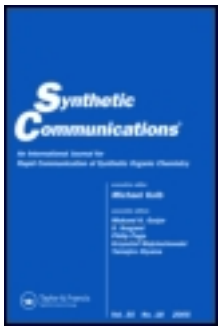


Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

Concise Synthesis and X-Ray Crystal Structure of N-Benzyl-2-(pyrimidin-4′-ylamino)-thiazole-4-carboxamide ('Thiazovivin'), a Small-Molecule Tool for Stem Cell Research

Oliver Ries [a] , Markus Granitzka [b] , Dietmar Stalke [b] & Christian Ducho [a c]

[a] Georg-August-University Göttingen, Department of Chemistry, Institute of Organic and Biomolecular Chemistry , Göttingen , Germany

[b] Georg-August-University Göttingen, Department of Chemistry, Institute of Inorganic Chemistry , Göttingen , Germany

[c] University of Paderborn, Department of Chemistry , Paderborn , Germany

Accepted author version posted online: 31 May 2013.

To cite this article: Oliver Ries , Markus Granitzka , Dietmar Stalke & Christian Ducho (2013): Concise Synthesis and X-Ray Crystal Structure of N-Benzyl-2-(pyrimidin-4′-ylamino)-thiazole-4-carboxamide ('Thiazovivin'), a Small-Molecule Tool for Stem Cell Research, Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, DOI:10.1080/00397911.2012.745567

To link to this article: http://dx.doi.org/10.1080/00397911.2012.745567

Concise Synthesis and X-ray Crystal Structure of *N*-Benzyl-2-(pyrimidin-4'-ylamino)-thiazole-4-carboxamide ('Thiazovivin'), a Small-Molecule Tool for Stem Cell Research

Oliver Ries[1], Markus Granitzka[2], Dietmar Stalke[2], Christian Ducho[1,3]

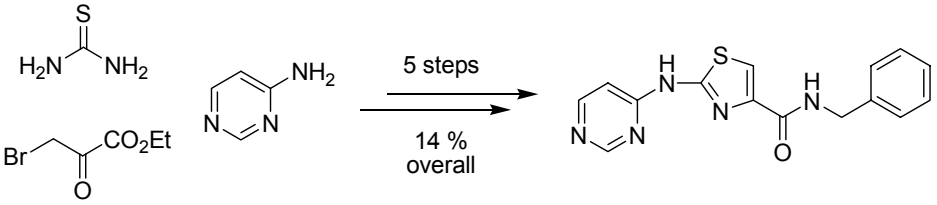
[1]Georg-August-University Göttingen, Department of Chemistry, Institute of Organic and Biomolecular Chemistry, Göttingen, Germany, [2]Georg-August-University Göttingen, Department of Chemistry, Institute of Inorganic Chemistry, Göttingen, Germany, [3]University of Paderborn, Department of Chemistry, Paderborn, Germany

Address correspondence to Christian Ducho, University of Paderborn, Department of Chemistry, Warburger Str. 100, 33 098 Paderborn, Germany. Phone: +49-(0)5251-60-5770. Fax: +49-(0)5251-60-3245. E-mail: christian.ducho@uni-paderborn.de

Abstract

Stem cell research is one of the most promising fields of modern biomedical research and regenerative medicine. Limited availability and ethical concerns suggest the renouncement of embryonic stem cells (ESCs), thus raising the need for more efficient procedures for the generation of stem cells, ideally through reprogramming of mammalian cells. The small molecule *N*-benzyl-2-(pyrimidin-4'-ylamino)-thiazole-4-carboxamide ('Thiazovivin') is known to improve the generation of human induced pluripotent stem cells (iPSCs) from human fibroblasts. We herein describe a highly efficient procedure for the synthesis of Thiazovivin over just five steps, which should be suitable for a large-scale application, and the first X-ray crystal structure of the target compound.

[Supplementary materials are available for this article. Go to the publisher's online edition of *Synthetic Communications*® for the following free supplemental resource(s): Full experimental and spectral details.]



KEYWORDS: Chemical biology; stem cells; heterocycles

INTRODUCTION

Within the last three decades, stem cell research has evolved to one of the most promising, though controversially discussed, fields of biomedical research.[1] Stem cells offer a great opportunity for the treatment of previously incurable diseases and novel future therapies.[2] One major drawback is the need for at least pluripotent stem cells (PSCs), which can form all of the human body's cell lineages.[3] Human embryonic stem cells (hESCs) represent an ethically problematic source for PSCs. A preferred option is the reprogramming of functionally heterogeneous cells of a multicellular organism to induced pluripotent stem cells (iPSCs).[4] Several advances in the generation of iPSCs have been made,[5–7] but the process takes up to four weeks and furnishes only a small amount of heterogeneous cell populations.

In 2009, Ding and coworkers described a chemical method that boosted the efficiency of

iPSC generation from human fibroblasts by a factor >200. They have achieved this vast improvement by the application of *N*-benzyl-2-(pyrimidin-4'-ylamino)-thiazole-4-carboxamide **1** ('Thiazovivin', Fig. 1).[8] Addition of Thiazovivin to iPSC colonies promoted their survival after trypsinization and led to high levels of endogenous mRNA and protein expression of pluripotent markers. The iPSC colonies created by this protocol could be grown under conventional hESC culture conditions. In terms of morphology, typical pluripotency marker expression and differentiation potential, the colonies likewise come close to hESCs. Recently, Thiazovivin **1** has been employed for the generation of fat-laden adipocytes from porcine embryonic fibroblasts (PEFs).[9] We herein describe an efficient and concise synthesis of **1** which is expected to be suitable for the large scale. Furthermore, an unprecedented X-ray crystal structure of **1** has been obtained.

RESULTS AND DISCUSSION

Thiazovivin has originally been obtained by a solid-phase supported synthesis involving a Pd-catalyzed key step.[10] In contrast, our approach employs standard solution-phase methodology and does not require transition metal catalysis. Using this novel route, the condensation of thiourea **2** with ethyl 3-bromopyruvate **3** led to aminothiazole **4**,[11,12] which could be recrystallized from ethanol, in 83 % yield (Scheme 1). Subsequent Sandmeyer-type bromination gave bromothiazole **5**[11,13] in 68 % yield after column chromatography. Saponification of the ethyl ester furnished carboxylic acid **6**[11,14] in 62 % yield after recrystallisation from water, followed by amide coupling with benzyl amine and EDC-HOBt to afford thiazole amide **7** (73 % yield). The final reaction

providing thiazovivin **1** was accomplished by the nucleophilic aromatic substitution of **7** with 4-aminopyrimidine **8** in the presence of sodium hydride. Purification by column chromatography and preparative HPLC in order to obtain highly pure material for cell applications afforded thiazovivin **1** in 53 % yield. In summary, thiazovivin **1** was prepared with an overall yield of 14 % from inexpensive starting materials over five steps (Scheme 1).

A single crystal of **1** was obtained by crystallization of the purified material after evaporation of residual solvent (dichloromethane). Thiazovivin **1** crystallizes in the tetragonal space group $I4_1/a$ with one molecule in the asymmetric unit (Fig. 2). The hydrogen atoms H31 and H51 bonded to the nitrogen atoms N3 and N5 generate hydrogen bonds to O1 and N1 of neighbouring molecules (Fig. 3). The hydrogen bond distances are N3–H31···O1A 190.0(16) pm and N5–H51···N1A 221.0(17) pm, respectively.

In summary, we have developed a convenient synthetic route providing thiazovivin **1** without the use of expensive heavy metal catalysts, thus making this concise synthesis potentially useful for large-scale applications.

EXPERIMENTAL

Chemicals were purchased from Sigma-Aldrich, Alfa Aesar, Fluorochem, Merck, Roth, Acros Organics and GL Biochem. Reactions involving moisture sensitive reagents were

carried out under an atmosphere of argon using anhydrous solvents. Anhydrous solvents were obtained in the following manner: acetonitrile was dried over P_2O_5 and distilled. THF was dried over sodium/benzophenone and distilled. DMF was dried over activated molecular sieves (4 Å). All other solvents were of technical quality and distilled prior to their use, and deionized water was used throughout. Column chromatography was carried out on silica gel 60 (0.040-0.063 mm, 230-400 mesh ASTM, VWR) under flash conditions. TLC was performed on aluminum plates precoated with silica gel 60 F_{254} (VWR). Visualization of the spots was carried out using UV light (254 nm) and/or staining under heating (staining solution: 1 g $KMnO_4$, 6 g K_2CO_3 and 1.5 mL 5 % $NaOH_{(aq)}$ (w/v), all dissolved in 100 mL H_2O). Preparative HPLC was carried out on a Jasco system equipped with PU-1587 Intelligent Prep. Pumps, a UV-1575 Intelligent UV/Vis detector and a Merck LiChroCART column (250 x 10 mm, LiChrospher 100 RP 18, 10 µm). 300 MHz-^{1}H as well as 75 MHz-, 76 MHz- and 126 MHz-^{13}C NMR spectra were recorded on Varian UNITY 300, MERCURY 300 and INOVA 500 spectrometers. All ^{13}C NMR spectra are ^{1}H-decoupled. All spectra were recorded at room temperature or at 35 °C (DMSO-d_6) and were referenced internally to solvent reference frequencies. Chemical shifts (δ) are quoted in ppm, and coupling constants (*J*) are reported in Hz. Assignment of signals was carried out using ^{1}H,^{1}H-COSY, HSQC and HMBC spectra obtained on the spectrometers mentioned above. Low resolution ESI mass spectrometry was performed on a Varian MAT 311 A spectrometer operating in positive or negative ionization mode. High resolution (HR) ESI mass spectrometry was carried out on a Bruker microTOF spectrometer or a Bruker 7 T FTICR APEX IV spectrometer. Melting

points (mp) were measured on a Büchi instrument and are not corrected. Infrared spectroscopy (IR) was performed on a Perkin-Elmer Vektor 22 spectrometer with solids being measured as KBr pills. Wavenumbers (ν) are quoted in cm^{-1}. UV spectroscopy was carried out on a Perkin-Elmer Lambda 2 spectrometer. Wavelengths of maximum absorption (λ_{max}) are reported in nm with the corresponding logarithmic molar extinction coefficient (log ε, $\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$) given in parenthesis.

_N_-Benzyl-2-Bromothiazol-4-Carboxamide (7)

A solution of **6** (101 mg, 0.486 mmol) in THF (2.5 mL) was treated with HOBt (78 mg, 0.58 mmol), EDC hydrochloride (110 mg, 0.574 mmol) and DIPEA (0.10 mL, 0.59 mmol). The solution was stirred at room temperature for 30 min, and then a solution of benzyl amine (80 µL, 0.73 mmol) in THF (1 mL) was added dropwise. The reaction mixture was stirred at room temperature for 18 h and was then diluted with EtOAc (2.5 mL) and water (2 mL). The organic layer was washed with 0.5 M HCl (1 x 5 mL), sat. $NaHCO_3$ solution (2 x 5 mL) and water (2 x 5 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure, and column chromatography of the resultant crude product (petroleum ether-EtOAc 4:1) gave 106 mg (0.357 mmol, 73 %) of **7** as a white solid. R_f = 0.41 (petroleum ether-EtOAc 1:1); mp = 78 °C; ^{1}H NMR (300 MHz, C_6D_6): δ = 4.32 (d, J = 6.3 Hz, 2H, benzyl-CH_2), 6.96-7.17 (m, 5H, aryl-H), 7.19 (br s, 1H, NH), 7.51 (s, 1H, 5-H); ^{13}C NMR (75 MHz, C_6D_6): δ = 43.25 (benzyl-CH_2), 127.08 (C-5), 127.41 (aryl-CH), 128.00 (aryl-CH), 128.72 (aryl-CH), 135.66 (C-2), 138.92 (aryl-C), 150.51 (C-4), 159.24 (C-6); IR (KBr): ν (cm^{-1}) = 3388, 3092, 1665,

1542, 1487, 1429, 1239, 1014, 764, 718, 605, 577; UV (MeOH): λ_{max} (log ε) = 203 (4.42), 235 (4.07); MS (ESI): *m/z* = 319.0 $[M+Na]^+$, 321.0 $[M+Na]^+$; HRMS (ESI): *m/z* calcd. for $C_{11}H_9BrN_2OS$ $[M+Na]^+$ 320.9491, found 320.9487.

Thiazovivin (1)

Sodium hydride (60 % dispersion in mineral oil, 20 mg, 0.50 mmol) was added to a solution of **7** (50 mg, 0.17 mmol) and 4-aminopyrimidine **8** (48 mg, 0.50 mmol) in DMF (3 mL). The solution was stirred for 3 h at 140 °C and then cooled to room temperature. The solvent was removed under reduced pressure and the residue was co-evaporated with toluene and CH_2Cl_2. Column chromatography (EtOAc) and preparative HPLC purification (MeOH/water 70:30, flow 8 mL/min, UV detection at 310 nm) gave 28 mg (90 μmol, 53 %) of **1** as a yellow-brownish solid. R_f = 0.20 (EtOAc); mp = 216 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 4.49 (d, *J* = 6.2 Hz, 2H, benzyl-CH_2), 7.15 (dd, *J* = 5.9 Hz, *J* = 1.1 Hz, 1H, 5'-H), 7.20-7.38 (m, 5H, aryl-H), 7.79 (s, 1H, 5-H), 8.33 (t, *J* = 6.2 Hz, 1H, amide-NH), 8.47 (d, *J* = 5.9 Hz, 1H, 6'-H), 8.84 (d, *J* = 1.1 Hz, 1H, 2'-H), 11.79 (br s, 1H, 2-NH); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 42.20 (benzyl-CH_2), 107.70 (C-5'), 117.06 (C-5), 126.62 (aryl-CH), 127.07 (aryl-CH), 128.11 (aryl-CH), 139.16 (aryl-C), 144.40 (C-4), 155.97 (C-6'), 156.38 (C-4'), 156.95 (C-2'), 158.00 (C-2), 160.53 (C-6); IR (KBr): ν (cm^{-1}) = 3403, 3086, 1655, 1615, 1554, 1527, 1495, 1445, 1393, 1327, 765, 698, 565; UV (MeOH): λ_{max} (log ε) = 210 (4.39), 225 (4.27), 258 (4.07), 299 (4.24), 345 (3.05). MS (ESI): *m/z* = 312.1 $[M+H]^+$, 334.1 $[M+Na]^+$; HRMS (ESI): *m/z* calcd. for $C_{15}H_{13}N_5OS$ $[M+Na]^+$ 334.0733, found 334.0733.

X-RAY STRUCTURE DETERMINATION OF 1

Single crystals of **1** were selected from a flask and covered with perfluorated polyether oil on a microscope slide. An appropriate crystal was selected using a polarize microscope, mounted on the tip of a MITEGEN®MicroMount fixed to a goniometer head and shock cooled by the crystal cooling device.

The data for **1** were collected from shock-cooled crystals at 100(2) K[15] on a INCOATEC Mo Microsource[16] (used MoK$_\alpha$ radiation, λ = 71.073 pm) with mirror optics and APEX II detector with a D8 goniometer. They were integrated with SAINT[17] and an empirical absorption correction (SADABS)[18] was applied. The structure was solved by direct methods (SHELXS-97)[19a] and refined by full-matrix least-squares methods against F^2 (SHELXL-97)[19b,c] within the ShelXle GUI. All non-hydrogen-atoms were refined with anisotropic displacement parameters. The hydrogen atoms, besides H31 and H51, were refined isotropically on calculated positions using a riding model with their U_{iso} values constrained to equal to 1.5 times the U_{eq} of their pivot atoms for terminal sp^3 carbon atoms and 1.2 times for all other carbon atoms. Only the hydrogen atoms of the nitrogen atoms (H31 and H51) were refined freely and their positions were found from the difference density map. The disordered dichloromethane solvent molecule was refined using bond length and angle restraints and anisotropic displacement parameter restraints. Data of the X-ray crystal structure of **1** were deposited at the Cambridge Crystallographic Data Centre (excluding structure factors) and are available online. The CCDC numbers,

crystal data and experimental details for the X-ray measurements are listed in Table S1 (Supplementary Material).

SUPPLEMENTARY MATERIAL

Supplementary Material: Syntheses of compounds **4-6**, ^{1}H and ^{13}C NMR spectra of all compounds, data on the X-ray crystal structure of **1**. This material can be found via the "Supplementary Content" section of this article's Web page. Copies of the crystallographic data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif or from the corresponding author.

ACKNOWLEDGMENTS

The authors thank Dr. Benjamin Groß (Hannover Medical School, Germany) for helpful discussions and the Fonds der Chemischen Industrie (FCI, Sachkostenzuschuss) for financial support. M.G. and D.S. thank the Danish National Research Foundation (DNRF) funded Center for Materials Crystallography (CMC).

REFERENCES

1. DeWitt, N. Stem-cell Biology. *Nature* **2006**, *441*, 1059.

2. Williams, D. A. Rapid Development of Pluripotent Stem Cells as a Potential Therapeutic Modality. *Mol. Ther.* **2009**, *17*, 929–930.

3. Smith, A. A glossary for stem-cell biology. *Nature* **2006**, *441*, 1060.

4. Hochedlinger, K.; Jaenisch, R. Nuclear reprogramming and pluripotency. *Nature* **2006**, *441*, 1061–1067.

5. Takahashi, K.; Tanabe, K.; Ohnuki, M.; Narita, M.; Ichisaka, T.; Tomoda, K.; Yamanaka, S. Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. *Cell* **2007**, *131*, 861–872.

6. Yu, J.; Vodyanik, M. A.; Smuga-Otto, K.; Antosiewicz-Bourget, J.; Frane, J. L.; Tian, S.; Nie, J.; Jonsdottir, G. A.; Ruotti, V.; Stewart, R.; Slukvin, I. I.; Thomson, J. A. Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells. *Science* **2007**, *318*, 1917-1920.

7. Müller, L. U. W.; Daley, G. Q.; Williams, D. A. Upping the Ante: Recent Advances in Direct Reprogramming. *Mol. Ther.* **2009**, *17*, 947-953.

8. Lin, T.; Ambasudhan, R.; Yuan, X.; Li, W.; Hilcove, S.; Abujarour, R.; Lin, X.; Hahm, H. S.; Hao, E.; Hayek, A.; Ding, S. A chemical platform for improved induction of human iPSCs. *Nat. Methods* **2009**, *6*, 805-808.

9. Zhu, J.; Pang, D.; Zhou, Y.; Tang, X.; Huang, Y.; Xie, W.; Gao, F.; Lai, L.; Zhang, M.; Ouyang, H. Direct Conversion of Porcine Embryonic Fibroblasts into Adipocytes by Chemical Molecules. *Cell. Reprogramming* **2012**, *14*, 99-105.

10. Xu, Y.; Zhu, Y.; Hahm, H. S.; Wei, W.; Hao, E.; Hayek, A.; Ding, S. Revealing a core signalling regulatory mechanism for pluripotent stem cell survival and self-renewal by small molecules. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 8129-8134.

11. Chen, H.; Wang, W.; Wang, G.; Shi, L.; Gu, M.; Ren, Y.; Hou, L.; He, P.; Zhu, F.; Zhong, X.; Tang, W.; Zuo, J.; Nan, F. Rational Design and Synthesis of 2,2-

Bisheterocycle Tandem Derivatives as Non-Nucleoside Hepatitis B Virus Inhibitors. *ChemMedChem* **2008**, *3*, 1316-1321.

12. Plouvier, B.; Houssin, R.; Bailly, C.; Henichart, J. P. Synthesis and DNA-binding study of a thiazole-containing analog of netropsin. *J. Heterocycl. Chem.* **1989**, *26*, 1643-1647.

13. Delgado, O.; Muller, H. M.; Bach, T. Concise total synthesis of the thiazolyl peptide antibiotic GE2270A. *Chem. Eur. J.* **2008**, *14*, 2322-2339.

14. Silberg, A.; Ursu, A. Preparation and properties of 2-halo-4-halomethylthiazoles. *Rev. Roum. Chim.* **1965**, *10*, 897-902.

15. a) Kottke, T.; Stalke, D. Crystal handling at low temperatures. *J. Appl. Crystallogr.* **1993**, *26*, 615-619; b) Kottke, T.; Lagow, R. J.; Stalke, D. Low-cost conversion of a coaxial nozzle arrangement into a stationary low-temperature attachment. *J. Appl. Crystallogr.* **1996**, *29*, 465-468; c) Stalke, D. Cryo crystal structure determination and application to intermediates. *Chem. Soc. Rev.* **1998**, *27*, 171-178.

16. Schulz, T.; Meindl, K.; Leusser, D.; Stern, D.; Graf, J.; Michaelsen, C.; Ruf, M.; Sheldrick, G. M.; Stalke, D. A comparison of a microfocus X-ray source and a conventional sealed tube for crystal structure determination. *J. Appl. Crystallogr.* **2009**, *42*, 885-891.

17. SAINT v7.68A in Bruker APEX v2011.9, Bruker AXS Inst. Inc., Madison, USA, **2008**.

18. Sheldrick, G. M. *SADABS 2008/2*; Georg-August-University Göttingen: Germany, 2008.

19. a) Sheldrick, G. M. Phase annealing in *SHELX*-90: direct methods for larger structures. *Acta Crystallogr. Sect. A* **1990**, *46*, 467-473; b) Sheldrick, G. M. A short history of *SHELX*. *Acta Crystallogr*. *Sect. A* **2008**, *64*, 112-122; c) Müller, P.; Herbst-Irmer, R.; Spek, A. L.; Schneider, T. R.; Sawaya M. R. in *Crystal Structure Refinement - A Crystallographer's Guide to SHELXL*, IUCr Texts on Crystallography, Vol. 8 (Ed.: P. Müller), Oxford University Press, Oxford, UK, **2006**; d) Hübschle, C. B.; Sheldrick, G. M.; Dittrich, B. *ShelXle*: a Qt graphical user interface for *SHELXL*. *J. Appl. Crystallogr*. **2011**, *44*, 1281-1284.

Scheme 1. Synthesis of target compound **1**.

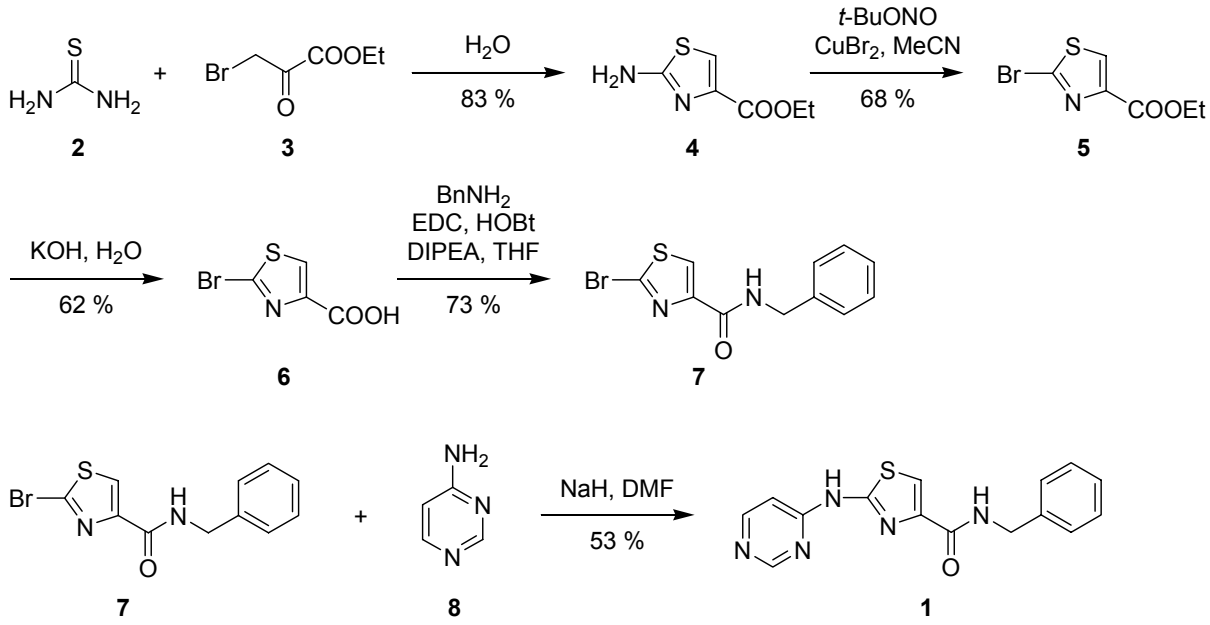
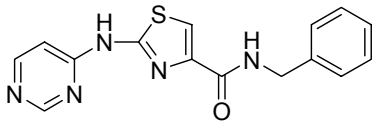


Figure 1. Structure of Thiazovivin **1**, the target compound of this study.



1

Figure 2. Molecular structure of Thiazovivin **1**. Anisotropic displacement parameters are depicted at the 50 % probability level. Atom color code: carbon = black, nitrogen = blue, oxygen = red, sulfur = yellow and hydrogen = white. The other hydrogen atoms and the disordered solvent molecule dichloromethane are omitted for clarity.

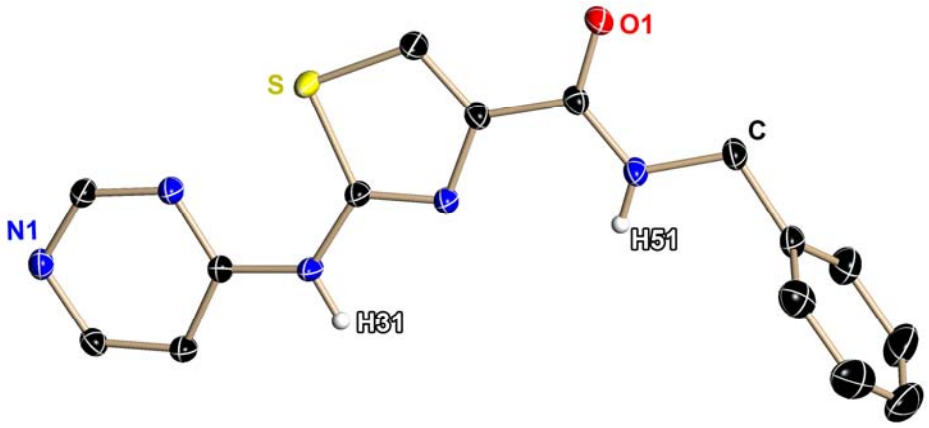


Figure 3. Packing plot and hydrogen bonds of Thiazovivin **1**. Atom color code: carbon = black, nitrogen = blue, oxygen = red, sulfur = yellow and hydrogen = white. Symmetry generated molecules are shown in wireframe projection for clarity.