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Original article

Synthesis and biological evaluation of benzenesulfonamide-substituted 4-(6-alkylpyridin-2-yl)-5-(quinoxalin-6-yl)imidazoles as transforming growth factor-β type 1 receptor kinase inhibitors

Dae-Kee Kim [a,*], Sun Hee Jung [a], Ho Soon Lee [b], Purushottam M. Dewang [a]

[a] College of Pharmacy, Ewha Womans University, 11-1 Daehyun-dong, Seodaemun-gu, Seoul 120-750, South Korea
[b] In2Gen Co., Ltd., 608 Daerung Posttower II Building, 182-13 Guro-dong, Guro-gu, Seoul 152-050, South Korea

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Abstract

A series of benzenesulfonamide-substituted 4-(6-alkylpyridin-2-yl)-5-(quinoxalin-6-yl)imidazoles (**15a–l**) have been synthesized and evaluated for their ALK5 inhibitory activity in cell-based luciferase reporter assays. Among them, 4-[5-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1*H*-imidazol-2-ylmethyl]benzenesulfonamide (**15b**) and 4-[5-(6-ethylpyridin-2-yl)-4-(quinoxalin-6-yl)-1*H*-imidazol-2-ylmethyl]benzenesulfonamide (**15c**) showed more than 90% inhibition at 0.5 μM in a luciferase reporter assay using HaCaT cells transiently transfected with p3TP-luc reporter construct, but inhibited p38α MAP kinase activity only 11 and 8% at a concentration of 10 μM, respectively.

Keywords: Transforming growth factor-β (TGF-β); ALK5 inhibitors; Fibrosis; Cancer

1. Introduction

The TGF-β superfamily of ligands includes TGF-β, activins, and bone morphogenetic proteins (BMPs) and regulates a wide range of responses including cell proliferation, differentiation, adhesion, migration, and apoptosis. Three TGF-β isoforms (TGF-β1, TGF-β2, and TGF-β3) are expressed in mammals, and each is encoded by a unique gene and expressed in a tissue-specific manner. TGF-β1 is the prototypic member of this family of cytokines that signals through two types of transmembrane receptor serine/threonine kinases, the type I and type II TGF-β receptors (TβR-I and TβR-II, respectively). Generally, ligand interaction with a homodimer of type II receptors recruits and activates homodimers of type I receptors or activin receptor-like kinase 5 (ALK5). Activated ALK5 phosphorylates a subset of downstream signaling molecules, the receptor-activated Smad2/Smad3 proteins, which then enable their binding to the common mediator Smad4. This complex is shuttled into the nucleus and regulates transcription of specific target genes involved in cell growth, differentiation, development, and the immune response [1–4]. Dysregulated signaling of TGF-β has been implicated in the pathogenesis of a number of diseases, including fibrosis and carcinogenesis [4–6]. TGF-β also plays important roles in wound healing and is possibly deregulated in the related pathologies of hypertrophic scars and keloids [7,8]. Attempts to block the effects of TGF-β contributed to the development of molecules that inhibit TGF-β binding to its receptor including decorin [9], soluble chimeric TGF-β receptor [10], and neutralizing antibodies [11]. The extensive knowledge regarding TGF-β-mediated ALK5-dependent signaling pathway as an initiating point at the receptor level has highlighted the therapeutic potential of TGF-β signaling antagonist. Recent studies have shown that several small molecule ATP-competitive ALK5 inhibitors (Fig. 1) such as **1** (SB-431542) [12], **2** (SB-505124) [13], **3** (SB-525334) [14], **4** (A-83-01) [15], **5** (GW6604) [16], **6** (LY580276) [17], and SD-208 [18,19] inhibited autophosphorylation of ALK5 and

* Corresponding author. Tel.: +82 2 3277 3025; fax: +82 2 3277 2467.
E-mail address: dkkim@ewha.ac.kr (D.-K. Kim).

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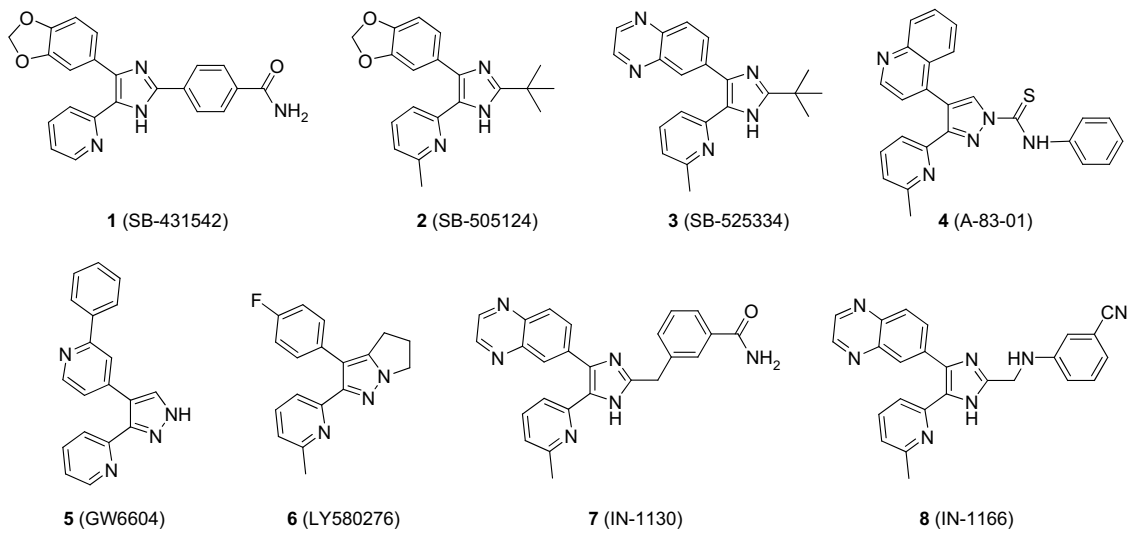


Fig. 1. Small molecule ATP-competitive ALK5 inhibitors.

TGF-β-induced transcription of matrix genes in reporter assays at submolar concentrations. Among them, **3**, **5** and SD-208 effectively retarded progressive fibrosis in kidney, liver and lung, respectively, and SD-208 also strongly inhibited growth and invasiveness of cancer cells in animal models. Earlier reports from our laboratory described several 2-pyridyl-substituted triazole [20,21], imidazole [22–24], and thiazole derivatives [25]. These derivatives possess phenyl moiety as one of the substituents on the central 5-membered heterocycle attached directly or through methylene or aminomethylene linkage. We have demonstrated that incorporation of a short linkage, either methylene [21] or aminomethylene [22,25], between a central triazole, an imidazole, or a thiazole and a phenyl ring significantly enhances ALK5 inhibitory activity along with selectivity. Moreover, introduction of a carbonitrile or a carboxamide group at *meta*- or *para*-position on the phenyl ring leads to the similar activity and selectivity enhancing effects. These structural modifications have culminated in the discovery of compound **8** (IN-1166), a highly potent and selective ALK5 inhibitor [22]. Based on similar modifications, inhibitor **7** (IN-1130) was designed and synthesized. To our delight, **7** effectively suppressed fibrogenic process of unilateral ureteral obstruction in rats underscoring the potential clinical benefits in the treatment of renal fibrosis [26] and ameliorated experimental autoimmune encephalomyelitis (EAE) in SBE-luc and GFAP-luc mice immunized with MOG_{35-55} [24].

Hence being encouraged with these results, it was of our immediate interest to observe the effect of introduction of a sulfonamide group as a bioisoster for a carboxamide on the phenyl substitution. It was previously observed by us that compounds with a carboxamide substituent in the *meta*-position on the phenyl ring showed slightly higher ALK5 inhibition compared to the corresponding compounds that have that substituent in the *para*-position [22,25]. To prepare compounds with a sulfonamide substituent in the *meta*-position on the phenyl ring, according to our synthetic scheme shown in Scheme 1, 3-(2-oxoethyl)benzenesulfonamide and 3-(3-oxopropyl)benzenesulfonamide are requisite. However, these unknown sulfonamides are not readily accessible, therefore, we decided to prepare compounds having a sulfonamide substituent in the *para*-position. In combination with this structural modification, the impact of linkage, either methylene (**15a–f**) or ethylene (**15g–l**), between the central imidazole and a phenyl ring was evaluated. Based on the previous observations [22,25,27], 2-pyridyl substitution on the central imidazole was also modified by incorporating a small alkyl functionality at 6-position.

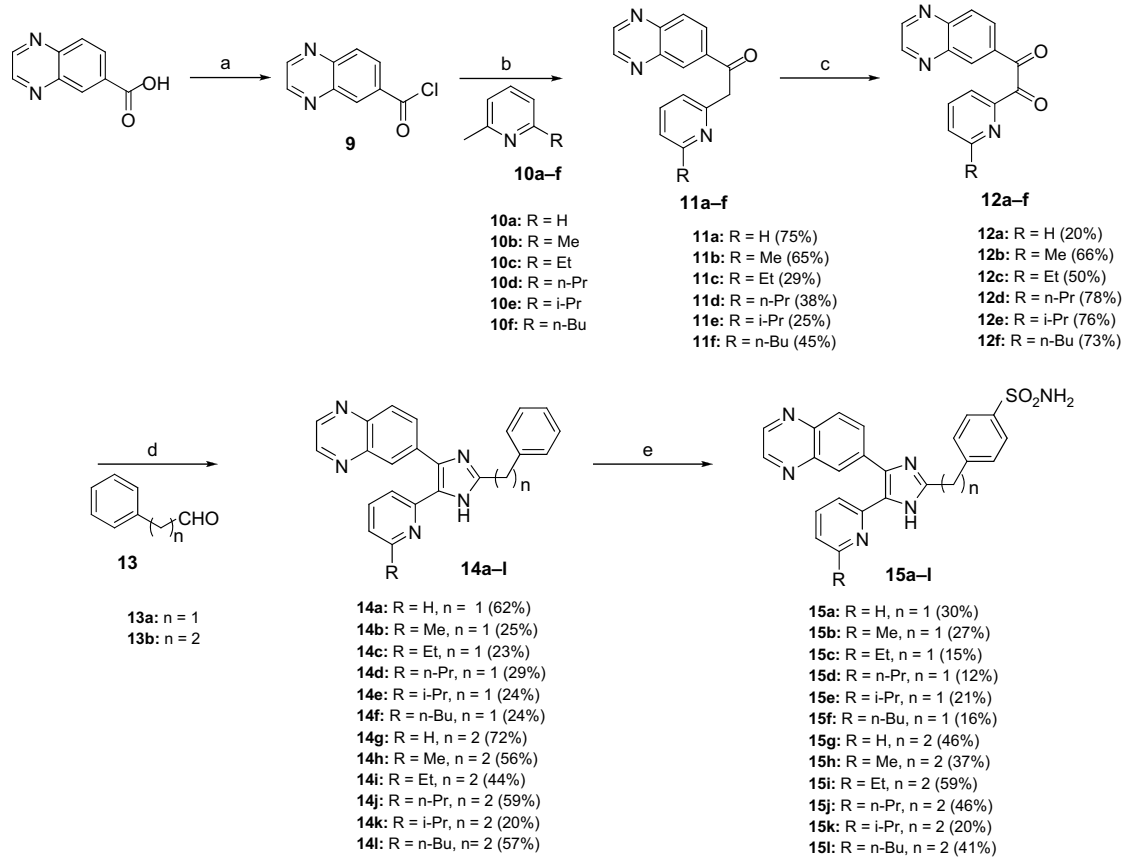
2. Results and discussion

2.1. Chemistry

A series of benzenesulfonamide-substituted 4-(6-alkylpyridin-2-yl)-5-(quinoxalin-6-yl)imidazoles, **15a–l**, were prepared as shown in Scheme 1. Quinoxaline-6-carboxylic acid was reacted with $(COCl)_2$ in toluene to give quinoxaline-6-carbonyl chloride (**9**) in quantitative yield. Coupling of **9** with the pyridines **10a–f** in anhydrous THF in the presence of base (*n*-BuLi and Et_2AlCl in hexane) was carried out to obtain the quinoxalinyl monoketones **11a–f** in 25–75% yields. Oxidation of **11a–f** in DMSO with HBr (48 wt.% in water) gave the quinoxalinyl diketones **12a–f** in 20–78% yields, which were subsequently cyclized in MeOH/*t*-BuOMe with phenylacetaldehyde (**13a**) or hydrocinnamaldehyde (**13b**) in the presence of NH_4OAc to afford the pyridin-2-ylimidazoles **14a–l** in 23–72% yields. Chlorosulfonylation of **14a–l** with $ClSO_3H$ in CH_2Cl_2 followed by amination of the resulting sulfonyl chlorides with NH_4OH yielded the pyridin-2-ylimidazole sulfonamides **15a–l** in 12–59% yields.

2.2. Luciferase reporter assay and p38α MAP kinase assay

To evaluate TGF-β-induced downstream transcriptional activation to ALK5 signaling, cell-based luciferase activity of **15a–l** was measured using HaCaT cells transiently transfected

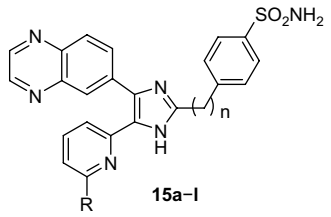


Scheme 1. Reagents and conditions: (a) $(COCl)_2$, toluene, reflux, 2 h; (b) *n*-BuLi, Et_2AlCl, anhydrous THF, −60 °C; (c) HBr (48 wt.% in water), DMSO, 60–70 °C, 2 h; (d) NH_4OAc, MeOH, *t*-BuOMe, 60–70 °C, 2 h; (e) (i) $ClSO_3H$, CH_2Cl_2, rt, 2 h, Ar atmosphere; (ii) NH_4OH (28%, aq.), dioxane, THF, rt, 2 h.

with two different luciferase receptor genes, p3TP-luciferase reporter [28] and ARE-luciferase reporter [29], at a concentration of 0.5 μM. The selectivity of the synthesized compounds was determined against p38α MAP kinase because this was the only kinase to be significantly affected by SB-431542 in vitro on a panel of 24 kinases unrelated to the ALKs [12].

A close look at the inhibitory activity (Table 1) as the effect of change in length of linkage joining the central imidazole ring and a phenyl ring reflects that generally the methylene ($n = 1$) linkage is favored over the ethylene linkage ($n = 2$) with the exception of compound **15d**. In the case of **15d**, where *n*-propyl moiety was present on the pyridyl substitution the reverse effect was observed. The activity was increased with the lengthening of methylene (**15d**) to ethylene linkage (**15j**). In another approach to optimize these analogues, we concentrated on pyridyl substitution. A small alkyl group at the 6-position of pyridyl ring has shown crucial influence on the binding affinity in the cases of several other closely related ALK5 inhibitors [22,27]. Motivated by these reports, we wished to judge the optimal size of an alkyl moiety best suitable to this series for the 6-position of pyridyl substitution. Hence, the 6-position was derivatized with different small alkyl moieties. The activities of resulted compounds (**15b–f** and **15h–l**) were compared within themselves and with unsubstituted analogues (**15a** and **15g**). Incorporation of a methyl substituent resulted in excellent increase in both sets, one with methylene (**15b**) and another with ethylene (**15b**) linkage, as compared, respectively, with their counterpart compound (**15a** or **15g**) which lack alkyl substitutions. Extending methyl substitution further to ethyl (**15c** and **15i**) imparted additional binding interactions that led to improvement in inhibitory activity. However, propyl groups were too big to be accommodated beneficially and hence the activities were dropped when ethyl (**15c**) was replaced with *n*-propyl (**15d**) and *i*-propyl (**15e**) groups. The similar drop in activity was observed in the case of **15j** and **15k** albeit to different order of magnitudes as compared to the corresponding ethyl derivative (**15i**). The excellent improvements in activity as evidenced by methyl (**15b** and **15h**) and ethyl (**15c** and **15i**) analogues proved that in this series also 6-position on pyridyl ring offers a room for appending alkyl substitutions to increase activity. Nevertheless, the limitations of the available space were indicated by lower activity of compounds having propyl groups (**15d**, **15e**, **15j** and **15k**) than the corresponding methyl (**15b** and **15h**) and ethyl (**15c** and **15i**) appended compounds. This was further confirmed by *n*-butyl derivatives (**15f** and **15l**) that exhibited significant loss in ALK5 inhibitory activity compared to *n*-propyl analogues (**15d** and **15j**). Study of activities in this series conclusively reveals that the methyl-, ethyl-, or *n*-propyl-substituted analogues are more potent than parent unsubstituted (**15a** or **15g**) derivatives.

Table 1
Inhibitory activity of benzenesulfonamide-substituted 4-(6-alkylpyridin-2-yl)-5-(quinoxalin-6-yl)imidazoles, **15a–l**, on ALK5



15a–l

Compound	R	*n*	Activity[a] (% control)		
			p3TP-luciferase[b]	ARE-luciferase[b]	p38α MAP kinase[c]
Mock			2	2	
TGF-β			100 ± 15	100 ± 14	
15a	H	1	47 ± 4	90 ± 9	95 ± 5
15b	Me	1	7 ± 1	40 ± 4	89 ± 6
15c	Et	1	6	38 ± 4	92 ± 4
15d	*n*-Pr	1	40 ± 5	107 ± 11	105 ± 9
15e	*i*-Pr	1	88 ± 8	128 ± 13	98 ± 6
15f	*n*-Bu	1	70 ± 5	109 ± 16	104 ± 10
15g	H	2	60 ± 5	93 ± 15	97 ± 6
15h	Me	2	13 ± 2	64 ± 6	91 ± 8
15i	Et	2	9 ± 2	46 ± 10	96 ± 9
15j	*n*-Pr	2	16 ± 2	68 ± 7	103 ± 12
15k	*i*-Pr	2	102 ± 15	114 ± 13	105 ± 9
15l	*n*-Bu	2	123 ± 8	147 ± 12	97 ± 8
1			25 ± 4	76 ± 7	49 ± 5

[a] Activity is given as the mean ± SD of three independent experiments run in triplicate relative to control incubations with DMSO vehicle.
[b] Luciferase activity was determined at a concentration of 0.5 μM of inhibitor.
[c] Kinase activity was determined at a concentration of 10 μM of inhibitor.

After achieving improvements in activities on introducing alkyl substitutions in this series, it was of paramount interest to know the selectivity of these analogues. As mentioned above, to determine the selectivity p38α MAP kinase was chosen. In vitro kinase assay of this enzyme was performed in the presence of 10 μM of inhibitors. The most outstanding observation regarding selectivity profile was that all the compounds, **15a–l**, exhibited weak or no measurable inhibition (<11%) for p38α MAP kinase. This shows remarkably higher selectivity than SB-431542 (**1**) which caused 51% inhibition of p38α MAP kinase. The selectivity profile of these derivatives was not affected to a great extent either by introduction of alky moiety on pyridyl substitution or by change in the length of linkage that attaches benzenesulfonamide group to central imidazole scaffold.

The above series provided us with compounds **15b** and **15c** which exhibited, respectively, 93 and 94% inhibition at 0.5 μM in a p3TP-luciferase reporter assay. Moreover, these compounds possess high selectivity against p38α MAP kinase causing only 11 (**15b**) and 8% (**15c**) inhibition. Hence, compounds **15b** and **15c** were selected for further studies and their inhibitory activity was determined at four different concentrations (0.0625, 0.125, 0.25, and 0.5 μM) using HaCaT cells transiently transfected with p3TP-luciferase reporter construct. The results were compared with two different known small molecule ALK5 inhibitors, **1** and **7**. The regulatory preclinical studies of compound **7** are currently under way. Compounds **15b** and **15c** inhibited

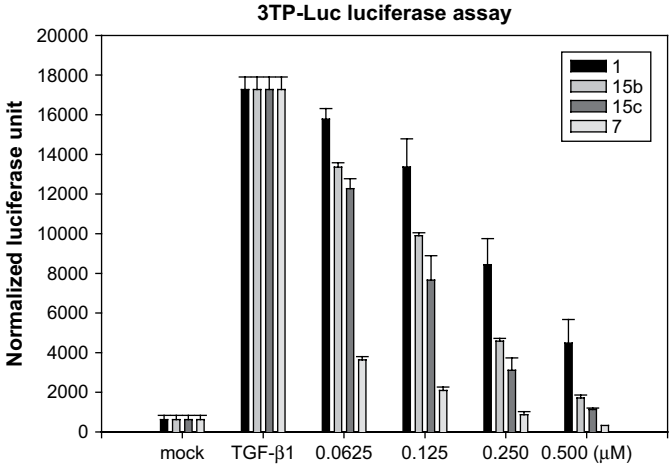


Fig. 2. Effect of **15b** and **15c** on the activity of TGF-β-induced ALK5. HaCaT cells were transiently transfected with p3TP-luciferase reporter construct. Luciferase activity was determined in the presence of different concentrations of each compound and is given as the mean ± SD of three independent experiments run in triplicate relative to control.

ALK5 in a dose-dependent manner as shown in Fig. 2. Also these compounds were more potent than **1** at all four concentrations tested. Compound **15c** at concentrations of 0.125, 0.25 and 0.5 μM was at least two-fold more potent than **1**. Also, the inhibition at 0.5 μM concentration exhibited by **15b** was twice of that shown by compound **1**. The comparison of ALK5 inhibitory activity of **15b** and **15c** with **7** demonstrates roughly the influence of bioisosteric replacement of carboxamide with sulfonamide functionality. It can be realized from the higher activity of **7** that *m*-carboxamide moiety present on the phenyl substitution interacts more favorably with the ATP binding pocket of ALK5 than the corresponding *p*-sulfonamide moiety.

3. Conclusion

In this report, on the backdrop of the introduction of a *p*-sulfonamide group on the phenyl substitution in tri-substituted imidazole-based ALK5 inhibitors other two crucial structural variations were investigated systematically. The structure–activity relationships (SARs) regarding the optimal length of an alkylene linkage between a phenyl and the central imidazole ring revealed that commonly a methylene is superior to an ethylene linkage. Although our recent reports [22,25] and other [27] suggest that a methyl is generally the best optimal substituent for the 6-position of pyridyl ring, an ethyl substituent is also equivalently beneficial in this series of compounds. These modifications furnished compounds **15b** and **15c** that are more potent than the known inhibitor **1**. Albeit, compound **7** that contains a *m*-carboxamide group on the phenyl substitution was more potent in ALK5 inhibition than these *p*-sulfonamide analogues. All synthesized compounds were more selective for ALK5 inhibition than p38α MAP kinase inhibition compared to **1**. This manuscript reports the first exclusive attempt to use *p*-sulfonamide functionality on the phenyl substitution and ethylene linkage in the design of ATP-competitive inhibitors of ALK5. The SAR disclosed here shall

provide valuable insights to the medicinal chemists for design and development of imidazole-based ALK5 inhibitors.

4. Experimental section

4.1. Chemistry

^{1}H NMR spectra were recorded on a Varian Unity 400 spectrophotometer. The chemical shifts are reported in parts per million (ppm) relative to internal tetramethylsilane in $CDCl_3$. Infrared spectra were recorded on an FT-infrared spectrometer (Bio-Rad). Electrospray ionization mass spectra (ESI-MS) were obtained on a Q-Tof2 mass spectrometer (Micromass). All melting points were taken in Pyrex capillaries using an electrothermal digital melting point apparatus (Buchi) and are not corrected. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60F-254 glass plates. Medium-pressure liquid chromatography (MPLC) was performed using Merck silica gel 60 (230–400 mesh) with a YFLC-540 ceramic pump (Yamagen). Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer.

4.1.1. General procedure for the preparation of the 2-(pyridin-2-yl)-1-(quinoxalin-6-yl)ethanones (**11a–f**)

A stirred solution of pyridine **10a–f** (13 mmol) in anhydrous THF (100 mL) at −60 °C under Ar atmosphere was treated dropwise with a solution of *n*-BuLi in hexanes (2.0 M, 13 mmol). After 30 min, a solution of Et_2AlCl in hexane (1.0 M, 14 mmol) was added dropwise to the reaction mixture, and the reaction mixture was allowed to warm to room temperature. The reaction mixture was cooled to −60 °C and transferred via cannula to a stirred solution of quinoxaline-6-carbonyl chloride (**9**) (10.3 mmol) in anhydrous THF (100 mL) at −60 °C. Stirring was continued for 20 min and the reaction mixture was quenched with saturated aqueous NH_4Cl solution. The mixture was filtered through a pad of Celite and the filtered residue was washed with EtOAc (100 mL). The combined filtrate was concentrated under reduced pressure and the residue was purified by MPLC to afford the title compound **11a–f** as a solid.

*4.1.1.1. 2-(Pyridin-2-yl)-1-(quinoxalin-6-yl)ethanone (**11a**).* Yield 75%; ^{1}H NMR ($CDCl_3$) δ 7.06 (ddd, 1H, J = 5.2, 2.4, 1.2 Hz), 7.17 (d, 1H, J = 8.0 Hz), 7.68 (td, 1H, J = 8.4, 1.2 Hz), 8.12 (d, 1H, J = 8.8 Hz), 8.24 (dd, 1H, J = 8.8, 2.0 Hz), 8.36 (d, 1H, J = 4.8 Hz), 8.61 (d, 1H, J = 1.6 Hz), 8.84 (d, 1H, J = 1.6 Hz), 8.88 (d, 1H, J = 2.0 Hz).

*4.1.1.2. 2-(6-Methylpyridin-2-yl)-1-(quinoxalin-6-yl)ethanone (**11b**) [30].* Yield 65%; mp 139–140 °C; ^{1}H NMR ($CDCl_3$) δ 2.59 (s, 3H), 6.88 (d, 1H, J = 7.8 Hz), 6.97 (d, 1H, J = 7.8 Hz), 7.57 (t, 1H, J = 7.8 Hz), 8.12 (d, 1H, J = 8.8 Hz), 8.25 (dd, 1H, J = 8.8, 2.0 Hz), 8.60 (d, 1H, J = 2.0 Hz), 8.83 (d, 1H, J = 1.8 Hz), 8.88 (d, 1H, J = 1.8 Hz); IR (neat) 1636 (CO) cm^{-1}; MS (EIS) *m/z* 264 (MH^+).

*4.1.1.3. 2-(6-Ethylpyridin-2-yl)-1-(quinoxalin-6-yl)ethanone (**11c**).* Yield 29%; mp 109–110 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (t, 3H, J = 7.6 Hz), 2.88 (q, 2H, J = 7.6 Hz), 6.89 (d, 1H, J = 7.8 Hz), 6.98 (d, 1H, J = 7.8 Hz), 7.60 (t, 1H, J = 7.8 Hz), 8.12 (d, 1H, J = 8.8 Hz), 8.26 (dd, 1H, J = 8.8, 2.0 Hz), 8.60 (d, 1H, J = 1.6 Hz), 8.83 (d, 1H, J = 2.0 Hz), 8.88 (d, 1H, J = 1.6 Hz); IR (neat) 1631 (CO) cm^{-1}; MS (EIS) *m/z* 278 (MH^+).

*4.1.1.4. 2-(6-n-Propylpyridin-2-yl)-1-(quinoxalin-6-yl)ethanone (**11d**).* Yield 38%; mp 133–134 °C; ^{1}H NMR ($CDCl_3$) δ 1.03 (t, 3H, J = 7.4 Hz), 1.84–1.90 (m, 2H), 2.81 (t, 2H, J = 7.8 Hz), 6.92 (d, 1H, J = 7.6 Hz), 6.98 (d, 1H, J = 7.6 Hz), 7.58 (t, 1H, J = 7.8 Hz), 8.12 (d, 1H, J = 8.8 Hz), 8.25 (dd, 1H, J = 8.8, 2.0 Hz), 8.60 (d, 1H, J = 2.0 Hz), 8.83 (d, 1H, J = 2.0 Hz), 8.87 (d, 1H, J = 2.0 Hz); MS (EIS) *m/z* 292 (MH^+).

*4.1.1.5. 2-(6-Isopropylpyridin-2-yl)-1-(quinoxalin-6-yl)ethanone (**11e**).* Yield 25%; mp 129–130 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (d, 6H, J = 7.2 Hz), 3.05–3.15 (m, 1H), 6.29 (s, 1H), 6.90 (d, 1H, J = 7.2 Hz), 6.97 (dd, 1H, J = 8.0, 0.8 Hz), 7.59 (t, 1H, J = 8.0 Hz), 8.11 (d, 1H, J = 8.8 Hz), 8.25 (dd, 1H, J = 8.8, 2.0 Hz), 8.60 (d, 1H, J = 2.0 Hz), 8.82 (d, 1H, J = 2.0 Hz), 8.86 (d, 1H, J = 2.0 Hz); MS (EIS) *m/z* 292 (MH^+).

*4.1.1.6. 2-(6-n-Butylpyridin-2-yl)-1-(quinoxalin-6-yl)ethanone (**11f**).* Yield 45%; mp 89–90 °C; ^{1}H NMR ($CDCl_3$) δ 0.99 (t, 3H, J = 7.2 Hz), 1.42–1.47 (m, 2H), 1.77–1.85 (m, 2H), 2.83 (t, 2H, J = 8.0 Hz), 6.87 (d, 1H, J = 8.0 Hz), 6.97 (d, 1H, J = 8.0 Hz), 7.58 (t, 1H, J = 8.0 Hz), 8.12 (d, 1H, J = 8.8 Hz), 8.26 (dd, 1H, J = 8.8, 2.0 Hz), 8.60 (d, 1H, J = 2.0 Hz), 8.83 (d, 1H, J = 2.0 Hz), 8.87 (d, 1H, J = 2.0 Hz); MS (EIS) *m/z* 306 (MH^+).

4.1.2. General procedure for the preparation of the 1-(pyridin-2-yl)-2-(quinoxalin-6-yl)ethane-1,2-diones (**12a–f**)

A stirred suspension of **11a–f** (13 mmol) in DMSO was treated dropwise with HBr (48 wt.% in water, 6 mL) and the mixture was heated to 60–70 °C. After 2 h, the reaction mixture was cooled to 0 °C, poured onto ice water and brought to pH 10 with K_2CO_3. The mixture was extracted with EtOAc (30 mL × 3), and the EtOAc solution was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by MPLC to afford the title compound **12a–f** as a solid.

*4.1.2.1. 1-(Pyridin-2-yl)-2-(quinoxalin-6-yl)ethane-1,2-dione (**12a**).* Yield 20%; ^{1}H NMR ($CDCl_3$) δ 7.55 (ddd, 1H, J = 7.6, 4.8, 1.2 Hz), 7.99 (td, 1H, J = 7.6, 1.6 Hz), 8.27 (d, 1H, J = 8.8 Hz), 8.28 (dq, 1H, J = 7.6, 1.2 Hz), 8.43 (dd, 1H, J = 8.8, 2.0 Hz), 8.55 (d, 1H, J = 1.6 Hz), 8.65 (dq, 1H, J = 4.8, 1.2 Hz), 8.92 (d, 1H, J = 1.8 Hz), 8.95 (d, 1H, J = 1.8 Hz).

*4.1.2.2. 1-(6-Methylpyridin-2-yl)-2-(quinoxalin-6-yl)ethane-1,2-dione (**12b**) [30,31].* Yield 66%; mp 123–124 °C; ^{1}H NMR ($CDCl_3$) δ 2.46 (s, 3H), 7.39 (dd, 1H, J = 8.0, 0.4 Hz), 7.84 (t, 1H, J = 7.6 Hz), 8.06 (dd, 1H, J = 7.2, 0.4 Hz), 8.26 (d, 1H, J = 8.8 Hz), 8.41 (dd, 1H, J = 8.8, 2.0 Hz), 8.54 (d, 1H, J = 1.6 Hz), 8.92 (d, 1H, J = 2.0 Hz), 8.95 (d, 1H, J = 2.0 Hz); IR (neat) 1698 (CO), 1684 (CO) cm^{-1}; MS (EIS) *m/z* 278 (MH^+).

*4.1.2.3. 1-(6-Ethylpyridin-2-yl)-2-(quinoxalin-6-yl)ethane-1,2-dione (**12c**).* Yield 50%; mp 115–116 °C; ^{1}H NMR ($CDCl_3$)

δ 1.07 (t, 3H, $J = 7.4$ Hz), 2.72 (q, 2H, $J = 7.2$ Hz), 7.39 (dt, 1H, $J = 7.6$, 0.6 Hz), 7.85 (t, 1H, $J = 7.6$ Hz), 8.07 (dd, 1H, $J = 7.6$, 0.6 Hz), 8.26 (d, 1H, $J = 8.8$ Hz), 8.40 (dd, 1H, $J = 8.8$, 2.0 Hz), 8.53 (d, 1H, $J = 1.6$ Hz), 8.91–8.92 (d, 1H, $J = 2.0$ Hz), 8.95–8.96 (d, 1H, $J = 2.0$ Hz); IR (neat) 1687 (CO) cm^{-1}; MS (EIS) *m/z* 292 (MH^+).

*4.1.2.4. 1-(6-n-Propylpyridin-2-yl)-2-(quinoxalin-6-yl)ethane-1,2-dione (**12d**).* Yield 78%; mp 87–88 °C; ^1H NMR ($CDCl_3$) δ 0.73 (t, 3H, $J = 7.4$ Hz), 1.48–1.57 (m, 2H), 2.66 (t, 2H, $J = 7.6$ Hz), 7.36 (dt, 1H, $J = 8.0$, 0.6 Hz), 7.85 (t, 1H, $J = 7.8$ Hz), 8.06 (dt, 1H, $J = 7.6$, 0.6 Hz), 8.26 (d, 1H, $J = 8.8$ Hz), 8.40 (dd, 1H, $J = 8.8$, 2.0 Hz), 8.52 (d, 1H, $J = 2.0$ Hz), 8.92 (d, 1H, $J = 2.0$ Hz), 8.95 (d, 1H, $J = 2.0$ Hz); IR (neat) 1684 (CO) cm^{-1}; MS (EIS) 306 *m/z* (MH^+).

*4.1.2.5. 1-(6-Isopropylpyridin-2-yl)-2-(quinoxalin-6-yl)ethane-1,2-dione (**12e**).* Yield 76%; mp 95–96 °C; ^1H NMR ($CDCl_3$) δ 1.05 (d, 6H, $J = 6.4$ Hz), 2.91–2.98 (m, 1H), 7.42 (d, 1H, $J = 7.6$ Hz), 7.87 (t, 1H, $J = 8.0$ Hz), 8.06 (dd, 1H, $J = 8.0$, 1.2 Hz), 8.26 (d, 1H, $J = 8.8$ Hz), 8.39 (dd, 1H, $J = 8.8$, 2.0 Hz), 8.53 (d, 1H, $J = 2.0$ Hz), 8.92 (d, 1H, $J = 1.6$ Hz), 8.95 (d, 1H, $J = 1.6$ Hz); IR (neat) 1683 (CO) cm^{-1}; MS (EIS) *m/z* 306 (MH^+).

*4.1.2.6. 1-(6-n-Butylpyridin-2-yl)-2-(quinoxalin-6-yl)ethane-1,2-dione (**12f**).* Yield 73%; mp 59–62 °C; ^1H NMR ($CDCl_3$) δ 0.64 (t, 3H, $J = 7.4$ Hz), 1.08–1.14 (m, 2H), 1.41–1.49 (m, 2H), 2.68 (t, 2H, $J = 7.6$ Hz), 7.37 (dd, 1H, $J = 7.6$, 1.2 Hz), 7.84 (t, 1H, $J = 7.6$ Hz), 8.06 (dd, 1H, $J = 7.6$, 1.2 Hz), 8.26 (d, 1H, $J = 8.8$ Hz), 8.40 (dd, 1H, $J = 8.8$, 2.0 Hz), 8.51 (d, 1H, $J = 2.0$ Hz), 8.92 (d, 1H, $J = 1.6$ Hz), 8.95 (d, 1H, $J = 1.6$ Hz); IR (neat) 1704 (CO) cm^{-1}; MS (EIS) *m/z* 320 (MH^+).

*4.1.3. General procedure for the preparation of the 6-[2-benzyl-5-(pyridin-2-yl)-1H-imidazol-4-yl]quinoxalines (**14a–f**) and 6-[2-phenethyl-5-(pyridin-2-yl)-1H-imidazol-4-yl]quinoxalines (**14g–l**)*

To a stirred solution of **12a–f** (1.44 mmol) in *t*-BuOMe (9 mL) and MeOH (6 mL) were added ammonium acetate (7.20 mmol) and phenylacetaldehyde (**13a**) (4.32 mmol) or hydrocinnamaldehyde (**13b**) (4.32 mmol) at room temperature. The mixture was heated at 60–70 °C for 2 h and then cooled to room temperature. The reaction mixture was concentrated under reduced pressure and the residue was neutralized with saturated aqueous $NaHCO_3$ solution (pH 8) at 0 °C. The mixture was diluted with water and extracted with CH_2Cl_2 (15 mL × 3). The CH_2Cl_2 solution was washed with brine (25 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by MPLC to afford the title compound **14a–l** as a solid.

*4.1.3.1. 6-[2-Benzyl-5-(pyridin-2-yl)-1H-imidazol-4-yl]quinoxaline (**14a**).* Yield 62%; mp 59–62 °C; ^1H NMR ($CDCl_3$) δ 4.21 (s, 2H), 7.07–7.09 (m, 1H), 7.22–7.24 (m, 1H), 7.29–7.32 (m, 4H), 7.51 (br m, 2H), 8.14 (s, 2H), 8.40–8.43 (br m, 2H), 8.83–8.85 (m, 2H); MS (EIS) *m/z* 364 (MH^+).

*4.1.3.2. 6-[2-Benzyl-5-(6-methylpyridin-2-yl)-1H-imidazol-4-yl]quinoxaline (**14b**).* Yield 25%; mp 61–62 °C; ^1H NMR ($CDCl_3$) δ 2.47 (s, 3H), 4.23 (s, 2H), 6.95 (d, 1H, $J = 7.2$ Hz), 8.14 (br s, 2H), 8.42 (br s, 1H), 8.83 (d, 1H, $J = 1.6$ Hz), 8.83 (d, 1H, $J = 2.0$ Hz), 8.84 (d, 1H, $J = 2.0$ Hz); MS (EIS) *m/z* 378 (MH^+).

*4.1.3.3. 6-[2-Benzyl-5-(6-ethylpyridin-2-yl)-1H-imidazol-4-yl]-quinoxaline (**14c**).* Yield 23%; mp 139–140 °C; ^1H NMR ($CDCl_3$) δ 1.27 (t, 3H, $J = 7.6$ Hz), 2.77 (q, 2H, $J = 7.6$ Hz), 4.25 (s, 2H), 6.96 (d, 1H, $J = 7.6$ Hz), 7.28–7.40 (m, 7H), 8.15–8.16 (m, 2H), 8.44 (d, 1H, $J = 0.8$ Hz), 8.83 (d, 1H, $J = 2.0$ Hz), 8.85 (d, 1H, $J = 2.0$ Hz), 9.91 (br s, 1H); MS (EIS) *m/z* 392 (MH^+).

*4.1.3.4. 6-[2-Benzyl-5-(6-n-propylpyridin-2-yl)-1H-imidazol-4-yl]quinoxaline (**14d**).* Yield 29%; mp 68–69 °C; ^1H NMR ($CDCl_3$) δ 0.95 (t, 3H, $J = 7.2$ Hz), 1.68–1.74 (m, 2H), 2.71 (t, 2H, $J = 7.6$ Hz), 4.25 (s, 2H), 6.95 (d, 1H, $J = 7.2$ Hz), 7.31–7.38 (m, 7H), 8.15 (m, 2H), 8.43 (s, 1H), 8.83 (d, 1H, $J = 2.0$ Hz), 8.84 (d, 1H, $J = 2.0$ Hz), 9.92 (br s, 1H); MS (EIS) *m/z* 406 (MH^+).

*4.1.3.5. 6-[2-Benzyl-5-(6-isopropylpyridin-2-yl)-1H-imidazol-4-yl]quinoxaline (**14e**).* Yield 24%; mp 66–67 °C; ^1H NMR ($CDCl_3$) δ 1.27 (d, 6H, $J = 6.8$ Hz), 3.00–3.03 (m, 1H), 4.26 (s, 2H), 6.98 (d, 1H, $J = 7.2$ Hz), 7.31–7.41 (m, 7H), 8.15–8.16 (m, 2H), 8.44 (br s, 1H), 8.83 (d, 1H, $J = 2.0$ Hz), 8.85 (d, 1H, $J = 2.0$ Hz), 9.82 (br s, 1H); MS (EIS) *m/z* 406 (MH^+).

*4.1.3.6. 6-[2-Benzyl-5-(6-n-butylpyridin-2-yl)-1H-imidazol-4-yl]quinoxaline (**14f**).* Yield 24%; mp 61–62 °C; ^1H NMR ($CDCl_3$) δ 0.92 (t, 3H, $J = 7.2$ Hz), 1.33–1.39 (m, 2H), 1.60–1.67 (m, 2H), 2.72 (t, 2H, $J = 7.6$ Hz), 4.24 (s, 2H), 6.94 (d, 1H, $J = 7.2$ Hz), 7.31 (d, 1H, $J = 7.2$ Hz), 7.36–7.38 (m, 6H), 8.15 (s, 1H), 8.16 (d, 1H, $J = 1.6$ Hz), 8.43 (d, 1H, $J = 1.2$ Hz), 8.83 (d, 1H, $J = 2.0$ Hz), 8.85 (d, 1H, $J = 2.0$ Hz), 10.02 (br s, 1H); MS (EIS) *m/z* 420 (MH^+).

*4.1.3.7. 6-[2-Phenethyl-5-(pyridin-2-yl)-1H-imidazol-4-yl]quinoxaline (**14g**).* Yield 72%; mp 62–65 °C; ^1H NMR ($CDCl_3$) δ 3.15 (s, 4H), 7.11 (dd, 1H, $J = 9.0$, 4.6 Hz), 7.23 (dd, 3H, $J = 12.0$, 7.0 Hz), 7.31 (q, 2H, $J = 7.4$ Hz), 7.50 (d, 2H, $J = 3.6$ Hz), 8.14 (dd, 2H, $J = 12.0$, 8.4 Hz), 8.42 (s, 1H), 8.51 (dt, 1H, $J = 5.2$, 1.4 Hz), 8.85 (d, 2H, $J = 5.2$ Hz); MS (EIS) *m/z* 378 (MH^+).

*4.1.3.8. 6-[5-(6-Methylpyridin-2-yl)-2-phenethyl-1H-imidazol-4-yl]quinoxaline (**14h**).* Yield 56%; mp 47–49 °C; ^1H NMR ($CDCl_3$) δ 2.54 (s, 3H), 3.17 (s, 4H), 6.97 (d, 1H, $J = 7.2$ Hz), 7.23–7.41 (m, 7H), 8.13 (s, 2H), 8.42 (s, 1H), 8.83 (d, 1H, $J = 2.0$ Hz), 8.85 (d, 1H, $J = 2.0$ Hz); MS (EIS) *m/z* 392 (MH^+).

*4.1.3.9. 6-[5-(6-Ethylpyridin-2-yl)-2-phenethyl-1H-imidazol-4-yl]quinoxaline (**14i**).* Yield 44%; mp 48–49 °C; ^1H NMR ($CDCl_3$) δ 1.32 (t, 3H, $J = 7.6$ Hz), 2.81 (q, 2H, $J = 7.6$ Hz), 3.19 (s, 4H), 6.98 (d, 1H, $J = 7.2$ Hz), 7.27–7.41 (m, 7H),

8.13 (s, 2H), 8.42 (s, 1H), 8.83 (d, 1H, $J = 2.0$ Hz), 8.85 (d, 1H, $J = 2.0$ Hz); MS (EIS) *m/z* 406 (MH^+).

*4.1.3.10. 6-[2-Phenethyl-5-(6-n-propylpyridin-2-yl)-1H-imidazol-4-yl]quinoxaline (**14j**).* Yield 59%; mp 53–54 °C; ^{1}H NMR ($CDCl_3$) δ 0.98 (t, 3H, $J = 7.4$ Hz), 1.69–1.76 (m, 2H), 2.73 (t, 2H, $J = 7.6$ Hz), 3.17 (q, 4H, $J = 2.8$ Hz), 6.96 (d, 1H, $J = 7.6$ Hz), 7.24–7.34 (m, 6H), 7.40 (t, 1H, $J = 7.6$ Hz), 8.13 (d, 2H, $J = 1.2$ Hz), 8.42 (s, 1H), 8.83 (d, 1H, $J = 2.0$ Hz), 8.85 (d, 1H, $J = 2.0$ Hz); MS (EIS) *m/z* 420 (MH^+).

*4.1.3.11. 6-[5-(6-Isopropylpyridin-2-yl)-2-phenethyl-1H-imidazol-4-yl]quinoxaline (**14k**).* Yield 20%; mp 58–60 °C; ^{1}H NMR ($CDCl_3$) δ 1.30 (d, 6H, $J = 6.4$ Hz), 3.01–3.05 (m, 1H), 3.18 (t, 4H, $J = 4.6$ Hz), 6.98 (d, 1H, $J = 7.6$ Hz), 7.23–7.37 (m, 6H), 7.41 (t, 1H, $J = 7.6$ Hz), 8.14 (s, 2H), 8.42 (s, 1H), 8.83 (d, 1H, $J = 1.6$ Hz), 8.85 (d, 1H, $J = 1.6$ Hz); MS (EIS) *m/z* 420 (MH^+).

*4.1.3.12. 6-[5-(6-n-Butylpyridin-2-yl)-2-phenethyl-1H-imidazol-4-yl]quinoxaline (**14l**).* Yield 57%; mp 54–55 °C; ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H, $J = 7.4$ Hz), 1.39 (m, 2H), 1.64–1.72 (m, 2H), 2.75 (t, 2H, $J = 7.8$ Hz), 3.16 (br s, 4H), 6.96 (d, 1H, $J = 7.2$ Hz), 7.23–7.34 (m, 6H), 7.40 (t, 1H, $J = 7.8$ Hz), 8.13 (d, 2H, $J = 0.8$ Hz), 8.42 (s, 1H), 8.83 (d, 1H, $J = 2.0$ Hz), 8.85 (d, 1H, $J = 2.0$ Hz); MS (EIS) *m/z* 434 (MH^+).

*4.1.4. General procedure for the preparation of the 4-[5-(pyridin-2-yl)-4-(quinoxalin-6-yl)-1H-imidazol-2-ylalkyl]benzenesulfonamides (**15a–l**)*

To a stirred solution of **14a–l** (0.14 mmol) in CH_2Cl_2 (3 mL) was added $ClSO_3H$ (3.00 mmol) at 0 °C under Ar atmosphere. After 10 min, the mixture was allowed to warm to room temperature and stirred for an additional 2 h. The reaction mixture was dropped into ice water and neutralized with 6 N NaOH, and it was extracted with CH_2Cl_2 (10 mL × 5). The CH_2Cl_2 solution was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to give the sulfonyl chloride derivatives as a brown foam, which was used in the next step without further purification. To a stirred solution of the sulfonyl chloride derivatives in dioxane (3 mL) and THF (3 mL) was added 28% aqueous ammonia solution (0.5 mL) at 0 °C. After 10 min, the mixture was allowed to warm to room temperature and stirred for an additional 2 h. The reaction mixture was concentrated under reduced pressure and then extracted with CH_2Cl_2 (10 mL × 3). The CH_2Cl_2 solution was washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by MPLC to afford the title compound **15a–l** as a solid.

*4.1.4.1. 4-[5-(Pyridin-2-yl)-4-(quinoxalin-6-yl)-1H-imidazol-2-ylmethyl]benzenesulfonamide (**15a**).* Two-step yield 30%; mp 137–144 °C; ^{1}H NMR ($CDCl_3$) δ 4.09 (s, 2H), 5.69 (br s, 2H), 5.88 (br s, 1H), 7.10 (td, 1H, $J = 7.2$, 1.2 Hz), 7.15–7.21 (br d, 2H, $J = 7.6$ Hz), 7.47–7.59 (m, 4H), 8.09 (br s, 2H), 8.27 (d, 1H, $J = 0.8$ Hz), 8.35 (br t, 1H), 8.80–8.82 (br m, 2H); IR (neat) 3282, 3175, 1334, 1158 cm^{-1}; MS (EIS) *m/z* 443 (MH^+). Anal. Calcd for $C_{23}H_{18}N_6O_2S$: C, 62.43; H, 4.10; N, 18.99. Found: C, 62.72; H, 4.02; N, 18.81.

*4.1.4.2. 4-[5-(6-Methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-imidazol-2-ylmethyl]benzenesulfonamide (**15b**).* Two-step yield 27%; mp 140–143 °C; ^{1}H NMR ($CDCl_3$) δ 2.38 (br s, 3H), 4.17 (br s, 2H), 6.99 (d, 1H, $J = 7.6$ Hz), 7.33 (d, 3H, $J = 7.6$ Hz), 7.43 (t, 1H, $J = 7.6$ Hz), 7.73 (br s, 2H), 8.15 (s, 2H), 8.35 (br s, 1H), 8.85–8.86 (br m, 2H); IR (neat) 3411, 3244, 1341, 1169 cm^{-1}; MS (EIS) *m/z* 457 (MH^+). Anal. Calcd for $C_{24}H_{20}N_6O_2S$: C, 63.14; H, 4.42; N, 18.41. Found: C, 62.99; H, 4.53; N, 18.25.

*4.1.4.3. 4-[5-(6-Ethylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-imidazol-2-ylmethyl]benzenesulfonamide (**15c**).* Two-step yield 15%; mp 155–156 °C; ^{1}H NMR ($CDCl_3$) δ 1.14 (t, 3H, $J = 7.6$ Hz), 2.69 (q, 2H, $J = 7.6$ Hz), 4.14 (s, 2H), 5.22 (br s, 2H), 7.02 (d, 1H, $J = 7.6$ Hz), 7.31 (br s, 2H), 7.34 (d, 1H, $J = 8.0$ Hz), 7.48 (t, 1H, $J = 8.0$ Hz), 7.70 (br s, 2H), 8.13 (s, 2H), 8.33 (s, 1H), 8.84–8.85 (m, 2H); IR (neat) 3046, 1325, 1153 cm^{-1}; MS (EIS) *m/z* 471 (MH^+). Anal. Calcd for $C_{25}H_{22}N_6O_2S$: C, 63.81; H, 4.71; N, 17.86. Found: C, 63.63; H, 4.85; N, 17.69.

*4.1.4.4. 4-[5-(6-n-Propylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-imidazol-2-ylmethyl]benzenesulfonamide (**15d**).* Two-step yield 12%; mp 150 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, $J = 8.0$ Hz), 1.50–1.68 (m, 2H), 2.62–2.70 (m, 2H), 4.17 (s, 2H), 5.08 (br s, 2H), 7.01 (d, 1H, $J = 7.2$ Hz), 7.34–7.36 (m, 3H), 7.46 (t, 1H, $J = 7.6$ Hz), 7.73 (br s, 2H), 8.13 (s, 2H), 8.35 (s, 1H), 8.85 (s, 2H); IR (neat) 3390, 1298, 1046 cm^{-1}; MS (EIS) *m/z* 485 (MH^+). Anal. Calcd for $C_{26}H_{24}N_6O_2S$: C, 64.44; H, 4.99; N, 17.34. Found: C, 64.53; H, 4.80; N, 17.29.

*4.1.4.5. 4-[5-(6-Isopropylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-imidazol-2-ylmethyl]benzenesulfonamide (**15e**).* Two-step yield 21%; mp 147–148 °C; ^{1}H NMR ($CDCl_3$) δ 1.26 (d, 6H, $J = 6.8$ Hz), 3.04 (br s, 1H), 4.25 (s, 2H), 4.94 (br s, 2H), 7.04 (d, 1H, $J = 7.6$ Hz), 7.36 (d, 1H, $J = 8.0$ Hz), 7.46–7.47 (m, 3H), 7.83 (br d, 2H, $J = 8.0$ Hz), 8.13 (s, 2H), 8.38 (s, 1H), 8.84–8.85 (m, 2H); IR (neat) 3406, 1030 cm^{-1}; MS (EIS) *m/z* 485 (MH^+). Anal. Calcd for $C_{26}H_{24}N_6O_2S$: C, 64.44; H, 4.99; N, 17.34. Found: C, 64.33; H, 5.10; N, 17.29.

*4.1.4.6. 4-[5-(6-n-Butylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-imidazol-2-ylmethyl]benzenesulfonamide (**15f**).* Two-step yield 16%; mp 135–136 °C; ^{1}H NMR ($CDCl_3$) δ 0.76 (t, 3H, $J = 7.6$ Hz), 1.15–1.24 (m, 2H), 1.40 (br s, 2H), 2.62 (br s, 2H), 4.05 (br s, 2H), 5.45 (br s, 2H), 7.01 (d, 1H, $J = 7.6$ Hz), 7.16 (br s, 2H), 7.32 (d, 1H, $J = 7.2$ Hz), 7.47 (t, 1H, $J = 7.6$ Hz), 7.56 (br s, 2H), 8.12 (br s, 2H), 8.30 (s, 1H), 8.83 (d, 1H, $J = 2.0$ Hz), 8.84 (d, 1H, $J = 2.0$ Hz); IR (neat)

3422, 1422, 1035 cm^{-1}; MS (EIS) *m/z* 499 (MH^+). Anal. Calcd for $C_{27}H_{26}N_6O_2S$: C, 65.04; H, 5.26; N, 16.86. Found: C, 65.23; H, 5.10; N, 16.69.

*4.1.4.7. 4-[2-[5-(Pyridin-2-yl)-4-(quinoxalin-6-yl)-1H-imidazol-2-yl]ethyl]benzenesulfonamide (**15g**).* Two-step yield 46%; mp 139–140 °C; ^1H NMR ($CDCl_3$) δ 3.07 (t, 2H, J = 7.2 Hz), 3.14 (t, 2H, J = 7.2 Hz), 7.14 (ddd, 1H, J = 6.0, 3.6, 1.2 Hz), 7.23 (d, 2H, J = 8.4 Hz), 7.52–7.58 (m, 2H), 7.74 (d, 2H, J = 8.4 Hz), 8.03 (d, 1H, J = 8.8 Hz), 8.09 (d, 1H, J = 8.8 Hz), 8.19 (br s, 1H), 8.45 (dt, 1H, J = 4.8, 1.2 Hz), 8.82–8.83 (m, 2H); IR (neat) 3275, 3180, 1331, 1158 cm^{-1}; MS (EIS) *m/z* 457 (MH^+). Anal. Calcd for $C_{24}H_{20}N_6O_2S$: C, 63.14; H, 4.42; N, 18.41. Found: C, 62.93; H, 4.60; N, 18.29.

*4.1.4.8. 4-[2-[5-(6-Methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-imidazol-2-yl]ethyl]benzenesulfonamide (**15h**).* Two-step yield 37%; mp 130–131 °C; ^1H NMR ($CDCl_3$) δ 2.56 (s, 3H), 3.17 (t, 2H, J = 6.8 Hz), 3.24 (t, 2H, J = 6.8 Hz), 7.01 (d, 1H, J = 8.0 Hz), 7.31–7.35 (m, 1H), 7.37 (d, 2H, J = 8.8 Hz), 7.44 (t, 1H, J = 8.2 Hz), 7.88 (d, 2H, J = 8.4 Hz), 8.07 (d, 1H, J = 8.4 Hz), 8.12 (d, 1H, J = 8.8 Hz), 8.22 (br s, 1H), 8.84–8.85 (m, 2H); IR (neat) 3460, 3320, 1336, 1158 cm^{-1}; MS (EIS) *m/z* 471 (MH^+). Anal. Calcd for $C_{25}H_{22}N_6O_2S$: C, 63.81; H, 4.71; N, 17.86. Found: C, 63.63; H, 4.90; N, 17.69.

*4.1.4.9. 4-[2-[5-(6-Ethylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-imidazol-2-yl]ethyl]benzenesulfonamide (**15i**).* Two-step yield 59%; mp 130 °C; ^1H NMR ($CDCl_3$) δ 1.25 (t, 3H, J = 7.6 Hz), 2.78 (q, 2H, J = 7.6 Hz), 3.11 (t, 2H, J = 6.4 Hz), 3.17 (t, 2H, J = 6.4 Hz), 7.03 (d, 1H, J = 8.0 Hz), 7.30 (d, 2H, J = 8.4 Hz), 7.35 (br s, 1H), 7.48 (t, 1H, J = 7.8 Hz), 7.80 (d, 2H, J = 8.0 Hz), 8.06–8.11 (m, 2H), 8.22 (br s, 1H), 8.84 (d, 1H, J = 2.0 Hz), 8.85 (d, 1H, J = 2.0 Hz); IR (neat) 3434, 1331, 1153 cm^{-1}; MS (EIS) *m/z* 485 (MH^+). Anal. Calcd for $C_{26}H_{24}N_6O_2S$: C, 64.44; H, 4.99; N, 17.34. Found: C, 64.53; H, 4.90; N, 17.29.

*4.1.4.10. 4-[2-[5-(6-n-Propylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-imidazol-2-yl]ethyl]benzenesulfonamide (**15j**).* Two-step yield 46%; mp 215–217 °C; ^1H NMR ($CDCl_3$) δ 0.98 (t, 2H, J = 7.6 Hz), 1.69–1.76 (m, 3H), 2.77 (t, 2H, J = 7.6 Hz), 3.19 (d, 2H, J = 6.2 Hz), 3.24 (d, 2H, J = 6.2 Hz), 7.01 (d, 1H, J = 7.2 Hz), 7.38 (d, 2H, J = 8.4 Hz), 7.44 (br s, 2H), 7.88 (d, 2H, J = 8.0 Hz), 8.11 (br s, 2H), 8.24 (br s, 1H), 8.84 (d, 1H, J = 2.0 Hz), 8.85 (d, 1H, J = 2.0 Hz); IR (neat) 3387, 3222, 1332, 1156 cm^{-1}; MS (EIS) *m/z* 499 (MH^+). Anal. Calcd for $C_{27}H_{26}N_6O_2S$: C, 65.04; H, 5.26; N, 16.86. Found: C, 65.23; H, 5.10; N, 16.79.

*4.1.4.11. 4-[2-[5-(6-Isopropylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-imidazol-2-yl]ethyl]benzenesulfonamide (**15k**).* Two-step yield 20%; mp 124–127 °C; ^1H NMR ($CDCl_3$) δ 1.31 (d, 6H, J = 6.4 Hz), 3.03–3.05 (m, 1H), 3.18 (d, 2H, J = 6.2 Hz), 3.23 (d, 2H, J = 6.2 Hz), 7.03 (d, 1H, J = 8.0 Hz), 7.37 (d, 2H, J = 8.4 Hz), 7.87 (d, 2H, J = 8.4 Hz), 8.10 (br s, 2H), 8.23 (br s, 1H), 8.84 (d, 1H, J = 2.0 Hz), 8.85 (d, 1H, J = 2.0 Hz); IR (neat) 3399, 3067, 1337, 1160 cm^{-1}; MS (EIS) *m/z* 499 (MH^+). Anal. Calcd for $C_{27}H_{26}N_6O_2S$: C, 65.04; H, 5.26; N, 16.86. Found: C, 64.83; H, 5.40; N, 16.79.

*4.1.4.12. 4-[2-[5-(6-n-Butylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-imidazol-2-yl]ethyl]benzenesulfonamide (**15l**).* Two-step yield 41%; mp 208–210 °C; ^1H NMR ($CDCl_3$) δ 0.94 (t, 3H, J = 7.2 Hz), 1.40 (m, 2H), 1.69 (m, 2H), 2.78 (t, 2H, J = 7.6 Hz), 3.17 (d, 2H, J = 6.6 Hz), 3.22 (d, 2H, J = 6.6 Hz), 7.01 (d, 1H, J = 7.6 Hz), 7.36 (d, 2H, J = 8.4 Hz), 7.24–7.49 (m, 2H), 7.86 (d, 2H, J = 8.0 Hz), 8.09–8.12 (m, 2H), 8.23 (br s, 1H), 8.84 (d, 1H, J = 2.0 Hz), 8.85 (d, 1H, J = 2.0 Hz); IR (neat) 3411, 3062, 1333, 1156 cm^{-1}; MS (EIS) *m/z* 513 (MH^+). Anal. Calcd for $C_{28}H_{28}N_6O_2S$: C, 65.60; H, 5.51; N, 16.39. Found: C, 65.93; H, 5.30; N, 16.09.

4.2. Biological activity testing

4.2.1. Luciferase reporter assay

Biological activity of the test compounds was determined by measuring their ability to inhibit TGF-β-induced p3TP-luciferase reporter activity and ARE-luciferase reporter activity in HaCaT cells. HaCaT cells were seeded at concentrations of 5×10^4 in 24-well plates. And the next day, when they reach approximately 90% confluence, the cells were transfected with 0.1 μg of p3TP-Luc reporter construct or ARE-Luc reporter construct and 0.1 μg of β-galactosidase using Lipofectamine 2000 (Invitrogen). At 24 h after transfection, ALK5 inhibitors of various concentrations were added to the cells. After 2 h, the cells were treated with 5 ng/mL of TGF-β for 18–24 h. Cell lysates were harvested according to the manufacturer's instruction and luminescence was measured by a luminometer VICTOR (Perkin–Elmer Life).

4.2.2. p38α MAP kinase inhibition assay

p38α kinase assay was performed according to the instruction manual of assay kit provided by the manufacturer (Upstate Biotechnology). Briefly, to activate MAPKAP kinase-2, 10 μL of reaction mixture containing 200 ng of inactive MAPKAP kinase-2, 0.06 U of purified p38α kinase and 2 μL of magnesium/ATP cocktail (75 mM $MgCl_2$/500 μM cold ATP) were mixed by vortexing and incubated for 15 min at 30 °C with agitation. Before activation reaction was started, inhibitors dissolved in DMSO were added to the reaction tube with 10 μM of final concentration. After activation, 10 μCi [γ-^{32}P]ATP and 10 μL of 0.86 mM MAPKAP kinase-2 substrate were added, and the mixture was incubated for 10 min at 30 °C with agitation. Then, 40 μL of reaction mixture was transferred into P81 phosphocellulose paper. After extensively washing the paper three times with 40 mL of 0.75% phosphoric acid, the bound radioactivity was determined by liquid scintillation counter.

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