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PII: S0223-5234(19)31046-3

DOI: https://doi.org/10.1016/j.ejmech.2019.111894

Reference: EJMECH 111894

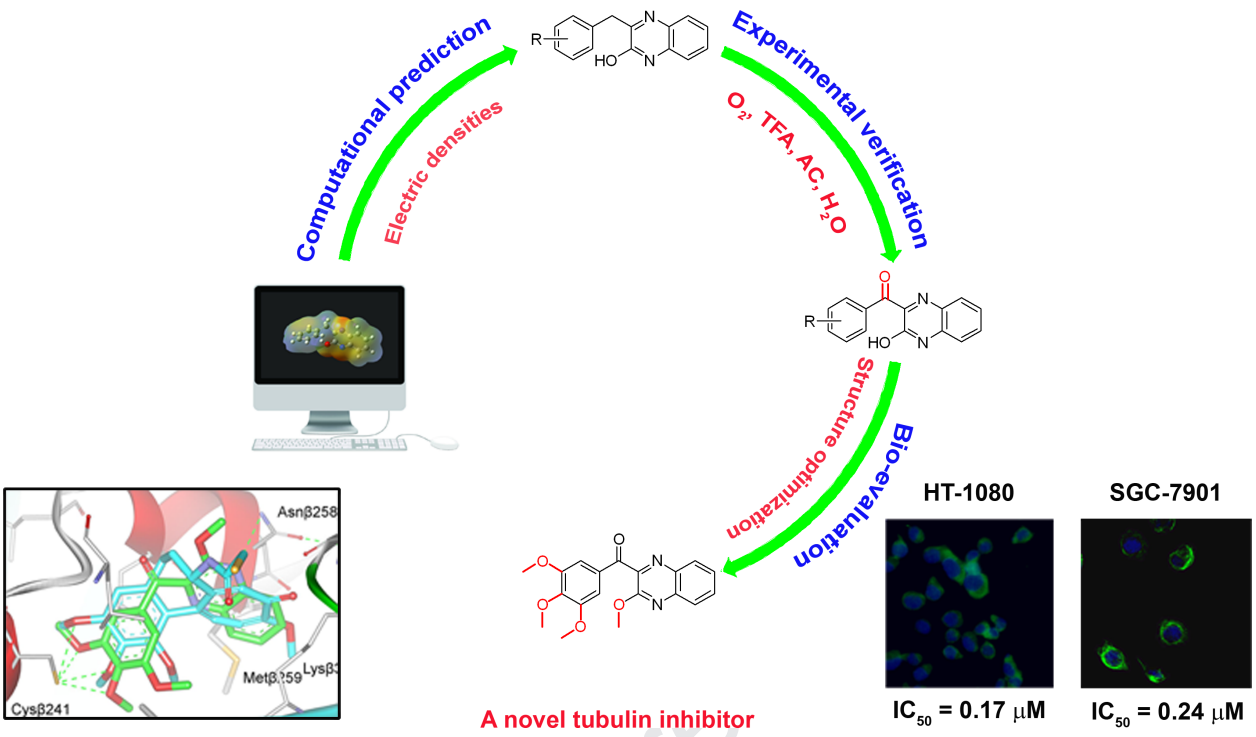
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 7 October 2019

Revised Date: 31 October 2019

Accepted Date: 14 November 2019

Please cite this article as: Q. Guan, L. Cong, Q. Wang, C. Yu, K. Bao, K. Zhou, L. Wu, W. Zhang, Activated carbon/Brønsted acid-promoted aerobic benzylic oxidation under "on-water" condition: Green and efficient synthesis of 3-benzoylquinoxalinones as potent tubulin inhibitors, *European Journal of Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.ejmech.2019.111894.



Activated Carbon/Brønsted Acid-Promoted Aerobic Benzylic Oxidation under "On-Water" Condition: Green and Efficient Synthesis of 3-Benzoylquinoxalinones as Potent Tubulin Inhibitors

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Abstract

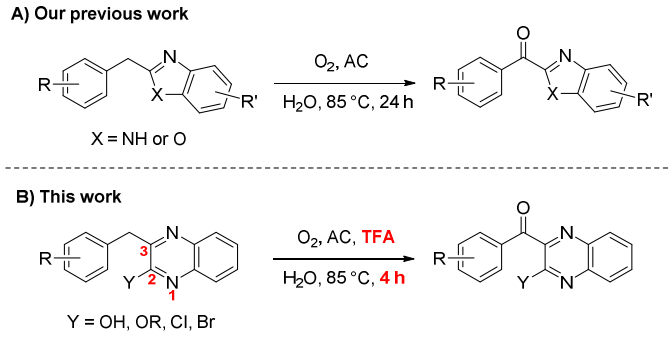
Green chemistry is becoming the favored approach to preparing drug molecules in pharmaceutical industry. Herein, we developed a clean and efficient method to synthesize 3-benzoylquinoxalines via activated carbon promoted aerobic benzylic oxidation under "on-water" condition. Moreover, biological studies with this class of compounds reveal an antiproliferative profile. Further structure modifications are performed and the investigations exhibited that the most active **12a** could inhibit the microtubule polymerization by binding to tubulin and thus induce multipolar mitosis, G2/M phase arrest, and apoptosis of cancer cells. In addition, molecular docking studies allow the rationalization of the pharmacodynamic properties observed. Our systematic studies provide not only guidance for applications of O_2/AC/H_2O system, but also a new scaffold targeting tubulin for antitumor agent discovery.

Keywords: Green chemistry, on-water, 3-benzoylquinoxalinone, tubulin inhibitor, molecular modelling.

1. Introduction

In the last decades, green chemistry gave medicinal chemists a new way of looking at the design of drug molecules and the processes to make them that reduces or eliminates the impact on the environment. Green chemistry principles have now become a strategic focus for pharmaceutical industry. Benzylic oxidation is one of the most important synthetic transformations in organic chemistry, as generated products may act as valuable building blocks for the manufacture of pharmaceuticals.[1] The development of oxidants for benzylic oxidation has therefore attracted considerable interest. Therein, molecular oxygen is an ideal oxidant, as water is the sole by-product. During the past few years, the benzylic oxidation of alkylaromatics to the corresponding carbonyl compounds using molecular oxygen as oxidant has been reported frequently, but organic solvents were generally used as the reaction medium.[2-9] Recent trends in organic synthesis involve reactions of water-insoluble organic compounds that take place under "on-water" condition have received a great deal of attention because of their high efficiency and operational convenience.[10-12] However, benzylic oxidation via the direct utility of molecular oxygen from air as oxidant under "on-water" condition, which is one of the requirements for the realization of green chemical processes, remains a tremendous challenge in current organic synthesis and industrial chemistry. This leads us to development of a novel catalytic process to accomplish the green or greener synthesis.

Recent efforts in our laboratory revealed that activated carbon (AC) could promote the aerobic oxidation of 2-benzylbenzimidazoles and 2-benzylbenzoxazoles to the corresponding carbonyl products in the "on water" heterogeneous system (Scheme 1A).[13] While the AC is found to be an ideal catalytic system for the aerobic oxidation under on-water condition, both reaction efficiency and substrate scope remain to be substantially improved. In our previous work, we also have observed that the oxidation yield is related to the electric density at the benzylic position of the substrate. This prompts us to verify whether the rule of computational electric density can be used to search the substrate that is suitable for our approach.



Scheme 1. Our previous work and this work.

The molecular scaffolds of quinoxaline are of great interest in medicinal chemistry since they are found in a large number of natural products and possess a wide range of biological activities.[14-20] Moreover, 3-benzoylquinoxalines, as an important structural motif, have been also widely used as a synthon for the synthesis of bioactive products. General methods for the preparation of 3-benzoylquinoxalines are oxidation of benzyl to corresponding carbonyl compounds. Up to date, only few literatures have reported to achieve this transformation, and yet all these methods have serious environmental impacts because of the required using (super)stoichiometric quantities of Cr(VI) oxide as oxidant in organic solvent.[21-23] We found that the theoretically calculated electric density at the benzylic position of 3-benzylquinoxaline (**3**) was well close with the data of 2-benzylbenzimidazole (**1**) and 2-benzylbenzoxazole (**2**) (Fig. 1). Based on this result and our previous work, it is tempting to speculate that the O_2/AC/H_2O may be a proper oxidation system for this oxidation transformation and we wish to explore the effect of pH on this transformation.[24] Even more intriguing, we noticed that 3-benzoylquinoxalinone framework is as similar as some tubulin inhibitors, such as phenstatin (**4**),[25] BNC-105P (**5**),[26] BPR0L075 (**6**),[27] and **7** (Fig. 2),[28] which are receiving growing attention in anticancer drug discovery. As a continuation of an ongoing research on the development of "green and sustainable" methodologies for the synthesis of potential functional molecules, we herein wish to report a novel strategy to extend the applications of AC promoted aerobic benzylic oxidation and develop a clean method to synthesize 3-benzoylquinoxalines. This would not only offer potential biological applications but also represent a significant contribution to green medicinal chemistry.

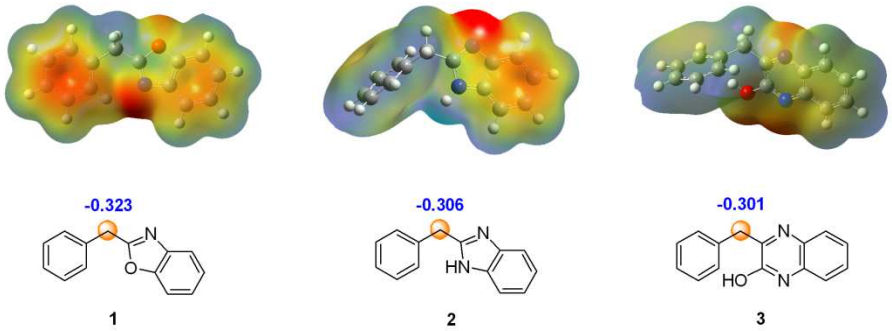


Figure 1. Connolly surface (probe radius = 1 Å) mapped with the molecular electrostatic potential (MEP), quantum chemical calculations performed with Gaussian 09; Blue/red areas represent negative/positive electrostatic potentials.

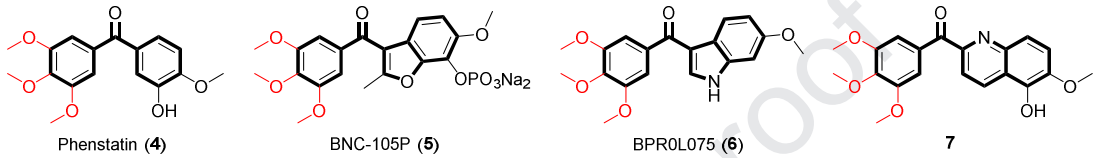


Figure 2. Structures of some tubulin inhibitors.

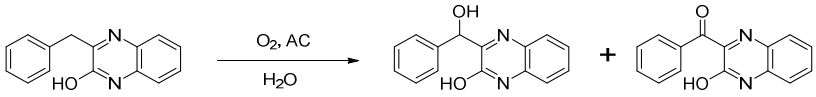
2. Results and Discussion

Chemistry and biological studies.

In order to validate our hypothesis, 3-benzylquinoxalin-2-ol (**8a**) was initially selected as the model substrate to explore the predicted reactivity (Table 1). To our delight, the reaction proceeded to afford selectively the desired product **9a** in 83.4% yield when the suspension of AC and the insoluble substrate **8a** in water under oxygen atmosphere was stirred at 85 °C for 24 h (Table 1, entry 3). To improve the efficiency of this transformation, various Brønsted-acids as additives were also screened to examine their effects on the reaction. As shown in Table 1, we were glad to see that the yield of **9a** could be obviously improved to 71.9% after adding 10.0 equivalents of AcOH to the above aqueous solution at 85 °C for 4 h (Table 1, entry 4). Replacement of AcOH with other acids, including HCOOH, H_2SO_4, TsOH and TFA, could also accelerate the aerobic oxidation process (Table 1, entries 5–8). Remarkably, the yield of **9a** could be improved to 94.4% in the presence of 10.0 equivalents of TFA (Table 1, entry 8). Further optimization revealed that this TFA-promoted transformation could be performed using lower additive loadings in aqueous solution (Table 1, entries 9–11) while still giving **9a** with a high yield (>85%). Gratifyingly, increase of the reaction temperature to 100 °C led to completion of the process in 4 h with 91.2% yield of **9a** but no **9a′** was observed (Table 1, entry 13). Extending the reaction time was unfavorable for the reaction (Table 1, entry 14). Based on these results, the

reaction could be directed cleanly to the desired products, 3-benzoylquinoxalines, by optimization of the reaction conditions. Most importantly, this "on water" reaction is operationally straightforward.

Table 1. Optimization of the Reaction Conditions[a]



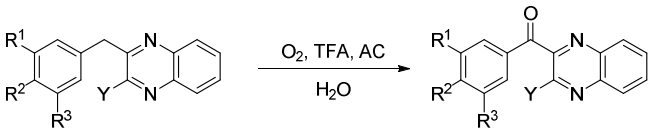
8a **9a′** **9a**

Entry	Additive (equiv)	Temperature (°C)	Time (h)	Ratio (**9a′**: **9a**)[c]
1[a]	none	85	4	NR
2[b]	none	85	4	21%: 19.1%
3[b]	none	85	24	4.5%: 83.4%
4[b]	AcOH (10.0)	85	4	15.0%: 71.9%
5[b]	HCOOH (10.0)	85	4	9.8%: 76.9%
6[b]	H_2SO_4 (10.0)	85	4	5%: 67.5%
7[b]	TsOH (10.0)	85	4	5.2%: 90.4%
8[b]	TFA (10.0)	85	4	1.2%: 94.4%
9[b]	TFA (5.0)	85	4	0.9%: 87.6%
10[b]	TFA (3.0)	85	4	1.1%: 86.7%
11[b]	TFA (1.0)	85	4	1.4%: 85.8%
12[b]	TFA (1.0)	70	4	6.5%: 79.4%
13[b]	**TFA (1.0)**	**100**	**4**	**0%: 91.2%**
14[b]	TFA (1.0)	100	6	0%: 88.1%

[a]Reaction conditions: 3-benzylquinoxalin-2-ol (0.2 mmol) in H_2O (3.0 mL) under O_2 atmosphere. [b]Reaction conditions: 3-benzylquinoxalin-2-ol (0.2 mmol), 50 wt % of AC, and additive in H_2O (3.0 mL) under O_2 atmosphere. [c]Yields and the ratio of **9a′** to **9a** were determined by HPLC analysis of the crude reaction mixture. NR = no reaction.

Having established the optimal reaction conditions, we subsequently turned our focus to explore the substrate scope of this protocol. The results are presented in Table 2. It was found that AC-TFA pairing could effectively promote the aerobic oxidation of 3-benzylquinoxaline derivatives to the corresponding carbonyl products in the "on water" heterogeneous system with moderate to acceptable isolated yield (65–85%). The presence of an electron-withdrawing group at the phenyl ring somewhat lowered the yields (entries 3, 6, 7, 9 and 10). On the other hand, the substrates with electron-donating groups at the 2-position of the quinoxaline ring showed higher reactivity than those with electron-withdrawing groups on the same position (entries 8–10 vs 5–7).

Table 2 Substrate Scope[a] and Their Antiproliferative Activities *in Vitro*[b]

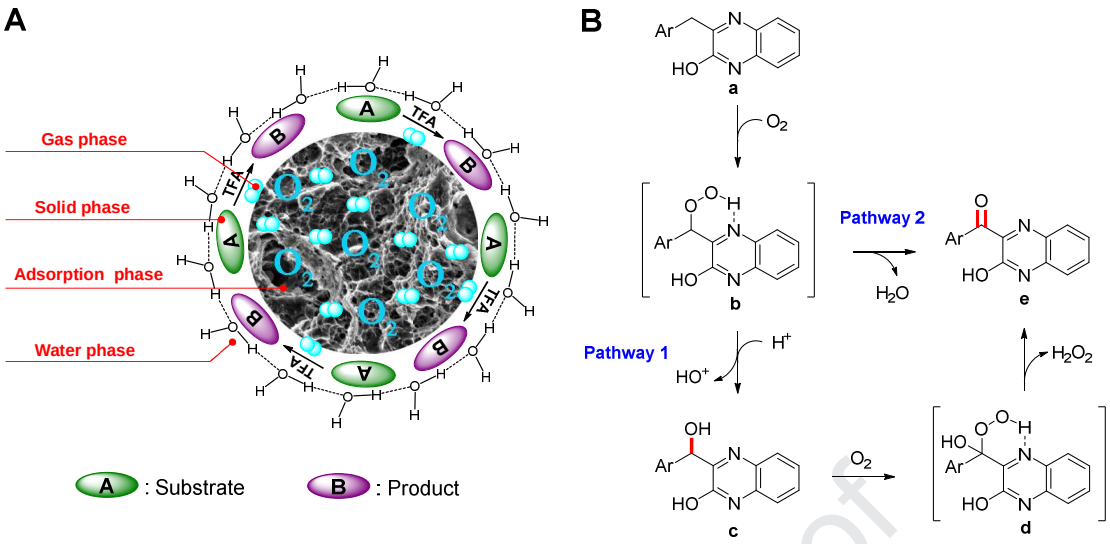


8a~j **9a~j**

Entry	Product	R^1	R^2	R^3	Y	Yield[c] (%)	IC_{50} (μM)
1	**9a**	H	H	H	OH	71	>30
2	**9b**	H	OCH_3	H	OH	80	>30
3	**9c**	H	Cl	H	OH	77	>30
4	**9d**	OCH_3	OCH_3	OCH_3	OH	65[d]	0.47 ± 0.02
5	**9e**	H	OCH_3	H	Cl	75	>30
6	**9f**	H	Cl	H	Cl	73	>30
7	**9g**	H	Br	H	Cl	72	>30
8	**9h**	H	OCH_3	H	OCH_3	85	>30
9	**9i**	H	Cl	H	OCH_3	83	>30
10	**9j**	H	Br	H	OCH_3	81	>30

[a]Reaction conditions: 3-benzylquinoxalines (0.2 mmol), 50 wt % of AC, and TFA (0.2 mmol) in H_2O (3.0 mL) at 100 °C for 4 h. [b]A549 cell line. [c]Isolated yield. [d]Reaction conditions: **8d** (0.2 mmol), 50 wt % of AC, and TFA (0.6 mmol) in H_2O (3.0 mL) at 85 °C for 4 h.

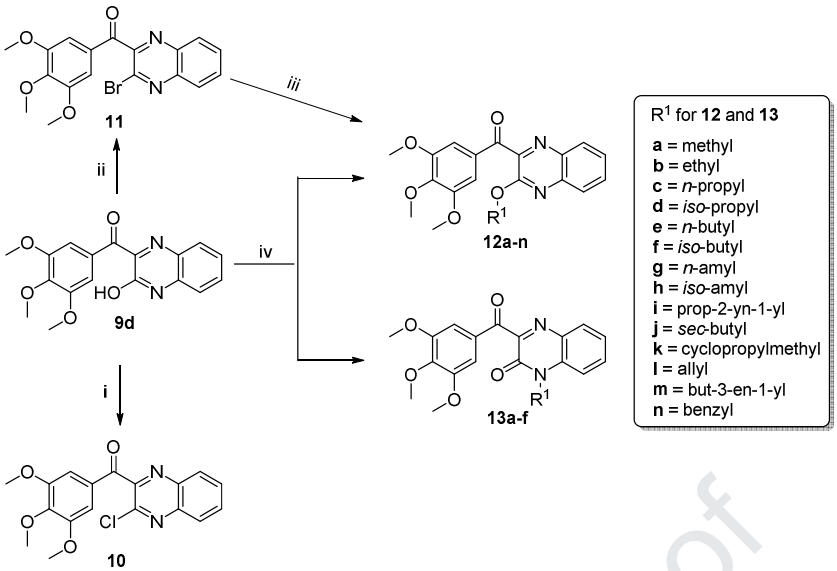
Generally, the reaction process of AC-TFA promoted aerobic benzylic oxidation under "on-water" condition is depicted in Scheme 2A. We speculate that the substrates are adsorbed on the surface of the superhydrophobic porous activated carbon and the reaction is carried out at air-liquid-solid joint interfaces where the hydrogen-bonding activation, absorption and hydrophobic effects work together to achieve the accelerating effect. In addition, it was reported that molecular oxygen could be activated and improved dissolution in aqueous TFA solution.[24] Hence, a plausible mechanism for the formation of 3-benzoylquinoxalinones is proposed in Scheme 2B. These two pathways might both involve the formation of peroxide (**b**) as a key intermediate. Under the acidic conditions, **b** spontaneously evolved to the products **c** and **f**, respectively. The hydroxyl product **c** could be further converted into the corresponding carbonyl product **f** via oxidative dehydrogenation.



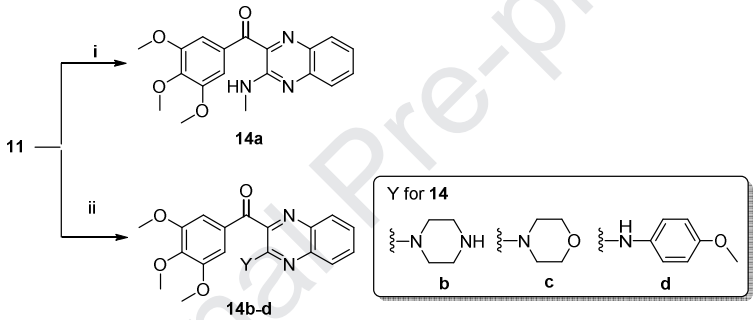
Scheme 2. Proposed reaction model (A) and mechanism (B) of AC-TFA promoted aerobic benzylic oxidation under "on-water" condition.

The screening of potential biological activity in synthetic or natural products is a significant step in drug discovery and development process. Since they are structurally correlated with CSBIs, the newly synthesized compounds **9a–j** were first screened for antiproliferative activities against human lung adenocarcinoma cell line (A549). The result was shown in Table 2. As anticipated, **9d** showed potent antiproliferative activity and it suggested the essential role of 3,4,5-trimethoxy substituents on the phenyl ring. This finding is consistent with results from most of the known tubulin inhibitors in which the 3,4,5-trimethoxy substituted compound was most potent (Fig. 2).

To investigate the activity further, **9d** was chosen as a lead compound for further structure modification (Scheme 3 and 4). We retained the 3,4,5-trimethoxyphenyl group of **9d** and mainly focused on modifications to the substituent at the 2- and 3-positions of the quinoxaline ring. The general synthesis of the modified derivatives of **9d** is outlined in Schemes 1–2. Firstly, chlorination of **9d** with neat $POCl_3$ gave **10** in 91% yield,[29] and **9d** was converted into **11** by treatment with Bu_4NBr and P_2O_5 in refluxing toluene for 5 h in 75% yield.[30] Subsequently, **11** was substituted with different alcoholate or amino derivatives to form the corresponding products (**12a–n** and **14a–d**). On the other hand, **9d** reacted with the appropriately alkylating reagent in the presence of KI and Na_2CO_3 in DMF, affording N-alkyl products (**13a–f**).[29]



Scheme 3. Reagents and conditions: (i) $POCl_3$, reflux, 3 h; (ii) P_2O_5, TBAB, toluene, reflux, 5 h; (iii) Na, ROH, reflux, 0.5 h; (iv) R^1X, Na_2CO_3, KI, DMF, 40~80 °C, 4 h.



Scheme 4. Reagents and conditions: (i) CH_3NH_2, THF, 50 °C, overnight; (ii) HY, microwave, 150 °C, 0.5 h.

Antiproliferative activity assays and SAR analysis. The synthesized compounds **10–14** were evaluated for their antiproliferative activities in three different human cancer cell lines (HT-1080, SGC-7901 and A549). Combretastatin A-4 (CA-4), a well-known tubulin inhibitor, was included in the assay as positive control. The results are summarized in Table 3. As shown in Table 3, **12a** displayed the most active compound. As compared to **12a**, **12b** had about 10-fold decrease in activity likely due to its one-carbon longer alkoxy chain. Similarly, the longer and bulkier group at 2-postion of the quinoxaline ring, such as *n*-pentyloxy (**12g**), *i*-pentyloxy (**12h**), benzyloxy (**12n**) and but-3-en-1-yloxy (**12m**), was tolerated but with a significant activity decline. Whereas replacing the alkoxy chains with electron-withdrawing groups resulted in complete loss of antiproliferative activities (**11** and **12**). Additionally, it is noted that the incorporation of a comparatively polar amine did not increase the activity significantly (**14a–d**). The activity variation of the substituent at 2-postion of the quinoxaline ring suggests that the activity drop is truly related to steric hindrance and electrical effect but not solubility. One interesting

phenomenon is that the modifications at 1-postion of the quinoxaline ring almost abrogated activity (**13a–f**).

Table 3. Growth Inhibition IC_{50} Values of Synthesized Quinoxaline Derivatives *in Vitro*

Compound	IC_{50} (μM)[a]		
	HT-1080	SGC-7901	A549
9d	**0.74 ± 0.05**	**0.88 ± 0.07**	**0.47 ± 0.02**
10	>30	>30	>30
11	>30	15.20 ± 1.35	>30
12a	**0.17 ± 0.01**	**0.24 ± 0.03**	**0.28 ± 0.01**
12b	1.60 ± 0.23	1.48 ± 0.11	2.96 ± 0.16
12c	2.30 ± 0.31	**0.66 ± 0.17**	3.23 ± 0.21
12d	2.06 ± 0.16	**0.68 ± 0.08**	**0.41 ± 0.05**
12e	6.94 ± 0.35	4.58 ± 0.29	2.09 ± 0.13
12f	2.13 ± 0.19	**0.97 ± 0.04**	**0.72 ± 0.06**
12g	6.35 ± 0.42	3.43 ± 0.29	2.43 ± 0.14
12h	6.13 ± 0.38	3.56 ± 0.18	2.16 ± 0.12
12i	**0.26 ± 0.07**	**0.84 ± 0.10**	1.28 ± 0.33
12j	1.90 ± 0.10	1.55 ± 0.22	**0.79 ± 0.15**
12k	2.56 ± 0.13	1.81 ± 0.19	1.32 ± 0.14
12l	1.18 ± 0.09	2.20 ±0.14	**0.78 ± 0.06**
12m	5.56 ± 0.36	4.65 ± 0.26	0.82 ± 0.03
12n	6.50 ± 0.27	4.46 ± 0.16	6.96 ± 0.44
13a	>30	>30	>30
13b	>30	>30	>30
13c	>30	9.66 ± 0.92	>30
13d	10.86 ± 1.19	>30	>30
13e	>30	>30	>30
13f	>30	>30	14.97 ± 1.06
14a	1.42 ± 0.22	7.35 ± 0.31	8.67 ± 0.28
14b	6.07 ± 0.35	2.21 ± 0.29	1.62 ± 0.17
14c	2.11 ± 0.21	**0.68 ± 0.14**	1.28 ± 0.13
14d	25.83 ± 1.37	15.47 ± 1.05	9.67 ± 0.44
CA-4[c]	0.016 ± 0.003	0.024 ± 0.003	0.012 ± 0.002

Bold values show IC_{50} of < 1 μM. [a]IC_{50}: Concentration of the compound (μM) producing 50% cell growth inhibition after 72 h of drug exposure, as determined by the MTT assay. Each experiment was carried out in triplicate. [b]pIC_{50} values of A549 cell line. The pIC_{50} values are used 4.52 when the obtained IC_{50} is greater than 30μM. [c]Used as a positive control.

Inhibition of Tubulin Polymerization. As we mentioned before, the quinoxaline derivatives were originally desired to inhibit the tubulin polymerization and induce apoptosis. To validate this biological target, the most active compound **12a** was evaluated by microtubule dynamics assay in parallel with taxol and CA-4 as reference. The result in this assay is shown in Fig. 3. At 1 μM, **12a**

showed a weak antitubulin polymerization activity. When the concentration of **12a** was increased to 3.3 μM, a clear inhibition of polymerization was noted, and the rate of assembly as well as the final amount of microtubules was lower than that in the control experiment. In contrast, CA-4 at 2 μM nearly completely inhibited tubulin polymerization. These data confirmed that the mechanism of action of **12a** was to inhibit tubulin polymerization.

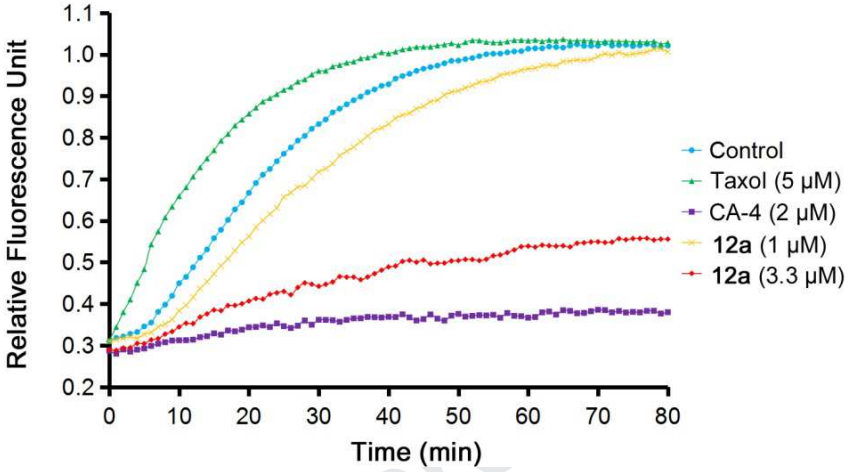


Figure 3. Effect of **12a** on microtubule dynamics**.**

Analysis of immunofluorescence staining. Next, we performed immunocytochemistry studies to test effect of **12a** on the cellular microtubule structure stained for DNA (blue) and α-tubulin (green). Untreated cells were used as a negative control, and cells treated with CA-4 were used as a positive control. As shown in Fig. 4, the microtubule network in HT-1080 and SGC-7901 cells exhibited normal arrangement and organization in the absence of drug treatment. In contrast, either **12a** or CA-4 caused dramatic reduction of the interphase microtubule network wrapped around the cell nucleus. These results indicated that **12a** was like CA-4 as a microtubule-destabilizing agent.

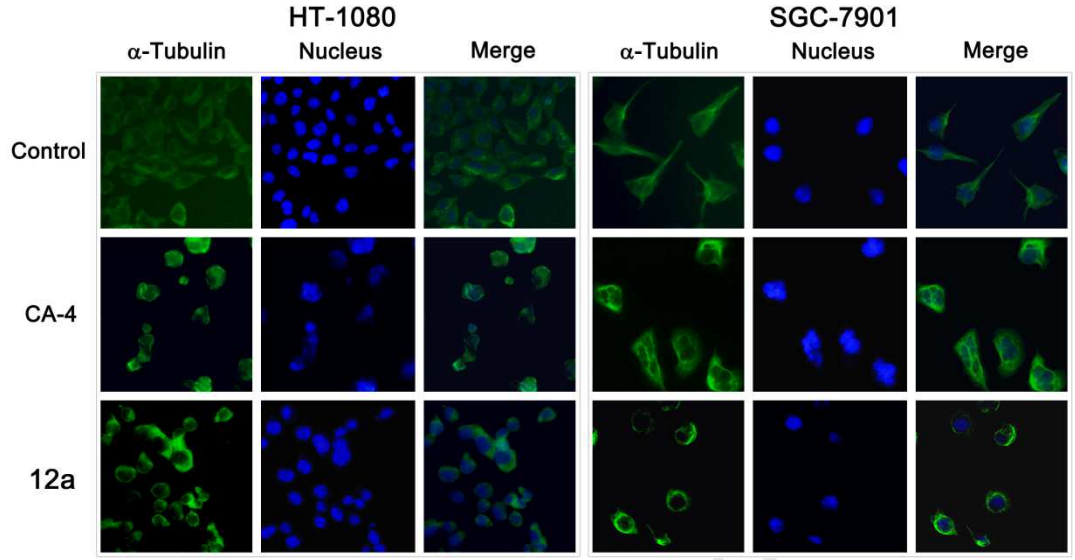


Figure 4. Effects of **12a** (0.34 μM) and CA-4 (0.032 μM) on the cellular microtubule network and microtubule reassemble by immunofluorescence. HT-1080 and SGC-7901 cells were treated with **12a** or CA-4 for 48 h, and then direct microscopy detection of the fixed and stained cell was performed. The cellular microtubules were stained with anti-α-tubulin-FITC specific antibodies (green). DNA was stained by 4′,6-diamidino-2-phenylindole (DAPI, blue).

Cell cycle analysis. Because of the above results, **12a** was also subjected to analysis for its effect on cell cycle distribution by flow cytometry. HT-1080 cells were treated with different concentrations of **12a** for 12 and 24 h, respectively. The tested data are summarized in Fig. 5 and Fig. 6. Cells treated with either **12a** or CA-4 were observed an obvious G2/M phase arrest, whereas control cells were mainly in the G0/G1 phase. The effect of **12a** on cell cycle distribution patterns was similar to the positive control CA-4, and it showed dose-dependent and time-dependent clearly.

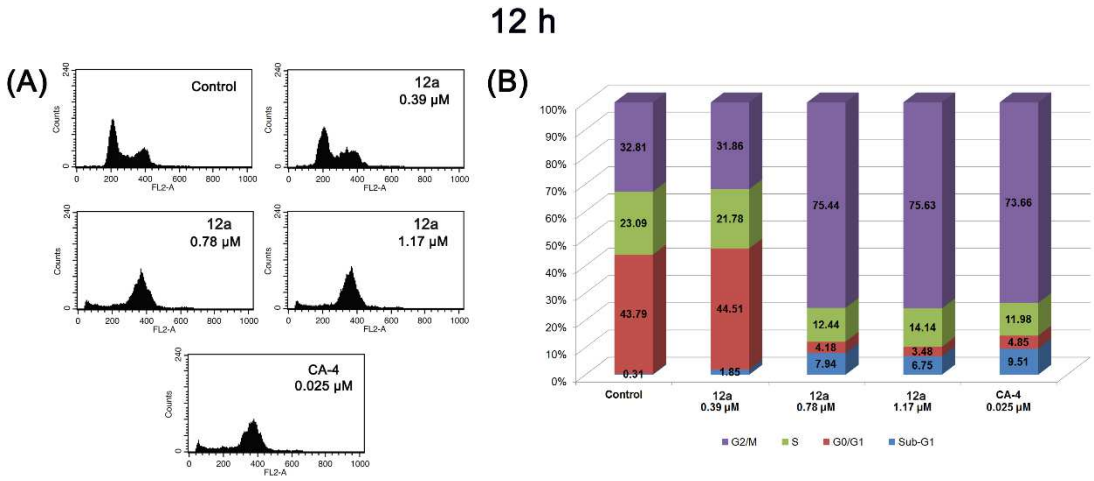


Figure 5. (A) Cell cycle distribution of HT-1080 cells with or without treatment of **12a** for 12 h. (B) The statistical histogram of the tested data.

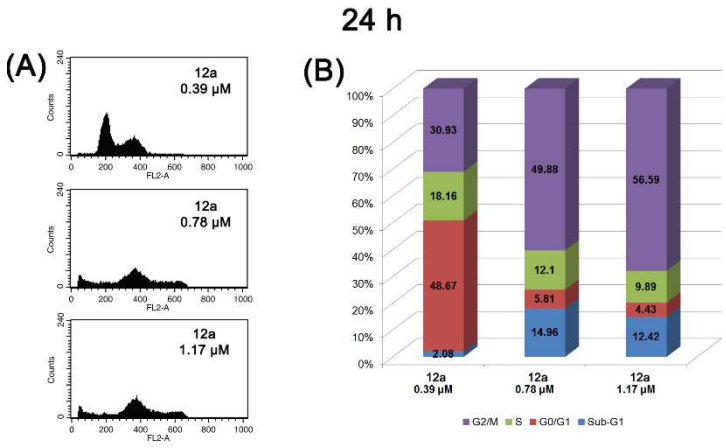


Figure 6. (A) Cell cycle distribution of HT-1080 cells with or without treatment of **12a** for 24 h. (B) The statistical histogram of the tested data.

Molecular docking. To rationalize our experimental data from a structure-based perspective, a series of molecular docking simulations of these new compounds in the colchicine site of tubulin were performed by using a procedure reported previously.[31, 32] As shown in Fig. 7A, **12a** displayed a binding conformation with low energy (-45.13 kcal/mol). It was found that the binding conformation of **12a** generated in the docking simulation was very similar to the conformation of Phenstatin and the native ligand (Fig. 7D). Colchicine derivatives with groups substituted at the methoxy positions can cross-link with Cys-β241.[33] By examining the ligand interactions for the docked compounds, it becomes apparent that the trimethoxyphenyl moieties of Phenstatin and **12a** were well overlapped with each other, and occupied a hydrophobic region in the binding site and established a series of interactions with Cys-β241, Val-β238, and Leu-β255 (Fig. 7B). It has been noted that, for many CA-4 analogues and Phenstatin, the 3-hydroxy-4-methoxyphenyl moiety was essential for activity and the docked solution presented here indicated that the *p*-methoxy group on the 3-hydroxy-4-methoxyphenyl of Phenstatin played a role in sterically interacting with the Thr-α179 (Supporting Information, Fig. S1). For **12a**, the quinoxaline moiety was well overlapped with the 3-hydroxy-4-methoxyphenyl moiety of Phenstatin, and formed van der Waals forces with the hydrophobic pocket near α-tubulin, and Asn-β258 formed a hydrogen bond with the nitrogen atom at 1-position on the quinoxaline ring. The key hydrogen bond could stabilize the interaction of **12a** with the binding pocket, and this might explain why alkylation at this position showed a dramatic loss in activity. From the results in Table 2, a larger group, such as **12e**, **12g** and **12n**, at 2-position on the quinoxaline ring was unfavorable to activity. Indeed, the methoxy was the group with optimal volume for interaction with the binding pocket as shown in Fig. 7C, and the docking

simulations of larger group compounds were not able to generate a reasonable binding pose. In general, these docking results were in accordance with the data in the cell lines tested, and they provided a possible structural justification of the SARs observed.

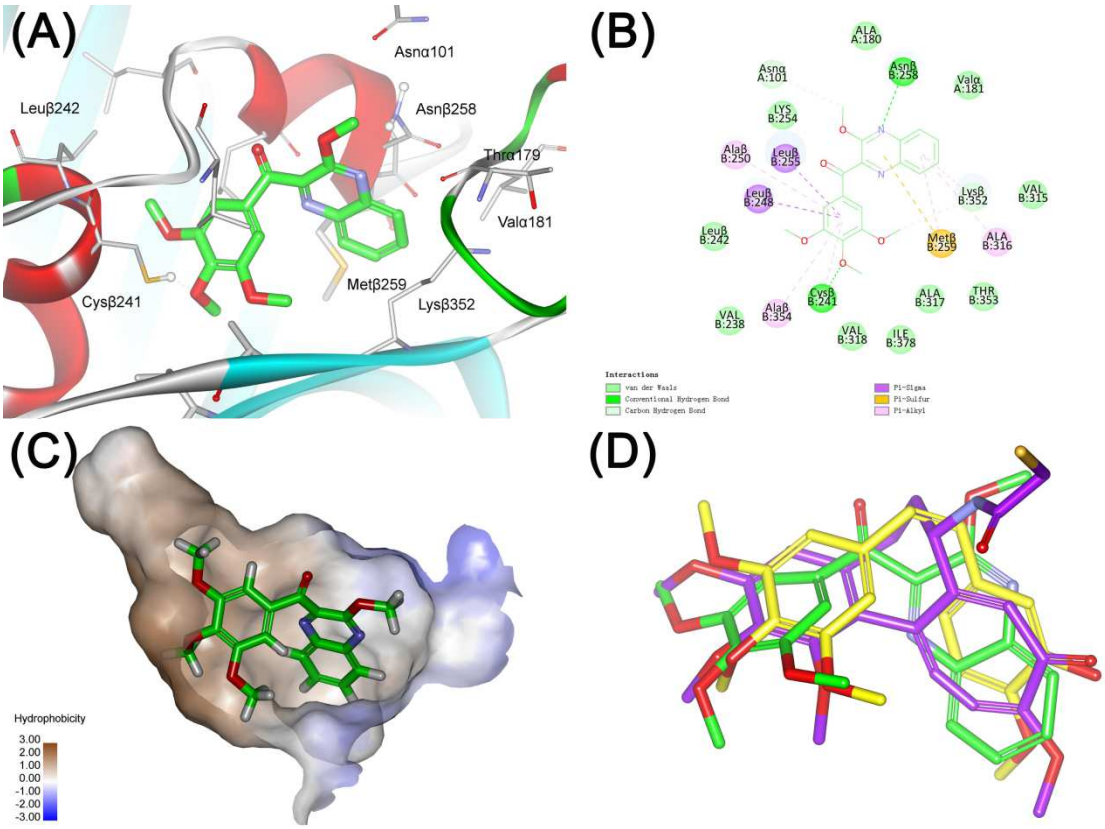


Figure 7. (A) Proposed binding for **12a** (green) in the colchicine site. Residues within 3.5 Å of **12a** are represented as the thicker sticks. Hydrogen bonds (distance of < 3 Å) are shown as dotted green lines. (B) A interaction sketch between **12a** and its binding site. (C) The hydrophobicity surface on the receptor. (D) Superposition of **12a** (green) with the docking modes of CA-4 (gray) and co-crystallized DAMA-colchicine (purple).

3. Conclusion

In summary, a full account of approach for searching the proper substrate, which is suitable for O_2/AC/H_2O system promoted aerobic benzylic oxidation, has been presented by analysis the structure computationally. Use of 3-benzylquinoxalinones as substrates provides an experimental proof, which may be applicable to our strategy. Data indicated that a Brønsted acid, especially TFA, is able to significantly improve the efficiency of the reaction process and achieve high yield. Among the resulting products, **9d** exhibited excellent antiproliferative activity and was chosen to structure modification. The *in vitro* immunofluorescence staining and tubulin polymerisation assay demonstrated that **12a** as an analogue of **9d** inhibited tubulin polymerisation into microtubules and caused microtubule destabilisation, which suggests that **12a** is a novel tubulin inhibitor. Further investigations are in progress in our laboratory to evaluate the strategy with a

broader range of substrates, and to synthesize more complex products that comprises privileged scaffolds displaying diverse range of biological activities. We hope to provide more green versions of the reactions for medicinal chemists to construct molecules in the led-seeking or candidate-seeking stages of drug discovery.

4. Experimental section

4.1. Chemistry

All of reagents and solvents were purchased from chemical company. The AC was purchased from Tokyo Chemical Industry (TCI) (catalogue number: C2194). ^{1}H-NMR and ^{13}C-NMR spectra were tested in $CDCl_3$ or d_6-DMSO with TMS as the internal reference on a Bruker AVANCE 400 or 600 (^{1}H at 400 or 600 MHz, ^{13}C at 150 MHz). Mass spectra (MS) were measured on an Agilent 1100-sl mass spectrometer with an electrospray ionisation source from Agilent Co. Ltd. High resolution accurate mass determinations (HRMS) for all of the final target compounds were obtained on a Bruker Micromass Time of Flight mass spectrometer equipped with electrospray ionisation (ESI). TLC analysis was used for determining the extent of reactions under UV light (wavelength: 365 nm and 254 nm). Melting point was measured (uncorrected) on hot-stage microscope (Beijing Taike, X-4).

*4.1.1. General synthetic procedures for 3-benzoyl-2-methoxyquinoxaline (**9a–j**).*

A mixture of substrate (**8a–j**) (0.2 mmol), trifluoroacetic acid (0.2 mmmol) and 50 wt % of activated carbon in water (5 mL) was placed in a sealed tube (100 mL). The system was purged with molecular oxygen and sealed. The reaction mixture was heated to 100°C and stirred for 4 h. Upon completion, the reaction mixture was cooled to room temperature. After filtration of the suspension and washing of the solids with EtOAc, the crude product was obtained by removal of the solvent.

*4.1.1.1. 3-(Hydroxy(phenyl)methyl)quinoxalin-2(1H)-one (**9a'**)*

A mixture of **8a** (0.2 mmol) and 50 wt % of activated carbon in water (5 mL) was placed in a sealed tube (100 mL). The system was purged with molecular oxygen and sealed. The reaction mixture was heated to 85°C and stirred for 4 h. Upon completion, the reaction mixture was cooled to room temperature, filtered and washed with EtOAc. The removal of the solvent yielded a residue that was purified by silica gel column chromatography (*n*-hexane: EtOAc=8:1 as eluent) on silica gel to afford pure product. White solid (12% yield); Mp: 192–194°C; ^{1}H NMR (600 MHz,

DMSO-d_6): δ=12.38 (s, 1H), 7.72 (d, 1H, J=7.7 Hz), 7.47 (m, 1H), 7.27 (m, 2H), 6.65 (s, 2H), 4.05 (s, 2H), 3.72 (s, 6H), 3.61 (s, 3H); HRMS: calcd. for $C_{15}H_{13}N_2O_2$ $[M+H]^+$ 253.0977, found 253.0979.

*4.1.1.2. 3-Benzoylquinoxalin-2(1H)-one (**9a**)*

Yellowish solid (71% yield); Mp: 179–181°C; ^{1}H NMR (600 MHz, DMSO-d_6): δ=12.38 (s, 1H), 7.72 (d, 1H, J=7.7 Hz), 7.47 (m, 1H), 7.27 (m, 2H), 6.65 (s, 2H), 4.05 (s, 2H), 3.72 (s, 6H), 3.61 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ=129.9, 156.7, 153.9, 135.1, 135.0, 133.2, 132.2, 131.7, 130.1 (×2), 129.6, 129.5 (×2), 124.3, 116.4; HRMS: calcd. for $C_{15}H_{10}N_2NaO_5$ $[M+Na]^+$ 273.0640, found 273.0638.

*4.1.1.3. 3-Benzoylquinoxalin-2(1H)-one (**9b**)*

Yellowish solid (80% yield); Mp: 244–245°C; ^{1}H NMR (600 MHz, DMSO-d_6): δ=12.68 (s, 1H), 7.93 (d, J=8.6 Hz, 2H), 7.81 (d, J=7.8 Hz, 1H), 7.63 (t, 1H), 7.38 (m, 2H), 7.07 (d, J=8.6 Hz, 2H), 3.8 (s, 3H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ=191.2, 157.1, 164.8, 153.9, 133.1, 132.7 (×2), 131.9, 131.7, 129.5, 128.0, 124.3, 116.3 (×2), 114.8, 56.2; HRMS: calcd. for $C_{16}H_{11}N_2O_3$ $[M–H]^–$ 279.0770, found 279.0774.

*4.1.1.4. 3-(4-Chlorobenzoyl)quinoxalin-2(1H)-one (**9c**)*

Yellowish solid (77% yield); Mp: 242–244°C; ^{1}H NMR (600 MHz, DMSO-d_6): δ=8.41 (s, 1H), 7.98 (d, J=8.5 Hz, 2H), 7.79 (d, J=8.1 Hz, 1H), 7.62 (m, 3H), 7.45 (d, J=8.1 Hz, 1H), 7.34 (t, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ=191.8, 156.1, 153.8, 140.1, 133.8, 133.3, 133.4, 132.4, 132.0 (×2), 129.7, 129.6 (×2), 124.3, 116.4; HRMS: calcd. for $C_{15}H_8ClN_2O_2$ $[M–H]^–$ 283.0274, found 283.0270.

*4.1.1.5. 3-(3,4,5-Trimethoxybenzoyl)quinoxalin-2(1H)-one (**9d**)*

A mixture of substrate (**8d**) (0.2 mmol), trifluoroacetic acid (0.6 mmmol) and 50 wt % of activated carbon in water (3 mL) was placed in a sealed tube (100 mL). The system was purged with molecular oxygen and sealed. The reaction mixture was heated to 85°C and stirred for 4 h. Upon completion, the reaction mixture was cooled to room temperature. After filtration of the suspension and washing of the solids with EtOAc, the crude product was obtained by removal of the solvent. Yellowish solid (83% yield); Yellowish solid (65% yield); Mp: 222–224°C; ^{1}H NMR (600 MHz, DMSO-d_6): δ=12.78 (s, 1H), 7.82 (d, J=8.0 Hz, 1H), 7.64 (t, J=7.3 Hz, 1H), 7.40 (d, J=8.0 Hz, 1H), 7.36 (t, J=7.3 Hz, 1H), 7.27 (s, 2H), 3.80 (s, 6H), 3.78 (s, 3H); ^{13}C NMR (125

MHz, DMSO-d_6): δ=191.6, 156.5, 153.9, 153.4 (×2), 143.7, 133.3, 132.1, 131.8, 130.4, 129.6, 124.2, 116.3, 107.9 (×2), 60.8, 56.8 (×2); HRMS: calcd. for $C_{18}H_{17}N_2O_5$ $[M+H]^+$ 341.1137, found 341.1136.

*4.1.1.6. (3-Chloroquinoxalin-2-yl)(4-methoxyphenyl)methanone (**9e**)*

Yellowish solid (75% yield); Mp: 120–121°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.13 (m, 2H), 7.87 (m, 4H), 6.98 (d, J=8.7 Hz, 2H), 3.90 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ= 189.4, 164.9, 150.7, 142.0, 139.8, 132.9 (×3), 132.1, 130.9, 129.5, 128.5, 127.8, 114.2 (×2), 55.7; HRMS: calcd. for $C_{16}H_{12}ClN_2O_2$ $[M+H]^+$ 299.0587, found 299.0591.

*4.1.1.7. (4-Chlorophenyl)(3-chloroquinoxalin-2-yl)methanone (**9f**)*

Yellowish solid (73% yield); Mp: 143–146°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.14 (m, 2H), 7.92 (m, 1H), 7.88 (m, 3H), 7.50 (d, J=8.7 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=190.0, 149.6, 144.0, 142.2, 141.3, 139.6, 133.2, 132.5, 131.7 (×2), 131.1, 129.5, 129.3 (×2), 128.5; HRMS: calcd. for $C_{15}H_9Cl_2N_2O$ $[M+H]^+$ 303.0092, found 303.0088.

*4.1.1.8. (4-Bromophenyl)(3-chloroquinoxalin-2-yl)methanone (**9g**)*

Yellowish solid (72% yield); Mp: 124–126°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.12 (m, 2H), 7.80 (m, 2H), 7.79 (d, J=8.7 Hz, 2H), 7.66 (d, J=8.7 Hz, 2H); ^{13}C NMR (125MHz, $CDCl_3$): δ=190.2, 149.5, 143.9, 142.2, 139.6, 133.5, 132.5, 132.3 (×2), 131.8 (×2), 131.1, 130.2, 129.5, 128.5; HRMS: calcd. for $C_{15}H_9BrClN_2O$ $[M+H]^+$ 346.9587, found 346.9589.

*4.1.1.9. (4-Methoxyphenyl)(3-methoxyquinoxalin-2-yl)methanone (**9h**)*

Yellowish solid (85% yield); Mp: 134–136°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.05 (dd, J=7.93, J=0.59, 1H), 7.92 (d, J=8.8, 2H), 7.91 (d, J=7.9, 1H), 7.74 (m, 1H), 7.61 (m, 1H), 6.95 (d, J=8.8, 2H), 4.09 (s, 3H), 3.87 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=190.5, 164.5, 155.5, 145.5, 140.8, 137.5, 132.8 (×2), 131.1, 129.3, 128.4, 127.2, 127.0, 114.0 (×2), 55.6, 54.2; HRMS: calcd. for $C_{17}H_{15}N_2O_3$ $[M+H]^+$ 295.1083, found 295.1087.

*4.1.1.10. (4-Chlorophenyl)(3-methoxyquinoxalin-2-yl)methanone (**9i**)*

Yellowish solid (83% yield); Mp: 140–142°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.05 (dd, J_1=8.3 Hz, J_2=1.0 Hz, 1H), 7.93 (dd, J_1=8.3 Hz, J_2=1.0 Hz, 1H), 7.90 (d, J=8.6 Hz, 2H), 7.77 (m, 1H), 7.63 (m, 1H), 7.46 (d, J=8.6 Hz, 2H), 4.10 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=190.7, 155.5, 144.2, 141.0, 140.7, 137.5, 133.8, 131.7 (×2), 131.5, 129.4, 129.0 (×2), 127.4, 127.2, 54.2; HRMS: calcd. for $C_{16}H_{12}ClN_2O_2$ $[M+H]^+$ 299.0587, found 299.0588.

*4.1.1.11. (4-Bromophenyl)(3-methoxyquinoxalin-2-yl)methanone (**9j**)*

Yellowish solid (81% yield); Mp: 136–137°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.04 (d, *J*=8.2 Hz, 1H), 7.92 (d, *J*=8.2 Hz, 1H), 7.82 (d, *J*=8.37 Hz, 2H), 7.64 (d, *J*=8.4 Hz, 2H), 7.60 (m, 2H), 4.10 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=190.9, 155.5, 144.1, 141.0, 137.5, 134.2, 132.0, 131.8 (×2), 131.5 (×2), 129.6, 129.4, 127.4, 127.2, 54.2; HRMS: calcd. for $C_{16}H_{12}BrN_2O_2$ $[M+H]^+$ 343.0082, found 343.0077.

*4.1.1.12. (3-Chloroquinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**10**)*

Yellow solid (78% yield); Mp: 174–175°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.15 (m, 1H), 7.90 (m, 2H), 7.18 (m, 2H), 3.97 (s, 3H), 3.84 (m, 6H); ^{13}C NMR (150 MHz, $CDCl_3$): δ=190.1, 153.3 (×2), 150.2, 144.2 (×2), 142.1, 139.6, 132.3, 131.1, 129.7, 129.5, 128.5, 108.0 (×2), 61.1, 56.4 (×2); HRMS: calcd. for $C_{18}H_{16}ClN_2O_4$ $[M+H]^+$ 359.0799, found 359.0796.

*4.1.1.13. (3-Bromoquinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**11**)*

Yellowish solid (74% yield); Mp: 193–194°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.15 (m, 2H), 7.90 (m, 2H), 7.17 (s, 2H), 3.97 (s, 3H), 3.85 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$): δ=190.7, 153.3 (×2), 151.8, 144.2, 143.0, 139.7, 135.5, 132.2, 131.2, 130.9, 129.5, 128.6, 108.0 (×2), 61.1, 56.4 (×2); HRMS: calcd. for $C_{18}H_{16}BrN_2O_4$ $[M+H]^+$ 403.0293, found 403.0295.

*4.1.2. General procedure for the synthesis of **12a–n**.*

In a round-bottomed flask, a mixture of sodium (12 mmol) and corresponding alcohols (12 mmol) was dissolved in 10 mL of dry THF and heated at reflux for 0.5 h. The solution of (3-Bromoquinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**11**) (5 mmol) in dry THF (2 mL) was added dropwise to the mixture, and the contents of the flask were stirred for 1 h at reflux. Then water (30 mL) was added and the aqueous layer was extracted with EtOAc. The combined organic extracts were dried with brine and Na_2SO_4. After filtration and the evaporation of the solvent, the crude product was purified by column chromatography (*n*-hexane: EtOAc=7:1 as eluent) on silica gel to afford pure product.

*4.1.2.1. (3-Methoxyquinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**12a**)*

Yellowish solid (86% yield); Mp: 135–137°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=7.94 (dd, 1H, J_1=1.5 Hz, J_2=8.4 Hz), 7.69 (m, 1H), 7.43 (m, 2H), 7.26 (s, 2H), 3.94 (s, 3H), 3.87 (s, 6H), 3.76 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=190.7, 155.6, 153.1 (×2), 144.7, 143.8, 140.9, 137.4, 131.3, 130.3, 129.3, 127.4, 127.1, 108.0 (×2), 61.0, 56.3 (×2), 54.2; HRMS: calcd. for $C_{19}H_{19}N_2O_5$

$[M+H]^+$ 355.1294, found 355.1285.

*4.1.2.2. (3-Ethoxyquinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**12b**)*

Yellowish solid (82% yield); Mp: 132–134°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.05 (d, 1H, J=11.6 Hz), 7.90 (d, 1H, J=8.2 Hz), 7.74 (m, 1H), 7.61 (m, 1H), 7.22 (s, 2H), 4.60 (m, 2H), 3.95 (s, 3H), 3.84 (s, 6H), 1.41 (t, 3H, J_1=7.1 Hz, J_2=14.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ=190.8, 155.3, 153.2 (×2), 145.0, 143.7, 141.0, 137.4, 141.0, 137.4, 131.2, 130.5, 129.3, 127.2, 127.2, 107.9 (×2), 63.0, 31.0, 56.4 (×2), 14.3; HRMS: calcd. for $C_{20}H_{21}N_2O_5$ $[M+H]^+$ 369.1450, found 369.1442.

*4.1.2.3. (3-Propoxyquinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**12c**)*

Yellowish solid (84% yield); Mp: 114–115°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.06 (dd, 1H, J_1=0.8 Hz, J_2=8.2 Hz), 7.89 (m, 1H), 7.76 (m, 1H), 7.61 (m, 1H), 7.19 (s, 2H), 4.48 (t, 2H, J_1=6.6 Hz, J_2=13.1 Hz), 3.94 (s, 3H), 3.84 (s, 6H), 1.79 (m, 2H), 0.94 (t, 3H, J_1=7.4 Hz, J_2=14.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ=190.9, 155.4, 153.2 (×2), 145.2, 143.7, 141.0, 137.5, 131.2, 130.6, 129.4, 127.2, 127.1, 107.8 (×2), 68.6, 61.1, 56.4 (×2), 22.0, 10.5; HRMS: calcd. for $C_{21}H_{23}N_2O_5$ $[M+H]^+$ 383.1607, found 383.1597.

*4.1.2.4. (3-Isopropoxyquinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**12d**)*

Yellow oil (81% yield); ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.04 (m, 1H), 7.88 (d, 1H, J=8.3 Hz), 7.74 (m, 1H), 7.60 (m, 1H), 7.18 (s, 2H), 5.58 (m, 1H), 3.95 (s, 3H), 3.84 (s, 6H), 1.37 (d, 6H, J=6.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ=190.8, 154.6, 153.0 (×2), 145.4, 143.5, 140.9, 137.2, 131.0, 130.5, 129.1, 127.0, 126.9, 107.6 (×2), 70.0, 60.8, 56.2 (×2), 21.6 (×2); HRMS: calcd. for $C_{21}H_{23}N_2O_5$ $[M+H]^+$ 383.1607, found 383.1599.

*4.1.2.5. (3-Butoxyquinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**12e**)*

Yellowish solid (79% yield); Mp: 82–84°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.05 (1H, m), 7.90 (1H, d, J=8.0 Hz), 7.75 (1H, m), 7.61 (1H, m), 7.18 (2H, s), 4.52 (2H, t, J_1=6.5 Hz, J_2=13.1 Hz), 3.95 (3H, s), 3.84 (6H, s), 1.75 (2H, m), 1.38 (2H, m), 0.91 (3H, t, J_1=7.4 Hz, J_2=14.8 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ=191.0, 156.3, 153.2 (×2), 145.2, 143.8, 141.1, 136.8, 131.2, 130.7, 129.4, 127.2, 127.2, 106.5 (×2), 66.5, 60.9, 56.1 (×2), 31.0, 19.4, 13.9; HRMS: calcd. for $C_{21}H_{23}N_2O_5$ $[M+H]^+$ 397.1763, found 397.1758.

*4.1.2.6. (3-Isobutoxyquinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**12f**)*

Yellowish solid (82% yield); Mp: 94–95°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.06 (dd, 1H, J_1=0.6

Hz, J_2=8.2 Hz), 7.90 (d, 1H, *J*=7.9 Hz), 7.75 (m, 1H), 7.61 (m, 1H), 7.18 (s, 2H), 4.29 (d, 2H, *J*=6.6 Hz), 3.94 (s, 3H), 3.84 (s, 6H), 2.07 (m, 1H), 0.93 (d, 1H, *J*=6.7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ=191.0, 155.4, 153.2 (×2), 145.3, 143.7, 141.0, 137.6, 131.2, 130.7, 129.4, 127.2, 127.1, 107.7 (×2), 73.1, 61.0, 56.4 (×2), 27.8, 19.1 (×2); HRMS: calcd. for $C_{22}H_{25}N_2O_5$ $[M+H]^+$ 397.1763, found 397.1756.

*4.1.2.7. (3-(Pentyloxy)quinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**12g**)*

Yellowish solid (85% yield); Mp: 71–73°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ= 8.06 (d, 1H, *J*=8.2 Hz), 7.90 (d, 1H, *J*=8.3 Hz), 7.76 (m, 1H), 7.61 (t, 1H, J_1=7.2 Hz, J_2=15.2 Hz), 7.18 (s, 2H), 4.51 (t, 2H, J_1=6.6 Hz, J_2=13.3 Hz), 3.94 (s, 3H), 3.83 (s, 6H), 1.76 (m, 2H), 1.31 (m, 4H), 0.86 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=191.0, 155.4, 153.2 (×2), 145.2, 143.7, 141.0, 137.5, 131.2, 130.6, 129.4, 127.2, 127.1, 107.8 (×2), 67.1, 61.0, 56.4 (×2), 28.2, 28.0, 22.3, 14.0; HRMS: calcd. for $C_{23}H_{27}N_2O_5$ $[M+H]^+$ 411.1920, found 411.1909.

*4.1.2.8. (3-(Isopentyloxy)quinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**12h**)*

Yellowish solid (77% yield); Mp: 92–94°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.06 (d, 1H, *J*=8.2 Hz), 7.90 (d, 1H, *J*=8.3 Hz), 7.76 (t, 1H, J_1=7.2 Hz, J_2=15.3 Hz), 7.62 (t, 1H, J_1=7.2 Hz, J_2=15.2 Hz), 7.18 (s, 2H), 4.54 (t, 2H, J_1=6.2 Hz, J_1=12.4 Hz), 3.95 (s, 3H), 3.83 (s, 6H), 1.65 (m, 3H), 0.89 (d, 6H, *J*=5.8 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ=191.0, 155.4, 153.2 (×2), 145.3, 143.7, 141.1, 137.6, 131.3, 130.7, 129.4, 127.3, 127.2, 107.8 (×2), 65.7, 61.1, 56.4 (×2), 37.3, 25.1, 22.6 (×2); HRMS: calcd. for $C_{23}H_{27}N_2O_5$ $[M+H]^+$ 411.1920, found 411.1914.

*4.1.2.9. (3-(Prop-2-yn-1-yloxy)quinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**12i**)*

Yellowish solid (71% yield); Mp: 136–137°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.08 (d, 1H, *J*=8.0 Hz), 7.96 (d, 1H, *J*=8.1 Hz), 7.79 (t, 1H, J_1=7.3 Hz, J_2=14.3 Hz), 7.66 (m, 1H), 7.22 (s, 2H), 5.17 (s, 2H), 3.96 (s, 3H), 3.84, 2.45 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=190.4, 153.9, 153.3 (×2), 144.7, 144.0, 140.6, 138.0, 131.6, 130.4, 129.5, 127.9, 127.4, 108.2 (×2), 78.2, 75.2, 61.1, 56.5 (×2), 61.1, 56.5 (×2), 54.2; HRMS: calcd. for $C_{21}H_{19}N_2O_5$ $[M+H]^+$ 379.1294, found 379.1290.

*4.1.2.10. (3-(Sec-butoxy)quinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**12j**)*

Yellow oil (81% yield); ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.04 (dd, 1H, J_1=0.8 Hz, J_2=8.4 Hz), 7.87 (dd, 1H, J_1=0.6 Hz, J_2=8.4 Hz), 7.73 (m, 1H Hz), 7.59 (m, 1H Hz), 7.17 (s, 2H), 5.40 (m, 1H), 3.94 (s, 3H), 3.83 (s, 6H), 1.71 (m, 1H), 1.64 (m, 1H), 1.35 (d, 3H, *J*=6.2 Hz), 0.88 (t, 3H, J_1=7.4 Hz, J_2=14.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ=191.3, 155.1, 153.2 (×2), 145.8, 143.7, 141.1,

137.5, 131.1, 130.8, 129.4, 127.2, 127.1, 107.8 (×2), 74.8, 61.1, 56.5 (×2), 28.8, 19.2, 9.7; HRMS: calcd. for $C_{22}H_{25}N_2O_5$ $[M+H]^+$ 397.1763, found 397.1753.

*4.1.2.11. (3-(Cyclopropylmethoxy)quinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**12k**)*

Yellowish solid (74% yield); Mp: 133–138°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.05 (d, 1H, J=8.1 Hz), 7.88 (d, 1H, J=8.2 Hz), 7.75 (t, 1H, J_1=7.6 Hz, J_2=14.9 Hz), 7.61 (t, 1H, J_1=7.6 Hz, J_2=14.7 Hz), 7.21 (s, 2H), 4.38 (d, 2H, J=6.9 Hz), 3.95 (s, 3H), 3.84 (6H, s), 1.31 (s, 1H), 0.55 (d, 2H, J=7.0 Hz), 0.33 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=191.1, 155.5, 153.3 (×2), 145.3, 143.9, 141.1, 137.6, 131.3, 130.7, 129.5, 127.3, 127.2, 108.1 (×2), 71.7, 61.1, 56.5 (×2),9.9, 3.5 (×2); HRMS: calcd. for $C_{22}H_{23}N_2O_5$ $[M+H]^+$ 395.1607, found 395.1601.

*4.1.2.12. (3-(Allyloxy)quinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**12l**)*

Yellowish solid (76% yield); Mp: 105–107°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.06 (d, 1H, J=8.2 Hz), 7.91 (d, 1H, J=8.3 Hz), 7.77 (t, 1H, J_1=7.2 Hz, J_2=15.3 Hz), 7.63 (t, 1H, J_1=7.3 Hz, J_2=15.2 Hz), 7.21 (s, 2H), 6.06 (m, 1H), 5.37 (d, 1H, J=17.2 Hz), 5.24 (d, 1H, J=10.4 Hz), 5.05 (d, 1H, J=5.2 Hz), 3.95 (s, 3H), 3.83 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ=191.6, 170.8, 156.4, 153.9, 153.4 (×2), 143.7, 133.3, 132.1, 132.0, 131.8, 130.3, 129.6, 129.1, 124.2, 116.3, 107.9, 60.7, 60.2, 56.7 (×2); HRMS: calcd. for $C_{21}H_{21}N_2O_5$ $[M+H]^+$ 381.1450, found 381.1446.

*4.1.2.13. (3-(But-3-en-1-yloxy)quinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**12m**)*

Yellowish solid (71% yield); Mp: 67–70°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.07 (dd, 1H, J_1=1.2 Hz, J_2=7.9 Hz), 7.90 (dd, 1H, J_1=1.0 Hz, J_2=8.3 Hz), 7.76 (m, 1H), 7.62 (m, 1H), 7.18(s, 2H), 5.78 (m, 1H), 5.07 (m, 1H), 5.00 (m, 1H), 4.57 (t, 2H, J_1=6.8 Hz, J_2=13.5 Hz), 3.94 (s, 3H), 3.83 (s, 6H), 2.52 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=190.8, 155.9, 153.1 (×2), 145.1, 143.7, 140.9, 136.8, 134.4, 131.2, 130.6, 129.2, 127.3, 117.1, 106.5 (×2), 65.7, 60.8, 56.1 (×2), 33.3; HRMS: calcd. for $C_{22}H_{23}N_2O_5$ $[M+H]^+$ 395.1607, found 395.1597.

*4.1.2.14. (3-(benzyloxy)quinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**12n**)*

Yellowish solid (70% yield); Mp: 113–115°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.07 (dd, 1H, J_1=1.0 Hz, J_2=8.2 Hz), 7.93 (dd, 1H, J_1=0.9 Hz, J_2=8.3 Hz), 7.77 (m, 1H), 7.63 (m, 1H), 7.38 (d, 2H, J=7.2 Hz), 7.30 (m, 3H), 7.17 (s, 2H), 5.60 (s, 2H), 3.94 (s, 3H), 3.79 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$): δ=190.8, 171.3, 154.8, 153.1 (×2), 145.0, 143.6, 140.8, 137.7, 136.1, 131.3, 130.5, 129.3, 128.4 (×2), 128.0, 127.9 (×2), 127.4, 127.1, 107.8, 61.0, 56.3 (×2), 50.6; HRMS: calcd for $C_{25}H_{23}N_2O_5$ $[M+H]^+$ 431.1607, found 431.1599.

*4.1.3. General procedure for the synthesis of **13a–f**.*

To a solution of anhydrous K_2CO_3 (0.28 g, 0.002 mol), KI (0.016 g, 0.0001 mol) and the respective alkyl halide (0.002 mol) in DMF (5 mL) was added dropwise the solution of (3-Hydroxyquinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**9d**) (0.34 g, 0.001 mmol) in DMF (0.5 mL) and the mixture was heated under stirring at 30–80°C for 3h. The reaction was diluted with H_2O (100 mL) then extracted with EtOAc (3×100 mL) and the combined organic phases were washed with H_2O (3×100 mL) and brine (50 mL), dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography (0-15% EtOAc in *n*-hexane).

*4.1.3.1. 1-Methyl-3-(3,4,5-trimethoxybenzoyl)quinoxalin-2(1H)-one (**13a**)*

White solid (42% yield); Mp: 88–89°C; ^{1}H NMR (600 MHz, DMSO-d_6): δ=7.88 (dd, 1H, J_1=1.2 Hz, J_2=7.9 Hz), 7.77 (m, 1H), 7.70 (d, 1H, J=8.3 Hz), 7.46 (m, 1H), 7.27 (s, 2H), 3.79 (s, 6H), 3.78 (s, 3H), 3.67 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ=191.5, 155.0, 153.5 (×2), 153.4, 143.8, 132.4, 132.2, 130.4, 130.2, 124.3, 115.6, 108.0 (×2), 60.8, 56.8 (×2), 30.1; HRMS: calcd. for $C_{19}H_{19}N_2O_5$ $[M+H]^+$ 355.1294, found 355.1285.

*4.1.3.2. 1-Ethyl-3-(3,4,5-trimethoxybenzoyl)quinoxalin-2(1H)-one (**13b**)*

White solid (39% yield); Mp: 118–120°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=7.94 (d, 1H, J=7.9 Hz), 7.68 (t, 1H, J_1=7.9 Hz, J_2=15.5 Hz), 7.45 (d, 1H, J=8.5 Hz), 7.41 (t, 1H, J_1=7.6 Hz, J_2=15.2 Hz), 7.26 (s, 2H), 4.38 (q, 2H, J=7.1 Hz), 3.94 (s, 3H), 3.86 (s, 6H), 1.43 (t, 3H, J_1=7.1 Hz, J_2=14.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ=190.7, 154.5, 153.2 (×2), 152.9, 144.0, 133.0, 132.5, 132.1, 131.3, 130.0, 124.1, 113.9, 107.8 (×2), 61.0, 56.5 (×2), 37.5, 12.5; HRMS: calcd. for $C_{20}H_{21}N_2O_5$ $[M+H]^+$ 369.1450, found 369.1442.

*4.1.3.3. 1-Propyl-3-(3,4,5-trimethoxybenzoyl)quinoxalin-2(1H)-one (**13c**)*

White solid (37% yield); Mp: 135–136°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.95 (dd, 1H, J_1=1.2 Hz, J_2=8.3 Hz), 7.68 (m, 1H), 7.42 (m, 2H), 7.24 (s, 2H), 4.27 (t, 2H, J_1=7.7 Hz, J_2=15.5 Hz), 3.94 (s, 3H), 3.85 (s, 6H), 1.85 (m, 2H), 1.07 (t, 3H, J_1=7.4 Hz, J_2=14.8 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ=189.7, 153.5, 152.2 (×2), 152.1, 142.8, 132.1, 131.4, 130.9, 130.2, 129.0, 123.0, 113.0, 106.6 (×2), 59.9, 55.4 (×2), 42.8, 19.7, 10.3; HRMS: calcd. for $C_{21}H_{23}N_2O_5$ $[M+H]^+$ 383.1607, found 383.1597.

*4.1.3.4. 1-Isopropyl-3-(3,4,5-trimethoxybenzoyl)quinoxalin-2(1H)-one (**13d**)*

Colorless oil (37% yield); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.94 (d, 1H, J=8.0 Hz), 7.68 (d, 2H, J=3.7 Hz), 7.40 (m, 1H), 7.25 (s, 2H), 5.44 (m, 1H), 3.94 (s, 3H), 3.86 (s, 6H), 1.70 (d, 6H, J=7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ=190.8, 154.6, 153.0 (×2), 145.4, 143.5, 140.9, 137.2, 131.0, 130.5, 129.1, 127.0, 126.9, 107.6 (×2), 70.0, 60.8, 56.2 (×2), 21.6 (×2); HRMS: calcd. for $C_{21}H_{23}N_2O_5$ $[M+H]^+$ 383.1607, found 383.1599.

*4.1.3.5. 1-Butyl-3-(3,4,5-trimethoxybenzoyl)quinoxalin-2(1H)-one (**13e**)*

White solid (40% yield); Mp: 142–144°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.95 (dd, 1H, J_1=1.3 Hz, J_2=7.9 Hz), 7.68 (m, 1H), 7.42 (m, 2H), 7.24 (s, 2H), 4.30 (t, 2H, J_1=7.7 Hz, J_2=15.5 Hz), 3.93 (s, 3H), 3.86 (s, 6H), 1.80 (m, 2H), 1.50 (m, 2H), 1.00 (t, 3H, J_1=7.3 Hz, J_2=14.7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ=191.0, 155.4, 153.2 (×2), 149.2, 140.3, 137.6, 133.1, 129.4, 128.2, 126.8 (×2), 126.6, 107.9 (×2), 61.0, 56.4 (×2), 40.2, 30.7, 19.2, 13.8; HRMS: calcd. for $C_{21}H_{23}N_2O_5$ $[M+H]^+$ 397.1763, found 397.1758.

*4.1.3.6. 1-Isobutyl-3-(3,4,5-trimethoxybenzoyl)quinoxalin-2(1H)-one (**13f**)*

White solid (38% yield); Mp: 151–152°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.95 (d, 1H, J=8.0 Hz), 7.66 (t, 1H, J_1=8.2 Hz, J_2=16.1 Hz), 7.41 (m, 2H), 7.23 (s, 2H), 4.19 (d, 2H, J=7.4 Hz), 3.94 (s, 3H), 3.85 (s, 6H), 2.30 (m, 1H), 1.04 (d, 2H, J=6.6 Hz) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=190.9, 156.2, 153.1 (×2), 149.3, 140.2, 138.5, 136.6, 133.3, 129.2, 128.4, 126.7, 126.3, 106.2 (×2), 72.8, 60.8, 56.0 (×2), 40.4, 28.0, 19.3 (×2); HRMS: calcd. for $C_{22}H_{25}N_2O_5$ $[M+H]^+$ 397.1763, found 397.1756.

*4.1.4. The synthesis of **14a–d**.*

*4.1.4.1. (3-(Methylamino)quinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**14a**)*

A mixture of (3-Bromoquinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**11**) (500 mg, 1.24 mmol) and methylamine (2M solution in THF, 1.5 mL) in THF **13** (10 mL) was placed in a sealed tube (100 mL). The system was sealed and the reaction mixture was heated to 50°C and stirred overnight. Upon completion, the reaction mixture was cooled to room temperature. After removal of the solution, the residue was purified by silica gel column chromatography (*n*-hexane: EtOAc=5:1 as eluent) on silica gel to afford pure product. Brown solid (57% yield); Mp: 142–144°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=7.95 (d, 1H, J=8.2 Hz), 7.78 (d, 1H, J=8.2 Hz), 7.61 (t, 1H, J=7.3 Hz), 7.44 (t, 1H, J=7.4 Hz), 6.55 (s, 2H), 3.84 (s, 3H), 3.80 (s, 6H), 3.79 (s, 1H), 2.98 (d, 6H, J=4.7 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ=193.4, 152.8, 152.6 (×2), 142.8, 136.4, 135.1,

132.8, 131.9, 130.0 (×2), 126.2, 124.8, 109.2 (×2), 61.0, 56.3 (×2), 27.9; HRMS: calcd. for $C_{19}H_{20}N_3O_4$ $[M+H]^+$ 354.1454, found 354.1442.

*4.1.4.2. (3-(Piperazin-1-yl)quinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**14b**)*

(3-Bromoquinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**11**) (500 mg, 1.24 mmol) and piperazine (112 mg, 1.30 mmol) were added to a microwave tube and heated at 150°C for 30 minutes. The mixture was diluted with water and the aqueous layer was extracted with EtOAc. The combined organic layers were then dried with brine and Na_2SO_4. Evaporation of the solvent yielded 380 mg (75% yield) of a light brown solid. Mp: 99-100°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.92 (m, 1H), 7.79 (m, 1H), 7.67 (m, 1H), 7.47 (m, 1H), 7.31 (s, 1H), 3.97 (s, 3H), 3.87 (s, 6H), 3.54 (m, 4H), 2.92 (m, 4H); ^{13}C NMR (150 MHz, $CDCl_3$): δ=191.8, 167.7, 153.2 (×2), 143.9, 143.7, 141.6, 136.0, 131.3, 130.0, 129.1, 126.7, 126.1, 108.1 (×2), 61.0, 56.4 (×2), 49.0 (×2), 45.4 (×2); HRMS: calcd. for $C_{22}H_{25}N_4O_5$ $[M+H]^+$ 409.1876, found 409.1867.

*4.1.4.3. (3-Morpholinoquinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**14c**)*

Prepared according to procedure of **14b**. Orange solid (81% yield); Mp: 112–114°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=7.94 (m, 1H), 7.80 (m, 1H), 7.67 (m, 1H), 7.49 (m, 1H), 7.32 (s, 2H), 3.97 (s, 3H), 3.87 (s, 6H), 3.74 (m, 4H), 3.55 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=191.6, 153.3 (×2), 151.7, 144.0, 143.9, 140.9, 136.1, 131.7, 130.0, 129.2, 126.4, 126.4, 108.3 (×2), 66.5 (×2), 61.1, 56.5 (×2), 48.7 (×2); HRMS: calcd. for $C_{22}H_{24}N_3O_5$ $[M+H]^+$ 410.1716, found 410.1708.

*4.1.4.3. (3-((4-methoxyphenyl)amino)quinoxalin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**11e**)*

Prepared according to procedure of **14b**. Purple solid (66% yield); Mp: 156–157°C; ^{1}H NMR (600 MHz, $CDCl_3$): δ=10.06 (s, 1H), 7.91 (d, 1H, J=8.0 Hz), 7.81 (m, 3H), 7.70 (t, 1H, J_1=9.0 Hz, J_2=14.5 Hz), 7.48 (m, 3H), 6.97 (d, 2H, J=8.8 Hz), 4.00 (s, 3H), 3.92 (s, 6H), 3.85 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=193.8, 155.9, 152.7 (×2), 149.8, 143.1, 143.0, 136.3, 135.9, 133.1, 132.4, 131.9, 130.0, 126.9, 125.9, 122.3 (×2), 114.3 (×2), 109.5 (×2), 61.2, 56.4 (×2), 55.7; HRMS: calcd. for $C_{25}H_{24}N_3O_5$ $[M+H]^+$ 446.1716, found 410.1709.

4.2. Biological evaluation

4.2.1. Cell culture

The SGC-7901, HT-1080 and A549 were cultured in RPMI-1640 medium containing 10% FBS, 100 U/mL streptomycin and 100 U/mL penicillin at 37°C in a humidified atmosphere containing 5% CO_2. All cell lines were purchased from the American Type Culture Collection

(ATCC, Manassas, VA).

4.2.2. In vitro cell growth inhibitory assay.

The *in vitro* anti-proliferative activities of CA-4 (**1a**) and all of the target compounds were assayed by conventional 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) method. In brief, cells were seeded into 96-well plates at a density of 1-3 × 10^4/well (depending on the cell growth rate). 24 h later, cells were incubated with various concentrations of the test compounds for 72 h. Then the drug-containing medium was removed and replaced with 100 μL of fresh medium containing 5 mg/mL MTT solution. After 4 h of incubation, the medium with MTT was removed, and 100 μL of dimethyl sulphoxide (DMSO) was added to each well. The plates were gently agitated until the purple formazan crystals were dissolved, and the absorbance was measured at 570 nm by a microplate spectrophotometer (MK3, Thermo, Germany). The data were calculated and plotted as the per cent viability compared to the control. The 50% inhibitory concentration (IC_{50}) was defined as the concentration that reduced the absorbance of the untreated wells by 50% of the vehicle in the MTT assay.

4.2.3. In vitro tubulin polymerization assay.

The effects of **12a** and CA-4 on the polymerisation of tubulin were determined by employing a fluorescence-based tubulin polymerization assay kit (BK011P, Cytoskeleton, USA) according to the manufacturer's protocol. The tubulin reaction mix contained 2 mg/mL porcine brain tubulin (>99% pure), 2 mM $MgCl_2$, 0.5 mM EGTA, 1 mM GTP, and 15% glycerol. First, 96-well plate was incubated with 5 μL of inhibitors in various concentrations at 37°C for 1 min. Then 50 μL of the tubulin reaction mix was added. The samples were mixed well, and tubulin assembly was monitored (emission wavelength of 420 nm; excitation wavelength pf 360 nm) at 1 min intervals for 90 min at 37°C using a plate reader (FASCalibur, BD Biosciences, USA). The IC_{50} values were calculated after 20 min using the SPSS software.

4.2.4. Immunofluorescence staining

The SGC-7901 and HT-1080 cells were seeded in 24-well plate (with coverslips plated) at density of 1 × 10^4 cells. After overnight adherence, they were exposed to **12a** (0.34 μM) or **CA-4** (0.032 μM) respectively, for 48 h. The coverslips were fixed in ice-cold methanol/acetic acid (3:1) for 10 min and blocked with 3% bovine serum albumin for 20 min at room temperature. The primary α-tubulin antibody (Santa Cruz, CA) was diluted (1:100) with 2% BSA in PBS and

incubated overnight at 4 °C. The cells were washed with PBS to remove unbound primary antibody, and the cells were then incubated with FITC-conjugated antimouse secondary antibody and diluted (1:1000) with 2% BSA in PBS for 3 h at 37 °C. The cells were washed with PBS to remove unbound secondary antibody, the nuclei were stained with 4,6-diamino-2-phenolindol dihydrochloride (DAPI) and immunofluorescence was then detected using a fluorescence microscope (Olympus, Tokyo, Japan).

4.2.5. Cell cycle analysis

HT-1080 cells (8×10^4 cells) were incubated with various concentrations of CA-4, **12a** or 0.05% DMSO for the indicated times. The cells were collected by centrifugation, washed with PBS and fixed in ice-cold 70% ethanol. The fixed cells were harvested by centrifugation and treated with RNase A at 37 °C for 30 min, and incubated with PI solution (Solarbio, China) at 4 °C for 15 min. The samples were then analysed by FACScan flow cytometry (Becton-Dickinson, Franklin Lakes, NJ, USA). The experiments were repeated at least three times.

4.3. Molecular modelling

The crystal structure of tubulin in complex with DAMA-colchicine (PDB: 1SA0) was used as the template in the docking study. The protein structure was prepared using the Accelrys Discovery Studio 3.0. After extracting the ligand, hydrogen atoms were added to the crystal. Charges were added to biopolymer by CHARMm force field. Finally, **12a** and CA-4 were docked into the binding site using the CDOCKER protocol with the default settings.

Acknowledgement

We gratefully acknowledge the National Natural Science Foundation of China (81673293 and 81602969), Liaoning Revitalization Talents Program (XLYC1802072), Project funded by China Postdoctoral Science Foundation (2018M641715), Shenyang Science & Technology Bureau Item (17-231-1-41 and 18-014-4-01), and Plan for Development of Young Scholars of Shenyang pharmaceutical university (ZQN2018002) for the generous financial support. This work was also supported by the Program for Innovative Research Team of the Ministry of Education.

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1. A clean and green method to synthesize 3-benzoylquinoxalines under “on-water” condition was developed.
2. As anticipated, compound **9d** exerted potent antitumor efficacy.
3. Further structural optimization led to a promising compound **12a**, which is a novel tubulin inhibitor.
4. This work provides a green version for medicinal chemists to assemble molecules in the seeking stage of drug discovery

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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