C-1'), 118.72 (C-3'), 124.98 (C-6'), 135.72 (C-2'), 145.49 (C-5'), 149.58 (ov., C-4'), 165.32 (C-2), 167.61 (C-4) and 176.73 (C-6); MS (ESI) *m/z* 345 ([M + K]⁺, 40%), 329 ([M + Na]⁺, 100), 307 ([M + H]⁺, 77), 229 (24) and 185 (54); HRMS calcd for C₁₅H₁₈N₂O₃S + H (307.1111), found 307.1111.

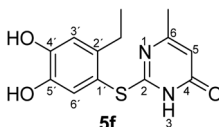


Synthesis and analytical data of 2-(4,5-dihydroxy-2-methylphenylthio)-5,6-dimethylpyrimidin-4(3H)-one (**5e**).



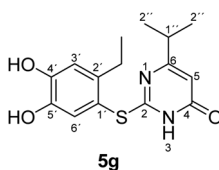
According to general procedure II, 4-methylcatechol (**1b**) (72 mg, 0.58 mmol), 2,3-dihydro-5,6-dimethyl-2-thioxopyrimidin-4(1H)-one (**2g**) (78 mg, 0.50 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U, 10 mg, *A. bisporus*) were reacted for 20 h. Workup gave 2-(4,5-dihydroxy-2-methylphenylthio)-5,6-dimethylpyrimidin-4(3H)-one (**5e**) as a pale yellow powder (120 mg, 86%), mp 229–331 °C; $R_f = 0.45$ ($\text{CH}_2\text{Cl}_2/\text{EtOAc}/\text{MeOH} = 3 : 3 : 0.1$); $\tilde{\nu}_{\text{max}}$ (atr)/ cm^{-1} 3420 (OH), 2926 (CH), 1625 (C=O), 1512 and 1264; δ_{H} (500 MHz; pyridine- d_5) 2.06 (3H, s, 5-CH₃), 2.13 (3H, s, 6-CH₃), 2.46 (3H, s, 2'-CH₃), 7.24 (1H, s, 3'-H) and 7.69 (1H, s, 6'-H); δ_{C} (125 MHz; pyridine- d_5) 10.87 (5-CH₃), 20.41 (2'-CH₃), 21.61 (6-CH₃), 113.56 (C-5), 116.85 (C-1'), 118.92 (C-3'), 125.00 (C-6'), 135.31 (C-2'), 145.61 (C-5'), 149.60 (C-4'), 161.78 (C-2), 162.20 (C-6) and 166.32 (C-4); MS (ESI) m/z 301 ([M + Na]⁺, 25%), 279 ([M + H]⁺, 100), 263 (25) and 157 (62); HRMS calcd for C₁₃H₁₄N₂O₃S + H (279.0798), found 279.0797.

Synthesis and analytical data of 2-(2-ethyl-4,5-dihydroxyphenylthio)-6-methylpyrimidin-4(3H)-one (**5f**).



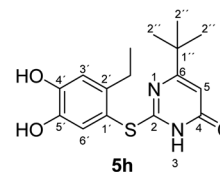
According to general procedure II, 4-ethylcatechol (**1c**) (80 mg, 0.58 mmol), 2,3-dihydro-6-methyl-2-thioxopyrimidin-4(1H)-one (**2a**) (71 mg, 0.50 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U, 10 mg, *A. bisporus*) were reacted for 14 h. Workup gave 2-(2-ethyl-4,5-dihydroxyphenylthio)-6-methylpyrimidin-4(3H)-one (**5f**) as a pale yellow powder (125 mg, 90%), mp 180–182 °C; $R_f = 0.31$ ($\text{CH}_2\text{Cl}_2/\text{EtOAc}/\text{MeOH} = 3 : 3 : 0.1$); $\tilde{\nu}_{\text{max}}$ (atr)/ cm^{-1} 3400 (OH), 2964 (CH), 1645 (C=O), 1503 (C=N) and 1278; δ_{H} (500 MHz; pyridine- d_5) 1.17 (3H, t, $^3J = 7.5$ Hz, 2'-CH₂CH₃), 2.13 (3H, s, 6-CH₃), 2.88 (2H, q, $^3J = 7.5$ Hz, 2'-CH₂CH₃), 6.31 (1H, s, 5-H), 7.27 (1H, s, 3'-H) and 7.69 (1H, s, 6'-H); δ_{C} (125 MHz; pyridine- d_5) 15.88 (2'-CH₂CH₃), 23.64 (6-CH₃), 27.30 (2'-CH₂CH₃), 105.54 (C-5), 116.11 (C-1'), 117.44 (C-3'), 125.45 (C-6'), 141.24 (C-2'), 145.68 (C-5'), 149.92 (C-4'), 166.67 (C-6), 167.74 (C-2) and 167.93 (C-4); MS (ESI) m/z 301 ([M + Na]⁺, 100%), 279 ([M + H]⁺, 21) and 143 (18); HRMS calcd for C₁₃H₁₄N₂O₃S + H (279.0798), found 279.0790.

Synthesis and analytical data of 2-(2-ethyl-4,5-dihydroxyphenylthio)-6-isopropylpyrimidin-4(3H)-one (**5g**).



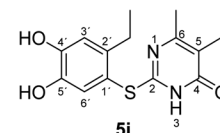
According to general procedure II, 4-ethylcatechol (**1c**) (80 mg, 0.58 mmol), 2,3-dihydro-6-isopropyl-2-thioxopyrimidin-4(1H)-one (**2d**) (85 mg, 0.50 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U, 10 mg, *A. bisporus*) were reacted for 12 h. Workup gave 2-(2-ethyl-4,5-dihydroxyphenylthio)-6-isopropylpyrimidin-4(3H)-one (**5g**) as a pale yellow powder (116 mg, 76%); mp 158–160 °C; $R_f = 0.50$ ($\text{CH}_2\text{Cl}_2/\text{EtOAc}/\text{MeOH} = 3 : 3 : 0.1$); $\tilde{\nu}_{\text{max}}$ (atr)/ cm^{-1} 3323 (OH), 2962 (CH), 1630 (C=O), 1514 and 1252; δ_{H} (300 MHz; pyridine- d_5) 1.09 (6H, d, $^3J = 7.0$ Hz, 2''-H), 1.16 (3H, t, $^3J = 7.5$ Hz, 2'-CH₂CH₃), 2.66 (1H, sep, $^3J = 7.0$ Hz, 1''-H), 2.86 (2H, q, $^3J = 7.5$ Hz, 2'-CH₂CH₃), 6.32 (1H, s, 5-H), 7.28 (1H, s, 3'-H) and 7.67 (1H, s, 6'-H); δ_{C} (75 MHz; pyridine- d_5) 15.80 (2'-CH₂CH₃), 21.25 (C-2''), 27.24 (2'-CH₂CH₃), 35.63 (C-1''), 103.06 (C-5), 116.20 (C-1'), 117.29 (C-3'), 125.44 (C-6'), 141.26 (C-2'), 145.57 (C-5'), 150.20 (ov., C-4'), 167.33 (C-2), 168.02 (C-4) and 175.01 (C-6); MS (ESI) m/z 345 ([M + K]⁺, 23%), 329 ([M + Na]⁺, 100), 307 ([M + H]⁺, 21) and 215 (14); HRMS calcd for C₁₅H₁₈N₂O₃S + H (307.1111), found 307.1103.

Synthesis and analytical data of 2-(2-ethyl-4,5-dihydroxyphenylthio)-6-tert-butylpyrimidin-4(3H)-one (**5h**).



According to general procedure II, 4-ethylcatechol (**1c**) (80 mg, 0.58 mmol), 6-tert-butyl-2,3-dihydro-2-thioxopyrimidin-4(1H)-one (**2e**) (92 mg, 0.50 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U, 10 mg, *A. bisporus*) were reacted for 16 h. Workup gave 2-(2-ethyl-4,5-dihydroxyphenylthio)-6-tert-butylpyrimidin-4(3H)-one (**5h**) as a pale yellow powder (130 mg, 81%), mp 153–155 °C; $R_f = 0.49$ ($\text{CH}_2\text{Cl}_2/\text{EtOAc}/\text{MeOH} = 3 : 3 : 0.1$); $\tilde{\nu}_{\text{max}}$ (atr)/ cm^{-1} 3410 (OH), 2962 (CH), 1632 (C=O), 1512 (C=N) and 1275; δ_{H} (300 MHz; pyridine- d_5) 1.15 (9H, s, 2''-H), 1.17 (3H, t, $^3J = 7.5$ Hz, 2'-CH₂CH₃), 2.84 (2H, q, $^3J = 7.4$ Hz, 2'-CH₂CH₃), 6.44 (1H, s, 5-H), 7.30 (1H, s, 3'-H) and 7.67 (1H, s, 6'-H); δ_{C} (75 MHz; pyridine- d_5) 15.79 (2'-CH₂CH₃), 27.24 (2'-CH₂CH₃), 28.75 (C-2''), 37.19 (C-1''), 102.12 (C-5), 116.14 (C-1'), 117.20 (C-3'), 125.47 (C-6'), 141.28 (C-2'), 145.52 (C-5'), 149.8 (ov., C-4'), 166.20 (C-2 or C-4), 167.80 (C-2 or C-4) and 176.82 (C-6); MS (ESI) m/z 343 ([M + Na]⁺, 100%), 321 ([M + H]⁺, 38) and 185 (25); HRMS calcd for C₁₆H₂₀N₂O₃S + H (321.1267), found 321.1260.

Synthesis and analytical data of 2-(2-ethyl-4,5-dihydroxyphenylthio)-5,6-dimethylpyrimidin-4(3H)-one (**5i**).

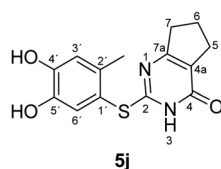


According to general procedure II, 4-ethylcatechol (**1c**) (80 mg, 0.58 mmol), 2,3-dihydro-5,6-dimethyl-2-thioxopyrimidin-4(1H)-one (**2g**) (78 mg, 0.50 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U,



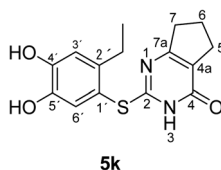
10 mg, *A. bisporus*) were reacted for 16 h. Workup gave 2-(2-ethyl-4,5-dihydroxyphenylthio)-5,6-dimethylpyrimidin-4(3*H*)-one (**5i**) as a pale yellow powder (133 mg, 91%), mp 185–187 °C; R_f = 0.43 ($\text{CH}_2\text{Cl}_2/\text{EtOAc}/\text{MeOH}$ = 3 : 3 : 0.1); $\tilde{\nu}_{\text{max}}$ (atr)/ cm^{-1} 3403 (OH), 2927 (CH), 1629 (C=O), 1523 (C=N) and 1252; δ_{H} (500 MHz; pyridine- d_5) 1.19 (3H, t, 3J = 7.5 Hz, 2'-CH₂CH₃), 2.06 (3H, s, 5-CH₃), 2.13 (3H, s, 6-CH₃), 2.88 (2H, q, 3J = 7.5 Hz, 2'-CH₂CH₃), 7.27 (1H, s, 3'-H) and 7.70 (1H, s, 6'-H); δ_{C} (125 MHz; pyridine- d_5) 10.82 (5-CH₃), 15.86 (2'-CH₂CH₃), 21.55 (6-CH₃), 27.27 (2'-CH₂CH₃), 113.42 (C-5), 116.19 (C-1'), 117.38 (C-3'), 125.40 (C-6'), 141.15 (C-2'), 145.64 (C-5'), 149.80 (ov., C-4'), 162.20 (C-6), 162.43 (C-2) and 166.34 (C-4); MS (ESI) m/z 315 ($[\text{M} + \text{Na}]^+$, 100%), 293 ($[\text{M} + \text{H}]^+$, 69) and 157 (41); HRMS calcd for $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_3\text{S} + \text{H}$ (293.0954), found 293.0949.

Synthesis and analytical data of 2-(4,5-dihydroxy-2-methylphenylthio)-6,7-dihydro-3*H*-cyclopenta[d]pyrimidin-4(5*H*)-one (5j**).**



According to general procedure II, 4-methylcatechol (**1b**) (72 mg, 0.58 mmol), 2,3,6,7-tetrahydro-2-thioxo-1*H*-cyclopenta[d]pyrimidin-4(5*H*)-one (**2i**) (84 mg, 0.50 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U, 10 mg, *A. bisporus*) were reacted for 14 h. Workup gave 2-(4,5-dihydroxy-2-methylphenylthio)-6,7-dihydro-3*H*-cyclopenta[d]pyrimidin-4(5*H*)-one (**5j**) as a pale yellow powder (130 mg, 90%), mp 240–242 °C; R_f = 0.30 ($\text{CH}_2\text{Cl}_2/\text{EtOAc}/\text{MeOH}$ = 3 : 3 : 0.1); $\tilde{\nu}_{\text{max}}$ (atr)/ cm^{-1} 3480 (OH), 2924 (CH), 1639 (C=O), 1512 and 1278; δ_{H} (500 MHz; pyridine- d_5) 1.72 (2H, quin, 6-H), 2.47 (3H, s, 2'-CH₃), 2.57 (2H, t, 3J = 7.7 Hz, 7-H), 2.75 (2H, t, 3J = 7.7 Hz, 5-H), 7.21 (1H, s, 3'-H) and 7.70 (1H, s, 6'-H); δ_{C} (125 MHz; pyridine- d_5) 20.42 (2'-CH₃), 21.37 (C-6), 27.41 (C-5), 34.89 (C-7), 115.94 (C-1'), 118.93 (C-3'), 119.48 (C-4a), 125.03 (C-6'), 135.35 (ov., C-2'), 145.71 (C-5'), 149.80 (ov., C-4'), 163.05 (C-4), 163.32 (C-2) and 171.02 (C-7a); MS (ESI) m/z 313 ($[\text{M} + \text{Na}]^+$, 93%), 291 ($[\text{M} + \text{H}]^+$, 100) and 169 (67); HRMS calcd for $\text{C}_{14}\text{H}_{14}\text{N}_2\text{O}_3\text{S} + \text{H}$ (291.0798), found 291.0788.

Synthesis and analytical data of 2-(2-ethyl-4,5-dihydroxyphenylthio)-6,7-dihydro-3*H*-cyclopenta[d]pyrimidin-4(5*H*)-one (5k**).**



According to general procedure II, 4-ethylcatechol (**1c**) (80 mg, 0.58 mmol), 2,3,6,7-tetrahydro-2-thioxo-1*H*-cyclopenta[d]pyrimidin-4(5*H*)-one (**2i**) (84 mg, 0.50 mmol), ethanol (2 mL), phosphate buffer (0.2 M, pH 6.0, 18 mL) and laccase (12 U, 10 mg, *A. bisporus*) were reacted for 14 h. Workup gave 2-(2-ethyl-4,5-dihydroxyphenylthio)-6,7-dihydro-3*H*-cyclopenta[d]pyrimidin-4(5*H*)-one (**5k**) as a pale yellow powder (115 mg, 76%), mp 178–180 °C; R_f = 0.36 ($\text{CH}_2\text{Cl}_2/\text{EtOAc}/\text{MeOH}$ = 3 : 3 : 0.1); $\tilde{\nu}_{\text{max}}$ (atr)/ cm^{-1}

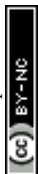
3500 (OH), 2962 (CH), 1639 (C=O), 1534 (C=N) and 1285; δ_{H} (500 MHz; pyridine- d_5) 1.20 (3H, t, 3J = 7.6 Hz, 2'-CH₂CH₃), 1.72 (2H, quin, 3J = 7.5 Hz, 6-H), 2.56 (2H, t, 3J = 7.5 Hz, 7-H), 2.75 (2H, t, 3J = 7.5 Hz, 5-H), 2.88 (2H, q, 3J = 7.6 Hz, 2'-CH₂CH₃), 7.24 (1H, s, 3'-H) and 7.72 (1H, s, 6'-H); δ_{C} (125 MHz; pyridine- d_5) 15.89 (2'-CH₂CH₃), 21.36 (C-6), 27.30 (2'-CH₂CH₃), 27.40 (C-5), 34.85 (C-7), 115.21 (C-1'), 117.41 (C-3'), 119.48 (C-4a), 125.41 (C-6'), 141.20 (C-2'), 145.76 (C-5'), 150.11 (C-4'), 163.06 (C-4), 163.85 (C-2) and 171.04 (C-7a); MS (ESI) m/z 327 ($[\text{M} + \text{Na}]^+$, 100%), 305 ($[\text{M} + \text{H}]^+$, 41) and 169 (14); HRMS calcd for $\text{C}_{15}\text{H}_{16}\text{N}_2\text{O}_3\text{S} + \text{H}$ (305.0954), found 305.0946.

Biology

Sulforhodamine assay. The human HepG2 cell line (kindly provided by Prof. Dr Lutz Graeve; Institut für Biologische Chemie und Ernährungswissenschaft, Universität Hohenheim) was grown in DMEM/Hams's medium (Merck) containing 10% fetal bovine serum (Merck) and 1% penicillin/streptomycin. Cells were inoculated into 96 well microtiter plates (Sarstedt) in 100 μL at plating densities $\sim$ 5000 cells per well. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO₂, 95% air and 100% relative humidity for 24 h prior to addition of compounds under test or doxorubicin (Sigma, cat. no. D2975000). Experimental drugs were prepared as 4 mM solution in DMSO and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate and pure DMSO were thawed and diluted with complete DMEM/Hams's medium containing 10% fetal bovine serum and 1% penicillin/streptomycin. An aliquot of 100 μL of 4% DMSO was added to the microtiter wells containing 100 μL of medium. An aliquot of 100 μL of the drug dilution was added to the appropriate microtiter wells, resulting in the required final drug concentrations (2.5–80 μM). Six wells were prepared for each individual dose. Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO₂, 95% air, and 100% relative humidity. The assay was terminated by the addition of cold trichloroacetic acid (TCA). Cells were fixed *in situ* by gentle addition of 50 μL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) (Sigma-Aldrich, cat. no. S1402) solution (100 μL) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing at least five times with 1% acetic acid and the plates were dried. Bound stain was subsequently solubilized with 10 mM Trizma base pH 10.5, and the absorbance was read on ELISA microplate reader at a wavelength of 515 nm. IC₅₀ was calculated for each experiment using four parameter logistic equation (Graph Pad, Prism Version 5). IC₅₀ results represent the mean of 2–5 experiments with the standard error of the mean indicating the variation.

Conclusions

To summarize, simple-to-perform, efficient and sustainable methods for the synthesis of regioisomeric pyrimidobenzo-thiazoles **3**, **4** as well as catechol thioethers **5** by laccase-catalyzed



domino reactions between catechols **1** and 2,3-dihydro-2-thioxopyrimidin-4-(1*H*)-ones **2** have been developed. The transformations rely on the laccase-catalyzed oxidation of a catechol **1** to the corresponding *o*-benzoquinone **8** which in turn undergoes reaction with a 2,3-dihydro-2-thioxopyrimidin-4-(1*H*)-one **2**. Depending on the substitution pattern of the 2,3-dihydro-2-thioxopyrimidin-4-(1*H*)-one **2**, the laccase-catalyzed reactions with unsubstituted catechol (**1a**) deliver either 7,8-dihydroxy-4*H*-pyrimido[2,1-*b*]benzothiazol-4-ones **3** and/or 7,8-dihydroxy-2*H*-pyrimido[2,1-*b*]benzothiazol-2-ones **4**. With 4-substituted catechols **1b**, **c**, catechol thioethers **5** are formed exclusively. All reactions can be performed under mild reaction conditions with aerial O₂ as the oxidant, and the products are formed with yields ranging between 76 and 97%. In addition, the cytotoxicity of selected pyrimidobenzothiazoles **3** and catechol thioethers **5** against HepG2 cell line is reported. A structure–activity relationship study reveals that the most potent compounds are **5c** (IC₅₀ = 7.77 μM) and **5g** (IC₅₀ = 2.74 μM) which carry an isopropyl group at C-6. The presence of an additional ethyl group at C-2' of **5g** increases the cytotoxic potency in comparison to **5c**.

Acknowledgements

We thank Mr M. Wolf (Institut für Chemie, Universität Hohenheim) for recording NMR spectra as well as Dipl.-Ing. (FH) J. Trinkner and D. Garnier (Institut für Organische Chemie, Universität Stuttgart) for recording mass spectra. Special thanks to Prof. Dr Lutz Graeve; (Institut für Biologische Chemie und Ernährungswissenschaft, Universität Hohenheim) for providing the HepG2 cell line. We acknowledge the help of A. Flaccus and D. Mvondo (Institut für Biologische Chemie und Ernährungswissenschaft, Universität Hohenheim) in carrying out the SRB assay. H. T. A.-M. is grateful to Science Technology Development Fund (STDF) for financial support (Project ID 11978).

Notes and references

- (a) V. Sharma, N. Chitranshi and A. K. Agarwal, *Int. J. Med. Chem.*, 2014, 202784; (b) K. S. Jain, T. S. Chitre, P. B. Miniyar, M. K. Kathiravan, V. S. Bendre, V. S. Veer, S. R. Shahana and C. J. Shishoo, *Curr. Sci.*, 2006, **90**, 793.
- A. Kleemann, J. Engel, B. Kutscher and D. Reichert, *Pharmaceutical Substances*, Thieme, Stuttgart, 5th edn, 2000.
- (a) E. Sherman, in *Lippincott Illustrated Reviews: Pharmacology*, ed. K. Whalen, Wolters Kluwer, Philadelphia, 6th edn, 2015, ch. 45, p. 567; (b) K. LaPlant and P. Louzon, in *Lippincott Illustrated Reviews: Pharmacology*, ed. K. Whalen, Wolters Kluwer, Philadelphia, 6th edn, 2015, ch. 46, p. 587; (c) R. Kaur, P. Kaur, S. Sharma, G. Singh, S. Mehndiratta, P. M. S. Bedi and K. Nepali, *Recent Pat. Anti-Cancer Drug Discovery*, 2015, **10**, 23.
- (a) R. Bandichhor, A. Bhattacharya, M. C. Bryan, A. Cosbie, A. Díaz-Rodríguez, L. Diorazio, Z. Fei, K. Fraunhoffer, J. Hayler, M. Hickey, L. Humphreys, P. Richardson, S. Roberts, T. White, S. Wuyts and J. Yin, *Org. Process Res. Dev.*, 2016, **20**, 1118; (b) F. Roschangar, R. A. Sheldon and C. H. Senanayake, *Green Chem.*, 2015, **17**, 752; (c) E. A. Peterson and J. B. Manley, *Green Chemistry Strategies for Drug Discovery*, in *RSC Drug Discovery Series*, Royal Society of Chemistry, Thomas Graham House, Cambridge, 2015; (d) P. Gupta and A. Mahajan, *RSC Adv.*, 2015, **5**, 26686; (e) H.-J. Federsel, *Green Chem.*, 2013, **15**, 3105.
- B. M. Trost, *Angew. Chem., Int. Ed.*, 1995, **34**, 259.
- (a) R. A. Sheldon, I. W. C. E. Arends and U. Hanefeld, *Green Chemistry and Catalysis*, WILEY-VCH, Weinheim, 2007; (b) P. T. Anastas and J. C. Warner, *Green Chemistry Theory and Practice*, Oxford University Press, New York, 2000; (c) K. Alfonsi, J. Colberg, P. J. Dunn, T. Fevig, S. Jennings, T. A. Johnson, H. P. Kleine, C. Knight, M. A. Nagy, D. A. Perry and M. Stefaniak, *Green Chem.*, 2008, **10**, 31; (d) D. J. C. Constable, P. J. Dunn, J. D. Hayler, G. R. Humphrey, J. L. Leazer Jr, R. J. Linderman, K. Lorenz, J. Manley, B. A. Pearlman, A. S. Wells, A. Zaks and T. Y. Zhang, *Green Chem.*, 2007, **9**, 411.
- (a) R. Dhanasekaran, A. Limaye and R. Cabrera, *Hepatic Med.*, 2012, **4**, 19; (b) S. Mittal and H. B. El-Serag, *J. Clin. Gastroenterol.*, 2013, **47**, S2; (c) E. Carr and J. A. Killman, *Basics of Cancer Chemotherapy (Clinical Nursing Series)*, Western Schools Press, Canada, 3rd edn, 1995.
- (a) S.-I. Shoda, H. Uyama, J.-I. Kadokawa, S. Kimura and S. Kobayashi, *Chem. Rev.*, 2016, **116**, 2307; (b) D. Monti, G. Ottolina, G. Carrea and S. Riva, *Chem. Rev.*, 2011, **111**, 4111; (c) F. Hollmann, I. W. C. E. Arends, K. Buehler, A. Schallmeyer and B. Bühler, *Green Chem.*, 2011, **13**, 226.
- (a) O. V. Morozova, G. P. Shumakovich, M. A. Gorbacheva, S. V. Shleev and A. I. Yarpolov, *Biochemistry (Moscow)*, 2007, **72**, 1136; (b) H. Claus, *Micron*, 2004, **35**, 93; (c) A. M. Mayer and R. C. Staples, *Phytochemistry*, 2002, **60**, 551; (d) C. F. Thurston, *Microbiology*, 1994, **140**, 19.
- (a) S. Witayakran and A. J. Ragauskas, *Adv. Synth. Catal.*, 2009, **351**, 1187; (b) A. Mikolasch and F. Schauer, *Appl. Microbiol. Biotechnol.*, 2009, **82**, 605; (c) A. Kunamneni, S. Camarero, C. García-Burgos, F. J. Plou, A. Ballesteros and M. Alcalde, *Microb. Cell Fact.*, 2008, **7**, 32.
- (a) J. González-Sabín, N. Ríos-Lombardía, I. García, N. M. Vior, A. F. Braña, C. Méndez, J. A. Salas and F. Morís, *Green Chem.*, 2016, **18**, 989; (b) K. Kędziora, A. Díaz-Rodríguez, I. Lavandera, V. Gotor-Fernández and V. Gotor, *Green Chem.*, 2014, **16**, 2448; (c) H. T. Abdel-Mohsen, K. Sudheendran, J. Conrad and U. Beifuss, *Green Chem.*, 2013, **15**, 1490; (d) H. T. Abdel-Mohsen, J. Conrad and U. Beifuss, *Green Chem.*, 2012, **14**, 2686; (e) H. Leutbecher, M.-A. Constantin, S. Mika, J. Conrad and U. Beifuss, *Tetrahedron Lett.*, 2011, **52**, 604; (f) A. Coniglio, C. Galli, P. Gentili and R. Vadalà, *J. Mol. Catal. B: Enzym.*, 2008, **50**, 40; (g) F. d'Acunzo, P. Baiocco and C. Galli, *New J. Chem.*, 2003, **27**, 329; (h) M. Fabbrini, C. Galli, P. Gentili and D. Macchitella, *Tetrahedron Lett.*, 2001, **42**, 7551; (i) A. Potthast, T. Rosenau, C.-L. Chen and J. S. Gratzl, *J. Org. Chem.*, 1995, **60**, 4320.
- (a) A. Llevot, E. Grau, S. Carlotti, S. Grelier and H. Cramail, *J. Mol. Catal. B: Enzym.*, 2016, **125**, 34; (b) C. Engelmann,



- S. Illner and U. Kragl, *Process Biochem.*, 2015, **50**, 1591; (c) M.-A. Constantin, J. Conrad and U. Beifuss, *Green Chem.*, 2012, **14**, 2375; (d) M.-A. Constantin, J. Conrad, E. Merişor, K. Koschorreck, V. B. Urlacher and U. Beifuss, *J. Org. Chem.*, 2012, **77**, 4528; (e) B. Pickel, M.-A. Constantin, J. Pfannstiel, J. Conrad, U. Beifuss and A. Schaller, *Angew. Chem., Int. Ed.*, 2010, **49**, 202; (f) S. Ncanana, L. Baratto, L. Roncaglia, S. Riva and S. G. Burton, *Adv. Synth. Catal.*, 2007, **349**, 1507; (g) C. Ponzoni, E. Beneventi, M. R. Cramarossa, S. Raimondi, G. Trevisi, U. M. Pagnoni, S. Riva and L. Forti, *Adv. Synth. Catal.*, 2007, **349**, 1497; (h) S. Ciecholewski, E. Hammer, K. Manda, G. Bose, V. T. H. Nguyen, P. Langer and F. Schauer, *Tetrahedron*, 2005, **61**, 4615.
- 13 (a) S. Emirdağ-Öztürk, S. Hajdok, J. Conrad and U. Beifuss, *Tetrahedron*, 2013, **69**, 3664; (b) J. Pietruszka and C. Wang, *Green Chem.*, 2012, **14**, 2402; (c) J. Pietruszka and C. Wang, *ChemCatChem*, 2012, **4**, 782; (d) S. Hajdok, J. Conrad and U. Beifuss, *J. Org. Chem.*, 2012, **77**, 445.
- 14 (a) V. Hahn, A. Mikolasch, C. Kuhlisch and F. Schauer, *J. Mol. Catal. B: Enzym.*, 2015, **122**, 56; (b) S. Herter, D. Michalik, A. Mikolasch, M. Schmidt, R. Wohlgemuth, U. Bornscheuer and F. Schauer, *J. Mol. Catal. B: Enzym.*, 2013, **90**, 91; (c) K. W. Wellington and N. I. Kolesnikova, *Bioorg. Med. Chem.*, 2012, **20**, 4472; (d) S. Herter, A. Mikolasch, D. Michalik, E. Hammer, F. Schauer, U. Bornscheuer and M. Schmidt, *Tetrahedron*, 2011, **67**, 9311; (e) V. Hahn, T. Davids, M. Lalk, F. Schauer and A. Mikolasch, *Green Chem.*, 2010, **12**, 879; (f) A. Mikolasch, A. Matthies, M. Lalk and F. Schauer, *Appl. Microbiol. Biotechnol.*, 2008, **80**, 389.
- 15 (a) M. Schlippert, A. Mikolasch, V. Hahn and F. Schauer, *J. Mol. Catal. B: Enzym.*, 2016, **126**, 106; (b) H. T. Abdel-Mohsen, J. Conrad and U. Beifuss, *Green Chem.*, 2014, **16**, 90; (c) K. W. Wellington, G. E. R. Gordon, L. A. Ndlovu and P. Steenkamp, *ChemCatChem*, 2013, **5**, 1570; (d) K. W. Wellington, R. Bokako, N. Raseroka and P. Steenkamp, *Green Chem.*, 2012, **14**, 2567.
- 16 (a) A. C. Sousa, M. F. M. M. Piedade, L. O. Martins and M. P. Robalo, *Green Chem.*, 2015, **17**, 1429; (b) M. D. Cannatelli and A. J. Ragauskas, *J. Mol. Catal. B: Enzym.*, 2015, **119**, 85; (c) A. C. Sousa, M. C. Oliveira, L. O. Martins and M. P. Robalo, *Green Chem.*, 2014, **16**, 4127; (d) H. T. Abdel-Mohsen, J. Conrad and U. Beifuss, *J. Org. Chem.*, 2013, **78**, 7986; (e) K. W. Wellington, G. E. R. Gordon, L. A. Ndlovu and P. Steenkamp, *ChemCatChem*, 2013, **5**, 1570; (f) M. Kidwai, A. Jain, A. Sharma and R. C. Kuhad, *Catal. Sci. Technol.*, 2013, **3**, 230; (g) H. Leutbecher, G. Greiner, R. Amann, A. Stolz, U. Beifuss and J. Conrad, *Org. Biomol. Chem.*, 2011, **9**, 2667; (h) S. Hajdok, J. Conrad, H. Leutbecher, S. Strobel, T. Schleid and U. Beifuss, *J. Org. Chem.*, 2009, **74**, 7230; (i) H. Leutbecher, S. Hajdok, C. Braunberger, M. Neumann, S. Mika, J. Conrad and U. Beifuss, *Green Chem.*, 2009, **11**, 676; (j) S. Witayakran, L. Gelbaum and A. J. Ragauskas, *Tetrahedron*, 2007, **63**, 10958; (k) S. Hajdok, H. Leutbecher, G. Greiner, J. Conrad and U. Beifuss, *Tetrahedron Lett.*, 2007, **48**, 5073; (l) S. Witayakran, A. Zettili and A. J. Ragauskas, *Tetrahedron Lett.*, 2007, **48**, 2983; (m) S. Witayakran and A. J. Ragauskas, *Green Chem.*, 2007, **9**, 475; (n) H. Leutbecher, J. Conrad, I. Klaiber and U. Beifuss, *Synlett*, 2005, 3126.
- 17 (a) M. Barmaki, G. Valiyeva, A. A. Maharramovm and M. M. Allaverdiyev, *J. Chem.*, 2013, 176213; (b) K.-P. Shao, X.-Y. Zhang, P.-J. Chen, D.-Q. Xue, P. He, L.-Y. Ma, J.-X. Zheng, Q.-R. Zhang and H.-M. Liu, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 2741.
- 18 S. Nicotra, A. Intra, G. Ottolina, S. Riva and B. Danieli, *Tetrahedron: Asymmetry*, 2004, **15**, 2927.
- 19 Structure solution and refinement using programs SHELXT and SHELXL (a) G. M. Sheldrick, *Acta Crystallogr., Sect. A: Found. Adv.*, 2015, **71**, 3; (b) G. M. Sheldrick, *Acta Crystallogr., Sect. C: Struct. Chem.*, 2015, **71**, 3. CCDC 1514742 (3f) and 1514743 (5a) contain the supplementary crystallographic data for this paper.
- 20 (a) P. Anastas and N. Eghbali, *Chem. Soc. Rev.*, 2010, **39**, 301; (b) S. L. Y. Tang, R. L. Smith and M. Poliakoff, *Green Chem.*, 2005, **7**, 761; (c) R. A. Sheldon, *Chem. Soc. Rev.*, 2012, **41**, 1437; (d) R. A. Sheldon, *Chem. Commun.*, 2008, 3352.
- 21 R. A. Sheldon, *Green Chem.*, 2007, **9**, 1273.
- 22 (a) R. K. Henderson, C. Jiménez-González, D. J. C. Constable, S. R. Alston, G. G. A. Inglis, G. Fisher, J. Sherwood, S. P. Binks and A. D. Curzons, *Green Chem.*, 2011, **13**, 854; (b) P. G. Jessop, *Green Chem.*, 2011, **13**, 1391; (c) C. Capello, U. Fischer and K. Hungerbühler, *Green Chem.*, 2007, **9**, 927.
- 23 P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney and M. R. Boyd, *J. Natl. Cancer Inst.*, 1990, **82**, 1107.
- 24 (a) G. W. Anderson, I. F. Halverstadt, W. H. Miller and R. O. Roblin, *J. Am. Chem. Soc.*, 1945, **67**, 2197; (b) W. H. Miller, A. M. Dessert and G. W. Anderson, *J. Am. Chem. Soc.*, 1948, **70**, 500.

