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Design, Synthesis, and Antiproliferative Activity Assessment of Non-ATP-Competitive Fibroblast Growth Factor Receptor 1 Inhibitors[1]

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Abstract—Fibroblast growth factor receptor 1 (FGFR1) is considered a therapeutic target for multiple cancers, including gastric cancer. FGFR1 inhibitors, being ATP competitors, can prevent the kinase domain and the downstream signaling cascade from phosphorylation and thus have the potential to treat cancers associated with aberrant FGFR1 activation. However, untargeted inhibition may cause numerous side effects. Thus, a non-ATP competitive FGFR1 inhibitor should be urgently identified and explored. In this study, we designed and synthesized 17 derivatives of nordihydroguaiaretic acid (NDGA), a known ATP-independent FGFR3 inhibitor. In the kinase activity assay, 3,5-bis(2-fluorobenzylidene)piperidin-4-one (**1B**) showed the highest kinase inhibitory activity among all derivatives and was thus identified as a non-ATP-competitive FGFR1 inhibitor. In the biological effect evaluation, **1B** restrained the FGFR−FRS2−ERK signaling pathway in a dose-dependent manner and inhibited the growth of two gastric cancer cell lines. Overall, **1B** can be considered as a potential candidate for treating gastric cancer and as an outstanding lead compound for the discovery of novel non-ATP-competitive FGFR1 inhibitors.

Keywords: antitumor activity, design and synthesis, FGFR1 inhibitors, gastric cancer, NDGA analogs, non-ATP-competitive protein kinase inhibitor

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INTRODUCTION

Fibroblast growth factor receptors (FGFRs), a well-known subfamily of receptor tyrosine kinases (RTKs), include four members, namely, FGFR1 to FGFR4, which are composed of an extracellular receptor domain, a single-pass trans-membrane domain, and a carboxy-terminal cytoplasmic domain [1]. Ligand binding induces FGFR dimerization, which causes the phosphorylation of intracellular receptor kinase domains and activates the intracellular FGFR substrate 2α (FRS2α) and phospholipase Cγ1 (PLCγ1) [2]. Activated FRS2α initiates downstream signaling through the RAS-MAPK-ERK pathway or the PI3K-AKT pathway to induce a series of biological effects, including cell proliferation, growth, differentiation, migration, and survival [3]. As a result of over-expression or mutation, the deregulation of FGFR activity could be closely associated with the development of different types of tumors, such as breast cancer [4], lung cancer [5], oral squamous cell carcinoma [6], and gastric cancer (GC) [7], by increasing cell proliferation and angiogenesis while inhibiting cell apoptosis.

Based on the important effect of FGFR on tumorigenesis, FGFR is considered to have great potential for targeted tumor therapy [8]. The blockage action of the kinase domain gives rise to the inhibition of the phosphorylation of FGFR and its substrate, such that the pathological state caused by aberrant FGFR activation is mitigated [9, 10]. Small molecule inhibitors of FGFR are universally becoming increasingly accepted to possess a high therapeutic

[1] The text was submitted by the authors in English.

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significance [11]. Since the phosphorylation of the receptor kinase domain is triggered by ATP binding, the major FGFR inhibitors generally imitate the structure of ATP, and then compete with ATP for the binding site, resulting in the inactivation of kinase conformation. However, almost all RTKs encoded by the human genome possess a similar 3D structure of the intercellular kinase domain. As a consequence of the untargeted inhibition among different RTKs, ATP competitive inhibitors have an inevitable defect in that they induce a series of adverse effects. Given the poor selectivity between the subtype FGFRs of ATP competitive inhibitors, the discovery of novel non-ATP competitive inhibitors may overcome the problem via binding to the atypical allosteric site or generating a hydrophobic interaction with the specific residues [12]. The development of this class of inhibitor presents good prospects.

Nordihydroguaiaretic Acid (NDGA), obtained from the creosote bush *Larrea divaricatta*, has been reported to exhibit strong activity as a non-ATP competitive inhibitor of FGFR3, inhibiting the autophosphorylation of FGFR3 both in vivo and in vitro [13]. Increasing concentrations of ATP did not affect K_m at various concentrations of NDGA, indicating that NDGA restricted the activation of FGFR in an ATP-independent manner. Notably, among all the potent non-ATP competitive inhibitors, NDGA is the only one obtained from a natural source and has a low toxicity and could thus be used as a leading compound for further structural modification and medicinal chemistry research.

On the basis of high homology between FGFR3 and FGFR1, our research group determined the FGFR1 inhibitory potential of NDGA and found it to be more preferably active than its FGFR3 inhibitory activity in our early study. In this research, we designed and synthesized two classes of NDGA derivatives. Through the kinase activity screening of 17 NDGA derivatives, we found that **1B** not only had a lower IC_{50} relative to FGFR1 kinase in an ATP-dependent manner compared with NDGA, but also showed excellent anti-tumor activity against GC.

RESULTS AND DISCUSSION

Design and synthesis. In this research, we designed and synthesized two classes NDGA derivatives (**A** and **B** class). Firstly, in consideration of the isolation and purification of chemicals containing chiral atom were always difficult, we deleted the chiral carbon atoms (Fig. 1) In the light of literature reported that the biological activity of small molecular inhibitor of kinase may greatly enhance via a Micheal addition reaction with the particular cysteine residue within the ATP-binding site after adding electrophilic functional groups into its structure such as α,β-unsaturated carbonyls [14]. One famous example was Afatinib (BIBW2992), an irreversible covalent ErbB Family Blocker. Thus, we introduced a Micheal addition receptor into the middle linker [14]. Furthermore, we also added one nitrogen atom into the previous aliphatic ring, which might provide site that forms added hydrogen bond with the protein residues and improve their water solubility, as well as be convenient for the next Structure-Activity Relationship (SAR) Analysis. The synthesis procedure was a simple, one-step reaction, and the yield ranged from 44 to 82.7%. The structural information of all compounds was shown in (Table 1).

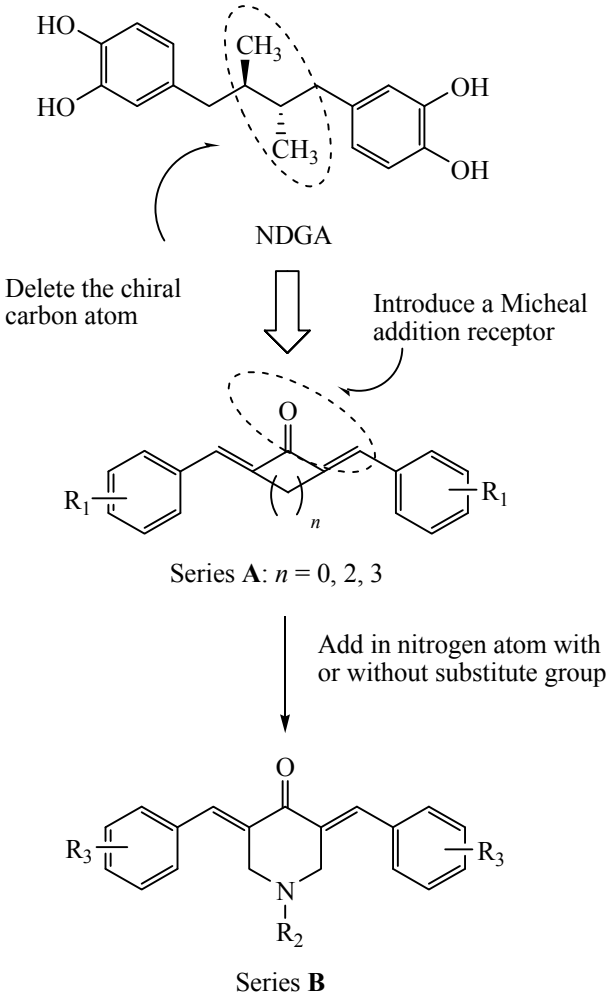
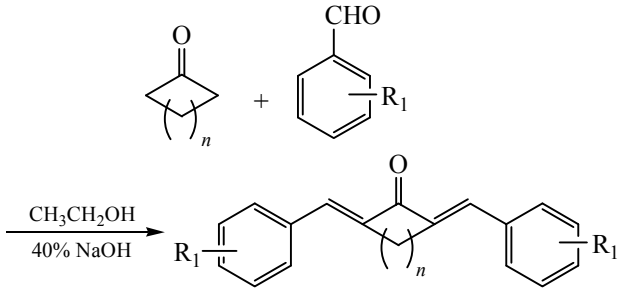
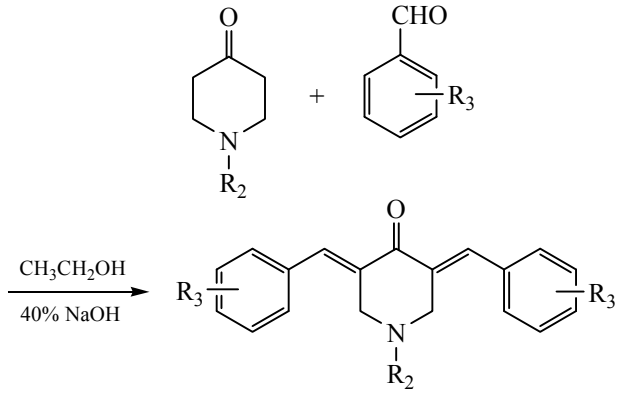


Fig. 1. Design of series **A** and **B** compounds on the basis of NDGA structure.

Table 1. Synthetic process and chemical structures of series **A** and **B** compounds



A



B

Comp. no.	R_1	n[a]	Comp. no.	R_2	R_3
1A	2,4-Cl	2	**1B**	H	2-F
2A	2-Cl	2	**2B**	H	2,6-F
3A	2-F	2	**3B**	H	2-CF_3
4A	2-Br	2	**4B**	CH_3	2-Cl
5A	2,3-Cl	2	**5B**	CH_3	4-F
6A	2,5-F	2	**6B**	CH_2CH_3	2-F
7A	2,4-Cl	3			
8A	2,3-Cl	3			
9A	2,4-Cl	0			
10A	2-Cl	0			
11A	2-Br	0			

[a] When n is equal to 2, 3, or 0, the middle connecting ring respectively corresponds to cyclopentanone, cyclohexanone, or acetone.

Screening for the inhibitory activity for FGFR1 kinase. By screening for the inhibition of FGFR1 kinase, we assessed the inhibitory effects of 17 compounds (Table 2). Compounds **11A** and **1B** exhibited considerable inhibitory activity, with the corresponding half maximal inhibitory concentration values (IC_{50}) of 11 and 14 μM, respectively. In contrast to NDGA, which has an IC_{50} of 24.5 μM, our active compound possessed approximately twice as much activity of its lead compound at the kinase level. By analyzing the result of the inhibitory effect, our preliminary SAR analysis was performed. Among the **A** class compounds, **4A** and **11A** both had relative better potent FGFR1 kinase inhibitory activity, whereas the compound with 2-bromine substituent may be superior to the other series **A** compounds. As shown in Table 2, only **1B** exhibited a particular FGFR1 inhibitory effect among the **B** class compounds, whereas others all had a negligible effect. The detailed SAR has to be performed after we synthesize more B class compounds and determine their inhibitory action in the future.

1B inhibits the activity of FGFR1 kinase in a non-ATP competitive manner. Given that NDGA is noncompetitive with ATP, we suppose that **1B** might be similar to its lead compound. To confirm the action mechanism of **1B** with FGFR1, an ATP-competitive assay was performed by using a kinase inhibitory activity screening assay. In the absence of a compound, the ATP concentration was gradually increased. The percentage conversion of the FGFR1 substrate steadily rode and became constant when the ATP concentration reached 2000 μM. When the compound of **1B** was added, FGFR1 phosphorylation was inhibited. The percentage conversion recovered because of the increased ATP concentration, but the initial no-compound status can no longer be achieved. Although the ATP concentration reached a level of 4000 μM, no influence of the inhibitory activity caused by the compound could be observed (Fig. 2a).

As a standard index to describe the biological activity of an inhibitor, the curve of graph B (Fig. 2b) shows that the IC_{50} value of **1B** was irrelevant to the increase in ATP concentration. All results indicated that the inhibitory effect of **1B** of FGFR1 phosphorylation occurred in a non-ATP competitive manner, which was similar to the case of NDGA. Moreover, the enzyme kinetic parameters (α value and K_i value, as shown in Fig. 2) more intuitively identified with our prediction.

Inhibitory activity of 1B relative to the intercellular FGFR1 kinase and its downstream signaling protein in GC cell lines. The intercellular

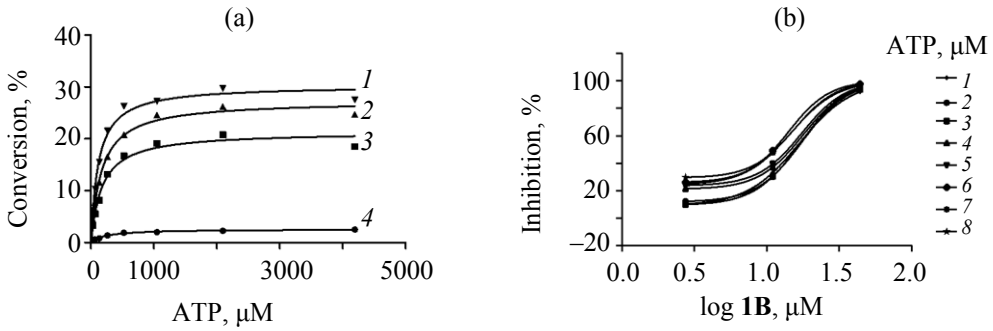


Fig. 2. Inhibitory mode of **1B** relative to FGFR1 kinase was identified as independent of ATP concentration by kinase inhibitory activity screening assay. The conversion data were fitted with GraphPad for global fitting using "mixed model inhibition." (a) Influence of increasing ATP concentration on the conversion of substrate peptide inhibited by **1B**, μM: (*1*) 0.00, (*2*) 2.75, (*3*) 11.00, (*4*) 44.00. (b) **1B** Maintained a stable capability to inhibit the phosphorylation of FGFR1 at all ATP concentrations, as indicated by similar IC_{50} values: (*1*) 4192, (*2*) 2096, (*3*) 1048, (*4*) 524, (*5*) 262, (*6*) 131, (*7*) 66, (*8*) 33. Inhibitory kinetics parameters of **1B**: v_{max} = 30.32 μM/s, α = 2.411, K_i = 20.91 μM, K_m = 117.43 μM.

FGFR1 kinase terminal was the core section of the whole receptor for exerting its biological effects and activating its downstream signaling pathways. Studies found that FGFR1 expression was amplified in SGC-7901 cells, which was thus selected for further research [15]. Among the 17 NDGA derivatives, **1B** showed the most potent inhibitory activity relative to FGFR1 according to kinase activity screening. Thus, **1B** was selected as the active compound for the following anti-proliferative evaluation and determination of the effect of downstream signaling. By Western blot analysis, Our data (Fig. 3a) reveal that compound **1B** exhibited an apparent inhibitory effect on the bFGF-induced phosphorylation of FGFR1 at a concentration of 5 μM. In previous studies on FGFR signaling, FRS2 has been universally recognized as being phosphorylated at multiple sites by activated FGFR to initiate downstream signaling, and the activation of the downstream MAPK/ERK pathway could be crucial to cell proliferation. Thus, we tested the effects of **1B** on the activation of FRS2 and ERK1/2 in SGC-7901 cells. Figure 3b intuitively shows that **1B** also remarkably inhibits the phosphorylation of the downstream signaling protein, such as FRS2 and ERK, in a dose-dependent manner.

1B Inhibited the growth of GC cell lines. The FGF-FGFR signaling pathway has been demonstrated to be significant to the survival, angiogenesis, proliferation, and migration of tumors. Wen reported that microRNA-133b could serve as a tumor-suppressive gene to down-regulate FGFR1 expression and suppress GC cell growth [15]. Therefore, two GC cells lines (MGC-803 and SGC-7901) were chosen for the determination of the anti-proliferative capability of compound **1B**. With the use of an MTT assay, all GC cell lines (4000 cells/well) were treated with various concentrations of **1B** for 72 h. The viability of tumor cells significantly declined (data not shown). Furthermore, we selected these GC cell lines to test the anti-proliferative activity of NDGA for a comparison with **1B**. The result (Table 3) shows that **1B** displays excellent anti-proliferative effect on the GC cell lines with an IC_{50} value of 1.5 μM for SGC-7901, and 1.6 μM for MGC-803. Meanwhile, the value of NDGA was over 30-fold higher than that of **1B** (Table 3).

Table 2. Inhibition rate (IR) and the IC_{50} value of series **A** and **B** compounds

Comp.	IR, %[a]	IC_{50},	Comp.	IR, %	IC_{50},
1A	59.1±3.5	74	**10A**	32.7±8.2	–
2A	95.3±2.1	86	**11A**	91.2±0.7	11
3A	41.5±3.3	>100	**1B**	80.9±1.6	14
4A	99.8±0.2	20	**2B**	24.2±0.8	–
5A	25.7±4.2	–[b]	**3B**	–6.0±3.0	–
6A	39.6±2.8	–	**4B**	–3.4±6.6	–
7A	54.2±4.8	>100	**5B**	–1.1±5.6	–
8A	41.9±7.5	–	**6B**	13.4±7.7	–
9A	58.1±6.4	29			

[a] IR indicates the inhibition rate of compounds relative to the FGFR1 kinase at a concentration of 20 μM. [b] (–) indicates that the IC_{50} value was undetermined.

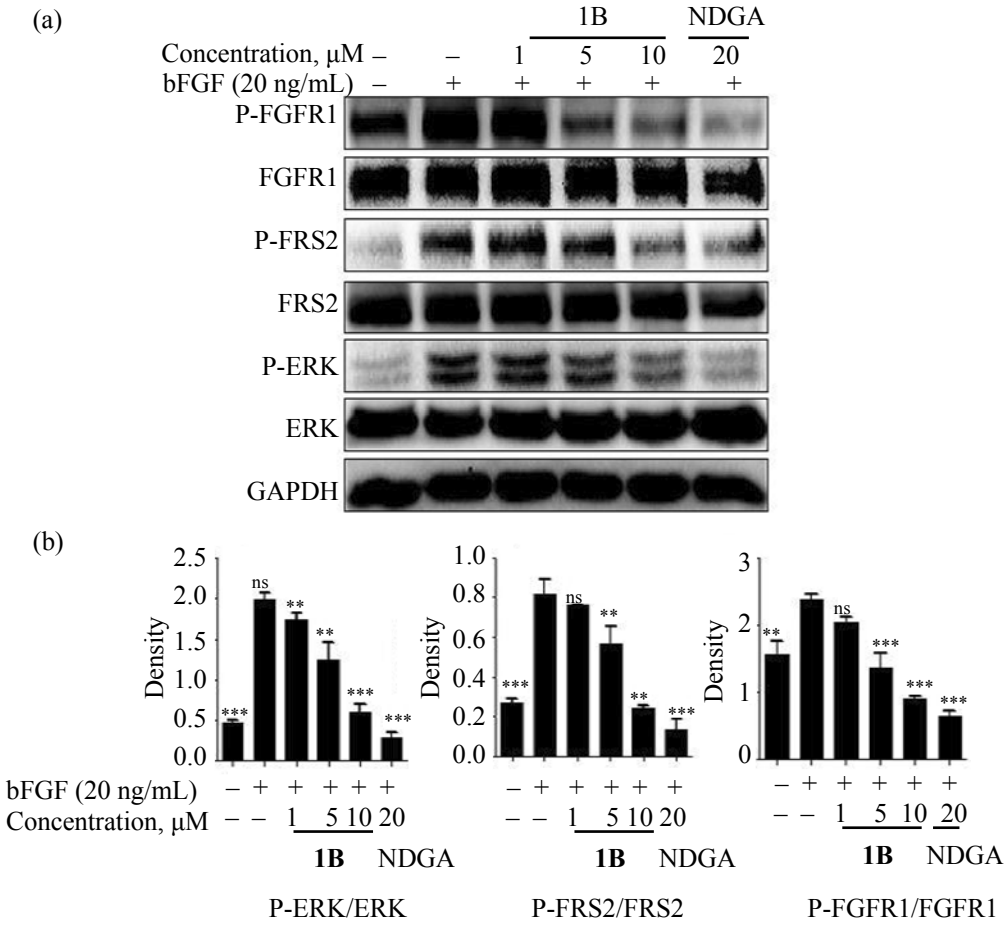


Fig. 3. 1B Inhibited the phosphorylation of FGFR1, FRS2, and ERK dependent on ATP concentration. (a) SGC-7901 cells were treated by **1B** for 1 h after being starved for 24 h and then stimulated with 20 ng/mL of bFGF for 10 min. Phosphorylation levels of FGFR1, FRS2, and ERK in cell lysates were measured by Western blot analysis. (b) Column figures show the normalized optical density as a percentage of total control.

Over the past two decades, the exploitation of FGFR inhibitors has attracted considerable attention from various research institutions and pharmaceutical companies. Numerous small-molecule FGFR inhibitors that bind to the intercellular kinase domain have been designed and synthesized [8, 9]. Among these inhibitors, some excellent ones are being evaluated in clinical trials to determine their anti-tumor activity. By analyzing the protein–ligand crystal structure, a selective FGFR1-3 inhibitor AZD4547 was designed and investigated for potential treatment capability against cancer under a phase 2 clinical trial (NCT01795768) [16]. Another highly active and selective FGFR inhibitor, FIIN-1, displays high inhibitory activity in various FGFR-dependent tumor cells by forming a covalent bond with Cys486 in the FGFR1 ATP-binding domain [17]. Despite these highly active inhibitors, we note that they both work through an ATP-dependent mechanism. However, the ATP-binding pocket of FGFRs is highly similar to that of other RTKs, such as VEGFR and PDGFR. The poor selectivity between distinct RTKs may cause numerous

Table 3. 1B exhibited excellent inhibition of the growth of the GC cells. Two GC cells were treated with 1B at concentrations (0.48, 2.4, 5, 12, and 60 μM) for 72 h. The MTT assay provides the respective IC_{50} values of the compounds. In contrast to NDGA, 1B showed over 30-fold activity upgrade

Compound	IC_{50}, μM	
	MGC-803	SGC-7901
1B	1.6±0.2	1.5±0.6
NDGA	46.9±7.1	65.1±5.8

side effects, such as nausea, diarrhea, as well as a decrease in thrombocytes and erythrocytes [9]. In addition, the effect of these ATP structural imitators may be offset by the high physiological or intracellular concentrations of ATP. These inevitable weaknesses have caused researchers to look toward non-ATP competitive inhibitors. Given that such inhibitors will be less likely to compete with ATP for binding; their selectivity may be significantly improved.

GC is the second most common cause of cancer death worldwide [18]. Several recent studies proved that over-expression of FGFR1 was closely associated with the formation and development of GC. Some studies discovered that over-expression of the mRNA and protein of FGFR1 in GC tissue was observed in 12 (50%) of 24 and 37 (61%) of 61 separately [19]. Conversely, knockdown of FGFR1 or the use of microRNA for the negative regulation of FGFR1 activity could successfully inhibit the growth of GC cells. Developing an anti-GC drug by targeting FGFR1 is becoming increasingly urgent and much promising. In this research, we successfully discovered an excellent leading compound of FGFR1 inhibitor for treating GC. At the cellular level, **1B** was found to show excellent inhibitory activity in FGFR1 over-expressing GC cells via a potent mechanism that suppresses the FGFR1/FRS2α/ERK1/2 pathway. This compound also exhibits better anti-tumor effects than NDGA, not only in the level of FGFR1 kinase, but also in the cellular level. Thus, **1B** may be a promising candidate for treating GC.

EXPERIMENTAL

Synthesis of series A and B compounds. Reagents and solvents for the synthesis were commercially available and obtained from Sigma-Aldrich (St Louis, Missouri, USA) and Aladdin (Shanghai, China), which were used without further purification. Silica gel (GF254) for column chromatography (200–300 mesh) was obtained from Aladdin. Melting points were measured on a Fisher-Johns melting apparatus and were uncorrected. Electron-spray ionization mass spectra (ESI-MS) data were collected in positive mode on a Bruker Esquire 3000t spectrometer. The 1H-NMR spectra data was recorded on a 600 MHz spectrometer (Bruker Corporation, Switzerland) with TMS as an internal standard.

Brief general procedure of synthesis (for details, see [20–22]). Corresponding ketone (2 mmol) was dissolved in 10 mL of ethanol (piperidin-4-one hydrochloride was dissolved in a 10 : 1 mixture of ethanol and distilled water). Corresponding benzaldehyde (4 mmol) was added to the solution at 5 to 8°C, and 0.5 mL of 40% aqueous NaOH was added dropwise as a catalyst with stirring. The reaction was monitored by TLC on silica gel. When the reaction was complete, distilled water was added to precipitate the product completely, after which it was filtered off in a vacuum and purified by column chromatography used eluent petroleum ether–ethyl acetate as eluent (6 : 1–2 : 1).

The structures were characterized by ^{1}H-NMR spectroscopy and confirmed by mass spectrometry. The spectral data of known products (**1A–11A, 1B, 4B**, and **5B**) are consistent with those reported in the published literature by our research team [20–22]. Unreported compounds (**2B, 3B, 6B**) are shown below.

(3*E*,5*E*)-3,5-Bis(2,6-difluorobenzylidene)piperidin-4-one (2B). Light yellow powder, yield 33%, mp 164.1–166.9°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J,* Hz): 7.602 s (2H, 2Ar-CH=CH), 7.322–7.372 m (2H, 2H^4), 6.949 d (4H, 2H^3, 2H^5; J = 7.8), 3.994 s (4H, 2N–CH_2). ESI MS spectrum, *m/z*: 348.0 $[M + 1]^+$. Found, %: C 65.51; H 3.83; F 21.68; N 4.13; O 4.81. $C_{19}H_{15}F_2NO$. Calculated, %: C 65.71; H 3.77; F 21.88; N 4.03; O 4.61. *M* 347.31.

(3*E*,5*E*)-3,5-Bis[2-(trifluoromethyl)benzylidene]-piperidin-4-one (3B). Yellow powder, yield 82%, mp 153.5–155.7°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J,* Hz): 7.996 s (2H, 2Ar-CH=C), 7.735 d (2H, 2Ar-H^3, J = 7.8), 7.563 t (2H, 2Ar-H^5, J = 7.8), 7.462 t (2Ar-H^4, J = 7.8), 7.254 d (2H, 2Ar-H^6, J = 7.8), 3.872 s (4H, CH_2–N–CH_2). ESI-MS *m/z*: 412.1 $[M + 1]^+$. Found, %: C 61.34; H 3.65; F 27.51; N 3.61; O 3.87. $C_{21}H_{15}F_6NO$. Calculated, %: C 61.32; H 3.68; F 27.71; N 3.41; O 3.89. *M* 411.34.

(3*E*,5*E*)-3,5-Bis(2-fluorobenzylidene)-1-ethylpiperidin-4-one (6B). Light yellow powder, yield 76%, mp 130.0–133.17°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J,* Hz): 7.907 s (2H, 2Ar-CH=C), 7.367–7.382 m (2H, 2Ar-H^6), 7.344–7.357 m (2H, 2Ar-H^4), 7.175–7.199 m (2H, 2Ar-H^5), 7.114–7.144 m (2H, Ar-H^3) , 3.717 d (4H, 2N–CH_2–C, J = 7.8), 2.567 q (2H, N–CH_2, J = 7.2), 1.010 t (3H, CH_3, J = 7.2). ESI-MS *m/z*: 340.7 $[M + 1]^+$. Found, %: C 74.13; H 5.89; F 11.08; N 4.31; O 4.64. $C_{21}H_{19}F_2NO$ Calculated, %: C 74.32; H 5.64; F 11.20; N 4.13; O 4.71. *M* 339.38.

Kinase activity screening. The kinase activity of FGFR1 was determined by Mobility Shift Assay on a

Caliper Life Sciences Labchip EZ Reader. The enzyme solution was prepared in a 1.25×kinase base buffer (62.5 mM HEPES, 0.001875% Brij-35, 12.5 mM $MgCl_2$, 2.5 mM DTT). Stop buffer (100 mM HEPES, 0.015% Brij-35, 0.2% coating reagent no. 3, 50 mM EDTA) was got ready. A test compound was dissolved in DMSO and then diluted to a specific concentration with water in a 384-well plate. 5 μL of compound solution were diluted with 10 μL of 2.5× enzyme solution, in wells. 10 mM EDTA was used as the low control. The mixture was incubated at room temperature for 10 min, after which 10 μL of peptide solution (2.5×, FAM-P22, add FAM-labeled peptide and ATP in the 1.25× kinase base buffer) was added to each well of the 384-well assay plate. After incubation at 28°C for 1 h, 25 μL of stop buffer was added into the mixture to stop the reaction. Conversion data were then collected on the EZ reader, the percent inhibition of kinase activity was calculated by the following formula:

$$\text{Inhibition \%} = (\text{max}-\text{conversion})/(\text{max}-\text{min}) \times 100\%,$$

where (max) stands for the DMSO control, (min) stands for low control, (conversion) means the average of two experimental values given by EZ reader.

To determine the half maximal inhibitory concentration of the test compound relative to kinase activity, 10 gradient concentrations of the compound (100, 33.330, 11.110, 3.700, 1.230, 0.410, 0.140, 0.046, 0.015, and 0.005 μM) were set up. The inhibition ratios for different concentrations relative to kinase were determined and calculated, and the concentration–inhibition rate curve was fit using the GraphPad Prism software.

ATP competitive test. The same kinase inhibition assay with four concentrations of the test compounds (0 to 100 μM) and eight concentrations of ATP (12.500, 6.250, 3.125, 1.563, 0.781, 0.391, 0.195, and 0.098 mM) was used to determine the conversion of the peptide substrate, catalyzed by FGFR1 kinase, within 1 h. The inhibition curve was fit using the GraphPad Prism software.

Cells and reagents. Human GC cells (SGC-7901, and MGC-803) were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All cells were maintained in RPMI 1640 medium (Invitrogen, CA, USA) with 10% FBS (HyClone, Logan, UT, USA); then incubated at 37ºC in an atmosphere of 5% CO_2. Methylthiazoletetrazolium (MTT) and dimethylsulfoxide (DMSO) were from Sigma-Aldrich (St. Louis, MO,USA). Forty percent acrylamide, Coomassie Brilliant Blue, TEMED, Tris, glycine, sodium dodecyl sulfate (SDS), pre-stained protein marker, and nonfat dry milk were from Bio-Rad (Germany). pFRS2α (Tyr 196) was purchased from Cell Signaling Technology (Beverly, MA, USA). pFGFR1, p-ERK1/2, ERK1/2, FRS2, and GAPDH were obtained from Santa Cruz Biotechnology, Inc. (CA, USA).

MTT cell proliferation assay. MGC803 and SGC7901 Cells (4000 cells/well) were seeded on a 96-well plate. The next day, cells were treated with different concentrations (0, 0.480, 2.400, 5.000, 12.000, and 60.000 μM) of the test compound. After 72-h incubation, 25 μL of MTT (5 mg/mL) were added to each well for 4 hours at 37°C in the dark. The MTT solution was removed, and 150 μL of DMSO was added to each well to solubilize the MTT metabolic product; then the absorbance was read at 490 nm.

Western blot analysis. SGC-7901 cells (treated with the test compound after 24-h starvation, and then stimulated with 20 ng/mL bFGF for 10 min) were lysed in a lysis buffer for 15 min on ice. The cell lysates were centrifuged at 4°C to remove insoluble components. Protein (90 μg) from each sample was subjected to SDS-PAGE and transferred onto PVDF membranes. After incubation with 5% nonfat dry milk in TBST, the membranes were incubated with primary antibodies (anti-pFGFR1, anti-FGFR1, anti- pFRS2, anti-FRS2, anti-pERK, anti-ERK, anti-GAPDH, Santa Cruz) at 4ºC overnight and then with the horseradish peroxidase-labeled secondary antibody at room temperature for 1 h. The results were analyzed by Quantity One software to determine the relative ratio.

CONCLUSIONS

In conclusion, we have designed and synthesized two types of compounds employing NDGA as the lead compound. Among these compounds, **1B** exhibits a satisfactory effect in terms of inhibiting the kinase activity of FGFR1 and suppressing the proliferation of two GC cell lines in vitro. This study discovered a potent non-ATP competitive FGFR1 inhibitor, as well as a new FGFR1 kinase inhibitor scaffold–3,5-dibenzylidenepiperidin-4-one. This scaffold was first recommended as an FGFR inhibitor and potential candidate for GC treatment applications.

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