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SYNTHESIS AND BIOLOGICAL EVALUATION OF NMDI14 DERIVATIVES AS ANTI-MESOTHELIOMA AGENTS

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Abstract – Mesothelioma is a severe tumor formed in pleura and peritoneum, for which no useful molecular-targeting therapy is available. We synthesized several derivatives of NMDI14, which is a reported inhibitor for non-sense mediated mRNA decay, and evaluated the activity of the NMDI14 derivatives as potential anti-mesothelioma agents. Some of the synthesized compounds showed promising activity in terms of cytotoxicity toward mesothelioma model cells and promotion of GAS5 expression selectively in mesothelioma cells. These results indicate that the NMDI14 derivatives may be useful for further developing clinically effective anti-mesothelioma drugs.

Malignant mesothelioma is a refractory pleural-derived tumor mainly caused by asbestos exposure, and there is a concern that the number of cases with malignant mesothelioma will increase in the future.[1] This cancer is extremely resistant to chemotherapy and radiation therapy, and there are no effective molecular target drugs to date. Hence, the development of molecular targeted drugs for mesothelioma cancer is strongly required. Murakami *et al*. found that mesothelioma is related to mutations of LATS (large tumor suppressor homolog) family proteins in the Hippo signaling pathway, which controls organ size by regulating cell proliferation and apoptosis.[2] The authors found that LATS1/2 plays a critical role in

regulating cell proliferation and/or survival of the cancerous mesothelium. When LATS1/2 is mutated, the tumor-suppressive Hippo signaling pathway is activated (Figure 1A). The authors also reported that knockdown of LATS2 significantly blocked phosphorylation of YAP with neurofibromatosis type 2 (NF2) transduction, suggesting that LATS2 is a crucial mediator of the Hippo signaling pathway.[2]

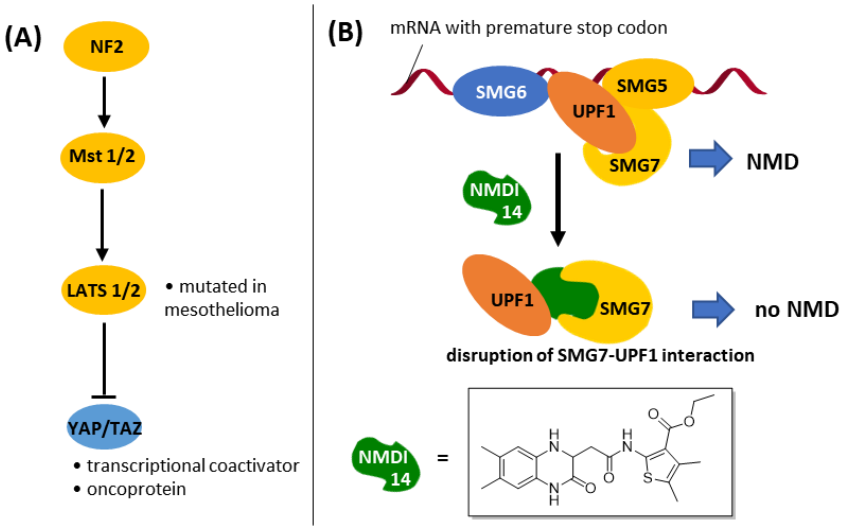


Figure 1. (A) Simplified overview of the Hippo signaling pathway. (B) Molecular mechanism of non-sense mediated mRNA decay (NMD).

One of the characteristics of mesothelioma cells is that they show lower expression of growth arrest specific transcript 5 (GAS5), a non-coding RNA relevant to the cell cycle, compared with normal cells. Renganathan *et al.* reported that GAS5 upregulation is accompanied by cell growth arrest and knockdown of GAS5 resulted in shortening of the cell cycle.[3] Based on these reports, we envisioned that molecules that upregulate GAS5 expression may be molecular target drugs for mesothelioma. Furthermore, Colombo *et al*. reported that inhibition of nonsense-mediated mRNA decay (NMD)[4] led to the upregulation of GAS5.[5] Gardner *et al*. reported that the small molecule NMDI14 is a potent inhibitor for NMD, and NMDI14 induced readthrough of premature termination codons (PTC) in cells with PTC-mutated p53 and restored full-length p53 production.[6] The authors proposed that NMDI14 binds to SMG7, a key protein in the NMD mechanism, thus preventing interactions with the UPF1 RNA helicase,[7] one of the important components in NMD (Figure 1B).[8] Based on these studies, here we explored derivatives of NMDI14 with the aim of developing new types of anti-mesothelioma agents.

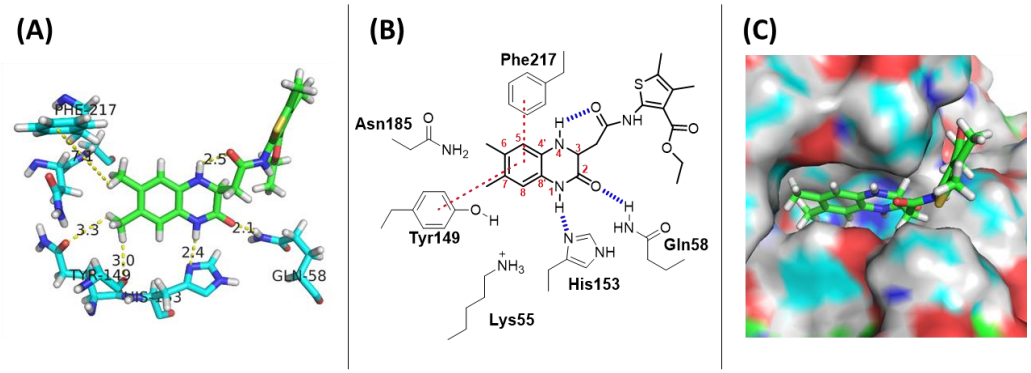


Figure 2. (A) Expected interaction between SMG7 and NMD14. The distance between the structures as described as value with Å unit. (B) Notable interaction between NMDI14 and amino acid side chains in NMDI14. Hydrophobic interaction and hydrogen bonding interaction are depicted as a red dashed line and blue dashed line, respectively. (C) Calculated binding mode of NMDI14 to SMG7. SMG7 is depicted in van der Waals surface format with red as electronically positive sites, blue as electronically negative sites, and light blue as hydrophobic sites.

A previous study reported that NMDI14 binds to SMG7 for NMD inhibition, but the precise NMD14-binding site in SMG7 was not identified.[6] To determine the moiety essential for NMDI14 binding to SMG7, we performed molecular docking study using the reported crystallographic structure of SMG7.[8a] The calculated binding mode of NMDI14 and SMG7 is described in Figure 2. The predicted binding mode suggested that the phenyl ring in quinoxalin-2(1*H*)-one might have π-π stacking interaction with Phe217 and Tyr149 (Figures 2A, 2B). Furthermore, the amino in the 1-position serves as the hydrogen donor to His153, while the nearby carboxyl group in the 2-position behaves as the hydrogen acceptor for Gln58. Those two hydrogen bonding interactions might be important for NMDI14 to bind to SMG7. Additionally, the amino group in the 4-position might have intramolecular hydrogen bonding with the carbonyl group in the amide side chain. This intramolecular hydrogen bonding would fix the conformation of NMDI, which is preferable for binding to SMG7. However, the function of the amide side chain in this binding interaction has not been determined (Figure 2C).

Based on these results, we presumed that the heterocycle quinoxalin-2(1*H*)-one represents the core structure for NMDI14 to bind with SMG7. A previous study[6] reported that NMDI14 binding to SMG7 interrupted the interaction between SMG7 and UPF-1, which leads to NMD inhibition. Although the presumed binding mode did not reveal the role of the amide side chain, we hypothesized that the amide side chain moiety may play an important role in preventing the interaction between UPF1 and SMG7. Therefore, a series of NMDI14 derivatives with 6,7-dimethyl-3,4-dihydroquinoxalin-2(1*H*)-one as the

core structure were designed and synthesized for higher NMD inhibitory activity (Figure 3A).

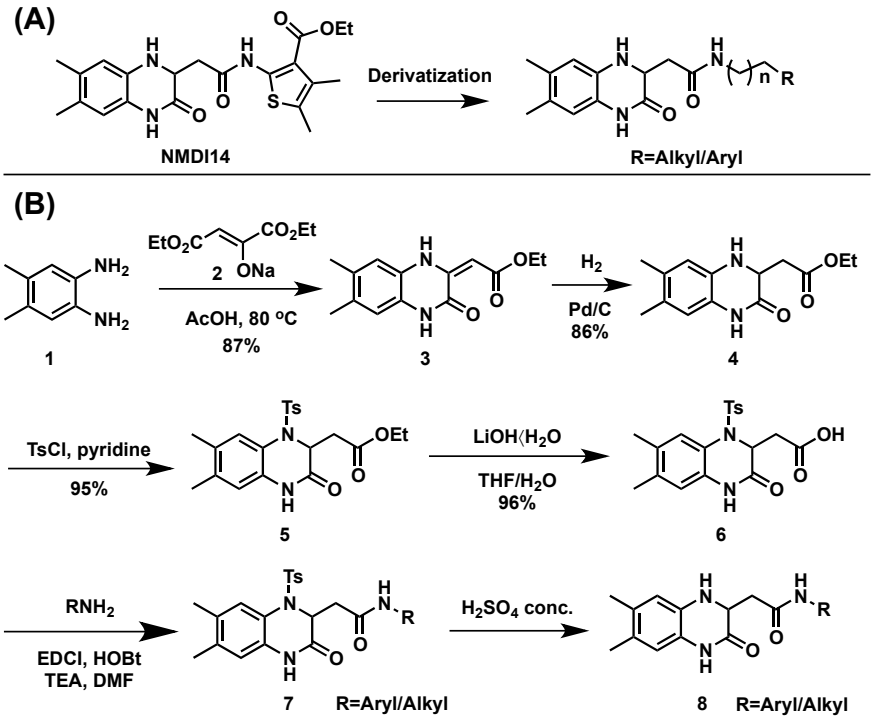
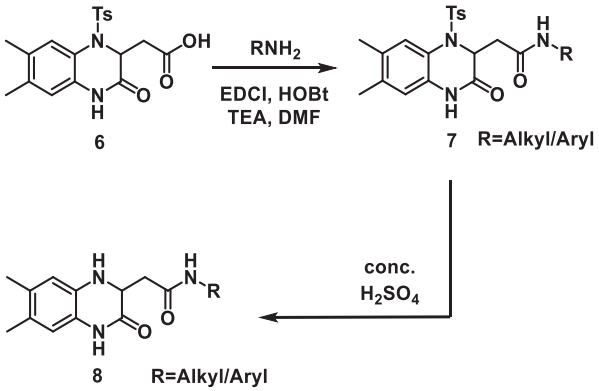


Figure 3. (A) Derivatization of NMDI14 at amide side chains. (B) Synthetic schemes for NMDI14 derivatives.

We proposed a synthetic route with 6-step conversion with 4,5-dimethylphenyl-1,2-diamine as the commercially available starting material (Figure 3B). To determine at an early stage whether the derivatization of side chains is effective for inhibitory activity, the target derivatives were planned to be synthesized as a racemic mixture in this study. There are many synthetic methods for the quinoxalin-2(1*H*)-one structure.[9] We selected the most straightforward methods for preparation of the core structure, a condensation reaction with 4,5-dimethylphenyl-1,2-diamine **1** and 1,2-dicarbonyl species or its equivalents. For the first trial, we performed a condensation reaction between 4,5-dimethylphenyl-1,2-diamine **1** with maleic anhydride under refluxing in THF as the solvent and BHT (15 mol%) as the antioxidant to prevent oxidative decarboxylation (Scheme S1).[10] However, during the purification process, the expected product quinoxalin-2-one **S1** was not obtained and instead the decarboxylation product **S2** was the main reaction product. This result suggested that the quinoxalin-2-one **S1** is very unstable under air, which was contradictory to some previous studies.[10] After several trials, diethyl oxaloacetate was condensed with 4,5-dimethylphenylenediamine to give stable product **3** as a yellow solid with excellent yield.[11] In the subsequent step, compound **3** was hydrogenated

with H_2, Pd/C (15% w/w) to obtain intermediate **4** in quantitative yield. Because compound **4** easily decomposes under air, presumably because of the free H at the N^4-position, there is a need to introduce a protecting group to the amino group. However, the similarity between N^1 and N^4 as well as the air-sensitivity of **4** makes it difficult to choose the proper protecting group. Nosyl was first chosen as the protecting group because of its tolerance to both acidic and basic conditions and mild deprotection conditions. However, the reaction consumed the starting material but yielded none of the desired product. We next tried to employ carbamates as the protection group. In the first trial, Boc was chosen because of its ease of introduction and its inertness to many nucleophilic reagents. However, the reaction with Boc_2O under several conditions consumed the starting material but gave no desired product. When increasing the equivalent of reagent (Boc_2O) and base, as well as lengthening the reaction time, amide-protected product was obtained as the major product. In the second trial, carboxybenzyl (Cbz) was chosen. However, similarly to the Boc reaction, no desired product was obtained, and instead only decomposed product was generated. Finally, tosyl was introduced using pyridine as the solvent to obtain the intermediate **5** with a high yield.

In the next step, ester **5** was hydrolyzed under basic conditions to obtain carboxylic acid **6** in high yield. As summarized in Figure 4, intermediate **6** was subjected to the coupling reaction with various commercially available amines to give the coupling adducts. Initially, the amide coupling reaction was performed with the simple *n*-propylamine to afford the desired product **7a**, followed by coupling with some (hetero) aromatic amines. Finally, deprotection of tosyl group by concentrated sulfuric acid yielded NMDI14 derivatives. Unfortunately, compound **8a** was too unstable even for storage, thus its biological activity could not be evaluated.



Entry	R	7 (yield)	8 (yield)
1		**7a** (75%)	**8a** (78%)
2		**7b** (69%)	**8b** (94%)
3		**7c** (55%)	**8c** (24%)
4		**7d** (69%)	**8d** (43%)
5		**7e** (73%)	**8e** (59%)
6		**7f** (64%)	**8f** (75%)

Figure 4. Reactions for the synthesis of NMDI14 derivatives **8a**–**8f** and reaction yields

With five NMDI14 derivatives in hand, cellular experiments were performed. First, to evaluate the

anticancer activity of the NMDI14 derivatives, viability tests were performed with normal mesothelial HOMC D4 cells in which LATS1/2 was knocked down with shRNA (mesothelioma model cells). The cells were treated with 5 or 10 μM of NMDI14 analogs and viability was assessed (Figure 5).

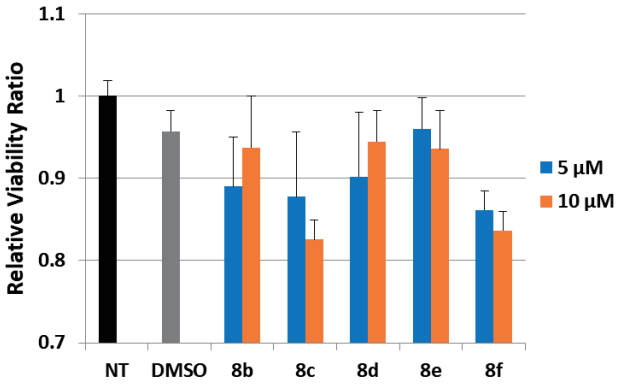


Figure 5. Evaluation of NMDI14 derivatives for cytotoxicity in mesothelioma model cells

Although the activity was not so obvious, compounds **8c** and **8f** showed dose-dependent cytocide activity toward the mesothelioma model cells, suggesting that these compounds are promising candidates. Thus, we performed further investigations of the effects of the two agents on GAS5 expression. In the control stable cell line with nontarget shRNA treatment, NMDI14 strongly promoted GAS5 expression, while **8c** and **8f** moderately induced GAS5 expression (Figure 6).

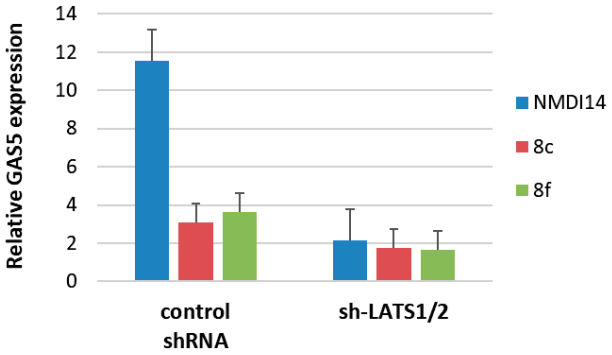


Figure 6. Evaluation of NMDI14 derivatives for effects on GAS5 expression. Control shRNA, cells treated with non-targeting shRNA; sh-LATS1/2, cells treated with LATS1/2 shRNA.

In the mesothelioma model cells, GAS5 expression was moderately increased to similar levels in response to NMDI14, **8c**, and **8f**. Upregulation of GAS5 in normal cells is not desirable because it would result in cell growth arrest; hence, compounds **8c** and **8f** are preferable as anti-mesothelioma agents

compared with NMDI14, because they resulted in weak upregulation of GAS5 expression in normal cells. The compounds **8c** and **8f** are promising in terms of GAS5 upregulation profiles; therefore, if we could obtain derivatives of **8c** and **8f** with stronger inhibition on cell viability, they would be promising anti-mesothelioma drugs without side effects like cytotoxicity to normal cells.

In summary, we developed new NMDI14 derivatives that moderately upregulate GAS5 expression in mesothelioma model cells. The promising agents **8c** and **8f** showed much lower induction of GAS5 expression in normal cells compared with their parent compound NMDI14, a feature that is desirable for anti-mesothelioma agents in showing lower negative side effects in normal cells. Thus, **8c** and **8f** are promising lead compounds for anti-mesothelioma agents. Investigations on further derivatization for higher activity and the mechanism of difference in activity profiles of **8c**, **8f** and NMDI are currently ongoing. In the future study for molecular optimization, the effect of chirality on activity will also be investigated in detail.

EXPERIMENTAL

All the reactions were carried out under Argon atmosphere. Unless otherwise noted, all the reagent obtained from commercial sources were used without purification. Dichrolomethane (DCM), pyridine, *N,N*-dimethylformamide (DMF), and acetic acid used as a reaction solvent were dried with molecular sieves and used. Commercially available Argon gas was used as it was. Thin-layer chromatography (TLC) were performed on Wako Silicagel 70 F-254 precoated silica gel plates. Column chromatography was carried out with Kishida neutral spherical silicagel 32 to 63 μm (230 to 430 mesh). The TLC plates were visualized with UV lamp (254 nm and 366 nm) and/or with TLC visualizing solutions activated with heat including: *p*-anisaldehyde solution. Mass spectrometry (MS) spectra were measured using Bruker Daltonics micrOTOF-QII (ESI-electrospray ionization). NMR spectra were measured using JEOL NMR-ECS 400-A (400 MHz) and JEOL NMR-ECS 400-B (400 MHz). The chemical shift value of ^{1}H NMR was calculated by using tetramethylsilane (TMS, $Si(Me)_4$ 0.00 ppm) as the internal standard, and deuterated chloroform ($CDCl_3$ 7.26 ppm) and DMSO-d_6 (2.50 ppm) as the measuring solvents. The chemical shift value of ^{13}C NMR were indicated as signals derived from the measuring solvent ($CDCl_3$ 77.00 ppm, DMSO-d_6 39.52 ppm). Chemical shifts were reported in parts per million (ppm). The following abbreviations were used to explain the multiplicities: s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet. Data were reported as follow: chemical shift, multiplicity, coupling constants (Hertz: Hz), and integration.

Ethyl 2-(6,7-Dimethyl-3-oxo-3,4-dihydroquinoxalin-2-yl)acetate (3): To a solution of sodium diethyl oxaloacetate (2.31 g, 11.01 mmol, 1 eq.) in acetic acid (5 mL) was added

4,5-dimethyl-1,2-phenylenediamine (1.5 g, 11.01 mmol, 1 eq.) under Ar atmosphere. The mixture was heated to 80 ^{o}C for 2.5 h. During the reaction, stirring was stopped upon complete dissolution of the starting compounds. After cooling to room temperature, Et_2O was added (100 mL) to the suspension, before it was shaken vigorously. The acicular product was filtered off by suction, washed with Et_2O (2×7 mL), water (3×7 mL) and Et_2O (4×7 mL). Finally, the solvent was removed under vacuum, give the 2.49 g product as the yellow solid (Yield 87%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.97 (s, 1H, NH), 7.10 (s, 1H, aromatic), 6.81 (s, 1H, aromatic), 5.43 (s, 1H), 4.14 (t, *J* = 7.1 Hz, 1H), 4.07 (t, *J* = 7.1 Hz, 2H), 3.74 (s, 2H), 2.27 (s, 3H, CH_3), 2.16 (s, 3H, CH_3). ^{13}C NMR (99 MHz, DMSO-*d*$_6$) δ 170.41, 166.47, 131.58, 130.11, 125.47, 123.81, 116.05, 115.19, 60.15, 52.85, 39.52, 36.49, 19.05, 18.69, 14.06. HRMS (ESI) calcd. for $C_{14}H_{16}N_2O_3$ $(M+H)^+$; 261.1233, found; 261.0360. Various other spectral data showed good agreement with the data described in the reference.[11]

Ethyl 2-(6,7-Dimethyl-3-oxo-1,2,3,4-tetrahydroquinoxalin-2-yl)acetate (4): 1.2 g of compound **3** (4.58 mmol, 1 eq.) was dissolved in 40 mL of DCM and MeOH (DCM:MeOH = 3:1), then 120 mg of Pd/C (15% w/w) was added. The resulting solution was then bubbled with H_2 in 1 h and kept stirring in H_2 atmosphere overnight. The reaction mixture was filtered through a pad of celite and concentrated in vacuo to give compound **4** as the bright yellow solid (m = 1.024 g, yield 85%), which was used without further purification. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.17 (bs, 1H, NH), 6.51 (s, 1H, aromatic), 6.49 (s, 1H, aromatic), 5.75 (bs, 1H, aromatic), 4.05 (q, *J* = 7.1 Hz, 3H, H_3, CH_2), 2.68 (dd, *J* = 15.8, 5.7 Hz, 1H, CH_2), 2.56 (dd, *J* = 15.8, 6.6 Hz, 1H, CH_2), 2.05 (d, *J* = 3.6 Hz, 6H, $2CH_3$), 1.17 (t, *J* = 7.1 Hz, 3H, CH_3) ^{13}C NMR (99 MHz, DMSO-*d*$_6$) δ 170.41, 166.47, 131.58, 130.11, 125.47, 123.81, 116.05, 115.19, 60.15, 52.85, 39.52, 36.49, 19.05, 18.69, 14.06. HRMS (ESI) calcd. for $C_{14}H_{18}N_2O_3$ $(M+H)^+$; 263.1389, found; 263.0617.

Ethyl 2-(6,7-Dimethyl-3-oxo-1-tosyl-1,2,3,4-tetrahydroquinoxalin-2-yl)acetate (5): A solution of **4** (1.00 g, 3.81 mmol, 1 eq.) in 20 mL anhydrous pyridine was added tosyl chloride (1.45 g, 7.63 mmol, 2 eq.). The resulting solution was stirred at room temperature overnight. The reaction mixture was concentrated to reduce solvent, followed by diluting with DCM (20 mL) then extracted with aqueous $CuSO_4$ 10% (1×20 mL), saturated aqueous $NaHCO_3$ solution (1×20 mL), H_2O (1×20 mL) and brine (1×20 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered off, and evaporated to obtain crude product. The residue was purified by short column chromatography (Hex/EtOAc = 2/1) to give 1.51g compound **5** as the off-white solid (Yield 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H, NH), 7.44 (s, 1H, aromatic), 7.31 (d, *J* = 8.4 Hz, 2H, SO_2Ph), 7.08 (d, *J* = 8.4 Hz, 2H, SO_2Ph), 6.43 (s, 1H, aromatic), 5.18 (q, *J* = 5.0 Hz, 1H, H_3), 4.22-4.13 (m, 2H, CH_2), 2.55 (dd, *J* = 14.5, 4.8 Hz, 1H, CH_2),

2.38 (dd, *J* = 14.7, 10.2 Hz, 1H, CH_2), 2.27 (s, 6H, 2 CH_3), 2.23 (s, 3H, CH_3), 1.32-1.26 (t, *J* = 7.1 Hz, 3H, CH_3), ^{13}C NMR (99 MHz, $CDCl_3$) δ 168.55, 167.09, 144.39, 137.49, 134.25, 133.01, 129.56, 129.12, 127.26, 119.44, 116.77, 61.52, 56.32, 36.01, 21.63, 19.71, 19.55, 14.25. HRMS (ESI) calcd. for $C_{21}H_{24}N_2O_5S$ $(M+H)^+$; 417.1478, found; 417.1405.

2-(6,7-Dimethyl-3-oxo-1-tosyl-1,2,3,4-tetrahydroquinoxalin-2-yl)acetic acid (6): 1.51 g of compound **5** (3.63 mmol, 1 eq.) was dissolved in 30 mL of THF and H_2O solvent mixture (THF: H_2O = 2:1), and then 358 mg of $LiOH{\cdot}1H_2O$ (7.27 mmol, 1eq.) was added. The reaction mixture was stirred at room temperature overnight until the reaction was completed by TLC. After that, the mixture was neutralized with 1M HCl solution until the pH~2 and the residue was extracted with EtOAc (3×20 mL). The combined organic layers were washed with brine (40 mL) and dried over anhydrous Na_2SO_4 to obtain the compound **6** as the orange solid (m = 1.36 g, yield 96%). The crude product was directly used without further purification. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.23 (s, 1H, NH), 7.32-7.24 (m, 3H, aromatic, SO_2Ph), 7.20 (d, *J* = 8.5 Hz, 2H, SO_2Ph), 6.54 (s, 1H, aromatic), 4.86-4.78 (m, 1H, H_3), 2.44 (dd, *J* = 15.0, 4.5 Hz, 1H, CH_2), 2.33 (s, 3H, CH_3), 2.27 (d, *J* = 7.2 Hz, 1H, CH_2), 2.21 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), ^{13}C NMR (99 MHz, DMSO-*d*$_6$) δ 169.91, 165.30, 144.27, 136.82, 133.67, 130.95, 130.48, 129.71, 128.47, 126.73, 118.53, 116.69, 56.12, 25.47, 21.07, 19.29, 19.04. HRMS (ESI) calcd. for $C_{19}H_{20}N_2O_5S$ $(M+H)^+$; 389.1165, found; 389.1992.

General procedure for amide coupling reaction (Compound 7): To a solution of compound **6** (70 mg, 0.19 mmol, 1 eq.) in anhydrous DMF (0.5 mL) at room temperature was added triethylamine (70 μL, 0.56 mmol, 1 eq.), followed by 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI·HCl, 58.10 mg, 0.37 mmol, 2 eq.), 1-hydroxy-7-azabenzotriazole (HOBt, 57.30 mg, 0.37 mmol, 2 eq.), and aryl amine (0.37 mmol, 2 eq.). After stirring overnight at room temperature, the reaction mixture was diluted with DCM (10 mL) and water (15 mL), and then extracted with DCM (3×10 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered off and concentrated under reduced pressure. The crude residue was subjected to silica gel chromatography to give product **7**.

General procedure for deprotection reaction (Compound 8): Compound **7** (1 eq.) was dissolved in concentrated sulfuric acid (3 mL) at 0 °C for 1.5 h. After warming up gradually to room temperature, the reaction mixture was alkalized using solid sodium carbonate, and the resulting solution was extracted several times with DCM/MeOH solvent mixture (DCM : MeOH = 4 : 1). The combined organic phase was wash with brine, and dried over anhydrous Na_2SO_4, filtered off, concentrated to reduced solvent. The residue was purified by column chromatography (Hex/EtOAc solvent mixture) to obtain product **8**.

2-(6,7-Dimethyl-3-oxo-1-tosyl-1,2,3,4-tetrahydroquinoxalin-2-yl)-*N*-propylacetamide (7a): ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.22 (s, 1H, NH), 7.48 (s, 1H, aromatic), 7.32-7.24 (m, 2H, SO_2Ph), 7.20 (d, *J* = 8.5 Hz, 2H, SO_2Ph), 7.04 (s, 1H, aromatic), 5.75 (q, *J* = 5.1 Hz, 1H, H_3), 3.60 (t, *J* = 4.8 Hz, 2H, CH_2), 2.44 (dd, *J* = 15.0, 4.5 Hz, 1H, CH_2), 2.33 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 2.27 (s, 3H, CH_3) 2.22 (d, *J* = 7.2 Hz, 1H, CH_2), 1.38 (q, *J* = 7.2 Hz, 2H, CH_2), 0.82 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (99 MHz, $CDCl_3$) δ 168.04, 156.15, 144.73, 140.55, 139.29, 136.82, 133.67, 131.81, 130.95, 130.17, 129.71, 128.12, 118.54, 115.35, 56.99, 47.02, 38.00, 22.41, 19.78, 19.86, 19.29, 11.45. HRMS (ESI) calcd. for $C_{22}H_{27}N_3O_4S$ $(M+H)^+$; 430.1794, found; 430.1795.

2-(6,7-Dimethyl-3-oxo-1-tosyl-1,2,3,4-tetrahydroquinoxalin-2-yl)-*N*-phenylacetamide (7b): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H, NH), 7.98 (s, 1H, NH), 7.58-7.51 (m, 2H, aromatic), 7.43 (s, 1H, aromatic), 7.37 (d, *J* = 8.3 Hz, 2H, SO_2Ph), 7.31 (t, *J* = 8.0 Hz, 2H, SO_2Ph), 7.12 (t, *J* = 8.6 Hz, 3H, aromatic), 6.48 (s, 1H, aromatic), 5.15 (dd, *J* = 10.0, 4.4 Hz, 1H, H_3), 2.65 (dd, *J* = 15.7, 4.5 Hz, 1H, CH_2), 2.52 (dd, *J* = 15.7, 10.1 Hz, 1H, CH_2), 2.31 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 2.20 (s, 3H, CH_3). ^{13}C NMR (99 MHz, $CDCl_3$) δ 166.69, 165.89, 145.00, 137.99, 137.87, 133.71, 133.31, 129.88, 129.61, 129.05, 127.32, 124.67, 120.29, 117.02, 56.27, 37.97, 21.77, 19.75, 19.52. HRMS (ESI) calcd. for $C_{25}H_{25}N_3O_4S$ $(M+H)^+$; 464.1638, found; 464.1365.

2-(6,7-Dimethyl-3-oxo-1-tosyl-1,2,3,4-tetrahydroquinoxalin-2-yl)-*N*-(2-(thiophen-2-yl)ethyl)-acetamide (7c): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.32 (bs, 1H, NH), 8.07 (t, *J* = 5.5 Hz, 1H, aromatic), 7.46 (d, *J* = 8.1 Hz, 1H, aromatic), 7.34 (d, *J* = 4.9 Hz, 1H, aromatic), 7.27 (d, *J* = 8.1 Hz, 2H, SO_2Ph), 7.23 (d, *J* = 7.9 Hz, 1H, aromatic), 7.20 (d, *J* = 8.3 Hz, 2H, SO_2Ph), 6.54 (s, 1H, aromatic), 4.98 (dd, *J* = 9.2, 5.2 Hz, 1H, H_3), 3.33-3.15 (m, 2H, CH_2), 2.87 (t, *J* = 7.5 Hz, 2H, CH_2), 2.33 (s, 3H, CH_3), 2.26 (dd, *J* = 14.2, 5.0 Hz, 1H, CH_2), 2.17 (dd, *J* = 14.4, 9.2 Hz, 1H, CH_2), 2.20 (s, 3H, CH_3), 2.15 (s, 3H, CH_3). ^{13}C NMR (99 MHz, DMSO-d_6) 167.13, 165.99, 154.46, 144.09, 141.61, 136.54, 133.99, 130.68, 129.69, 128.67, 127.05, 125.28, 124.01, 118.91, 116.57, 59.84, 56.12, 29.13, 21.10, 20.83, 19.29, 19.12, 14.15. HRMS (ESI) calcd. for $C_{25}H_{27}N_3O_4S_2$ $(M+H)^+$; 498.1515, found; 498.1443.

2-(6,7-Dimethyl-3-xo-1-tosyl-1,2,3,4-tetrahydroquinoxalin-2-yl)-*N*-((furan-2-yl)methyl)-acetamide (7d): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (bs, 1H, NH), 8.33 (t, *J* = 5.6 Hz, 1H, aromatic), 7.68-7.61 (m, 1H, aromatic), 7.57 (d, *J* = 1.8 Hz, 1H, aromatic), 7.47 (t, *J* = 7.9 Hz, 2H, SO_2Ph), 7.32 (d, *J* = 8.4 Hz, 2H, SO_2Ph), 7.23 (s, 1H, aromatic), 6.53 (s, 1H, aromatic), 6.41-6.38 (bs, 1H, NH), 6.25 (d, *J* = 2.5 Hz, 1H, CH_2), 4.94 (dd, *J* = 9.4, 5.2 Hz, 1H, H_3), 3.27 (dd, *J* = 15.7, 5.6 Hz, 1H, CH_2), 3.18 (dd, *J* = 15.7, 5.2 Hz, 1H, CH_2), 2.31 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 2.16 (s, 3H, CH_3). ^{13}C NMR (99 MHz,

DMSO-d_6) δ 166.92, 165.80, 152.03, 142.07, 136.56, 133.74, 130.71, 129.20, 128.54, 126.67, 118.76, 116.54, 110.48, 106.78, 56.03, 36.18, 35.74, 19.27, 19.10, 14.20. HRMS (ESI) calcd. for $C_{24}H_{25}N_3O_5S$ $(M+H)^+$; 468.1587, found; 468.1514.

2-(6,7-Dimethyl-3-oxo-1-tosyl-1,2,3,4-tetrahydroquinoxalin-2-yl)-*N*-((5-methylfuran-2-yl)methyl)-acetamide (7e): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.14 (bs, 1H, NH), 8.26 (t, J = 5.5 Hz, 1H, aromatic), 7.69-7.61 (m, 1H, aromatic), 7.52-7.43 (m, 2H, SO_2Ph), 7.32 (d, J = 8.4 Hz, 2H, SO_2Ph), 7.22 (s, 1H, aromatic), 6.53 (s, 1H, aromatic), 6.11 (d, J = 2.9 Hz, 1H, CH_2), 5.99 (d, J = 2.9 Hz, 1H, CH_2), 4.93 (q, J = 4.8 Hz, 1H, H_3), 4.22 (dd, J = 15.5, 5.8, 1H, CH_2), 4.11 (dd, J = 15.5, 5.1 Hz, 1H, CH_2), 2.30 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 2.16 (s, 3H, CH_3). ^{13}C NMR (99 MHz, DMSO-d_6) δ 166.79, 165.79, 150.58, 150.14, 136.58, 133.70, 130.71, 130.63, 129.17, 128.50, 126.66, 118.77, 116.53, 107.51, 106.33, 55.99, 36.15, 35.77, 19.25, 19.06, 14.30, 13.28. HRMS (ESI) calcd. for $C_{24}H_{24}N_4O_4S$ $(M+H)^+$; 465.1590, found; 465.1618.

2-(6,7-Dimethyl-3-oxo-1-tosyl-1,2,3,4-tetrahydroquinoxalin-2-yl)-*N*-(pyridin-2-yl)acetamide (7f): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.95 (s, 1H, NH), 9.62 (s, 1H), 8.20 (d, J = 8.3 Hz, 1H, aromatic), 7.60 (dd, J = 16.3, 7.5 Hz, 2H, aromatic), 7.34 (s, 1H, aromatic), 7.25 (d, J = 8.3 Hz, 2H, SO_2Ph), 7.04 (d, J = 8.5 Hz, 2H, SO_2Ph), 6.67 (s, 1H, aromatic), 5.86 (bs, 1H, NH), 5.20 (dd, J = 10.8, 6.5 Hz, 1H, H_3), 2.83-2.79 (m, 1H, CH_2), 2.58-2.47 (m, 1H, CH_2), 2.30 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), 1.93 (s, 3H, CH_3).^{13}C NMR (99 MHz, $CDCl_3$) δ 167.35, 166.47, 146.28, 138.55, 134.02, 132.28, 129.50, 128.70, 127.17, 119.53, 116.63, 115.43, 60.52, 56.54, 39.14, 21.71, 19.85, 19.51, 14.31. HRMS (ESI) calcd. for $C_{25}H_{27}N_3O_5S$ $(M+H)^+$;482.1743, found for 482.1671.

(2-(6,7-Dimethyl-3-oxo-1,2,3,4-tetrahydroquinoxalin-2-yl)-*N*-propylacetamide (8a): ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (s, 1H, NH), 7.99 (s, 1H, NH), 7.48 (s, 1H, aromatic), 7.04 (s, 1H, aromatic), 3.60 (m, 2H, CH_2), 3.56 (t, J = 4.8 Hz, 1H, H_3), 3.08-3.04 (m, 1H, CH_2), 3.03-2.99 (m, 1H, CH_2), 2.30 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 1.42 (q, J = 7.2 Hz, 2H, CH_2), 0.86 (t, J = 7.4 Hz, 3H, CH_3). ^{13}C NMR (99 MHz, DMSO-d_6) δ 168.05, 156.15, 154.73, 140.55, 139.29, 131.81, 130.17, 128.12, 115.35, 47.03, 38.00, 22.41, 19.78, 18.99, 11.45. HRMS (ESI) calcd. for $C_{15}H_{21}N_3O_2$ $(M+H)^+$; 276.1706, found 276.1634.

2-(6,7-Dimethyl-3-oxo-1,2,3,4-tetrahydroquinoxalin-2-yl)-*N*-phenylacetamide (8b): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.18 (s, 1H, NH), 10.00 (s, 1H, NH), 7.59 (d, J = 8.8 Hz, 2H, aromatic), 7.29 (t, J = 8.0 Hz, 2H, aromatic), 7.03 (t, J = 7.4 Hz, 1H, aromatic), 6.54 (d, J = 7.2 Hz, 2H, aromatic), 5.72 (s, 1H, NH), 4.15-4.09 (m, 1H, H_3), 2.82 (dd, J = 15.3, 4.7 Hz, 1H, CH_2), 2.56 (dd, J = 15.4, 8.2 Hz, 1H, CH_2),

2.05 (s, 6H, 2 x CH_3). ^{13}C NMR (99 MHz, DMSO-d_6) δ 169.58, 167.06, 139.20, 131.63, 130.11, 128.70, 125.48, 123.91, 123.20, 119.20, 116.04, 52.96, 38.19, 19.06, 19.69. HRMS (ESI) calcd. for $C_{18}H_{19}N_3O_2$ $(M+H)^+$; 310.1549, found 310.1477.

2-(6,7-Dimethyl-3-oxo-1,2,3,4-tetrahydroquinoxalin-2-yl)-*N*-(2-(thiophen-2-yl)ethyl)acetamide (8c): ^1H NMR (400 MHz, DMSO-d_6) δ 10.27 (s, 1H, NH), 8.14 (t, J = 5.6 Hz, 1H, aromatic), 7.32 (d, J = 4.0 Hz, 1H, aromatic), 6.96 (s, 1H, aromatic), 6.89 (d, J = 3.4 Hz, 1H, aromatic), 6.79 (s, 1H, aromatic), 6.57 (s, 1H, NH), 5.80 (s, 1H, NH), 4.15 (q, J = 5.2 Hz, 1H, H_3), 3.34-3.26 (m, 2H, CH_2), 2.93 (t, J = 7.2 Hz, 2H, CH_2), 2.62 (dd, J = 15.1, 4.2 Hz, 1H, CH_2), 2.38-2.31 (m, 1H, CH2), 2.22 (s, 3H, CH_3), 2.17 (s, 3H, CH_3). ^{13}C NMR (99 MHz, DMSO-d_6) δ 169.63, 166.99, 141.51, 133.76, 127.07, 125.64, 124.03, 122.84, 118.29, 114.67, 114.24, 52. 74, 37.55, 20.67, 19.31, 19.04. HRMS (ESI) calcd. for $C_{18}H_{21}N_3O_2S$ $(M+H)^+$; 344.1426, found; 344.1964.

2-(6,7-Dimethyl-3-oxo-1,2,3,4-tetrahydroquinoxalin-2-yl)-*N*-((furan-2-yl)methyl)acetamide (8d): ^1H NMR (400 MHz, DMSO-d_6) δ 10.57 (s, 1H, NH), 9.67 (s, 1H, NH), 8.22 (t, J= 7.0 Hz, 1H, aromatic), 7.25 (d, J= 2.8 Hz, 1H, aromatic), 7.18 (d, J = 1.8 Hz, 1H, aromatic), 6.92 (s, 1H, aromatic), 6.58 (s, 1H, aromatic), 6.39 (s, 1H, NH), 4.75 (s, 2H, CH_2), 4.12 (q, J = 6.2 Hz, 1H, H_3), 2.74 (dd, J = 15.4, 6.0 Hz, 1H, CH_2), 2.53 (dd, J = 15.1, 5.6 Hz, 1H, CH_2), 2.35 (s, 3H, CH_3), 2.23 (s, 3H, CH_3). ^{13}C NMR (99 MHz, DMSO-d_6) δ 168.99, 166.34, 153.29, 141.63, 134.79, 129.56, 128.09, 127.05, 120.27, 116.67, 112.40, 112.21, 48.55, 35.99, 32.88, 19.27, 19.05. HRMS (ESI) calcd. for $C_{17}H_{19}N_3O_3$ $(M+H)^+$; 314.1498, found; 314.1426.

2-(6,7-Dimethyl-3-oxo-1,2,3,4-tetrahydroquinoxalin-2-yl)-*N*-((5-methylfuran-2-yl)methyl)acetamide (8e): ^1H NMR (400 MHz, DMSO-d_6) δ 10.38 (s, 1H, NH), 8.93 (s, 1H, NH), 7.13 (s, 1H, aromatic), 6.39 (s, 1H, aromatic), 6.12 (d, J = 7.5 Hz, 1H, aromatic), 6.01 (d, J = 7.3 Hz, 1H, aromatic), 5.91 (bs, 1H, NH), 4.95 (s, 1H, CH_2), 4.35 (q, J = 6.2 Hz, 1H, H_3), 2.74 (dd, J = 15.2, 5.9 Hz, 1H, CH_2), 2.52 (dd, J = 15.3, 4.9 Hz, 1H, CH_2), 2.35 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 2.17 (s, 3H, CH_3). ^{13}C NMR (99 MHz, DMSO-d_6) 169.05, 166.23, 150.96, 149.14, 134.69, 128.65, 127.06, 126.53, 121.45, 116.34, 112.78, 105.63, 49.55, 35.09, 34.91, 19.55, 19.01, 14.30. HRMS (ESI) calcd. for $C_{18}H_{21}N_3O_3$ $(M+H)^+$; 328.1655, found; 328.1583.

2-(6,7-Dimethyl-3-oxo-1,2,3,4-tetrahydroquinoxalin-2-yl)-*N*-(pyridin-2-yl)acetamide(8f): ^1H NMR (400 MHz, DMSO-d_6) δ 10.56 (bs, 1H, NH), 8.44 (m, 1H, aromatic) 8.30 (m, 1H, aromatic), 7.87 (dd, J = 16.5, 7.4 Hz, 1H, aromatic), 7.17 (m, 1H, aromatic), 6.90 (bs, 1H, NH), 6.68 (s, 1H,

aromatic), 6.40 (s, 1H, aromatic), 5.21 (bs, 1H, NH), 4.20 (1, J = 6.5 Hz, 1H, H_3), 2.79-2.74 (m, 1H, CH_2), 2.56-2.51 (m, 1H, CH_2), 2.28 (s, 3H, CH_3), 2.21 (s, 3H, CH_3), ^{13}C NMR (99 MHz, DMSO-d_6) δ 171.82, 166.34, 153.50, 148.20,138.83, 134.79, 129.56, 128.09, 127.04, 120. 34, 117.83, 116.60, 112.49, 50.01, 36.28, 20.14, 19.59, 18.20. HRMS (ESI) calcd. for $C_{17}H_{18}N_4O_2$ $(M+H)^+$; 311.1502, found; 311.1429.

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SUPPORTING INFORMATION

Supplementary data associated with this article can be found, in the online version, at URL; https://www.heterocycles.jp/newlibrary/downloads/PDFsi/26567/100/2.

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