Bioorganic & Medicinal Chemistry 20 (2012) 356–367

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis, and biological evaluation of novel (1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acids as selective inhibitors for AKR1B1

Daisuke Minehira [a], Daisuke Takeda [a], Hirokazu Urata [a], Atsushi Kato [a,*], Isao Adachi [a], Xu Wang [b], Yuji Matsuya [b], Kenji Sugimoto [b], Mayuko Takemura [c], Satoshi Endo [c], Toshiyuki Matsunaga [c], Akira Hara [c], Jun Koseki [d], Kayo Narukawa [d], Shuichi Hirono [d], Naoki Toyooka [b,e,f,*]

[a] Department of Hospital Pharmacy, University of Toyama, Toyama 930-0194, Japan
[b] Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama 930-0194, Japan
[c] Gifu Pharmaceutical University, Gifu 502-8585, Japan
[d] School of Pharmaceutical Sciences, Kitasato University, Tokyo 108-8641, Japan
[e] Graduate School of Science and Technology for Research, University of Toyama, Toyama 930-8555, Japan
[f] Graduate School of Innovative Life Science, University of Toyama, Toyama 930-8555, Japan

ARTICLE INFO

Article history:
Received 5 September 2011
Revised 23 October 2011
Accepted 25 October 2011
Available online 3 November 2011

Keywords:
AKR1B1
Diabetes mellitus
Aldose reductase
Molecular docking
Selectivity index

ABSTRACT

New substituted (1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acids were designed as the inhibitor of AKR1B1 based upon the structure of rhetsinine, a minor alkaloidal component of *Evodia rutaecarpa*, and twenty derivatives were synthesized and evaluated. The most active compound of the series was (2-benzyl-6-methoxy-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid (**7m**), which showed comparable inhibitory activity for AKR1B1 (IC_{50} = 0.15 μM) with clinically used epalrestat (IC_{50} = 0.1 μM). In the view of activity and selectivity, the most potent compound was (2-benzyl-6-carboxy-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid (**7t**), which showed strong inhibitory effect (IC_{50} = 0.17 μM) and very high selectivity for AKR1B1 against AKR1A1 (311:1) and AKR1B10 (253:1) compared with epalrestat.

1. Introduction

Diabetes mellitus is one of the most common chronic metabolic diseases, which occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. Today, the worldwide prevalence of diabetes is taking pandemic dimensions, as changing lifestyles lead to reduced physical activity, and increased obesity. In 2010, 285 million people were suffering from diabetes and this number is estimated to increase to 439 million by 2030.[1] Prolonged hyperglycemia is a primary causal factor of several diabetic complications. Many studies have revealed a correlation between glucose metabolism via the polyol pathway and long-term complications. Aldose reductase (EC 1.1.1.21) is the first and rate-limiting enzyme in this pathway responsible for converting glucose into sorbitol, which is further metabolized into fructose by sorbitol dehydrogenase (EC 1.1.1.14). The enzyme has a low affinity for glucose and the polyol pathway plays a minor role in glucose metabolism because it competes with the glucose hexokinase of the glycolytic pathway. Under hyperglycaemic conditions, however, glucose is rapidly metabolized through the polyol pathway, and the product, sorbitol, cannot cross the cell membrane easily and therefore causes swelling and cell dysfunction in a number of tissues. In addition, fructose can become phosphorylated to fructose-3-phosphate, which is broken down to 3-deoxyglucosone, ultimately forming advanced glycation end products that are capable of cellular damage.[2–4] These abnormal metabolic results have been reported to be factors responsible for diabetic complications such as cataracts,[5] retinopathy,[6] neuropathy,[7] and nephropathy.[8]

Mammalian aldose reductases belong to the aldo–keto reductase (AKR) 1B subfamily of the AKR superfamily, in which the human enzyme is named AKR1B1.[9] The inhibition of AKR1B1 is an important potential therapeutic mechanism in treating diabetic complications. At present, the inhibitors are divided into two different chemical classes. One is the spiro-hydantoin or -imido derivatives, such as sorbinil, fidarestat and ranirestat, and the other is the carboxylic acid derivatives, such as epalrestat and tolrestat. Although many laboratories have synthesized various structures of AKR1B1 inhibitors which appear to be promising during *in vitro* studies and in trials with animal models, they often failed to proceed any further because of their unexpected side effects and lack of selectivity towards the target enzyme AKR1B1. This lack of specificity is a major concern and has been attributed to the high degree of structural and sequence similarity of AKR1B1

* Corresponding authors.
E-mail addresses: kato@med.u-toyama.ac.jp (A. Kato), toyooka@eng.u-toyama.ac.jp (N. Toyooka).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.10.073

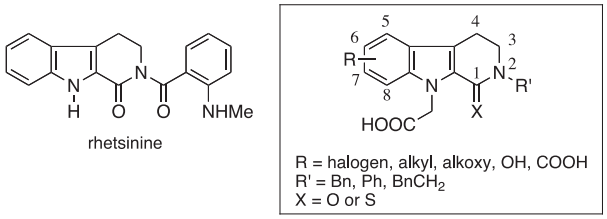


Figure 1. Structures of rhetsinine and (1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acids.

with human aldehyde reductase (AKR1A1, EC 1.1.1.2).[9] AKR1A1 is identical to D-glucuronate reductase in the glucuronic acid/uronate cycle of glucose metabolism,[10] and also plays an important role in the detoxification of 3-deoxyglucosone, a precursor of advanced glycation end products.[11] In addition to AKR1A1, AKR1B10 with amino acid sequence identity of 71% with AKR1B1[12] is inhibited by AKR1B1 inhibitors.[13] AKR1B10 exhibits board substrate specificity for the AKR1B1 substrates other than aldoses that are specifically reduced by AKR1B1.[12,14] AKR1B10 has specialized roles in the controlling levels of signaling molecules, all-*trans*-, 9-*cis* and 13-*cis*-retinoic acids,[13] and regulating isoprenoid metabolism[14] and fatty acid synthesis.[15] Therefore, it is necessary to elucidate the structural requirements for the design of selective AKR1B1 inhibitors without affecting to AKR1A1 and AKR1B10.

Many reports on the selectivity of inhibitors to AKR1B1 over AKR1A1 have been published,[16–19] however, no report has been made on the inhibitory selectivity to AKR1B1 over AKR1B10. In recent years, many kinds of AKR1B1 inhibitors have been found from natural sources, such as flavones, flavonoids, coumarins and so on.[20] In our search for AKR1B1 inhibitors, we found that a hot water extract of *Evodia rutaecarpa* exhibited significant inhibitory activity and reported that rhetsinine, which is a minor alkaloidal component of *E. rutaecarpa*, significantly suppressed sorbitol accumulation in human erythrocytes by 79.3% at 100 μM.[21] Here, we report the design, synthesis, and evaluation of novel (1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acids, based upon the structure of rhetsinine, as selective inhibitors of AKR1B1 (Fig. 1).

2. Results and discussion

2.1. Chemistry

The synthesis began with tricyclic lactam (**2a**), which was synthesized according to the known procedure by treatment with corresponding lactone (**1a**)[22] with 4-fluorobenzyl amine. Alkylation of nitrogen on the indole nuclei gave rise to the methyl ester (**3**), which was converted to desired acid (**4**) (Scheme 1).

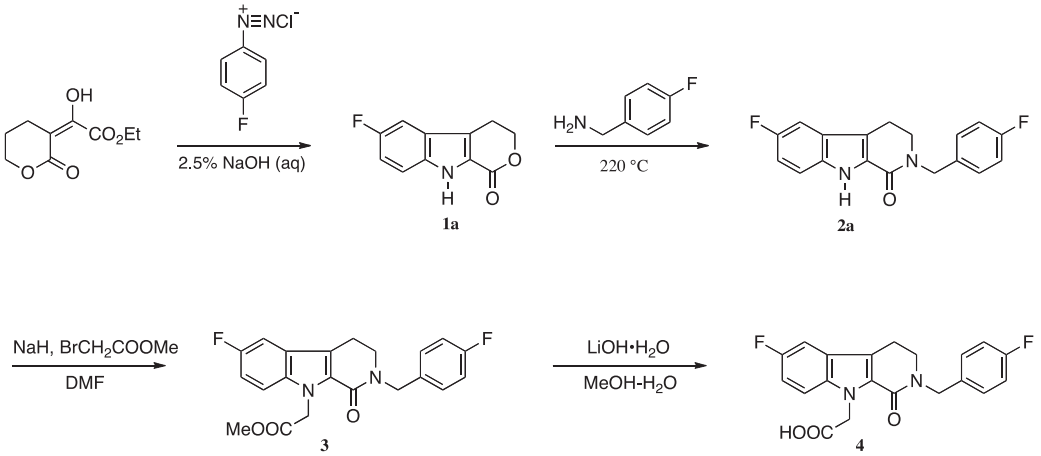
The inhibitory activity of **4** was not improved compared with benzoyl derivatives[23] (see Table 1). Next, we designed the thiolactam-type of tricyclic carboxylic acids (**7b**–**7s**). Reaction of lactams (**2b**–**r**), derived from lactones (**1b**–**1k**) same as in Scheme 1, with Lawesson's reagent proceeded smoothly to provide the corresponding thiolactams (**5b**–**5r**) in high yield, which were transformed into the esters (**6b**–**6r**). Finally, deprotection of *t*-butyl or methyl esters with TMSI or LiOH furnished desired acids (**7b**–**7r**). The OH (**7s**) and COOH (**7t**) derivatives were also synthesized from (**6m**) or (**6l**) by treatment with BBr_3 or Pd-catalyzed CO insertion reaction (**6t**) followed by hydrolysis, respectively, as shown in Scheme 2.

2.2. Biological evaluation

Many of newly synthesized compounds showed potent inhibition for AKR1B1 compared with the lead compound (**4**) (Table 1). The inhibitory potency was not affected in the introduction of halogen(s), alkyl, alkoxy, OH, or COOH substituent on 6- or 5,7-positions on the parent nuclei except for 5,7-difluoro-derivative (**7o**). Introduction of halogen (**7e**, **7g**, and **7j**) or trifluoromethyl group (**7d** and **7f**) on the benzene ring of benzyl moiety at the 2-position much decreased the inhibitory effect on AKR1B1, and these compounds were more selective on AKR1B10 rather than AKR1B1. Substitution on the nitrogen at the 2-position with phenyl (**7q**) or phenylethyl (**7r**) group instead of simple benzyl substituent did not improve the inhibitory potency for AKR1B1. Accordingly, the best structure on this position would be simple benzyl group. The IC_{50} values for several derivatives (**7l**–**7p**, **7s** and **7t**) were comparable to that of the known inhibitor, epalrestat that was determined under the same conditions. In addition, these synthesized compounds showed higher selectivity to AKR1B1 over both AKR1A1 and AKR1B10 than epalrestat. In particular, the COOH derivative (**7t**) showed the highest selectivity indexes of 312 and 253 in AKR1A1/AKR1B1 and AKR1B10/AKR1B1, respectively.

2.3. Kinetic analysis

The inhibition patterns of the potent derivatives (**7m** and **7t**) were non-competitive with respect to the substrate pyridine-3-aldehyde, showing the two inhibition constants (K_{is} = 79 ± 1 nM and K_{ii} = 180 ± 20 nM for **7m**, and K_{is} = 55 ± 7 nM and K_{ii} = 220 ± 57 nM for **7t**). The inhibition patterns are similar to those of known AKR1B1 inhibitors, which are demonstrated to bind to the active site of the enzyme–coenzyme binary complex by crystallographic



Scheme 1.

Table 1
Inhibitory potency and selectivity of tricyclic carboxylic acids for AKR1A1, AKR1Bl and AKR1B10

Compound	IC_{50} (μM)			Selectivity index	
	AKR1A1	AKR1B1	AKR1B10	(AKR1A1/AKR1B1)	(AKR1B10/AKR1B1)
4	ND	48.6	ND	—	—
7b	9.5 ± 0.2	0.22 ± 0.03	5.1 ± 0.8	43	23
7c	19 ± 1	0.23 ± 0.02	3.3 ± 0.4	83	14
7d	(27%)	10 ± 1.6	1.6 ± 0.2	—	0.2
7e	(45%)	1.7 ± 0.3	0.28 ± 0.02	—	0.2
7f	(45%)	14 ± 0.1	3.8 ± 0.1	—	0.3
7g	14 ± 2	5.9 ± 0.9	0.85 ± 0.1	2.4	0.1
7h	11 ± 1	0.32 ± 0.03	1.4 ± 0.3	34	4.4
7i	21 ± 1	0.24 ± 0.01	1.4 ± 0.1	88	5.8
7j	(45%)	4.2 ± 0.2	0.68 ± 0.07	—	0.2
7k	15 ± 1	0.35 ± 0.02	1.4 ± 0.2	43	4
71	11 ± 1	0.19 ± 0.01	1.4 ± 0.1	58	7.4
7m	12 ± 1	0.15 ± 0.01	2.9 ± 0.1	80	19
7n	16 ± 1	0.20 ± 0.01	3.4 ± 0.1	80	17
7p	10 ± 1	0.19 ± 0.02	6.1 ± 0.5	53	32
7o	4.7 ± 0.4	1.3 ± 0.1	0.48 ± 0.04	3.6	0.4
7q	(2%)	31 ± 2	21 ± 0.3	—	0.6
7r	(39%)	1.8 ± 0.2	0.40 ± 0.04	—	0.2
7s	(28%)	0.20 ± 0.01	1.9 ± 0.2	—	10
7t	53 ± 6	0.17 ± 0.02	40 ± 1	312	253
Epalrestat	2.6 ± 0.2	0.10 ± 0.01	0.33 ± 0.04	26	3.3

ND: Not determined. The values in parentheses are inhibition percentages by 20 μM.

studies.[24] The inhibition constants for **7m** and **7t** are comparable or superior to those for known inhibitors including epalrestat.[25,26]

2.4. Molecular modeling

The docking simulations of **7t** were performed for AKR1B1 and AKR1B10. Figure 2A and B shows the binding conformation of **7t** for AKR1B1 and for AKR1B10, respectively. In this figure, we can find that the binding orientation and conformation of **7t** for AKR1B1 differ from those for AKR1B10. In AKR1B1, the benzyl group of **7t** is located in the inner part of the ligand-binding pocket where is hydrophobic. The π–π stacking and CH–π interactions are formed between tricyclic group of **7t** and W219. In addition, the strong hydrogen bonding is formed between carboxyl group connected the nitrogen on the tricyclic group and S302.

On the other hand, **7t** binds to AKR1B10 with different orientation and conformation from in AKR1B1. In AKR1B10, the benzyl group of **7t** interacts with F123 in T-shape and with V301 through CH-π interaction. The poor hydrogen bonding is formed between carboxyl group (6-position) and Y49. As a whole, the interaction of **7t** with AKR1B10 (Glide Score: −7.76 kcal/mol) is weaker than that with AKR1B1 (Glide Score: −8.60 kcal/mol). The difference between the binding conformations of **7t** in AKR1B1 and in AKR1B10 seems to come from the difference between the binding pocket shape of AKR1B1 and that of AKR1B10. Figure 2C shows the binding pocket of AKR1B1 (green solid surface) and that of AKR1B10 (red wireframe). In Figure 2 we can find that the inner part of the binding pocket of AKR1B10 is narrower than that of AKR1B1. Therefore, the benzyl group of **7t** cannot be located in the inner part of the binding pocket of AKR1B10, and so it fails to form favorable hydrophobic and hydrogen bonding interactions with residues of AKR1B10.

3. Conclusion

In conclusion, a series of novel (1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acids (**7b–7t**) were designed, synthesized, and inhibitory activity for AKR1B1 was evaluated in vitro. Among the carboxylic acids, the MeO derivative (**7m**) showed the highest inhibitory activity for AKR1B1 (IC_{50} = 0.15 μM) comparable to that of epalrestat (IC_{50} = 0.10 μM), and the COOH derivative (**7t**) exhibited strong inhibitory potency (IC_{50} = 0.17 μM) with the highest selectivity for AKR1B1 (selectivity ratios to AKR1A1 and AKR1B10 were 311 and 253, respectively). According to the structure–activity relationship (SAR), non-substituted simple benzyl moiety on the 2-nitrogen was essential for high inhibitory activity (**7b** vs **7q**), and addition of substituent(s) such as halogen or trifluoromethyl on the phenyl ring of this moiety was not effected for the improvement of inhibitory activity (**7b** vs **7f**, **7g** or **7j**, **7c** vs **7d** or **7e**).

4. Experimental section

4.1. Chemistry

Typical procedure for lactones (**1a–1j**): The known lactones (**1a**, **1b**, **1c**, **1d**, **1e**, **1f**, and **1h**) and other lactones (**1g**, **1i**, **1j**, and **1k**) were prepared by the literature procedure.[27]

4.1.1. 6-Iodo-4,9-dihydro-3*H*-pyrano[3,4-*b*]indol-1-one (1g)

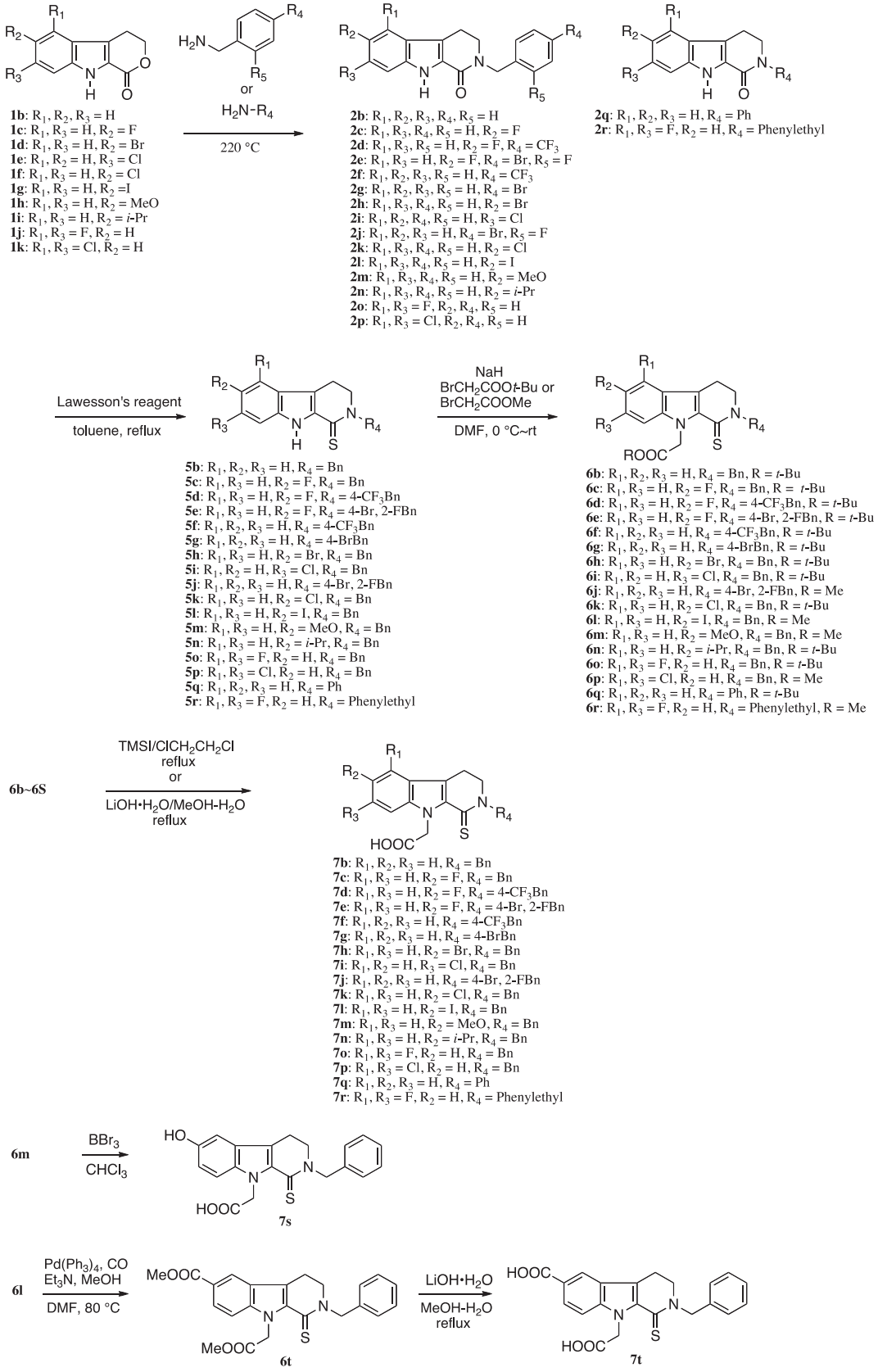
Yield: 52%; mp: 228–230 °C; IR (KBr): 3283, 1705 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.13 (1H, br), 8.00 (1H, s), 7.63 (1H, dd, *J* = 1.5, 8.4 Hz), 7.27 (1H, d, *J* = 8.4 Hz), 4.71 (2H, t, *J* = 6.3 Hz), 3.13 (2H, t, *J* = 6.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.93, 137.01, 134.99, 129.84, 128.99, 126.95, 121.90, 114.66, 84.16, 69.44, 21.27; MS (EI) *m/z* 313 (M^+); HRMS (EI) calcd for $C_{11}H_8O_2NI$: 312.9600 (M^+), found: 312.9620.

4.1.2. 6-Iso-propyl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indol-1-one (1i)

Yield: 62%; brown oil; IR (neat): 3276, 1689 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.64 (1H, br), 7.45 (2H, d, *J* = 8.8 Hz), 7.31–7.28 (1H, m), 4.71 (2H, t, *J* = 6.3 Hz), 3.17 (2H, t, *J* = 6.3 Hz), 3.03 (1H, sept, *J* = 6.9 Hz), 1.32 (6H, d, *J* = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.72, 141.28, 137.08, 126.33, 124.21, 122.79, 122.00, 116.87, 112.79, 69.48, 34.08, 24.37, 21.46; MS (EI) *m/z* 229 (M^+); HRMS (EI) calcd for $C_{14}H_{15}O_2N$: 229.1103 (M^+), found: 229.1096.

4.1.3. 5,7-Difluoro-4,9-dihydro-3*H*-pyrano[3,4-*b*]indol-1-one (1j)

Yield: 40%; mp: 186–187 °C; IR (KBr): 3275, 1697 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 7.03 (1H, dd, *J* = 2.1, 9.4 Hz), 7.00–6.96 (1H, m), 4.63 (2H, t, *J* = 6.2 Hz), 3.19 (2H, t, *J* = 6.2 Hz); ^{13}C



Scheme 2.

NMR (125 MHz, $CDCl_3$): δ 162.11 (dd, J = 12.5, 246.3 Hz), 160.60, 157.70 (dd, J = 15.3, 253.0 Hz), 139.25 (dd, J = 12.5, 15.3 Hz), 122.77 (d, J = 3.8 Hz), 121.47 (d, J = 1.9 Hz), 111.36 (d, J = 21.1 Hz), 97.03 (dd, J = 24.0, 29.7 Hz), 94.98 (dd, J = 4.8, 26.8 Hz), 69.48, 22.28; MS (EI) m/z 223 (M^+); HRMS (EI) calcd for $C_{11}H_7O_2NF_2$: 223.0445 (M^+), found: 223.0417.

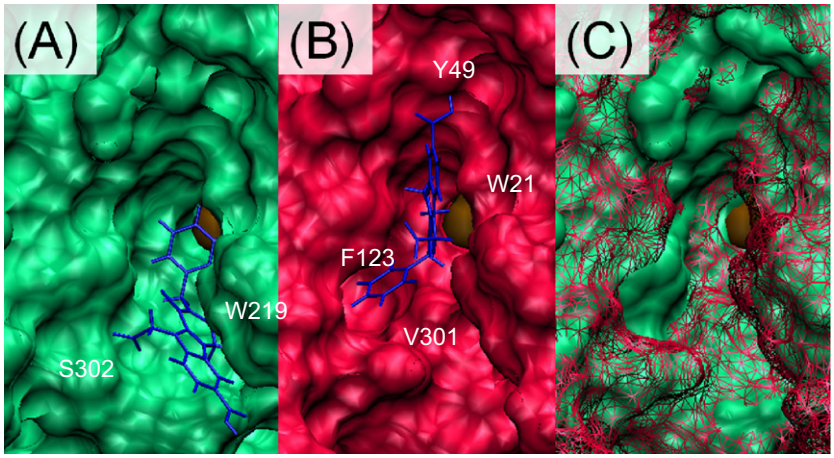


Figure 2. The binding conformation of the **7t** (blue stick) for AKR1B1 (A) and for AKR1B10 (B), and the difference of binding pocket (C) between AKR1B1 (green solid surface) and AKR1B10 (red wireframe).

4.1.4. 5,7-Dichloro-4,9-dihydro-3*H*-pyrano[3,4-*b*]indol-1-one (1k)

Yield: 42%; mp: 137–139 °C; IR (KBr): 3267, 1712 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.17 (1H, br), 7.38 (1H, d, J = 1.6 Hz), 7.17 (1H, d, J = 1.6 Hz), 4.71 (2H, t, J = 6.3 Hz), 3.44 (2H, t, J = 6.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.47, 139.60, 135.20, 128.49, 124.21, 122.08, 117.91, 111.32, 69.51, 24.60, 22.64; MS (EI) m/z 255 (M^+); HRMS (EI) calcd for $C_{11}H_7O_2NCl_2$: 254.9854 (M^+), found: 254.9856.

4.1.5. Typical procedure for lactams (2a–2s)

The known lactams (**2b**, **2c**, **2h**, **2i**, **2k**, **2m**, **2q**, **2s**) and other lactams (**2a**, **2d**, **2e**, **2f**, **2g**, **2j**, **2l**, **2n**, **2o**, **2p**, **2r**) were prepared by the literature procedure.[24]

4.1.6. 6-Fluoro-2-(4-fluorobenzyl)-2,3,4,9-tetrahydro-β-carbolin-1-one (2a)

Yield: 34%; mp: 211–213 °C; IR (KBr) 3216, 1635 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 11.8 (1H, br s), 7.40–7.36 (4H, m), 7.18 (2H, t-like, J = 9.0 Hz), 7.08 (1H, td, J = 2.6, 9.2 Hz), 4.68 (2H, s), 3.60 (2H, t, J = 7.0 Hz), 2.95 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 160.1, 133.9, 129.5 & 129.4, 128.4, 124.7, 117.4 & 117.3, 115.3, 115.0, 113.7 & 113.5, 112.8 & 112.4, 104.6 & 104.3, 48.0, 47.2, 20.0; MS 312 (M^+), 312 (100); HRMS calcd for $C_{18}H_{14}ON_2F_2$: 312.1074, found: 312.1046.

4.1.7. 6-Fluoro-2-(4-trifluoromethylbenzyl)-2,3,4,9-tetrahydro-β-carbolin-1-one (2d)

Yield: 35%; mp: 237–240 °C; IR (KBr): 3223, 1635 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 9.74 (1H, br), 7.61 (2H, d, J = 8.0 Hz), 7.48 (2H, d, J = 8.0 Hz), 7.35–7.32 (1H, m), 7.19 (1H, dd, J = 1.4, 9.2 Hz), 7.04 (1H, td, J = 2.3, 9.2 Hz), 4.86 (2H, s), 3.67 (2H, t, J = 6.9 Hz), 3.02 (2H, t, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.37, 157.97 (d, J = 236.7 Hz), 141.52, 134.07, 129.92 (q, J = 32.6 Hz), 128.09, 127.77, 125.71 (q, J = 3.8 Hz), 125.39, 125.28, 118.25 (d, J = 4.8 Hz), 113.96 (d, J = 26.8 Hz), 113.35 (d, J = 9.6 Hz), 104.67 (d, J = 24.0 Hz), 49.36, 47.72, 20.61; MS (EI) m/z 362 (M^+); HRMS (EI) calcd for $C_{19}H_{14}ON_2F_4$: 362.1042 (M^+), found: 362.1027.

4.1.8. 2-(4-Bromo-2-fluorobenzyl)-6-fluoro-2,3,4,9-tetrahydro-β-carbolin-1-one (2e)

Yield: 33%; mp: 235–238 °C; IR (KBr): 3217, 1637 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 9.53 (1H, br), 7.36–7.26 (4H, m), 7.19 (1H, dd, J = 2.3, 9.2 Hz), 7.05 (1H, td, J = 2.3, 9.2 Hz), 4.79 (2H, s), 3.71 (2H, t, J = 6.9 Hz), 3.01 (2H, t, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.50, 160.86 (d, J = 251.2 Hz), 157.95 (d, J = 236.8 Hz), 134.14, 131.53 (d, J = 4.8 Hz), 128.12, 127.83 (d, J = 3.8 Hz), 125.28 (d, J = 9.6 Hz), 123.58 (d, J = 14.4 Hz), 121.61 (d, J = 9.6 Hz), 119.14 (d, J = 24.9 Hz), 118.27 (d, J = 4.8 Hz), 113.93 (d, J = 26.8 Hz), 113.40 (d, J = 9.6 Hz), 104.64 (d, J = 24.0 Hz), 47.97, 43.08 (d, J = 2.9 Hz), 20.64; MS (EI) m/z 390 (M^+); HRMS (EI) calcd for $C_{18}H_{13}ON_2F_2Br$: 390.0179 (M^+), found: 390.0175.

4.1.9. 2-(4-Trifluoromethylbenzyl)-2,3,4,9-tetrahydro-β-carbolin-1-one (2f)

Yield: 37%; mp: 224–227 °C; IR (KBr): 3230, 1636 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.15 (1H, br), 7.62–7.57 (3H, m), 7.48 (2H, d, J = 7.7 Hz), 7.44 (1H, d, J = 8.2 Hz), 7.31 (1H, t, J = 7.1 Hz), 7.16 (1H, t, J = 7.1 Hz), 4.85 (2H, s), 3.66 (2H, t, J = 7.1 Hz), 3.06 (2H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.61, 141.72, 137.50, 129.83 (q, J = 32.6 Hz), 128.10, 126.55, 125.67 (q, J = 3.8 Hz), 125.24, 125.10, 122.74, 120.34, 120.20, 118.50, 112.45, 49.29, 47.76, 20.71; MS (EI) m/z 344 (M^+); HRMS (EI) calcd for $C_{19}H_{15}ON_2F_3$: 344.1137 (M^+), found: 344.1156.

4.1.10. 2-(4-Bromobenzyl)-2,3,4,9-tetrahydro-β-carbolin-1-one (2g)

Yield: 40%; mp: 249–252 °C; IR (KBr): 3218, 1635 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.96 (1H, br), 7.57 (1H, d, J = 7.7 Hz), 7.48–7.42 (3H, m), 7.34–7.22 (3H, m), 7.15 (1H, t, J = 7.7 Hz), 4.73 (2H, s), 3.63 (2H, t, J = 7.0 Hz), 3.03 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.25, 137.22, 136.49, 131.70, 129.57, 126.59, 125.22, 124.99, 121.30, 120.28, 120.12, 118.32, 112.29, 49.04, 47.54, 20.78; MS (EI) m/z 354 (M^+); HRMS (EI) calcd for $C_{18}H_{15}ON_2Br$: 354.0368 (M^+), found: 354.0323.

4.1.11. 2-(4-Bromo-2-fluorobenzyl)-2,3,4,9-tetrahydro-β-carbolin-1-one (2j)

Yield: 33%; mp: 217–219 °C; IR (KBr): 3223, 1635 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 11.66 (1H, s), 7.60–7.56 (2H, m), 7.43–7.32 (3H, m), 7.22 (1H, t, J = 8.1 Hz), 7.06 (1H, t, J = 8.1 Hz), 4.71 (2H, s), 3.65 (2H, t, J = 7.0 Hz), 3.00 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 160.09 (d, J = 249.0 Hz), 160.46, 137.21, 131.21 (d, J = 4.9 Hz), 127.57 (d, J = 3.7 Hz), 126.53, 124.56, 124.33, 124.10 (d, J = 4.9 Hz), 120.30 (d, J = 9.8 Hz), 120.01, 119.39, 118.59 (d, J = 25.6 Hz), 117.66, 112.44, 47.64, 42.61, 20.15; MS (EI) m/z 371 (M^+); HRMS (EI) calcd for $C_{18}H_{14}ON_2FBr$: 372.0274 (M^+), found: 372.0299.

4.1.12. 2-Benzyl-6-iodo-2,3,4,9-tetrahydro-β-carbolin-1-one (2l)

Yield: 45%; mp: 227–229 °C; IR (KBr): 3202, 1631 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 10.57 (1H, br), 7.78 (1H, s), 7.35 (1H, dd, J = 1.4 Hz, 8.7 Hz), 7.27–7.15 (5H, m), 7.08 (1H, d. J = 8.7 Hz), 4.73 (2H, s), 3.55 (2H, t, J = 7.1 Hz), 2.87 (2H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.43, 137.30, 136.73, 132.91, 128.97, 128.77, 127.88, 127.84, 127.60, 127.44, 117.07, 114.70, 83.29, 49.74, 47.47, 20.51; MS (EI) m/z 402 (M^+); HRMS (EI) calcd for $C_{18}H_{15}ON_2I$: 402.0230 (M^+), found: 402.0228.

4.1.13. 2-Benzyl-6-iso-propyl-2,3,4,9-tetrahydro-β-carbolin-1-one (2n)

Yield: 40%; mp: 205–207 °C; IR (KBr): 3214, 1635 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 9.42 (1H, br), 7.38–7.26 (7H, m), 7.19 (1H, dd, J = 1.6, 8.5 Hz), 4.82 (2H, s), 3.64 (2H, t, J = 6.9 Hz), 3.03–2.97 (3H, m), 1.30 (6H, d, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.64, 140.91, 137.64, 136.14, 128.66, 127.92, 127.42, 127.01, 125.31, 124.49, 118.00, 116.59, 112.28, 49.48, 47.44, 34.17, 24.49, 20.71; IR (KBr): 3214, 1635 cm^{-1}; MS (EI) m/z 318 (M^+); HRMS (EI) calcd for $C_{21}H_{22}ON_2$: 318.1732 (M^+), found: 318.1780.

4.1.14. 2-Benzyl-5,7-difluoro-2,3,4,9-tetrahydro-β-carbolin-1-one (2o)

Yield: 40%; mp: 167–169 °C; IR (KBr): 3193, 1626 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 10.62 (1H, br), 7.37–7.28 (5H, m), 6.89 (1H, dd, J = 1.8, 8.7 Hz), 6.58 (1H, dt, J = 1.8, 10.5 Hz), 4.83 (2H, s), 3.67 (2H, t, J = 7.1 Hz), 3.15 (2H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ161.17 (dd, J = 11.5, 242.5 Hz), 159.96, 157.49 (dd, J = 15.3, 251.1 Hz), 139.19 (dd, J = 13.4, 15.3 Hz), 137.51, 137.07, 129.12 (d, J = 3.8 Hz), 128.16, 127.54 (d, J = 3.8 Hz), 116.86, 111.94 (d, J = 21.1 Hz), 96.32 (dd, J = 22.5, 29.2 Hz), 95.13 (dd, J = 4.8, 25.9 Hz), 49.98, 47.78, 21.85; MS (EI) m/z 312 (M^+); HRMS (EI) calcd for $C_{18}H_{14}ON_2F_2$: 312.1074 (M^+), found: 312.1069.

4.1.15. 2-Benzyl-5,7-dichloro-2,3,4,9-tetrahydro-β-carbolin-1-one (2p)

Yield: 30%; mp: 248–250 °C; IR (KBr): 3188, 1633 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 10.04 (1H, br), 7.37–7.28 (6H, m), 7.09 (1H, d, J = 1.4 Hz), 4.81 (2H, s), 3.66 (2H, t, J = 7.1 Hz), 3.31 (2H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.96, 138.36, 137.04, 130.25, 128.81, 128.18, 128.00, 127.88, 127.68, 121.90, 121.21, 118.07, 111.23, 49.67, 47.38, 21.83; MS (EI) m/z 344 (M^+); HRMS (EI) calcd for $C_{18}H_{14}ON_2Cl_2$: 344.0483 (M^+), found: 344.0474.

4.1.16. 5,7-Difluoro-2-phenylethyl-2,3,4,9-tetrahydro-β-carbolin-1-one (2r)

Yield: 33%; mp: 220–222 °C; IR (KBr): 3161, 1629 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 10.51 (1H, br), 7.35–7.22 (5H, m), 6.98 (1H, dd, J = 1.9, 9.1 Hz), 6.60 (1H, dd, J = 1.9, 10.2 Hz), 3.84 (2H, t, J = 7.4 Hz), 3.58 (2H, t, J = 7.0 Hz), 3.07 (2H, t, J = 7.0 Hz), 3.01 (2H, t, J = 7.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.95 160.76 (dd, J = 11.5, 241.5 Hz), 157.23 (dd, J = 15.3, 251.1 Hz), 139.17 (dd, J = 12.9, 14.9 Hz), 138.92, 128.85, 128.66, 127.49 (d, J = 1.9 Hz), 126.57, 116.37 (d, J = 3.8 Hz), 111.52 (d, J = 1.9, 21.1 Hz), 95.82 (dd, J = 22.5, 29.2 Hz), 94.90 (dd, J = 4.8, 25.9 Hz), 49.03, 48.86, 34.64, 21.56; MS (EI) m/z 326 (M^+); HRMS (EI) calcd for $C_{19}H_{16}ON_2F_2$: 326.1231 (M^+), found: 326.1241.

4.1.17. [6-Fluoro-2-(4-fluorobenzyl)-1-oxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid methyl ester (3)

To a stirred solution of **2a** (193 mg, 0.62 mmol) in DMF (5 mL) was added NaH (60%, 37 mg, 0.93 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 30 min. To the mixture was added $BrCH_2COOMe$ (0.087 mL, 0.93 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 24 h. The reaction was quenched with H_2O (10 mL), and the aqueous mixture was extracted with Et_2O (10 mL × 3). The organic extracts were combined, dried over $MgSO_4$, and evaporated. The residue was chromatographed on SiO_2 (Hexane–Acetone = 8: 1) to give **3** (173 mg, 73%). Mp: 87–89 °C; IR (KBr) 1737, 1650 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.31–7.28 (2H, m), 7.22–7.19 (2H, m), 7.10 (1H, td, J = 2.6, 9.0 Hz), 7.02 (2H, t, J = 8.5 Hz), 5.44 (2H, s), 4.70 (2H, s), 3.77 (3H, s), 3.61 (2H, t, J = 7.0 Hz), 2.97 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.3, 160.9, 135.3, 133.0 & 132.9, 129.4 & 129.2, 126.9, 124.3 & 124.2, 118.9 & 118.8, 115.4 & 115.1, 113.8 & 113.5, 110.6 & 110.4, 105.0 & 104.7, 52.3, 48.6, 46.9, 45.7, 20.4; MS (EI) m/z 384 (M^+); HRMS (EI) calcd for $C_{21}H_{18}O_3N_2F_2$: 384.1286 (M^+), found: 384.1260.

4.1.18. [2-(4-Fluorobenzyl)-6-fluoro-1-oxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid (4)

To a stirred solution of **3** (173 mg, 0.45 mmol) in MeOH (3 mL) and H_2O (1 mL) was added $LiOH{\cdot}H_2O$ (37.2 mg, 0.90 mmol), and the resulting mixture was refluxed for 1 h. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with AcOEt (10 mL × 3). The organic extracts were combined, dried over $MgSO_4$, and evaporated. The residue was chromatographed on SiO_2 (Hexane–Acetone = 2: 1) to **4** (0.44 mmol, 162.9 mg, 98%). Mp: 148–150 °C; IR (KBr) 2929, 1724, 1647 cm $^{-1}$; ^1H NMR (300 MHz, DMSO-d_6): δ 7.61–7.58 (1H, m), 7.44–7.42 (1H, dd, J = 2.6, 9.4 Hz), 7.37–7.34 (2H, m), 7.19–7.14 (3H, m), 5.35 (2H, s), 4.65 (2H, s), 3.59 (2H, t, J = 7.0 Hz), 2.97 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 170.2, 160.2, 158.4, 155.7, 135.3, 133.9, 129.5 & 129.4, 126.9, 123.7, 118.6, 115.3 & 115.0, 113.2 & 112.9, 112.1 & 112.0, 104.8 & 104.5, 48.0, 47.0, 45.8, 19.9; MS 370 (M^+), 370 (100); HRMS calcd for $C_{20}H_{16}O_3N_2F_2$: 370.1129, found: 370.1124.

4.1.19. Typical procedure for thiolactams (5b–5r)

To a stirred solution of lactam (1 mmol) in toluene (5 mL) was added Lawesson's reagent (0.55 equiv), and the resulting mixture was refluxed for 20–24 h. After cooling, the solvent was removed, and the residue was chromatographed on SiO_2 (Hexane–Acetone = 25:1) to give thiolactam (**5b**–**5r**).

4.1.20. 2-Benzyl-2,3,4,9-tetrahydro-β-carboline-1-thione (5b)

Yield: 98%; mp: 197–198 °C; IR (KBr): 3319, 1556 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ11.32 (1H, br), 7.60–7.22 (8H, m), 7.05 (1H, t, J = 7.5 Hz), 5.38 (2H, s), 3.77 (2H, t, J = 7.0 Hz), 3.00 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ182.90, 138.31, 136.50, 132.32, 128.44, 127.39, 127.23, 124.52, 124.47, 120.61, 119.70, 112.66, 111.47, 54.94, 49.55, 19.65; MS (EI) m/z 292 (M^+); HRMS (EI) calcd for $C_{18}H_{16}N_2S$: 292.1034 (M^+), found: 292.1054.

4.1.21. 2-Benzyl-6-fluoro-2,3,4,9-tetrahydro-β-carboline-1-thione (5c)

Yield: 53%; mp: 127–130 °C; IR (KBr): 3320, 1555 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 11.43 (1H, br), 7.47 (1H, m), 7.41−7.26 (6H, m), 7.11 (1H, dt, J = 2.5, 9.4 Hz), 5.37 (2H, s), 3.77 (2H, t, J = 7.2 Hz), 2.97 (2H, t, J = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 182.70, 156.84 (d, J = 233.2 Hz), 136.35, 135.01, 133.75, 128.44, 127.39, 127.26, 124.47 (d, J = 9.8 Hz), 113.96 (d, J = 9.8 Hz), 113.31 (d, J = 26.9 Hz), 111.43 (d, J = 6.1 Hz), 104.84 (d, J = 23.2 Hz), 55.03, 49.60, 19.55; MS (EI) m/z 310 (M^+); HRMS (EI) calcd for $C_{18}H_{15}N_2FS$: 310.0940 (M^+), found: 310.0914.

4.1.22. 6-Fluoro-2-(4-trifluoromethylbenzyl)-2,3,4,9-tetrahydro-β-carboline-1-thione (5d)

Yield: 90%; mp: 180–182 °C; IR (KBr): 3309, 1558 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.08 (1H, br), 7.62 (2H, d, J = 8.1 Hz), 7.51 (2H, d, J = 8.1 Hz), 7.38–7.34 (1H, m), 7.19 (1H, d, J = 9.1 Hz),

7.08 (1H, td, J = 2.5, 9.1 Hz), 5.45 (2H, s), 3.78 (2H, t, J = 7.4 Hz), 3.02 (2H, t, J = 7.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.11, 157.88 (d, J = 236.8 Hz), 140.10, 134.68, 133.48, 130.03 (q, J = 33.0 Hz), 127.92, 125.69 (q, J = 3.7 Hz), 122.11, 118.50, 114.60 (d, J = 26.9 Hz), 113.10 (d, J = 9.8 Hz), 111.43 (d, J = 4.9 Hz), 105.19 (d, J = 23.2 Hz), 55.56, 49.80, 20.36; MS (EI) m/z 378 (M^+); HRMS (EI) calcd for $C_{19}H_{14}N_2F_4S$: 378.0814 (M^+), found: 378.0838.

4.1.23. 2-(4-Bromo-2-fluorobenzyl)-6-fluoro-2,3,4,9-tetrahydro-β-carboline-1-thione (5e)

Yield: 90%; mp: 128–131 °C; IR (KBr): 3310, 1554 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.07 (1H, br), 7.43 (1H, t, J = 8.1 Hz), 7.36–7.24 (3H, m), 7.19 (1H, dd, J = 1.6, 9.1 Hz), 7.07 (1H, td, J = 2.5, 9.1 Hz), 5.38 (2H, s), 3.82 (2H, t, J = 7.4 Hz), 3.01 (2H, t, J = 7.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 184.23, 160.51 (d, J = 278.0 Hz), 158.08 (d, J = 264.6 Hz), 134.70, 133.60, 131.34 (d, J = 3.8 Hz), 127.78 (d, J = 3.8 Hz), 125.55 (d, J = 9.6 Hz), 122.29 (d, J = 15.3 Hz), 121.86 (d, J = 9.6 Hz), 119.15 (d, J = 24.9 Hz), 114.55 (d, J = 26.8 Hz), 113.13 (d, J = 9.6 Hz), 111.48 (d, J = 5.8 Hz), 105.17 (d, J = 23.0 Hz), 49.99, 49.02 (d, J = 2.9 Hz), 20.19; MS (EI) m/z 406 (M^+); HRMS (EI) calcd for $C_{18}H_{13}N_2F_2SBr$: 405.9951 (M^+), found: 405.9924.

4.1.24. 2-(4-Trifluoromethylbenzyl)-2,3,4,9-tetrahydro-β-carboline-1-thione (5f)

Yield: 95%; mp: 187–190 °C; IR (KBr): 3340, 1557 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.08 (1H, br), 7.62 (2H, d, J = 8.5 Hz), 7.58 (1H, d, J = 8.2 Hz), 7.51 (2H, d, J = 8.5 Hz), 7.43 (1H, d, J = 8.2 Hz), 7.33 (1H, t, J = 7.5 Hz), 7.14 (1H, t, J = 7.5 Hz), 5.47 (2H, s), 3.79 (2H, t, J = 7.4 Hz), 3.06 (2H, t, J = 7.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 184.54, 140.40, 138.26, 132.27, 130.08 (q, J = 32.6 Hz), 128.00, 125.77 (q, J = 3.8 Hz), 125.50, 125.37, 122.66, 120.93, 120.74, 112.20, 118.78, 55.45, 49.78, 20.36; MS (EI) m/z 360 (M^+); HRMS (EI) calcd for $C_{19}H_{15}N_2F_3S$: 360.0908 (M^+), found: 360.0861.

4.1.25. 2-(4-Bromobenzyl)-2,3,4,9-tetrahydro-β-carboline-1-thione (5g)

Yield: 86%; mp: 171–174 °C; IR (KBr): 3334, 1558 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.08 (1H, br), 7.57 (1H, d, J = 8.0 Hz), 7.48 (2H, d, J = 8.2 Hz), 7.42 (1H, d, J = 8.5 Hz), 7.33 (2H, d, J = 8.2 Hz), 7.29 (1H, d, J = 8.5 Hz), 7.14 (1H, m), 5.35 (2H, s), 3.76 (2H, t, J = 7.4 Hz), 3.03 (2H, t, J = 7.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.03, 138.09, 135.28, 132.21, 131.80, 129.47, 125.56, 125.41, 121.66, 120.81, 120.59, 112.11, 111.61, 55.29, 49.59, 20.44; MS (EI) m/z 370 (M^+); HRMS (EI) calcd for $C_{18}H_{15}N_2SBr$: 370.0139 (M^+), found: 370.0139.

4.1.26. 2-Benzyl-6-bromo-2,3,4,9-tetrahydro-β-carboline-1-thione (5h)

Yield: 85%; mp: 176–178 °C; IR (KBr): 3220, 1550 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.94 (1H, br s), 7.82 (1H, s), 7.42−7.27 (7H, m), 5.36 (2H, s), 3.76 (2H, t, J = 7.2 Hz), 2.98 (2H, t, J = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 182.59, 136.79, 136.29, 133.23, 128.41, 127.39, 127.24, 126.97, 126.17, 122.87, 114.65, 112.05, 110.82, 55.03, 49.53, 19.44; MS (EI) 370 m/z (M^+); HRMS (EI) calcd for $C_{18}H_{15}N_2SBr$: 370.0139 (M^+), found: 370.0178.

4.1.27. 2-Benzyl-7-chloro-2,3,4,9-tetrahydro-β-carboline-1-thione (5i)

Yield: 99%; mp: 162–163 °C; IR (KBr): 3318, 1551, cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.45 (1H, br s), 7.62–7.30 (6H, m), 7.06 (2H, d-like, J = 6.6 Hz), 5.37 (2H, s), 3.79 (2H, t, J = 6.9 Hz), 3.00 (2H, t, J = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.40, 138.09, 136.02, 132.79, 131.19, 128.69, 127.76, 124.02, 121.66, 121.45, 111.90, 111.35, 55.87, 49.33, 20.21; MS (EI) m/z 326 (M^+); HRMS (EI) calcd for $C_{18}H_{15}N_2SCl$: 326.0644 (M^+), found: 326.0645.

4.1.28. 2-(4-Bromo-2-fluorombenzyl)-2,3,4,9-tetrahydro-β-carboline-1-thione (5j)

Yield: 68%; mp: 160–162 °C; IR (KBr): 3321, 1555 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.32 (1H, br s), 7.62–7.57 (2H, m), 7.49 (1H, d, J = 8.2 Hz), 7.42–7.21 (3H, m), 7.06 (1H, t, J = 7.4 Hz), 5.35 (2H, s), 3.86 (2H, t, J = 7.4 Hz), 3.05 (2H, t, J = 7.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.18, 160.42 (d, J = 250.3 Hz), 137.98, 132.11, 131.15 (d, J = 3.7 Hz), 127.62 (d, J = 3.7 Hz), 127.48, 125.27, 122.31 (d, J = 14.6 Hz), 121.62 (d, J = 8.5 Hz), 120.72, 120.49, 118.97 (d, J = 24.4 Hz), 112.00, 111.59, 49.98, 48.87 (d, J = 3.7 Hz), 20.36; MS (EI) m/z 389 (M^+); HRMS (EI) calcd for $C_{18}H_{14}N_2FSBr$: 388.0045 (M^+), found: 388.0075.

4.1.29. 2-Benzyl-6-chloro-2,3,4,9-tetrahydro-β-carboline-1-thione (5k)

Yield: 99%; mp: 194–196 °C; IR (KBr): 3317, 1551 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.53 (1H, br s), 7.69 (1H, s), 7.49 (1H, d, J = 9.4 Hz), 7.40–7.31 (4H, m), 7.30 (1H, t, J = 9.4 Hz), 7.23 (1H, d, J = 9.4 Hz), 5.37 (2H, s), 3.78 (2H, t, J = 7.3 Hz), 2.99 (2H, t, J = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 182.61, 136.58, 136.30, 133.42, 128.44, 127.39, 127.26, 125.45, 124.52, 124.12, 119.78, 114.27, 110.97, 55.03, 49.55, 19.42; MS (EI) 326 m/z (M^+); HRMS (EI) calcd for $C_{18}H_{15}N_2SCl$: 326.0645 (M^+), found: 326.0678.

4.1.30. 2-Benzyl-6-iodo-2,3,4,9-tetrahydro-β-carboline-1-thione (5l)

Yield: 99%; mp: 185–187 °C; IR (KBr): 3318, 1551 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.50 (1H, s), 8.01 (1H, s), 7.50–7.29 (7H, m), 5.36 (2H, s), 3.77 (2H, t, J = 7.6 Hz), 2.98 (2H, t, J = 7.6 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 182.56, 137.13, 136.33, 132.76, 132.31, 129.13, 128.46, 127.42, 127.27, 127.08, 115.04, 110.51, 83.40, 55.03, 49.56, 19.42; MS (EI) m/z 418 (M^+); HRMS (EI) calcd for $C_{18}H_{15}N_2SI$: 418.0001 (M^+), found: 418.0062.

4.1.31. 2-Benzyl-6-methoxy-2,3,4,9-tetrahydro-β-carboline-1-thione (5m)

Yield: 94%; mp: 173–174 °C; IR (KBr): 3318, 1548 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.18 (1H, br), 7.41–7.28 (6H, m), 7.04 (1H, s), 6.90 (1H, dd, J = 2.6, 9.0 Hz), 5.37 (2H, s), 3.75 (3H, s), 3.76 (2H, t, J = 7.5 Hz), 2.97 (2H, t, J = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 182.82, 153.57, 136.54, 133.76, 132.74, 128.42, 127.40, 127.23, 124.60, 116.08, 113.62, 111.14, 100.63, 55.23, 54.90, 49.60, 19.73; MS (EI) m/z 322 (M^+); HRMS (EI) calcd for $C_{19}H_{18}ON_2S$: 322.1140 (M^+), found: 322.1131.

4.1.32. 2-Benzyl-6-iso-propyl-2,3,4,9-tetrahydro-β-carboline-1-thione (5n)

Yield: 86%; mp: 161–164 °C; IR (KBr): 3326, 1558 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.03 (1H, br), 7.42–7.20 (8H, m), 5.41 (2H, s), 3.76 (2H, t, J = 7.4 Hz), 3.04–2.95 (3H, m), 1.30 (6H, d, J = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.06, 141.35, 136.90, 136.41, 132.61, 128.77, 127.83, 127.75, 125.59, 125.27, 117.26, 111.96, 111.46, 55.80, 49.49, 34.16, 24.41, 20.36; MS (EI) m/z 334 (M^+); HRMS (EI) calcd for $C_{21}H_{22}N_2S$: 334.1504 (M^+), found: 334.1500.

4.1.33. 2-Benzyl-5,7-difluoro-2,3,4,9-tetrahydro-β-carboline-1-thione (5o)

Yield: 93%; mp: 109–111 °C; IR (KBr): 3264, 1578 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.20 (1H, br), 7.39–7.30 (4H, m), 7.07 (1H, dd, J = 2.2, 9.6 Hz), 6.89 (1H, t, J = 10.6 Hz), 5.36 (2H, s),

3.79 (2H, t, J = 7.4 Hz), 3.09 (2H, t, J = 7.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.13, 161.30 (dd, J = 11.5, 244.4 Hz), 157.97 (dd, J = 15.3 Hz, 252.1 Hz), 139.05 (d, J = 1.9 Hz), 136.07, 132.70 (d, J = 3.8 Hz), 128.84, 127.91, 127.87, 109.74 (d, J = 1.9 Hz), 96.55 (dd, J = 22.5, 29.2 Hz), 94.45 (d, J = 4.8 Hz, 26.8 Hz), 55.84, 49.29, 21.07; MS (EI) m/z 328 (M^+); HRMS (EI) calcd for $C_{18}H_{14}N_2F_2S$: 328.0846 (M^+), found: 328.0829.

4.1.34. 2-Benzyl-5,7-dichloro-2,3,4,9-tetrahydro-β-carboline-1-thione (5p)

Yield: 98%; mp: 185–187 °C; IR (KBr): 3272, 1557 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.19 (1H, br), 7.39–7.30 (6H, m), 7.09 (1H, d, J = 1.6 Hz), 5.38 (2H, s), 3.76 (2H, t, J = 7.4 Hz), 3.30 (2H, t, J = 7.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ183.13, 138.56, 135.95, 133.29, 130.93, 128.86, 128.79, 127.95, 127.89, 122.26, 121.66, 111.30, 110.73, 55.92, 49.32, 21.37; MS (EI) m/z 360 (M^+); HRMS (EI) calcd for $C_{18}H_{14}N_2Cl_2S$: 360.0255 (M^+), found: 360.0273.

4.1.35. 2-Phenyl-2,3,4,9-tetrahydro-β-carboline-1-thione (5q)

Yield: 99%; mp: 175–176 °C; IR (KBr): 3395, 1556 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.31 (1H, br s), 7.66 (1H, d, J = 8.0 Hz), 7.52–7.32 (6H, m), 7.26 (1H, t, J = 8.0 Hz), 7.08 (1H, t, J = 8.0 Hz), 4.11 (2H, t, J = 7.3 Hz), 3.18 (2H, t, J = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 184.11, 146.04, 138.41, 132.95, 128.99, 127.14, 126.90, 125.36, 124.80, 120.85, 119.85, 113.33, 112.73, 54.01, 20.23; MS (EI) m/z 277 (M^+); HRMS (EI) calcd for $C_{17}H_{14}N_2S$: 278.0878 (M^+), found: 278.0890.

4.1.36. 5,7-Difluoro-2-phenylethyl-2,3,4,9-tetrahydro-β-carboline-1-thione (5r)

Yield: 99%; mp: 150–153 °C; IR (KBr): 3336, 1551 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.18 (1H, br), 7.36–7.22 (5H, m), 6.87 (1H, dd, J = 1.9, 9.1 Hz), 6.58 (1H, dd, J = 1.9, 10.4 Hz), 4.29 (2H, t, J = 7.7 Hz), 3.61 (2H, t, J = 7.4 Hz), 3.12 (2H, t, J = 7.7 Hz), 3.00 (2H, t, J = 7.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 181.95, 161.07 (dd, J = 11.5, 243.5 Hz), 157.84 (dd, J = 15.3, 252.1 Hz), 138.85 (dd, J = 12.9, 14.9 Hz), 138.46, 132.88 (d, J = 2.9 Hz), 128.90, 128.63, 126.68, 112.00 (d, J = 21.1 Hz), 109.52, 96.38 (dd, J = 22.5, 29.2 Hz), 94.45 (dd, J = 4.8, 24.9 Hz), 55.61, 51.35, 33.06, 20.87; MS (EI) m/z 342 (M^+); HRMS (EI) calcd for $C_{19}H_{16}N_2F_2S$: 342.1002 (M^+), found: 342.0992.

4.1.37. Typical procedure for esters (6b–6r)

To a stirred solution of thiolactam (1 mmol) in DMF (5 mL) was added NaH (60%, 1.2 equiv) at 0 °C, and the reaction mixture was stirred at 0 °C for 30 min. To the mixture was added $BrCH_2COO$*t*-Bu or $BrCH_2COOMe$ (1.2 equiv) at 0 °C, and the resulting mixture was stirred at room temperature for 20–24 h. The reaction was quenched with H_2O (10 mL), and the aqueous mixture was extracted with Et_2O (10 mL × 3). The organic extracts were combined, dried over $MgSO_4$, and evaporated. The residue was chromatographed on SiO_2 (Hexane–Acetone = 20:1) to give *t*-butyl ester (**6b**–**6i**, **6k**, **6n**, **6o**, and **6q**) or methyl ester (**6j**, **6l**, **6m**, **6p**, and **6r**).

4.1.38. (2-Benzyl-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid *t*-butyl ester (6b)

Yield: 66%; mp: 63–64 °C; IR (KBr): 1742 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56 (1H, d, J = 9.0 Hz), 7.27–7.39 (7H, m), 7.16 (1H, t. J = 7.5 Hz), 5.73 (2H, br), 5.47 (2H, s), 3.75 (2H, t, J = 7.3 Hz), 2.97 (2H, t, J = 7.3 Hz), 1.47 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.11, 167.91, 140.41, 136.22, 132.03, 128.31, 127.32, 127.19, 125.33, 122.89, 120.59, 120.51, 115.02, 109.88, 81.47, 55.22, 49.07, 47.28, 28.01, 20.37; MS (EI) m/z 406 (M^+); HRMS (EI) calcd for $C_{24}H_{26}O_2N_2S$: 406.1715 (M^+), found: 406.1745.

4.1.39. (2-Benzyl-6-fluoro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid t-butyl ester (6c)

Yield: 93%; mp: 54–56 °C; IR (KBr): 1742 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37–7.18 (7H, m), 7.10 (1H, dt, J = 2.5, 9.4 Hz), 5.71 (2H, br), 5.46 (2H, s), 3.75 (2H, t, J = 7.2 Hz), 2.92 (2H, t, J = 7.2 Hz), 1.47 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.17, 167.96, 157.98 (d, J = 237.5 Hz), 137.07, 136.19, 133.27, 128.49, 127.46, 127.41, 123.09 (d, J = 10.1 Hz), 114.70 (d, J = 5.5 Hz), 114.19 (d, J = 26.7 Hz), 111.04 (d, J = 9.2 Hz), 104.90 (d, J = 23.9 Hz), 81.72, 55.31, 49.07, 47.37, 27.92, 20.22; MS (EI) m/z 424 (M^+); HRMS (EI) calcd for $C_{24}H_{25}O_2N_2FS$: 424.1621 (M^+), found: 424.1602.

4.1.40. [6-Fluoro-1-thioxo-2-(4-trifluoromethylbenzyl)-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid t-butyl ester (6d)

Yield: 76%; mp: 53–55 °C; IR (KBr): 1740 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59 (2H, d, J = 8.0 Hz), 7.47 (2H, d, J = 8.0 Hz), 7.24–7.05 (3H, m), 5.68 (2H, br), 5.51 (2H, s), 3.76 (2H, t, J = 6.9 Hz), 2.96 (2H, t, J = 6.9 Hz), 1.47 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 184.00, 168.16, 158.30 (d, J = 237.7 Hz), 140.46, 137.37, 133.39, 129.89 (q, J = 32.6 Hz), 128.11, 128.00, 125.66 (q, J = 3.8 Hz), 114.99 (d, J = 4.8 Hz), 114.74 (d, J = 26.8 Hz), 113.15 (d, J = 9.6 Hz), 111.28 (d, J = 9.6 Hz), 105.17 (d, J = 23.0 Hz), 82.14, 55.32, 49.65, 47.58, 28.10, 20.58; MS (EI) m/z 492 (M^+); HRMS (EI) calcd for $C_{25}H_{24}O_2N_2F_4S$: 492.1495 (M^+), found: 492.1518.

4.1.41. [2-(4-Bromo-2-fluorobenzyl)-6-fluoro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid *t*-butyl ester (6e)

Yield: 95%; pale yellow oil; IR (neat): 1742 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.45–7.07 (6H, m), 5.65 (2H, br), 5.43 (2H, s), 3.79 (2H, t, J = 7.1 Hz), 2.96 (2H, t, J = 7.1 Hz), 1.46 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.85, 167.98, 160.48 (d, J = 250.3 Hz), 158.15 (d, J = 238.1 Hz), 137.24, 133.32, 130.96 (d, J = 4.9 Hz), 127.60 (d, J = 3.7 Hz), 123.18 (d, J = 9.8 Hz), 121.49 (d, J = 9.8 Hz), 119.10 (d, J = 24.4 Hz), 119.02 (d, J = 24.4 Hz), 114.95 (d, J = 6.1 Hz), 114.65 (d, J = 26.9 Hz), 111.20 (d, J = 8.5 Hz), 105.13 (d, J = 23.2 Hz), 82.11, 49.03 (d, J = 3.6 Hz), 47.63, 31.24, 28.20, 20.71; MS (EI) m/z 520 (M^+); HRMS (EI) calcd for $C_{24}H_{23}O_2FN_2SBr$: 520.0632 (M^+), found: 520.0612.

4.1.42. [2-(4-Trifluoromethylbenzyl)-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid *t*-butyl ester (6f)

Yield: 86%; pale yellow oil; IR (neat): 1735 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.61–7.57 (3H, m), 7.48 (2H, dd, J = 8.7 Hz), 7.41–7.30 (2H, m), 7.19–7.15 (1H, m), 5.70 (2H, br), 5.52 (2H, s), 3.77 (2H, t, J = 6.9 Hz), 3.01 (2H, t, J = 6.9 Hz), 1.47 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.25, 168.33, 132.34, 129.86 (q, J = 32.6 Hz), 128.01, 127.82, 125.94, 125.80, 125.77, 125.66 (q, J = 3.8 Hz), 123.20, 121.09, 120.87, 115.46, 110.25, 81.94, 55.29, 49.67, 47.46, 28.14, 20.72; MS (EI) m/z 474 (M^+); HRMS (EI) calcd for $C_{25}H_{25}O_2N_2F_3S$: 474.1589 (M^+), found: 474.1571.

4.1.43. [2-(4-Bromobenzyl)-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid *t*-butyl ester (6g)

Yield: 92%; pale yellow oil; IR (neat): 1744 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.57 (1H, d, J = 8.0 Hz), 7.49–7.44 (3H, m), 7.40–7.24 (3H, m), 7.19–7.14 (1H, m), 5.70 (2H, br), 5.41 (2H, s), 3.74 (2H, t, J = 7.1 Hz), 2.98 (2H, t, J = 7.1 Hz), 1.47 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.74, 168.14, 140.73, 135.55, 131.80, 131.69, 129.47, 129.31, 125.75, 123.11, 120.93, 120.73, 115.31, 110.15, 81.87, 50.01, 49.43, 47.52, 28.24, 20.78; MS (EI) m/z 484 (M^+); HRMS (EI) calcd for $C_{24}H_{25}O_2N_2SBr$: 484.0820 (M^+), found: 484.0832.

4.1.44. (2-Benzyl-6-bromo-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid *t*-butyl ester (6h)

Yield: 53%; mp: 157–158 °C; IR (KBr): 1742 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.69 (1H, s), 7.43–7.31 (6H, brm), 7.15 (1H, d, J = 8.7 Hz), 5.70 (2H, br s), 5.45 (2H, s), 3.74 (2H, t, J = 7.2 Hz), 2.92 (2H, t, J = 7.2 Hz), 1.47 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.11, 167.77, 139.05, 136.17, 128.65, 128.56, 128.21, 127.72, 127.55, 124.62, 122.98, 114.15, 113.71, 111.64, 82.00, 55.55, 49.20, 47.51, 28.15, 20.45; MS (EI) *m/z* 484 (M^+); HRMS (EI) calcd for $C_{24}H_{25}O_2N_2SBr$: 484.0820 (M^+), found: 484.0800.

4.1.45. (2-Benzyl-7-chloro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid *t*-butyl ester (6i)

Yield: 52%; mp: 156–158 °C; IR (KBr): 1741 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.49–7.46 (1H, m), 7.38–7.27 (6H, m), 7.14–7.10 (1H, m), 5.67 (2H, s), 5.45 (2H, s), 3.74 (2H, t, J = 6.9 Hz), 2.94 (2H, t, J = 6.9 Hz), 1.49 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.24, 167.85, 140.93, 136.33, 132.84, 131.56, 128.63, 127.61, 121.83, 121.75, 121.62 115.09, 110.20, 82.11, 55.58, 49.22, 47.63, 28.24, 20.61; MS (EI) *m/z* 440 (M^+); HRMS (EI) calcd for $C_{24}H_{25}O_2SN_2Cl$: 440.1325 (M^+), found: 440.1358.

4.1.46. [2-(4-Bromo-2-fluorobenzyl)-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid methyl ester (6j)

Yield: 58%; mp: 130–132 °C; IR (KBr): 1739 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.59 (1H, d, J = 8.1 Hz), 7.40–7.36 (2H, m), 7.30–7.24 (3H, m), 7.18 (1H, t, J = 7.5 Hz), 5.81 (2H, br s), 5.44 (2H, s), 3.81 (2H, t, J = 6.8 Hz), 3.76 (3H, s), 3.02 (2H, t, J = 6.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.82, 169.53, 160.44 (d, J = 249.0 Hz), 140.63, 131.96, 131.00 (d, J = 3.7 Hz), 127.60 (d, J = 3.7 Hz), 125.94, 123.10, 122.59 (d, J = 14.7 Hz), 121.45 (d, J = 9.8 Hz), 121.11, 120.83, 118.96 (d, J = 25.6 Hz), 115.62, 110.04, 52.31, 49.87, 48.90 (d, J = 3.7 Hz), 46.84, 20.74; MS (EI) *m/z* 460 (M^+); HRMS (EI) calcd for $C_{21}H_{18}O_2N_2FSBr$: 460.0256 (M^+), found: 460.0245.

4.1.47. (2-Benzyl-6-chloro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid *t*-butyl ester (6k)

Yield: 65%; mp: 34–35 °C; IR (KBr): 1743 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.53 (1H, d, J = 9.0 Hz), 7.31–7.28 (7H, m), 5.70 (2H, br), 5.46 (2H, s), 3.74 (2H, t, J = 7.1 Hz), 2.92 (2H, t, J = 7.1 Hz), 1.47 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.07, 167.78, 136.14, 132.98, 128.60, 128.52, 127.67, 127.50, 126.19, 125.68, 123.90, 119.78, 114.26, 111.24, 81.95, 55.50, 49.19, 47.51, 28.12, 20.40; MS (EI) *m/z* 440 (M^+); HRMS (EI) calcd for $C_{24}H_{25}O_2N_2SCl$: 440.1325 (M^+), found: 440.1313.

4.1.48. (2-Benzyl-6-iodo-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid methyl ester (6l)

Yield: 61%; mp: 70–72 °C; IR (KBr): 1749 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.92 (1H, s), 7.59 (1H, d, J = 9.0 Hz), 7.37–7.29 (5H, m), 7.05 (1H, d, J = 9.0 Hz), 5.83 (2H, br s), 5.44 (2H, s), 3.77 (3H, s), 3.74 (2H, t, J = 6.8 Hz), 2.92 (2H, t, J = 6.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.85, 169.18, 139.39, 136.14, 133.65, 132.16, 129.45, 128.56, 127.55, 125.49, 114.08, 111.96, 84.07, 55.50, 52.34, 49.06, 46.76, 20.42; MS (EI) *m/z* 490 (M^+); HRMS (EI) calcd for $C_{21}H_{19}O_2N_2SI$: 490.0212 (M^+), found: 490.0204.

4.1.49. (2-Benzyl-6-methoxy-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid methyl ester (6m)

Yield: 63%; mp: 117–118 °C; IR (KBr) 1751 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.37–6.93 (8H, m), 5.83 (2H, br s), 5.45 (2H, s), 3.85 (3H, s), 3.75 (2H, t, J = 5.6 Hz), 2.93 (2H, t, J = 7.1 Hz),; ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.8, 169.4, 154.5, 137.3, 136.2, 135.8, 132.1, 128.3, 127.5, 127.3, 127.2, 127.0, 126.9, 123.0, 116.7, 114.7, 110.8, 100.8, 55.4, 55.1, 52.0, 48.9, 46.6, 20.3; MS 394 (M^+), 394 (100); HRMS calcd for $C_{22}H_{22}O_3N_2S$: 394.1351, found: 394.1355.

4.1.50. (2-Benzyl-6-iso-propyl-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid *t*-butyl ester (6n)

Yield: 80%; pale yellow oil; IR (neat):1743 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.36–7.18 (8H, m), 5.70 (2H, br), 5.47 (2H, s), 3.74 (2H, t, J = 7.0 Hz), 3.04–2.94 (3H, m), 1.48 (9H, s), 1.29 (6H, d, J = 7.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.61, 168.30, 141.54, 139.53, 136.59, 128.58, 127.74, 127.59, 127.45, 125.34, 117.24, 115.12, 109.96, 81.74, 55.55, 49.37, 47.57, 38.67, 34.19, 28.25, 24.51, 20.81; MS (EI) *m/z* 448 (M^+); HRMS (EI) calcd for $C_{27}H_{32}O_2N_2S$: 448.2185 (M^+), found: 448.2164.

4.1.51. (2-Benzyl-5,7-difluoro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid *t*-butyl ester (6o)

Yield: 99%; mp: 118–121 °C; IR (KBr): 1743 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.37–7.28 (5H, m), 6.75 (1H, dd, J = 1.7, 9.4 Hz), 6.61 (1H, d, J = 10.9 Hz), 5.70 (2H, br), 5.44 (2H, s), 3.73 (2H, t, J = 7.0 Hz), 3.08 (2H, t, J = 7.0 Hz), 1.48 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.81, 167.76, 161.41 (dd, J = 12.5, 244.4 Hz), 158.00 (dd, J = 15.3, 252.1 Hz), 142.09 (dd, J = 12.0, 13.9 Hz), 136.31, 132.78 (d, J = 4.8 Hz), 128.81, 128.72, 127.88, 127.85, 127.68, 96.85 (dd, J = 23.0, 37.4 Hz), 92.92 (d, J = 4.8, 26.8 Hz), 82.30, 55.50, 49.40, 47.99, 28.10, 21.49; MS (EI) *m/z* 442 (M^+); HRMS (EI) calcd for $C_{24}H_{24}O_2N_2F_2S$: 442.1523 (M^+), found: 442.1546.

4.1.52. (2-Benzyl-5,7-dichloro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid *t*-butyl ester (6p)

Yield: 67%; pale yellow oil; IR (neat): 1754 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.36–7.32 (5H, m), 7.16–7.13 (2H, m), 5.80 (2H, br), 5.42 (2H, s), 3.79 (3H, s), 3.74 (2H, t, J = 6.9 Hz), 3.27 (2H, t, J = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.57, 169.11, 141.53, 136.25, 133.34, 131.32, 129.04, 128.79, 127.80, 127.76, 122.31, 119.77, 115.54, 109.05, 55.60, 52.52, 48.96, 47.16, 21.73; MS (EI) *m/z* 432 (M^+); HRMS (EI) calcd for $C_{21}H_{18}O_2N_2SCl_2$: 432.0466 (M^+), found: 432.0420.

4.1.53. (2-Phenyl-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid *t*-butyl ester (6q)

Yield: 30%; mp: 165–166 °C; IR (KBr): 1741 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.64 (1H, d, J = 7.7 Hz), 7.50–7.25 (7H, m), 7.19 (1H, t, J = 7.5 Hz), 5.66 (2H, br), 4.09 (2H, t, J = 6.9 Hz), 3.21 (2H, t, J = 6.9 Hz), 1.45 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.37, 167.91, 146.17, 140.60, 132.24, 129.24, 127.38, 126.96, 125.68, 123.00, 120.78, 120.73, 115.49, 110.02, 81.56, 53.52, 47.36, 28.06, 21.05; MS (EI) *m/z* 392 (M^+); HRMS (EI) calcd for $C_{23}H_{24}O_2N_2S$: 392.1559 (M^+), found: 392.1546.

4.1.54. (5,7-Difluoro-2-phenylethyl-1-thioxo-1,2,3,4-tetra-hydro-β-carbolin-9-yl)-acetic acid methyl ester (6r)

Yield: 60%; mp: 38–40 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 7.36–7.22 (5H, m), 6.72 (1H, dd, J = 1.9, 9.3 Hz), 6.62 (1H, dd, J = 1.9, 10.2 Hz), 5.77 (2H, br s), 4.32 (2H, t, J = 7.3 Hz), 3.78 (3H, br s), 3.57 (2H, t, J = 7.1 Hz), 3.10 (2H, t, J = 7.3 Hz), 2.96 (2H, t, J = 7.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 181.47, 169.18, 161.36 (dd, J = 11.5, 244.4 Hz), 159.18 (dd, J = 15.3, 253.0 Hz), 141.87 (dd, J = 12.5, 13.4 Hz), 138.56, 132.64 (d, J = 3.8 Hz), 128.91, 128.58, 126.60, 113.68, 109.51 (d, J = 21.1 Hz), 96.90 (dd, J = 22.5, 29.2 Hz), 92.82 (dd, J = 4.8, 24.0 Hz), 55.76, 52.39, 51.09, 47.19, 32.94, 21.22; IR (KBr): 1749 cm^{-1}; MS (EI) *m/z* 414 (M^+); HRMS (EI) calcd for $C_{22}H_{20}O_2N_2F_2S$: 414.1214 (M^+), found: 414.1241.

4.1.55. (2-Benzyl-6-methoxycarbonyl-1-thioxo-1,2,3,4-tetra-hydro-β-carbolin-9-yl)-acetic acid methyl ester (6t)

To a stirred solution of **6l** (105 mg, 0.21 mmol) in DMF (5 mL) was added $Pd(PPh_3)_4$ (25.5 mg, 0.021 mmol), and the resulting solution was stirred at room temperature under CO balloon pressure for 30 min. To the reaction mixture were added NEt_3

(0.06 mL, 0.84 mmol) and MeOH (0.21 mL, 8.4 mmol), and then the mixture was stirred at 70 °C under CO balloon pressure for 20 h. After cooling, the reaction mixture was diluted with H_2O (10 mL) and brine (5 mL), and the aqueous mixture was extracted with Et_2O (10 mL × 5). The organic extracts were combined, dried over $MgSO_4$, and evaporated to give pale yellow oil, which was chromatographed on SiO_2 (Hexane–Acetone = 8:1) to give **6t** (72.6 mg, 81%). Mp: 63–65 °C; IR (KBr): 1750, 1702 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.36 (1H, d, J = 1.3 Hz), 8.03 (1H, dd, J = 1.3, 9.0 Hz), 7.37–7.27 (6H, m), 5.87 (2H, br s), 5.45 (2H, s), 3.94 (3H, s), 3.79–3.76 (5H, m), 3.01 (2H, t, J = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 182.86, 169.11, 167.09, 142.58, 136.14, 133.05, 128.56, 127.55, 126.38, 123.79, 123.02, 122.82, 116.32, 109.78, 55.50, 52.34, 51.95, 49.04, 47.00, 20.45; MS (EI) m/z 422 (M^+); HRMS (EI) calcd for $C_{23}H_{22}O_4N_2S$: 422.1300 (M^+), found: 422.1311.

4.1.56. Typical procedure for carboxylic acids (7b–7t)

To a stirred solution of methyl ester (1 mmol) in MeOH (3 mL) and H_2O (1 mL) was added $LiOH{\cdot}H_2O$ (4 equiv), and the resulting mixture was refluxed for 1–4 h. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with $CHCl_3$ (10 mL × 3). The organic extracts were combined, dried over $MgSO_4$, and evaporated. The residue was chromatographed on SiO_2 (Hexane–Acetone = 2:1) to give carboxylic acid (**7l**, **7m**, and **7p**). To a stirred solution of NaI (4 equiv) in $ClCH_2CH_2Cl$ (3 mL) was added TMSCl (4 equiv), and the resulting mixture was stirred at room temperature for 15 min. To a solution of *t*-butyl ester (1 mmol) in $ClCH_2CH_2Cl$ (5 mL) was transferred a solution of TMSI, prepared above, via a cannula, and then the resulting mixture was refluxed for 1–2 days. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with $CHCl_3$ (10 mL × 3). The organic extracts were combined, dried over $MgSO_4$, and evaporated. The residue was chromatographed on SiO_2 (Hexane–Acetone = 2:1) to give carboxylic acid (**7b**–**7k**, **7n**–**7s**, and **7u**).

4.1.57. (2-Benzyl-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid (7b)

Yield: 78%; mp: 207–209 °C; IR (KBr): 1722 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.66 (1H, d, J = 7.5 Hz), 7.52 (1H, d, J = 7.5 Hz), 7.37–7.28 (6H, m), 7.15 (1H, t, J = 7.5 Hz), 5.77 (2H, br), 5.43 (2H, s), 3.76 (2H, t, J = 7.2 Hz), 2.99 (2H, t, J = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 182.14, 170.12, 140.17, 136.46, 131.51, 128.41, 127.19, 125.24, 122.48, 120.69, 120.54, 115.17, 110.90, 54.71, 49.48, 46.49, 19.81; MS (EI) m/z 350 (M^+); HRMS (EI) calcd for $C_{20}H_{18}O_2N_2S$: 350.1089 (M^+), found: 350.1085.

4.1.58. (2-Benzyl-6-fluoro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid (7c)

Yield: 85%; mp: 190–191 °C; IR (KBr): 1722 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.58 (1H, dd, J = 4.3, 9.0 Hz), 7.48 (1H, dd, J = 2.5, 9.4 Hz), 7.36 (4H, m), 7.30 (1H, m), 7.20 (1H, dt, J = 2.5, 9.4 Hz), 5.76 (2H, br), 5.42 (2H, s), 3.76 (2H, t, J = 7.3 Hz), 2.98 (2H, t, J = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.96, 170.00, 157.30 (d, J = 234.4 Hz), 136.84, 136.32, 132.69, 128.41, 127.19, 122.58 (d, J = 9.8 Hz), 114.94 (d, J = 4.9 Hz), 113.81 (d, J = 26.9 Hz), 112.49 (d, J = 9.8 Hz), 105.00 (d, J = 23.2 Hz), 54.81, 49.53, 46.68, 19.75; MS (EI) m/z 368 (M^+); HRMS (EI) calcd for $C_{20}H_{17}O_2N_2FS$: 368.0995 (M^+), found: 368.0952.

4.1.59. [6-Fluoro-1-thioxo-2-(4-trifluoromethylbenzyl)-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid (7d)

Yield: 98%; mp: 84–86 °C; IR (KBr): 1723 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.61 (2H, d, J = 8.0 Hz), 7.47 (2H, d, J = 8.0 Hz), 7.32–7.13 (3H, m), 5.78 (2H, br), 5.49 (2H, s), 3.76 (2H, t, J = 6.9 Hz), 2.96 (2H, t, J = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.51, 173.17, 158.53 (d, J = 238.7 Hz), 140.27, 137.44, 133.04, 130.07 (q, J = 3.8 Hz), 128.10, 127.88, 125.78 (q, J = 3.8 Hz), 115.64 (d, J = 4.8 Hz), 115.29 (d, J = 26.8 Hz), 111.48 (d, J = 9.6 Hz), 111.17 (d, J = 9.6 Hz), 105.39 (d, J = 24.0 Hz), 55.39, 49.48, 47.13, 20.56; MS (EI) m/z 436 (M^+); HRMS (EI) calcd for $C_{21}H_{16}O_2N_2SF_4$: 436.0869 (M^+), found: 436.0916.

4.1.60. [2-(4-Bromo-2-fluorobenzyl)-6-fluoro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid (7e)

Yield: 70%; mp: 173–175 °C; IR (KBr): 1712 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.41–7.13 (6H, m), 5.74 (2H, br), 5.42 (2H, s), 3.80 (2H, t, J = 7.1 Hz), 2.97 (2H, t, J = 7.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.51, 173.74, 160.66 (d, J = 251.2 Hz), 158.51 (d, J = 238.7 Hz), 137.37, 133.06, 131.24 (d, J = 4.8 Hz), 127.83 (d, J = 3.8 Hz), 123.36 (d, J = 9.6 Hz), 122.35 (d, J = 14.4 Hz), 121.82 (d, J = 9.6 Hz), 119.19 (d, J = 24.9 Hz), 115.67 (d, J = 4.8 Hz), 115.25 (d, J = 26.8 Hz), 111.42 (d, J = 9.6 Hz), 105.40 (d, J = 23.0 Hz), 49.83, 49.09 (d, J = 3.8 Hz), 47.09, 20.56; MS (EI) m/z 464 (M^+); HRMS (EI) calcd for $C_{20}H_{15}O_2F_2N_2SBr$: 464.0006 (M^+), found: 464.0016.

4.1.61. [1-Thioxo-2-(4-trifluoromethylbenzyl)-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid (7f)

Yield: 63%; mp: 148–151 °C; IR (KBr): 1723 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.61 (3H, m), 7.49 (2H, d, J = 8.0 Hz), 7.41 (2H, d, J = 6.0 Hz), 7.24–7.19 (1H, m), 5.77 (2H, br), 5.51 (2H, s), 3.78 (2H, t, J = 7.1 Hz), 3.02 (2H, t, J = 7.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.63, 173.50, 140.98, 140.47, 131.98, 129.99 (q, J = 31.6 Hz), 128.09, 127.88, 126.47, 125.75 (q, J = 3.8 Hz), 123.19, 121.55, 120.98, 116.18, 110.40, 55.32, 49.46, 47.06, 20.64; MS (EI) m/z 418 (M^+); HRMS (EI) calcd for $C_{21}H_{17}O_2N_2F_3SBr$: 418.0963 (M^+), found: 418.0929.

4.1.62. [2-(4-Bromobenzyl)-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid (7g)

Yield: 57%; mp: 183–185 °C; IR (KBr): 1710 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.58 (1H, d, J = 8.0 Hz), 7.48 (2H, d, J = 8.5 Hz), 7.40 (2H, d, J = 7.1 Hz), 7.28–7.18 (3H, m), 5.77 (2H, br), 5.39 (2H, s), 3.74 (2H, t, J = 7.0 Hz), 2.98 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.09, 173.30, 140.84, 135.33, 131.91, 131.78, 129.37, 126.32, 123.10, 121.59, 121.43, 120.88, 116.07, 110.35, 55.11, 49.27, 47.20, 20.73; MS (EI) m/z 428 (M^+); HRMS (EI) calcd for $C_{20}H_{17}O_2N_2SBr$: 428.0194 (M^+), found: 428.0189.

4.1.63. (2-Benzyl-6-bromo-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid (7h)

Yield: 65%; mp: 227–229 °C; IR (KBr): 1727 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.91 (1H, d, J = 2.1 Hz), 7.54 (1H, d, J = 9.0 Hz), 7.43 (1H, dd, J = 2.1, 9.0 Hz), 7.36 (4H, m), 7.32–7.27 (1H, m), 5.76 (2H, br), 5.42 (2H, s), 3.75 (2H, t, J = 7.2 Hz), 2.98 (2H, t, J = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.86, 169.89, 138.76, 136.30, 132.24, 128.44, 127.57, 127.24, 124.22, 122.97, 114.48, 113.17, 112.84, 54.82, 49.52, 46.67, 19.63; MS (EI) m/z 428 (M^+); HRMS (EI) calcd for $C_{20}H_{17}O_2N_2SBr$: 428.0194 (M^+), found: 428.0145.

4.1.64. (2-Benzyl-7-chloro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid (6i)

Yield: 50%; mp: 184–186 °C; IR (KBr): 1717 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.74 (1H, d, J = 1.8 Hz), 7.69 (1H, d, J = 8.5 Hz), 7.36 (4H, m), 7.32–7.27 (1H, m), 7.16 (1H, dd, J = 1.8, 8.5 Hz), 5.74 (2H, br), 5.42 (2H, s), 3.76 (2H, t, J = 7.3 Hz), 2.98 (2H, t, J = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.81, 169.91, 141.22, 140.48, 136.32, 132.16, 129.96, 128.41, 127.19, 122.21, 121.26, 121.06, 115.22, 110.93, 54.74, 49.42, 46.73, 19.65; MS

(EI) *m/z* 384 (M^+); HRMS (EI) calcd for $C_{20}H_{17}O_2N_2SCl$: 384.0699 (M^+), found: 384.0709.

4.1.65. [2-(4-Bromo2-fluorobenzyl)-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid (7j)

Yield: 93%; mp: 163–165 °C; IR (KBr): 1712 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 7.69 (1H, d, J = 8.1 Hz), 7.60 (1H, dd, J = 1.7, 9.8 Hz), 7.51 (1H, d, J = 8.5 Hz), 7.39 (1H, dd, J = 1.7, 8.5 Hz), 7.34 (1H, t, J = 7.5 Hz), 7.26 (1H, t, J = 8.3 Hz), 7.16 (1H, t, J = 7.5 Hz), 5.71 (2H, br), 5.38 (2H, s), 3.85 (2H, t, J = 7.0 Hz), 3.06 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 182.70, 170.07, 159.92 (d, J = 249.0 Hz), 140.27, 131.48, 130.36 (d, J = 6.1 Hz), 127.50, 125.43, 122.91 (d, J = 14.6 Hz), 122.50, 120.72 (d, J = 9.8 Hz), 120.32 (d, J = 9.8 Hz), 118.69 (d, J = 25.6 Hz), 115.53, 110.93, 50.20, 49.29, 46.51, 19.89; MS (EI) *m/z* 446 (M^+); HRMS (EI) calcd for $C_{20}H_{16}O_2N_2FSBr$: 446.0100 (M^+), found: 446.0099.

4.1.66. (2-Benzyl-6-chloro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid (7k)

Yield: 56%; mp: 228–229 °C; IR (KBr): 1728 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.77 (1H, s), 7.59 (1H, d, J = 9.0 Hz), 7.38−7.28 (6H, m), 5.76 (2H, br), 5.42 (2H, s), 3.76 (2H, t, J = 7.3 Hz), 2.98 (2H, t, J = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.88, 169.91, 138.52, 136.29, 132.44, 128.42, 127.23, 125.07, 124.96, 123.50, 119.85, 114.57, 112.78, 54.81, 49.52, 46.68, 19.63; MS (EI) *m/z* 384 (M^+); HRMS (EI) calcd for $C_{20}H_{17}O_2N_2SCl$: 384.0699 (M^+), found: 384.0662.

4.1.67. (2-Benzyl-6-iodo-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid (7l)

Yield: 99%; mp: 216–218 °C; IR (KBr): 1726 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.32 (1H, s), 8.06 (1H, d, J = 1.4 Hz), 7.56 (1H, d, J = 8.8 Hz), 7.39–7.28 (5H, m), 5.73 (2H, s), 5.42 (2H, s), 3.74 (2H, t, J = 6.9 Hz), 2.96 (2H, t, J = 6.9 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.91, 170.31, 139.18, 136.37, 132.81, 131.93, 129.09, 128.44, 127.26, 127.21, 125.03, 114.06, 113.54, 84.09, 54.79, 49.50, 46.91, 19.66; MS (EI) *m/z* 476 (M^+); HRMS (EI) calcd for $C_{20}H_{17}O_2N_2SI$: 476.0056 (M^+), found: 476.0044.

4.1.68. (2-Benzyl-6-methoxy-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid (7m)

Yield: 81%; mp: 126–128 °C; IR (KBr): 1711 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.44 (1H, d, J = 9.0 Hz), 7.35 (4H, m), 7.34–7.27 (1H, m), 7.12 (1H, d, J = 2.6 Hz), 6.98 (1H, dd, J = 2.6, 9.0 Hz), 5.73 (2H, br), 5.42 (2H, s), 3.78 (3H, s), 3.74 (2H, t, J = 7.3 Hz), 2.96 (2H, t, J = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 182.11, 170.18, 154.18, 136.53, 135.67, 131.85, 128.42, 127.23, 127.16, 122.68, 116.47, 114.77, 111.95, 100.90, 55.40, 54.69, 49.55, 46.55, 19.91; MS (EI) *m/z* 380 (M^+); HRMS (EI) calcd for $C_{21}H_{20}O_3N_2S$: 380.1195 (M^+), found: 380.1188.

4.1.69. (2-Benzyl-6-iso-propyl-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid (7n)

Yield: 63%; mp: 188–190 °C; IR (KBr): 1723 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.38–7.28 (8H, m), 5.76 (2H, br), 5.45 (2H, s), 3.74 (2H, t, J = 7.0 Hz), 3.05–2.93 (3H, m), 1.29 (6H, d, J = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.04, 173.55, 142.22, 139.78, 136.42, 135.49, 132.24, 128.75, 127.70, 126.02, 123.19, 117.39, 116.00, 110.30, 55.59, 49.14, 47.28, 34.08, 24.35, 20.61; MS (EI) *m/z* 392 (M^+); HRMS (EI) calcd for $C_{23}H_{24}O_2N_2S$: 392.1559 (M^+), found: 392.1533.

4.1.70. (2-Benzyl-5,7-difluoro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid (7o)

Yield: 47%; mp: 219–220 °C; IR (KBr): 1709 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.41 (1H, d, J = 10.2 Hz), 7.36–7.26 (5H, m), 6.99 (1H, t, J = 10.2 Hz), 5.76 (2H, br), 5.41 (2H, s), 3.76 (2H, t, J = 7.3 Hz), 3.08 (2H, t, J = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.62, 169.92, 160.48 (dd, J = 12.5, 240.6 Hz), 157.10 (dd, J = 15.8, 250.6 Hz), 141.72 (d, J = 14.4 Hz), 136.43, 132.37 (d, J = 2.9 Hz), 128.67, 127.44, 127.41, 113.15, 108.86 (d, J = 21.1 Hz), 96.60 (dd, J = 23.0, 29.7 Hz), 94.45 (dd, J = 4.3, 27.3 Hz), 54.84, 49.42, 47.36, 20.75; MS (EI) *m/z* 386 (M^+); HRMS (EI) calcd for $C_{20}H_{16}O_2N_2SF_2$: 386.0901 (M^+), found: 386.0902.

4.1.71. (2-Benzyl-5,7-dichloro-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid (7p)

Yield: 76%; mp: 208–210 °C; IR (KBr): 1727 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.37–7.28 (6H, m), 7.15 (1H, s), 5.75 (2H, br), 5.42 (2H, s), 3.73 (2H, t, J = 6.9 Hz), 3.27 (2H, t, J = 6.9 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 183.72, 172.28, 142.85, 137.78, 136.47, 134.82, 131.74, 129.59, 129.51, 128.69, 128.45, 122.58, 116.21, 110.62, 56.06, 50.09, 48.92 22.42; MS (EI) *m/z* 418 (M^+); HRMS (EI) calcd for $C_{20}H_{16}O_2N_2SCl_2$: 418.0310 (M^+), found: 418.0351.

4.1.72. (2-Phenyl-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)-acetic acid (7q)

Yield: 78%; mp: 200–202 °C; IR (KBr): 1721 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 7.74 (1H, d, J = 8.1 Hz), 7.53–7.46 (3H, m), 7.39–7.34 (4H, m), 7.19 (1H, t, J = 7.8 Hz), 5.75 (2H, br s), 4.04 (2H, t, J = 7.0 Hz), 3.20 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 183.38, 170.02, 146.22, 140.28, 131.58, 129.13, 127.14, 125.51, 122.53, 120.90, 120.66, 115.71, 110.95, 53.67, 46.46, 20.34; IR (KBr): 2914, 1721 cm^{-1}; MS (EI) *m/z* 336 (M^+); HRMS (EI) calcd for $C_{19}H_{16}O_2N_2S$: 336.0933 (M^+), found: 336.0926.

4.1.73. (5,7-Difluoro-2-phenylethyl-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid (7r)

Yield: 73%; mp: 199–201 °C; IR (KBr): 1718 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.35–7.22 (5H, m), 6.84 (1H, dd, J = 1.9, 9.2 Hz), 6.63 (1H, dd, J = 1.9, 10.1 Hz), 5.72 (2H, br), 4.32 (2H, t, J = 7.4 Hz), 3.55 (2H, t, J = 7.1 Hz), 3.11 (2H, t, J = 7.4 Hz), 2.95 (2H, t, J = 7.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 181.09, 172.47, 161.64 (dd, J = 12.5, 245.4 Hz), 157.95 (dd, J = 14.9, 253.5 Hz), 142.07 (dd, J = 12.0, 13.9 Hz), 138.45, 132.58 (d, J = 1.9 Hz), 128.96, 128.68, 126.73, 114.25 (d, J = 2.9 Hz), 109.57 (d, J = 21.1 Hz), 97.32 (dd, J = 22.5, 29.2 Hz), 93.19 (dd, J = 4.8, 26.8 Hz), 55.98, 51.17, 47.59, 33.00, 21.23; MS (EI) *m/z* 400 (M^+); HRMS (EI) calcd for $C_{21}H_{18}O_2N_2F_2S$: 400.1057 (M^+), found: 400.1030.

4.1.74. (2-Benzyl-6-hydoxy-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid (7s)

To a stirred solution of **6m** (485 mg, 1.19 mmol) in $CHCl_3$ (3 mL) was added BBr_3 (1 M in $CHCl_3$, 3.56 mL, 3.56 mmol), and the resulting mixture was stirred at room temperature for 4 h. The reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with $CHCl_3$ (10 mL × 3). The organic extracts were combined, dried over $MgSO_4$, and evaporated. The residue was chromatographed on SiO_2 (Hexane–Acetone = 3:1) to give **7s** (343.3 mg, 73%). Mp: 200–202 °C; IR (KBr): 1721 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 9.07 (1H, s), 7.36–7.29 (6H, m), 6.87–6.84 (2H, m), 5.66 (2H, br), 5.42 (2H, s), 3.72 (2H, t, J = 7.0 Hz), 2.89 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 179.61, 170.26, 160.78, 151.69, 136.58, 135.17, 131.80, 128.42, 127.21, 123.08, 116.50, 114.18, 111.60, 103.22, 54.66, 49.53, 46.64, 19.86; MS (EI) *m/z* 366 (M^+); HRMS (EI) calcd for $C_{20}H_{18}O_3N_2S$: 366.1038 (M^+), found: 336.1057.

4.1.75. (2-Benzyl-6-carboxy-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid (7t)

Yield: 94%; mp: 208–210 °C; IR (KBr): 1704 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 8.33 (1H, s), 7.88 (1H, d, J = 7.3 Hz), 7.60

(1H, d, J = 8.7 Hz), 7.35–7.27 (5H, m), 5.78 (2H, br), 5.41 (2H, s), 3.77 (2H, t, J = 7.0 Hz), 3.04 (2H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.83, 169.89, 167.51, 142.21, 136.33, 132.69, 127.47, 127.27, 125.79, 123.47, 123.21, 122.21, 116.35, 110.97, 54.82, 49.50, 46.86, 19.63; MS (EI) m/z 394 (M^+); HRMS (EI) calcd for $C_{21}H_{18}O_4N_2S$: 394.0987 (M^+), found: 394.0957.

4.2. Biological assays

4.2.1. Preparation of recombinant AKR1B1, AKR1B10 and AKR1A1

AKR1B10 with N-terminal 6-His tag, AKR1B1 and AKR1A1 without any additional amino acid were expressed in *Escherichia coli* BL21(DE3) pLysS cells transformed with the expression plasmids harboring their cDNAs, and purified to homogeneity as described previously.[14,28]

4.2.2. Inhibition assays

The reductase activities of AKRs (1B1, 1B10 and 1A1) were assayed by measuring the rate of change in NADPH absorbance (at 340 nm) accompanying the substrate reduction at 25 °C. The reaction mixture consisted of 0.1 M potassium phosphate buffer, pH 7.4, 0.1 mM NADPH, substrate and enzyme, in a total volume of 2.0 ml. The substrate in the assay of both AKR1B1 and AKR1B10 activities was 0.2 mM pyridine-3-aldehyde, and that of AKR1A1 was 10 mM D-glucuronate. In the determination of IC_{50} values, the inhibitors were dissolved into methanol, and added into the reaction mixture, in which the final concentration of methanol was less than 2.5%. Kinetic studies in the presence of inhibitors were carried out in pyridine-3-aldehyde reduction over a range of five substrate concentrations (0.2–5 $\times$ K_m) at the saturating concentration of NADPH. The inhibition pattern was analyzed by fitting the initial velocities in the Lineweaver–Burk plot, in which a noncompetitive inhibitor gave two inhibition constants, K_{is} (slope effect) and K_{ii} (intercept effect). The IC_{50} and K_i values are expressed as the means ± standard errors of at least three determinations.

4.2.3. Molecular docking

The docking simulations were performed with Glide in Schrödinger Suite 2009 (Schrödinger, LLC). The docking poses of each ligand were generated with Glide extra-precision (XP) method, and we selected the pose with the best Glide score as its binding poses for each ligand. The protein coordinates were taken from the Protein Data Bank whose entry code is 1ADS and 2PEV for AKR1B1 and 1ZUA for AKR1B10. Before the docking study of **7t**, we validated the application of Glide XP method using Fidarestat and Tolrestat, whose X-ray crystal structures complexed with AKR1B1 (2PEV) and AKR1B10 (1ZUA), respectively, had been known. Fidarestat was docked into AKR1B1s (2PEV, 1ADS) and Tolrestat was docked into AKR1B10 (1ZUA) using Glide XP method. The binding conformations obtained from the X-ray crystallography were reproduced within 2.0 Å RMSD errors for both compounds. In addition, we performed the docking simulations of epalrestat for AKR1A1, AKR1B1, and AKR1B10. We also confirmed that there is a good linear correlation between the Glide Docking Scores and pIC_{50} values as shown in Figure 3. The Glide Score seems to be good estimation of the binding affinities of ligands for AKR1A1, AKR1B1, and AKR1B10. We have performed the docking simulations for **7t**, after careful validations of the computational ligand-docking method. As previously mentioned, we selected one pose with the best Glide score as the binding pose of the compound **7t**. The best Glide scores for AKR1B1 (1ADS) and AKR1B10 (1ZUA) were −8.60 and −7.76 kcal/mol, respectively. Then, we analyzed the difference between the binding affinities of **7t** to AKR1B1 and to AKR1B10, comparing the binding modes of **7t** to them.

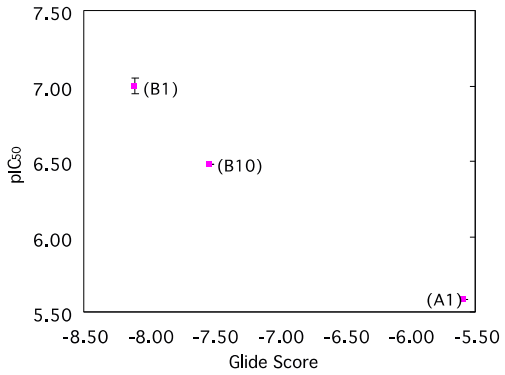


Figure 3. The correlation between Glide Score and pIC_{50} values for AKR1A1, AKR1B1, and AKR1B10.

Acknowledgment

This work was supported in part by Research for Promoting Technological Seeds, Japan Science and Technology Agency (JST). We gratefully acknowledge Dr. Ossama El-Kabbani, Monash Institute of Pharmaceutical Sciences, Medicinal Chemistry and Drug Action, for his helpful discussion.

References and notes

1. Shaw, J. E.; Sicree, R. A.; Zimmet, P. Z. *Diabetes Res. Clin. Pract.* **2010**, *87*, 4.
2. Szwergold, B. S.; Kappler, F.; Brown, T. R. *Science* **1990**, *247*, 451.
3. Gonzalez, A. M.; Sochor, M.; Hothersall, J. S.; McLean, P. *Diabetes* **1986**, *35*, 1200.
4. Wells-Knecht, K. J.; Brinkmann, E.; Wells-Knecht, M. C.; Litchfield, J. E.; Ahmed, M. U.; Reddy, S.; Zyzak, D. V.; Thorpe, S. R.; Baynes, J. W. *Nephrol. Dial. Transplant.* **1996**, *11*, 41.
5. Robinson, W. G., Jr.; Kador, P. F.; Kinoshita, J. H. *Science* **1983**, *221*, 1177.
6. Engerman, R. L. *Diabetes* **1989**, *38*, 1203.
7. Young, R. J.; Ewing, D. J.; Clarke, B. F. *Diabetes* **1983**, *32*, 938.
8. Dunlop, M. *Kidney Inter.* **2000**, *58*, S-3.
9. Jez, J. M.; Flynn, T. G.; Penning, T. M. *Biochem. Pharmacol.* **1997**, *54*, 639.
10. Linster, C. L.; Van Schaftingen, E. *FEBS J.* **2007**, *274*, 1.
11. Kanazu, T.; Shinoda, M.; Nakayama, T.; Deyashiki, Y.; Hara, A.; Awada, H. *Biochem. J.* **1991**, *279*, 903.
12. Cao, D.; Fan, S. T.; Chung, S. S. *J. Biol. Chem.* **1998**, *273*, 11429.
13. Gallego, O.; Ruiz, F. X.; Ardévol, A.; Dominguez, M.; Alvarez, S.; de Lera, A. R.; Rovira, C.; Farrés, J.; Fita, I.; Parés, X. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 20764.
14. Endo, S.; Matsunaga, T.; Mamiya, H.; Ohta, C.; Soda, M.; Kitade, Y.; Tajima, K.; Zhao, H. T.; El-Kabbani, O.; Hara, A. *Arch. Biochem. Biophys.* **2009**, *487*, 1.
15. Ma, J.; Yan, R.; Zu, X.; Cheng, J. M.; Rao, K.; Liao, D. F.; Cao, D. *J. Biol. Chem.* **2008**, *283*, 3418.
16. Van Zandt, M. C.; Doan, B.; Sawicki, D. R.; Sredy, J.; Podjarny, A. D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2006.
17. Chen, X.; Zhu, C.; Guo, F.; Qiu, X.; Yang, Y.; Zhang, S.; He, M.; Parveen, S.; Jing, C.; Li, Y.; Ma, B. *J. Med. Chem.* **2010**, *53*, 8330.
18. Maccari, R.; Ciurleo, R.; Giglio, M.; Cappiello, M.; Moschini, R.; Del Corso, A.; Mura, U.; Ottanà, R. *Bioorg. Med. Chem.* **2010**, *18*, 4049.
19. Maccari, R.; Del Corso, A.; Giglio, M.; Moschini, R.; Mura, U.; Ottanà, R. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 200.
20. Kato, A.; Kobayashi, K.; Narukawa, K.; Minoshima, Y.; Adachi, I.; Hirono, S.; Nash, R. J. *Bioorg. Med. Chem.* **2010**, *20*, 5630.
21. Kato, A.; Yasuko, H.; Goto, H.; Hollinshesd, J.; Nash, R. J. *Phytomedicine* **2009**, *16*, 258.
22. Lehmann, J.; Pohl, U. *Arch. Pharm.* **1987**, *320*, 1202.
23. Toyooka, N.; Takeda, D.; Minoshima, Y.; Kato, A.; Adachi, I. *Heterocycles* **2009**, *79*, 1073.
24. Petrash, J. M.; Tarle, I.; Wilson, D. K.; Quiocho, F. A. *Diabetes* **1994**, *143*, 955.
25. Grimshaw, C. E.; Shahbaz, M.; Jahangiri, G.; Putney, C. G.; McKercher, S. R.; Mathur *Eur. J. Biochem.* **1989**, *28*, 5343.
26. Ehrig, T.; Bohren, K. M.; Prendergast, F. G.; Gabbay, K. H. *Biochemistry* **1994**, *33*, 7157.
27. Lehmann, J.; Ghoneim, K. H.; El-Gendy, A. A. *Arch. Pharm.* **1987**, *320*, 22.
28. Endo, S.; Matsunaga, T.; Kuwata, K.; Zhao, H.-T.; El-Kabbani, O.; Kitade, Y.; Hara, A. *Bioorg. Med. Chem.* **2010**, *18*, 2485.