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Synthesis and structure–activity relationship of 2-phenyliminochromene derivatives as inhibitors for aldo–keto reductase (AKR) 1B10

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ABSTRACT

Inhibitors of a human member (AKR1B10) of the aldo–keto reductase superfamily are regarded as promising therapeutics for the treatment of cancer. Recently, we have discovered (*Z*)-2-(4-methoxyphenylimino)-7-hydroxy-*N*-(pyridin-2-yl)-2*H*-chromene-3-carboxamide (**1**) as the potent competitive inhibitor using the virtual screening approach, and proposed its 4-methoxy group on the 2-phenylimino moiety as an essential structural prerequisite for the inhibition. In this study, 18 derivatives of **1** were synthesized and their inhibitory potency against AKR1B10 evaluated. Among them, 7-hydroxy-2-(4-methoxyphenylimino)-2*H*-chromene-3-carboxylic acid benzylamide (**5n**) was the most potent inhibitor showing a K_i value of 1.3 nM. The structure–activity relationship of the derivatives indicated that the 7-hydroxyl group on the chromene ring, but not the 4-methoxy group, was absolutely required for inhibitory activity, The molecular docking of **5n** in AKR1B10 and site-directed mutagenesis of the enzyme residues suggested that the hydrogen-bond interactions between the 7-hydroxyl group of **5n** and the catalytic residues (Tyr49 and His111) of the enzyme, together with a π-stacking interaction of the benzylamide moiety of **5n** with Trp220, are important for the potent inhibition.

1. Introduction

A human member of the aldo–keto reductase (AKR) superfamily, AKR1B10, is a NADPH-dependent reductase, which was originally identified as an aldose reductase-like protein that is up-regulated in hepatocellular carcinomas.[1] Over-expression of AKR1B10 has been also observed in other tumors, such as smokers' non-small cell lung carcinomas,[2] uterine carcinomas,[3] cholangiocarcinomas,[4] pancreatic carcinoma,[5] and breast cancer.[6] The silencing of the AKR1B10 gene results in growth inhibition of cancer cells[5–8] and hepatocellular carcinoma xenografts in mice,[9] and its elevated expression in turn promotes proliferation of cancer cells,[10,11] indicating that the enzyme participates in tumor development. Furthermore, AKR1B10 is suggested to be implicated in developing colon cancer cell resistance to anticancer drugs such as mitomycin C[12] and oxaliplatin.[11] Due to its high catalytic efficiency towards aliphatic aldehydes, retinals and isoprenyl aldehydes,[1,6–8,13,14] the roles suggested for AKR1B10 in cell carcinogenesis and tumor development are the detoxification of cytotoxic carbonyls derived from lipid peroxidation,[6–8] decrease in retinoic acid synthesis,[13] and modulation of protein prenylation.[6,11,15] In addition, AKR1B10 is reported to promote fatty acid synthesis in cancer cells by blocking the ubiquitin-dependent degradation of acetyl CoA carboxylase.[8,16] Thus, this enzyme has been recognized not only as a potential diagnostic and/or prognostic marker, but also as a potential therapeutic target for the treatment of the above types of cancer and the colon cancer chemoresistance.

During the past five years, many synthetic and natural compounds that show inhibitory effects on AKR1B10 have been reported, as reviewed by Matsunaga et al.[11,17] Among them, (*Z*)-2-(4-methoxyphenylimino)-7-hydroxy-*N*-(pyridin-2-yl)-2*H*-chromene-3-carboxamide (**1**) is the most potent competitive inhibitor

Abbreviations: AKR, aldo–keto reductase; FBS, fetal bovine serum; SAR, structure–activity relationship.

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showing an IC_{50} value of 6.0 nM.[18] Molecular docking of **1** in AKR1B10 proposed that the interactions between the 4-methoxyl group on the 2-phenylimino moiety of **1** and the enzyme's active site residues, His111 and Trp112, are important for the tight binding (Fig. 1A). However, this compound almost equally inhibits the structurally similar human aldose reductase that is named AKR1B1 in the AKR superfamily. Because AKR1B1 plays distinct roles in glucose and prostaglandin metabolism,[19,20] selective inhibition of AKR1B10 is ideally required for the development of drugs targeting this enzyme. The structure–activity relationship (SAR) study on the chromene-3-carboxamide derivatives has not been also reported since its discovery by the virtual screening.[18] In this study, we designed and synthesized derivatives of **1** based on the molecular docking result, and evaluated them for enzyme inhibitory activity, in order to explore SAR of the **1**-derived compounds as potent inhibitors of ARK1B10.

2. Results and discussion

2.1. Chemistry

First, we synthesized seven **1**-based derivatives having different substituents on the 2-phenylimino moiety at the 4-position to confirm the interactions of the 4-methoxy group with His111 and Trp112 of AKR1B10, which was suggested by the previous docking model of **1** in this enzyme.[18] The Knoevenagel condensation of cyanoacetamide (**2**)[21] with 2,4-dihydroxybenzaldehyde (**3**) afforded the 2-iminochromene derivative (**4**). Reaction of **4** with anilines furnished the desired 2-phenyliminochromenes (**5a–g**) (Scheme 1). All of the derivatives potently inhibited AKR1B10, as evidenced by less than 2.2-fold increases in their IC_{50} values compared to that of **1** (See Table 1). Surprisingly, the des-methoxy derivative **5g** also showed potent inhibition, in contrast to the important interaction of the 4-methoxy group suggested by the docking model of **1**.[18] Next, we examined the effect of substituents of the 3-carboxamide moiety on AKR1B10 inhibition. Seven derivatives having isopropyl (**5h**) or acyclic alkyls (**5i–k**), hydroxyalkyls (**5l–m**), and benzyl (**5n**) substituent instead of the 2-pyridyl side chain of **1** were synthesized. Since these substitutions also did not significantly affect the inhibitory potency towards AKR1B10, we synthesized the different phenol derivatives (**5o–q**) and deoxy-derivative (**5r**) at the 7-position of the chromene ring system to examine the role of the 7-hydroxyl group on the chromene ring of **1** and the above derivatives. The same procedures for the synthesis of **5a–g** were applied for the synthesis of **5o–r**, as shown in Schemes 2 and 3.

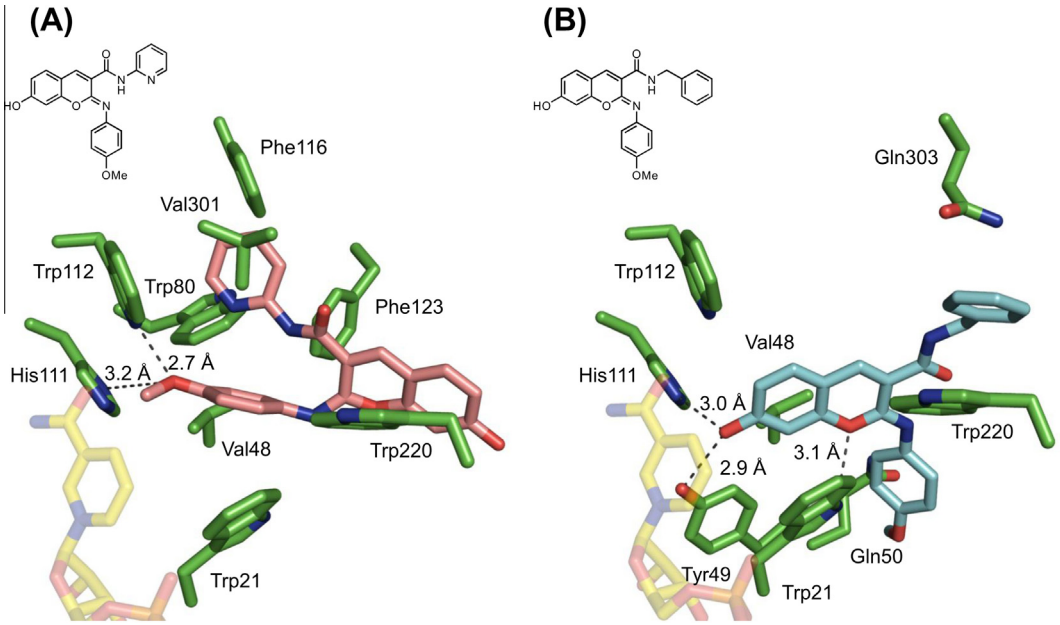
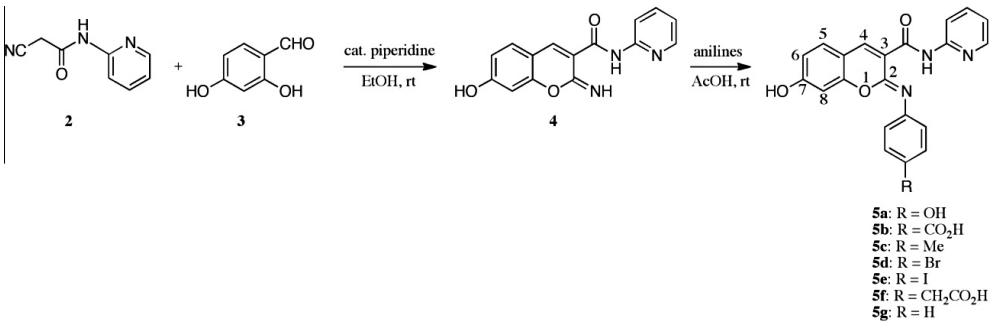


Figure 1. Structures of **1** (A) and **5n** (B), and their docked models in AKR1B10–$NADP^+$ complex. The portion of $NADP^+$ (yellow) and residues (green) within 4.0 Å from the inhibitors are depicted with possible hydrogen bond interactions, which are shown in dotted lines with distances. The docked model of **1** (A) is the same as that previously reported.[18]



Scheme 1. Synthesis of chromene derivatives **5a–g**.

Table 1
Inhibition of AKR1B10 and AKR1B1 by the **1**-based derivatives

Compound	IC_{50} (nM)		Ratio [a]
	AKR1B10	AKR1B1	
1	6.0 ± 0.1	11 ± 1.0	1.8
5a	6.8 ± 0.3	24 ± 1.1	3.5
5b	7.6 ± 1.0	19 ± 1.3	2.5
5c	8.6 ± 0.8	26 ± 3.0	3.0
5d	14 ± 0.8	25 ± 4.4	1.8
5e	13 ± 2.1	12 ± 1.5	0.9
5f	13 ± 1.6	20 ± 2.4	1.5
5g	12 ± 2.3	34 ± 1.5	2.8
5h	9.7 ± 1.6	6.7 ± 0.4	0.7
5i	18 ± 3.3	28 ± 2.7	1.6
5j	16 ± 2.1	28 ± 3.3	1.8
5k	11 ± 0.5	27 ± 4.6	2.5
5l	11 ± 0.5	20 ± 1.1	1.8
5m	8.8 ± 1.0	29 ± 0.4	3.3
5n	4.7 ± 0.04	24 ± 2.4	5.1
5o	>10,000 [b]	>10,000[b]	–
5p	>10,000[b]	>10,000[b]	–
5q	290 ± 30	>10,000[b]	–
5r	>10,000[b]	>10,000[b]	–

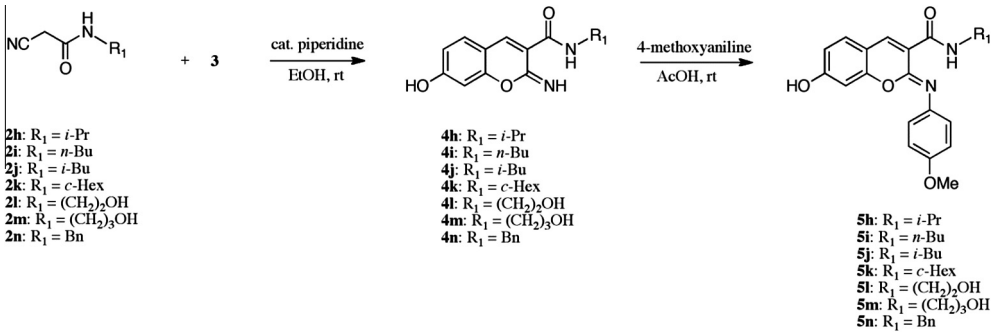
[a] Selectivity is expressed as a ratio of AKR1B1/AKR1B10.
[b] Inhibition percentages are less than 30% at 10,000 nM.

2.2. Biological evaluation and SAR of 5

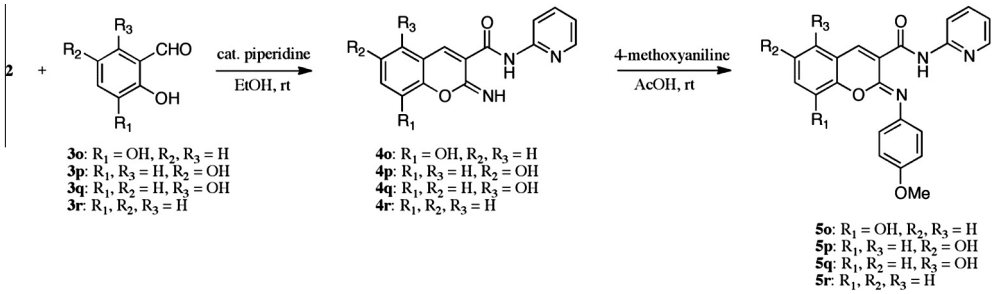
Newly synthesized compounds (**5**), except for **5o–r,** showed potent inhibition for AKR1B10, with less than 3-fold increases in the IC_{50} values compared with that of the original compound **1** (Table 1). While the replacement of the 4-methoxy group on the 2-phenylimino moiety of **1** with halogen (**5d**, **5e**) or CH_2CO_2H group (**5f**) slightly decreased the inhibitory potency, that with OH (**5a**), CO_2H (**5b**) or Me group (**5c**) had apparently no significant effect. This indicates that the 4-methoxy group is not an essential structural prerequisite for the tight binding of **1**, which is suggested by the previous dock model.[18] With respect to the moiety bound to the carboxyamide of the chromene ring, small alkyls (less than 3 carbons) or Bn substituent are better than *c*-Hex, *n*-Bu, or *i*-Bu as the substituent on the amide nitrogen. Among the synthesized compounds, the Bn derivative (**5n**) was the most potent and selective inhibitor of AKR1B10 (IC_{50} = 4.7 nM). The inhibition pattern of **5n** was competitive with respect to the alcohol substrate, geraniol, of AKR1B10, and its K_i value was 1.3 ± 0.3 nM (Fig. 2), which is the lowest among the values of the known inhibitors.[10,11,13,14,17,18] In contrast, the removal of the 7-hydroxyl group on the chromene ring (**5o–r**) resulted in drastic decreases in inhibitory potency, suggesting a crucial role of the 7-hydroxyl group in the tight binding of **1** to AKR1B10.

2.3. Docking model of 5n in AKR1B10

The above SAR studies of 2-phenyliminochromene derivatives (**5a–r**) revealed that the 7-hydroxyl group on the chromene ring is an essential moiety for their potent inhibition of AKR1B10. The underlying structural reasons for the high affinity of the 7-hydroxy-chromene derivatives were examined by constructing a model of docked **5n** in the AKR1B10-$NADP^+$ complex (Fig. 1B). In this model, **5n** occupied the substrate-binding site of the enzyme, in which its 7-hydroxyl group formed hydrogen-bond interactions with the catalytically important residues, Tyr49 and His111 (2.9 Å and 3.0 Å, respectively), and the chromene ring was surrounded by hydrophobic residues (Trp21, Val48, Trp112, and Trp220). An additional hydrogen-bond interaction between the oxygen in the chromene ring and the side chain of Trp21 (3.1 Å) was suggested. The orientation of the chromene ring of **5n** is contrast to that in the previous **1**-docked model (Fig. 1A), in which the 7-hydroxy-chromene ring is oppositely positioned. The benzylamide moiety of **5n** formed a π-stacking interaction with the side chain of Trp220 (3.5 Å), which may account for the higher inhibitory potency for AKR1B10 than other derivatives with alkylamide and *c*-hexylamide moieties (**5h–m**). In fact, the



Scheme 2. Synthesis of chromene derivatives **5h–n**.



Scheme 3. Synthesis of chromene derivatives **5o–r**.

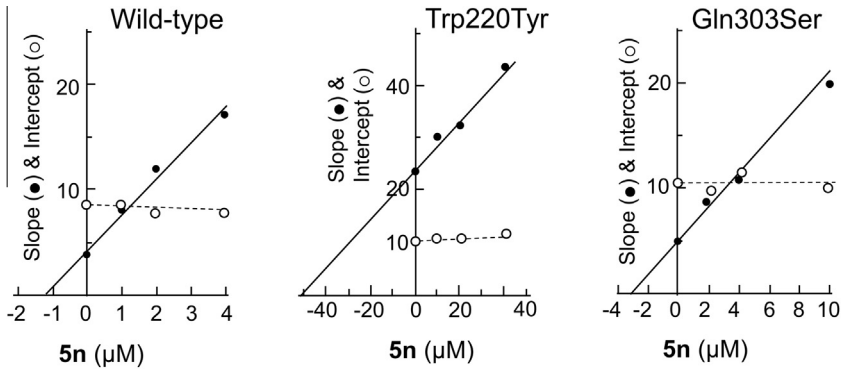


Figure 2. Inhibition of wild-type and mutant AKR1B10s by **5n**. The activity was determined with 0.25 mM $NADP^+$ and geraniol (25–200 μM for the wild-type and Gln303Ser enzymes; and 50–800 μM for Trp220Tyr enzyme) in the presence of the indicated concentrations of **5n**. The velocities were plotted double-reciprocally versus the substrate concentrations, and replots of the slopes (μM mL munit^{-1}) and intercepts (munit mL^{-1} × 10^2) versus the inhibitor concentrations are shown in the figure.

Trp220Tyr mutagenesis of the enzyme resulted in a 39-fold increase in the K_i value for **5n** (51.0 ± 1.0 nM) compared to that determined with the wild-type enzyme (Fig. 2). The benzylamide moiety of **5n** is also close to the side-chain of Gln303 (3.3 Å), which corresponds to Ser in AKR1B1, and the mutation of Gln303Ser decreased the affinity for **5n** (K_i = 3.8 ± 0.1 nM, Fig. 2) by 3-fold. Gln303 might be related to the highest AKR1B10-inhibitory selectivity of **5n** among the derivatives. The 4-methoxy group on the 2-phenylimino moiety of **5n** was located in the external region of the substrate-binding pocket, in which only the side-chain of Gln50 was close to the 4-methoxy group. This orientation may explain the insignificant alteration in the inhibitory potency following the replacement of the 4-methoxy group (**5a–g**).

3. Conclusion

In this study, a series of 2-phenyliminochromene derivatives (**5a–r**) were synthesized, and their inhibitory activities for AKR1B10 were evaluated. The SAR of the synthesized compounds revealed that the hydroxyl group on the chromene ring at the 7-position was essential to maintain the potent inhibitory effect. The necessity of the 7-hydroxyl group was in agreement with the molecular docking model of **5n** in complex with AKR1B10–$NADP^+$, in which the 7-hydroxyl group forms strong hydrogen bond interactions with catalytically important residues, Tyr49 and His111, of the active site of the enzyme.

4. Experimental section

4.1. Chemistry

4.1.1. General procedure for Knoevenagel condensation

To a stirred solution of cyanamide (**2**, 1.1 mmol) in ethanol (2 mL) were added 2-hydroxybenzaldehyde (1 mmol) and piperidine (catalytic amount), and the resulting mixture was stirred at room temperature for 12 h. The suspension was then filtered to give **4**, which was used directly in the next step.

4.1.1.1. 7-Hydroxy-2-imino-2*H*-chromene-3-carboxylic acid pyridin-2-ylamide (4). Yield: 88%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.59 (1H, s), 6.71 (1H, d, J = 8.5 Hz), 7.13 (1H, t, J = 8.9 Hz), 7.64 (1H, d, J = 8.5 Hz), 7.83 (1H, t, J = 8.9 Hz), 8.25 (1H, d, J = 8.9 Hz), 8.31–8.32 (1H, m), 8.50 (1H, s), 9.02 (1H, br), 13.14 (1H, s).

4.1.1.2. 7-Hydroxy-2-imino-2*H*-chromene-3-carboxylic acid isopropylamide (4h). Yield: 45%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.17 (6H, s), 4.05 (1H, sept, J = 6.5 Hz), 6.78 (1H, s), 6.86 (1H, dd, J = 1.3, 7.3 Hz), 7.89 (1H, dd, J = 1.3, 7.3 Hz), 8.44–8.45 (1H, m), 8.76 (1H, br), 11.02 (1H, br).

4.1.1.3. 7-Hydroxy-2-imino-2*H*-chromene-3-carboxylic acid butylamide (4i). Yield: 61%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 (3H, t, J = 7.1 Hz), 1.41 (2H, sext, J = 7.1 Hz), 1.60 (2H, quint, J = 7.1 Hz), 3.42 (2H, t, J = 7.1 Hz), 6.64 (1H, s), 6.72 (1H, dd, J = 2.4, 5.9 Hz), 7.33 (1H, d, J = 8.3 Hz), 8.81 (1H, s).

4.1.1.4. 7-Hydroxy-2-imino-2*H*-chromene-3-carboxylic acid isobutylamide (4j). Yield: 49%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94 (6H, d, J = 6.6 Hz), 1.81 (1H, m), 2.08 (2H, d, J = 6.6 Hz), 6.64 (1H, s), 6.72 (1H, dd, J = 2.4, 5.9 Hz), 7.33 (1H, d, J = 8.3 Hz), 8.81 (1H, s).

4.1.1.5. 7-Hydroxy-2-imino-2*H*-chromene-3-carboxylic acid cyclohexylamide (4k). Yield: 68%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25–1.97 (10H, m), 3.95 (1H, quint, J = 7.8 Hz), 6.64(1H, s), 6.73 (1H, dd, J = 2.4, 6.2 Hz), 7.30 (1H, d, J = 8.6 Hz), 8.35 (1H, s), 10.03 (1H, br).

4.1.1.6. 7-Hydroxy-2-imino-2*H*-chromene-3-carboxylic acid (2-hydroxyethyl)amide (4l). Yield: 81%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.25 (2H, t, J = 7.1 Hz), 3.46 (2H, t, J = 7.1 Hz), 4.77 (1H, br), 6.53 (1H, s), 6.65 (1H, d, J = 8.5 Hz), 7.55 (1H, d, J = 8.5 Hz), 8.3 (1H, s), 10.33 (1H, br).

4.1.1.7. 7-Hydroxy-2-imino-2*H*-chromene-3-carboxylic acid (3-hydroxypropyl)amide (4m). Yield: 43%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.62 (2H, quint, J = 6.5 Hz), 3.29 (2H, t, J = 6.5 Hz), 3.38 (2H, t, J = 6.5 Hz), 4.10 (1H, br), 6.54 (1H, s), 6.66 (1H, dd, J = 2.1, 6.2 Hz), 7.55 (1H, d, J = 8.3 Hz), 8.29 (1H, s), 8.74 (1H, br), 10.24 (1H, br), 10.63 (1H, br).

4.1.1.8. 7-Hydroxy-2-imino-2*H*-chromene-3-carboxylic acid benzylamide (4n). Yield: 98%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.50 (2H, d, J = 5.9 Hz), 6.55 (1H, dd, J = 2.2, 6.3 Hz), 7.23–7.35 (5H, m), 7.56 (1H, d, J = 8.5 Hz), 8.42 (1H, s), 10.66 (1H, br).

4.1.1.9. 8-Hydroxy-2-imino-2*H*-chromene-3-carboxylic acid pyridin-2-ylamide (4o). Yield: 78%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.07–7.24 (4H, m), 7.83 (1H, t, J = 7.5 Hz), 8.26 (1H, d, J = 8.5 Hz), 8.33 (1H, d, J = 7.5 Hz), 8.54 (1H, s), 9.14 (1H, br), 10.22 (1H, br), 13.26 (1H, br).

4.1.1.10. 6-Hydroxy-2-imino-2*H*-chromene-3-carboxylic acid pyridin-2-ylamide (4p). Yield: 86%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.99–7.17 (4H, m), 7.84 (1H, t, J = 8.4 Hz), 8.25 (1H, d,

J = 8.4 Hz), 8.3–8.34 (1H, m), 8.52 (1H, s), 9.06 (1H, br), 9.74 (1H, br).

4.1.1.11. 5-Hydroxy-2-imino-2*H*-chromene-3-carboxylic acid pyridin-2-ylamide (4q). Yield: 81%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.36 (1H, d, J = 7.9 Hz), 6.49 (1H, d, J = 8.4 Hz), 7.12 (1H, t, J = 8.4 Hz), 7.24 (1H, t, J = 7.9 Hz), 7.80 (1H, t, J = 7.9 Hz), 8.26–8.32 (2H, m), 13.19 (1H, br).

4.1.1.12. 2-Imino-2*H*-chromene-3-carboxylic acid pyridin-2-ylamide (4r). Yield: 89%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.04–7.27 (4H, m), 7.48–7.55 (2H, m), 7.74 (1H, t, J = 7.9 Hz), 8.36–8.39 (2H, m), 8.57 (1H, s), 13.09 (1H, br).

4.1.2. General procedure for synthesis of compounds (5a–r)

To a stirred solution of chromene (**4**, 1 mmol) in acetic acid (2 mL) was added aniline (1 mmol), and the resulting mixture was stirred at room temperature for 12 h. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel to give **5** as the yellow solid.

4.1.2.1. 7-Hydroxy-2-(4-hydroxyphenylimino)-2*H*-chromene-3-carboxylic acid pyridin-2-ylamide (5a: R = OH). Yield: 52%; mp: >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.62 (1H, s), 6.74 (1H, dd, J = 2.2, 6.1 Hz), 6.82 (2H, t, J = 8.8 Hz), 7.14 (1H, t, J = 7.6 Hz), 7.33 (2H, d, J = 8.8 Hz), 7.65 (1H, d, J = 8.3 Hz), 7.83 (1H, t, J = 7.6 Hz), 8.27 (1H, d, J = 7.6 Hz), 8.36–8.36 (1H, m), 8.54 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 100.11, 101.54, 110.94, 113.26, 113.69, 115.45, 115.95, 119.84, 125.24, 131.58, 134.89, 138.44, 147.58, 148.52, 151.62, 154.85, 154.98, 160.48, 163.15; IR (neat): 3312, 1661, 1574, 1506, 1437 cm^{-1}; IR (KBr): 3396, 1666, 1591, 1549, 1435 cm^{-1}; MS (EI): m/z 373 (M$^+$); HRMS: Calcd for $C_{21}H_{15}N_3O_4$ 373.1063. Found 373.1060.

4.1.2.2. 4-[7-Hydroxy-3-(pyridin-2-ylcarbamoyl)-chromen-2-ylideneamino]-benzoic acid (5b: R = CO_2H). Yield: 34%; mp: >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.51 (1H, s), 6.76 (1H, dd, J = 2.4, 6.1 Hz), 7.14 (1H, t, J = 7.8 Hz), 7.38 (2H, d, J = 8.6 Hz), 7.71 (1H, d, J = 7.8 Hz), 7.84 (1H, t, J = 7.8 Hz), 7.99 (2H, d, J = 8.6 Hz), 8.28–8.35 (2H, m), 8.68 (1H, s), 12.73 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 72.73, 93.77, 101.47, 111.00, 113.74, 115.35, 119.96, 123.09, 130.40, 131.89, 138.50, 143.72, 148.29, 148.47, 150.23, 151.45, 154.91, 160.30, 163.60, 167.17; IR (KBr): 3422, 1680, 1589, 1437 cm^{-1}; MS (EI): m/z 401 (M$^+$); HRMS: Calcd for $C_{22}H_{15}N_3O_5$ 401.1012. Found 401.1013.

4.1.2.3. 7-Hydroxy-2-*p*-tolylimino-2*H*-chromene-3-carboxylic acid pyridin-2-ylamide (5c: R = Me). Yield: 47%; mp: >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.33 (1H, s), 6.76 (1H, dd, J = 2.3, 6.1 Hz), 7.13–7.29 (5H, m), 7.70 (1H, d, J = 8.4 Hz), 7.84 (1H, t, J = 8.2 Hz), 8.28 (1H, d, J = 8.2 Hz), 8.34–8.36 (1H, m), 8.62 (1H, s), 13.04 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.67, 101.39, 110.94, 113.39, 113.71, 115.71, 119.85, 123.29, 129.39, 131.69, 133.78, 138.41, 141.06, 142.61, 148.46, 148.84, 151.54, 154.91, 160.38, 163.20; IR (KBr): 3421, 1676, 1593, 1551, 1437 cm^{-1}; MS (EI): m/z 371 (M$^+$); HRMS: Calcd for $C_{22}H_{17}N_3O_3$ 371.1270. Found 371.1273.

4.1.2.4. 2-(4-Bromophenylimino)-7-hydroxy-2*H*-chromene-3-carboxylic acid pyridin-2-ylamide (5d: R = Br). Yield: 49%; mp: >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.56 (1H, s), 6.78 (1H, d, J = 8.4 Hz), 7.15 (1H, t, J = 7.5 Hz), 7.31 (2H, d, J = 8.7 Hz), 7.60 (2H, d, J = 8.7 Hz), 7.72 (1H, d, J = 8.4 Hz), 7.85 (1H, t, J = 7.5 Hz), 8.28 (1H, d, J = 7.5 Hz), 8.32–8.34 (1H, m), 8.67 (1H, s), 12.80 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 101.45, 111.00, 113.60, 113.71, 115.53, 116.70, 119.92, 125.45, 131.78, 138.44, 143.21, 143.29, 148.44, 149.83, 149.85, 151.45, 154.84, 160.25, 163.32; IR (KBr): 3317, 1682, 1558, 1435 cm^{-1}; MS (EI): m/z 435 (M$^+$); HRMS: Calcd for $C_{21}H_{14}BrN_3O_3$ 435.0219. Found 435.0226.

4.1.2.5. 7-Hydroxy-2-(4-iodophenylimino)-2*H*-chromene-3-carboxylic acid pyridin-2-ylamide (5e: R = I). Yield: 60%; mp: >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.55(1H, s), 6.78 (1H, dd, J = 2.2, 6.3 Hz), 7.13–7.18 (3H, m), 7.71–7.77 (3H, m), 7.84 (1H, t, J = 8.1 Hz), 8.27 (1H, d, J = 8.1 Hz), 8.32–8.34 (1H, m), 8.66 (1H, s), 12.79 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 100.11, 101.46, 111.12, 113.60, 113.72, 115.56, 119.91, 125.72, 131.80, 137.65, 138.46, 143.28, 143.66, 148.45, 149.79, 151.45, 154.85, 160.27, 163.33; IR (KBr): 3442, 1686, 1595, 1541, 1435 cm^{-1}; MS (EI): m/z 483 (M$^+$); HRMS: Calcd for $C_{21}H_{14}IN_3O_3$ 483.0080. Found 483.0074.

4.1.2.6. {4-[7-Hydroxy-3-(pyridin-2-ylcarbamoyl)chromen-2-ylideneamino]phenyl}-acetic acid (5f: R = CH_2CO_2H). Yield: 61%; mp: >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.57 (2H, s), 6.51 (1H, s), 6.73 (1H, d, J = 8.7 Hz), 7.14 (1H, t, J = 7.3 Hz), 7.28–7.32 (4H, m), 7.67 (1H, d, J = 8.7 Hz), 7.83 (1H, t, J = 7.3 Hz), 8.27 (1H, d, J = 7.3 Hz), 8.33–8.35 (1H, m), 8.62 (1H, s), 13.00 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 40.92, 48.63, 101.52, 110.69, 113.71, 115.26, 119.81, 123.13, 129.90, 131.69, 131.79, 138.39, 142.15, 142.82, 148.46, 149.16, 151.57, 155.01, 160.42, 163.94, 173.34; IR (KBr): 3402, 1668, 1543, 1437, 1229 cm^{-1}; MS (EI): m/z 415 (M$^+$); HRMS: Calcd for $C_{23}H_{17}N_3O_5$ 415.1168. Found 415.1172.

4.1.2.7. 7-Hydroxy-2-phenylimino-2*H*-chromene-3-carboxylic acid pyridin-2-ylamide (5g: R = H). Yield: 52%; mp: 294–295 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.51 (1H, s), 6.76 (1H, dd, J = 2.4, 6.2 Hz), 7.13–7.21 (2H, m), 7.32–7.46 (4H, m), 7.70 (1H, d, J = 8.6 Hz), 7.86 (1H, t, J = 8.0 Hz), 8.29 (1H, d, J = 8.0 Hz), 8.32–8.34 (1H, m), 8.65 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 101.38, 110.86, 113.50, 113.71, 115.49, 119.80, 123.15, 124.52, 128.88, 131.68, 138.44, 142.95, 143.84, 148.32, 149.28, 151.52, 154.90, 160.34, 163.40; IR (KBr): 3442, 1684, 1582, 1541, 1437 cm^{-1}; MS (EI): m/z 357 (M$^+$); HRMS: Calcd for $C_{21}H_{15}N_3O_3$ 357.1113. Found 357.1110.

4.1.2.8. 7-Hydroxy-2-(4-methoxyphenylimino)-2*H*-chromene-3-carboxylic acid iso-propylamide (5h: R_1 = *i*-Pr). Yield: 46%; mp: 229–231 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.18 (6H, d, J = 6.6 Hz), 3.99 (1H, sept, J = 6.6 Hz), 6.55 (1H, s), 6.71 (1H, d, J = 8.3 Hz), 6.94 (2H, d, J = 8.5 Hz), 7.30 (2H, d, J = 8.5 Hz), 7.58 (1H, d, J = 8.3 Hz), 8.37 (1H, s), 10.18 (1H, d, J = 6.8 Hz), 10.72 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.35, 22.45, 55.21, 101.33, 111.01, 112.97, 114.05, 116.84, 124.88, 131.08, 136.79, 140.51, 148.14, 154.56, 156.36, 160.74, 162.34; IR (KBr): 3321, 1655, 1570, 1558, 1506, 1246 cm^{-1}; MS (EI): m/z 352 (M$^+$); HRMS: Calcd for $C_{20}H_{20}N_2O_4$ 352.1423. Found 352.1426.

4.1.2.9. 7-Hydroxy-2-(4-methoxyphenylimino)-2*H*-chromene-3-carboxylic acid butyl-amide (5i: R_1 = *n*-Bu). Yield: 40%; mp: 257–259 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.89 (3H, t, J = 7.1 Hz), 1.33 (2H, sext, J = 7.1 Hz), 1.49 (2H, quint, J = 7.1 Hz), 3.35 (2H, t, J = 7.1 Hz), 3.74 (3H, s), 6.51 (1H, s), 6.70 (1H, d, J = 8.1 Hz), 6.93 (2H, d, J = 8.9 Hz), 7.26 (2H, d, J = 8.9 Hz), 7.56 (1H, d, J = 8.1 Hz), 8.35 (1H, s), 10.15 (1H, d, J = 5.1 Hz), 10.64 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.56, 19.71, 30.98, 55.13, 55.19, 101.29, 110.97, 112.90, 113.93, 114.00, 116.86, 124.72, 131.00, 136.91, 140.50, 148.21, 154.52, 156.25, 161.53, 162.27, 164.80; IR (KBr): 3373, 1665, 1570, 1504, 1246 cm^{-1}; MS (EI): m/z 366 (M$^+$); HRMS: Calcd for $C_{21}H_{22}N_2O_4$ 366.1580. Found 366.1585.

4.1.2.10. 7-Hydroxy-2-(4-methoxphenylino)-2*H*-chromene-3-carboxylic acid isobutyl-amide (5j: R_1 = *i*-Bu). Yield: 62%; mp: >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94 (6H, d, J = 6.6 Hz), 1.81 (1H, m), 2.08 (2H, d, J = 6.6 Hz), 3.77 (3H, s), 6.54 (1H, s), 6.72 (1H, d, J = 8.7 Hz), 6.95 (2H, d, J = 7.7 Hz), 7.28 (2H, d, J = 7.7 Hz), 7.60 (1H, d, J = 8.7 Hz), 8.39 (1H, s), 10.25 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.12, 27.99, 30.71, 46.35, 55.22, 101.34, 110.83, 113.15, 114.06, 116.67, 124.66, 131.12, 136.95, 140.72, 148.46, 154.61, 156.28, 161.70, 162.76; IR (KBr): 3420, 1663, 1558, 1506, 1244 cm^{-1}; MS (EI): m/z 366 (M$^+$); HRMS: Calcd for $C_{21}H_{22}N_2O_4$ 366.1580. Found 366.1584.

4.1.2.11. 7-Hydroxy-2-(4-methoxy-phenylimino)-2*H*-chromene-3-carboxylic acid cyclohexylamide (5k: R_1 = *c*-Hex). Yield: 63%; mp: 240–242 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.31–1.84 (10H, m), 3.76 (3H, s), 3.79–3.81 (1H, m), 6.54 (1H, s), 6.71 (1H, dd, J = 2.2, 6.3 Hz), 6.95 (2H, d, J = 9.1 Hz), 7.29 (2H, d, J = 9.1 Hz), 7.59 (1H, d, J = 8.5 Hz), 8.38 (1H, s). 10.32 (1H, d, J = 7.8 Hz), 10.67 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.81, 25.22, 32.45, 55.22, 101.32, 111.04, 112.98, 114.09, 116.88, 124.81, 131.11, 136.79, 140.62, 148.28, 154.55, 156.35, 160.59, 162.33; IR (KBr): 3450, 1653, 1558, 1506 cm^{-1}; MS (EI): m/z 392 (M$^+$); HRMS: Calcd for $C_{23}H_{24}N_2O_4$ 392.1736. Found 392.1740.

4.1.2.12. 7-Hydroxy-2-(4-methoxyphenylimino)-2*H*-chromene-3-carboxylic acid (2-hydroxyethyl)amide (5l: $R_1 = (CH_2)_2$ OH). Yield: 80%; mp: 213–214 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.39 (2H, t, J = 5.2 Hz), 3.54 (2H, t, J = 5.2 Hz), 3.77 (3H, s), 4.86 (1H, br), 6.56 (1H, s), 6.71 (1H, dd, J = 2.2, 6.1 Hz), 6.94 (2H, d, J = 9.0 Hz), 7.37 (2H, d, J = 9.0 Hz), 7.60 (1H, d, J = 8.3 Hz), 8.40 (1H, s), 10.41 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 41.75, 55.19, 59.73, 101.33, 110.99, 112.92, 113.91, 116.97, 125.15, 131.08, 136.81, 140.49, 147.77, 154.59, 156.33, 161.70, 162.30; IR (KBr): 3368, 1661, 1559, 1506 cm^{-1}; MS (EI): m/z 354 (M$^+$); HRMS: Calcd for $C_{19}H_{18}N_2O_5$ 354.1216. Found 354.1213.

4.1.2.13. 7-Hydroxy-2-(4-methoxyphenylimino)-2*H*-chromene-3-carboxylic acid (3-hydroxypropyl)amide (5m: $R_1 = (CH_2)_3$-OH). Yield: 72%; mp: 229–231 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.67 (2H, quint, J = 6.6 Hz), 3.38 (2H, d, J = 6.6 Hz), 3.51 (2H, d, J = 6.6 Hz), 3.77 (3H, s), 4.56 (1H, br), 6.53 (1H, s), 6.71 (1H, dd, J = 2.3, 6.2 Hz), 6.95 (2H, d, J = 6.7 Hz), 7.32 (2H, d, J = 6.7 Hz), 7.60 (1H, d, J = 8.5 Hz), 8.39 (1H, s), 10.188 (1H, t, J = 5.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 32.11, 36.21, 55.16, 58.53, 101.29, 110.98, 112.91, 113.92, 113.94, 116.88, 124.83, 131.04, 136.93, 140.09, 148.09, 154.54, 156.25, 161.70, 162.28; IR (KBr): 3371, 1665, 1570, 1504, 1242 cm^{-1}; MS (EI): m/z 368 (M$^+$); HRMS: Calcd for $C_{20}H_{20}N_2O_5$ 368.1372. Found 368.1371.

4.1.2.14. 7-Hydroxy-2-(4-methoxyphenylimino)-2*H*-chromene-3-carboxylic acid benzylamide (5n: R_1 = Bn). Yield: 40%; mp: 257–259 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.75 (3H, s), 4.55 (2H, d, J = 5.9 Hz), 6.52 (1H, s), 6.71 (1H, dd, J = 2.2, 6.2 Hz), 6.91–6.93 (2H, m), 7.24–7.26 (3H, m), 7.32–7.36 (4H, m), 7.61 (1H, d, J = 8.4 Hz), 8.42 (1H, s), 10.55 (1H, t, J = 5.9 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 42.78, 55.20, 101.35, 110.93, 113.06, 113.96, 116.74, 124.81, 126.95, 127.25, 128.48, 131.17, 136.86, 139.07, 140.86, 148.21, 154.66, 156.28, 161.92, 162.58; IR (KBr): 3407, 1661, 1558, 1506 cm^{-1}; MS (EI): m/z 400 (M$^+$); HRMS: Calcd for $C_{24}H_{20}N_2O_4$ 400.1423. Found 400.1426.

4.1.2.15. 8-Hydroxy-2-(4-methoxy-phenylimino)-2*H*-chromene-3-carboxylic acid pyridin-2-ylamide (5o: R_1 = OH, R_2 = H, R_3 = H). Yield: 88%; mp: 253–255 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.80 (3H, s), 7.02 (2H, d, J = 8.9 Hz), 7.12–7.19 (3H, m), 7.27 (1H, d, J = 6.6 Hz), 7.75 (2H, d, J = 8.9 Hz), 7.87 (1H, t, J = 7.9 Hz), 8.41 (1H, d, J = 7.9 Hz), 8.41–8.43 (1H, m), 8.59 (1H, s), 13.48 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 55.26, 113.73, 114.05, 119.62, 119.66, 119.85, 119.98, 120.55, 124.60, 126.36, 135.96, 138.46, 141.54, 141.57, 144.37, 147.10, 148.60, 151.51, 153.14, 157.01, 159.99; IR (KBr): 3369, 1678, 1601, 1506, 1435 cm^{-1}; MS (EI): m/z 387 (M$^+$); HRMS: Calcd for $C_{22}H_{17}N_3O_4$ 387.1219. Found 387.1214.

4.1.2.16. 6-Hydroxy-2-(4-methoxy-phenylimino)-2*H*-chromene-3-carboxylic acid pyridin-2-ylamide (5p: R_1, R_3 = H, R_2 = OH). Yield: 59%; mp:>300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.79 (3H, s), 6.99–7.04 (3H, m), 7.15–7.18 (3H, m), 7.45 (2H, d, J = 8.9 Hz), 7.87 (1H, t, J = 8.1 Hz), 8.28 (1H, d, J = 8.1 Hz), 8.37–8.39 (1H, m), 8.60 (1H, s), 9.80 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 55.24, 113.77, 114.15, 114.27, 116.15, 119.14, 120.02, 120.52, 121.11, 125.14, 136.38, 138.49, 141.73, 146.30, 148.18, 148.55, 151.45, 153.96, 156.63, 160.03; IR (KBr): 3312, 1661, 1574, 1506 cm^{-1}; MS (EI): m/z 387 (M$^+$); HRMS: Calcd for $C_{22}H_{17}N_3O_4$. Found 387.1214.

4.1.2.17. 5-Hydroxy-2-(4-methoxy-phenylimino)-2*H*-chromene-3-carboxylic acid pyridin-2-ylamide (5q: R_1, R_2 = H, R_3 = OH). Yield: 49%; mp: 294–296 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.79 (3H, s), 6.71 (1H, d, J = 8.6 Hz), 6.74 (1H, d, J = 8.6 Hz), 7.01 (2H, d, J = 8.8 Hz), 7.16 (1H, t, J = 7.3 Hz), 7.39–7.45 (3H, m), 7.86 (1H, t, J = 7.3 Hz), 8.28 (1H, d, J = 7.3 Hz), 8.36–8.38 (1H, m), 8.75 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 55.22, 105.66, 107.98, 110.57, 113.73, 114.13, 117.86, 119.94, 125.09, 134.38, 136.28, 136.61, 138.45, 147.79, 148.52, 151.46, 153.82, 156.09, 156.65, 160.09; IR (KBr): 3319, 1684, 1541, 1506 cm^{-1}; MS (EI): m/z 387 (M$^+$); HRMS: Calcd for $C_{22}H_{17}N_3O_4$ 387.1219. Found 387.1221.

4.1.2.18. 2-(4-Methoxy-phenylimino)-2*H*-chromene-3-carboxylic acid pyridin-2-ylamide (5r: R_1, R_2, R_3 = H). Yield: 75%; mp: 249–250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.83 (3H, s), 6.94 (2H, d, J = 9.0 Hz), 7.03 (1H, t, J = 6.7 Hz), 7.14–7.24 (2H, m), 7.45–7.52 (4H, m), 7.72 (1H, t, J = 6.7 Hz), 8.32–8.34 (2H, m). 8.56 (1H, s), 13.25 (1H, br); ^{13}C NMR (100 MHz, DMSO-d_6) δ 55.46, 114.00, 114.78, 115.47, 119.07, 119.75, 121.47, 124.45, 125.55, 129.43, 132.85, 136.56, 137.80, 141.15, 147.42, 148.38, 151.89, 153.55, 157.17, 160.56; IR (KBr): 1676, 1541, 1506, 1437 cm^{-1}; MS (EI): m/z 371 (M$^+$); HRMS: Calcd for $C_{22}H_{17}N_3O_3$ 371.1270. Found 371.1271.

4.2. Biological assays

4.2.1. Preparation of recombinant enzymes

Recombinant AKR1B1[22] and AKR1B10 with the N-terminal 6-His tag,[14] and Trp220Tyr and Gln303Ser mutant AKR1B10s[23] were expressed in *Escherichia coli* cells harboring their cDNAs, and purified to homogeneity, as described previously.

4.2.2. Assay of enzyme activity

The reductase and dehydrogenase activities of the enzymes were determined at 25 °C by measuring the rate of change in NADPH absorbance (at 340 nm) and fluorescence (at 455 nm with an excitation wavelength of 340 nm), respectively.[14] The IC_{50} values for inhibitors were determined in the reaction mixture that consisted of 0.1 M potassium phosphate, pH 7.4, 0.1 mM NADPH, 0.2 mM pyridine-3-aldehyde (approximately 15 × K_m concentration), and enzyme, in a total volume of 2.0 mL. The inhibitor constant, K_i, for **5n** was determined by kinetic analysis in the NADP$^+$-linked geraniol oxidation, because the reaction of AKR1B10 follows an order bi bi mechanism[14] and many competitive inhibitors including **1** show mixed-type or noncompetitive

inhibition with respect to pyridine-3-aldehyde in the reduction reaction.[10,11,13,14,17,18] The K_i value was estimated from the replots of the slopes and intercepts of double reciprocal plot of the five geraniol concentrations versus velocities, which were determined in the presence of a saturating $NADP^+$ concentration (0.25 mM) and three concentrations of the inhibitor. The IC_{50} and K_i values are expressed as the means of at least three determinations.

4.2.3. Molecular modeling and energy minimization

The coordinates for AKR1B10 (PDB code: 1ZUA)[24] were obtained from the RCSB Protein Data Bank. The structure was prepared using the Maestro (Schrödinger, LLC, Portland, OR) software package Version 8.5, as described previously.[14] The docking calculations were performed using the program Glide 5.043 on a Linux workstation under the conditions described previously.[14] The docked models shown in Figure 1 were generated using PyMOL (DeLano Scientific, San Carlos, CA, USA).

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