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Synthesis of 3,5-Disubstituted Isoxazoles Containing Privileged Substructures with a Diverse Display of Polar Surface Area

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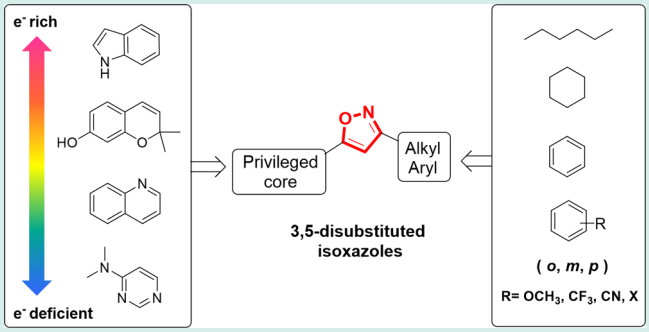
Supporting Information

ABSTRACT: We designed and synthesized the molecular framework of 3,5-disubstituted isoxazoles containing privileged substructures with various substituents which uniquely display polar surface area in a diverse manner. A library of 3,5-disubstituted isoxazoles were systematically prepared via 1,3-dipolar cycloaddition of alkynes with nitrile oxides prepared by two complementary synthetic routes; method A utilized a halogenating agent with a base and method B utilized a hypervalent iodine reagent. Through the biological evaluation of corresponding isoxazoles via three independent phenotypic assays, the different pattern of biological activities was shown according to the type of privileged substructure and substituent. These results demonstrated the significance of molecular design via introducing privileged substructures and various substituents to make a diverse arrangement of polar surface area within a similar 3-dimensional molecular framework.



KEYWORDS: *3,5-disubstituted isoxazoles, privileged substructure, polar surface area, 1,3-diopolar cycloaddition, nitrile oxide*

INTRODUCTION

A collection of drug-like small molecules have played a pioneering role in exploring untapped biological systems through specific perturbations of various biopolymers.[1] A broad spectrum of therapeutic targets have been revealed via the discovery of new bioactive small molecules, of which the identification was accelerated by an extensive campaign of high-throughput screening (HTS).[2] The maximum degree of molecular diversity and complexity of drug-like small molecules has been the major interest in unbiased screening approaches to probe the unexplored regions of bioactive chemical space. A school of synthetic chemists have applied diversity-oriented synthesis (DOS)[3] and privileged-substructure-based DOS (pDOS)[4] as guiding strategies for the efficient delivery of a drug-like compound library with a maximal molecular diversity. Nevertheless, there are always evolving challenges in designing novel structural motives and skeletons with high biological relevancy and selectivity.

Among various molecular frameworks, we paid attention to 3,5-disubstituted isoxazoles as a promising scaffold because of their wide range of chemical reactivities, synthetic accessibilities, and biological activities, including $GABA_\alpha$ agonist,[5] antibiotic,[6] apoptotic,[7] antiproliferative,[8] and antiviral[9] activities and nicotinic receptor modulators[10] (Figure 1). Therefore, we pursued the construction of 3,5-disubstiuted isoxazoles as a key skeleton in combination of privileged substructures for the construction of a new drug-like, small molecule library. In addition, we aimed to diversely display the polar surface area within similar 3-dimensional conformers. Thus, we adopted 1,3-dipolar cycloaddition[11] of nitrile oxides to terminal alkynes to access 3,5-disubstituted isoxazoles on behalf of the efficiency and robustness of this convergent synthetic route compared to other methods.[12] 1,3-Dipolar cycloaddition allows the construction of isoxazoles at the last stage of synthesis as a heterocyclic bridge between unique privileged structures and diverse substituents in a single molecular framework.[13]

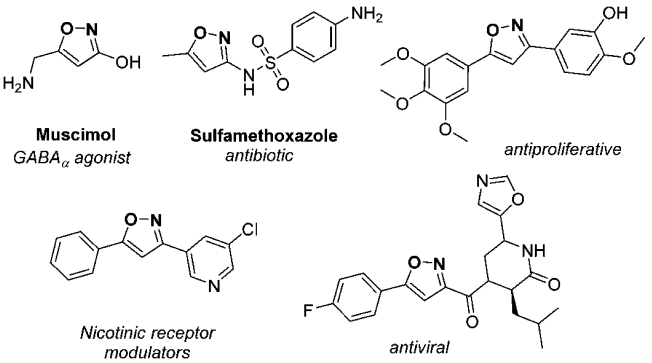


Figure 1. Examples of 3,5-disubstituted isoxazoles with pharmacological activities.

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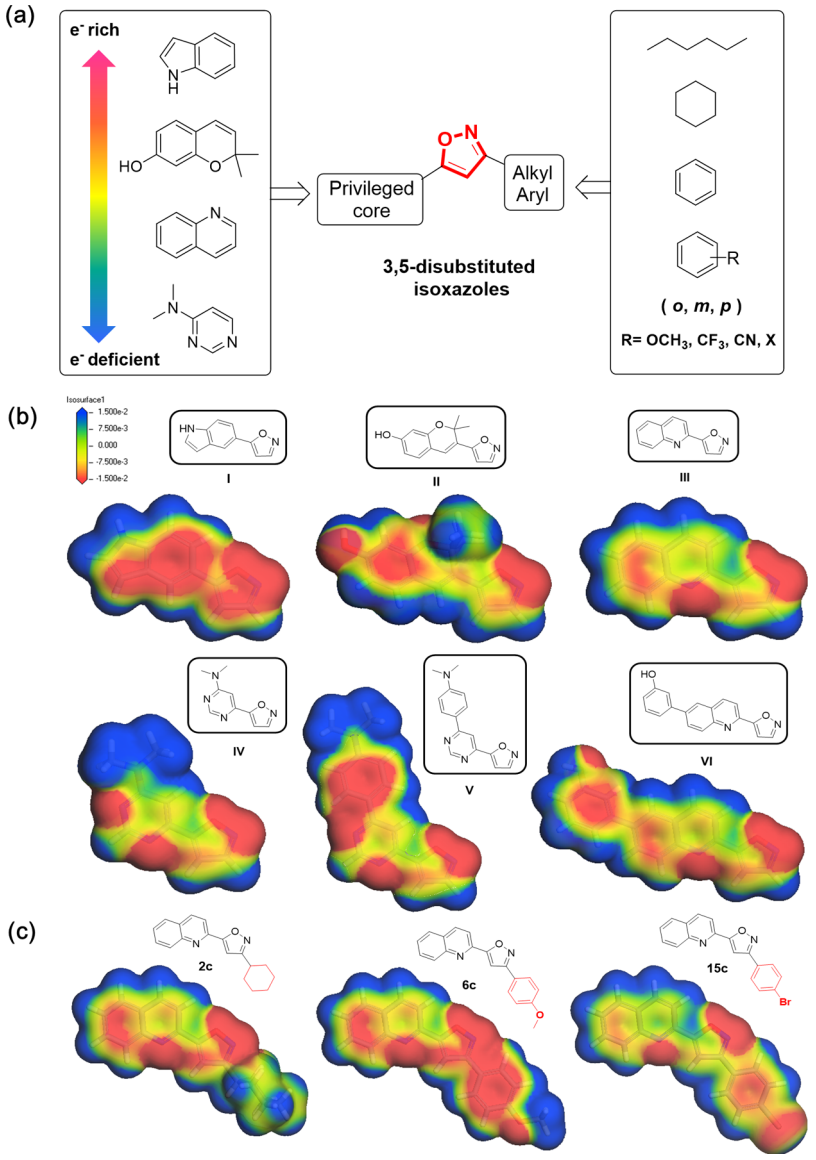

Figure 2. (a) Molecular framework of 3,5-disubstituted isoxazoles involving privileged substructures and diverse substituents such as alkyl and aryl groups. (b) Polar surface area (PSA) is illustrated with an isosurface diagram using energy-minimized conformers of the scaffolds **I**–**VI** based on the calculation of an electrostatic potential and electron density. (c) PSA of **2c**, **6c**, and **15c** using the same calculation method as b.

RESULTS AND DISCUSSION

The primary element of this molecular framework is the privileged substructures, such as indole, quinoline, 3,3-dimethylbenzopyran, and pyrimidine, that could ensure high biological relevancy, skeletal diversity, and unique charge distribution. Privileged structures are defined as a single molecular framework able to provide a series of ligands for diverse receptors and have been extensively utilized in rational drug design owing to their potent biological activities.[14] Therefore, the systematic conjugation of diverse privileged substructure with an isoxazole might enhance their potential bioactivities. In this study, we incorporated four privileged substructures having different levels of electronic properties ranging from electron-rich to deficient as follows: indole > 3,3-dimethylbenzopyran > quinoline > pyrimidine (Figure 2a). The variations in electronic properties of privileged substructures are expected to correlate with the diverse arrangement of electrostatic surface charge within a 3-dimensionally similar conformation.

For assessing the molecular diversity of the devised molecular framework, electrostatic polar surface area of energy-minimized conformers as well as the isosurface diagram of each scaffold (**I**–**VI**) containing six different privileged substructures were obtained by the calculation of electrostatic polar potentials and electron density. As shown in Figure 2b, six scaffolds have a distinguishable display of electrostatic polar surface area because of the differentiation in electronic properties of each privileged substructure. For instance, scaffolds **I** and **III** containing an electron-rich indole and an electron-deficient quinolone, respectively, were well-differentiated by their unique display of polar surface area. The introduction of additional aryl moieties on privileged substructures in scaffolds **V** and **VI** also allows the distinct layout of polar surface area, compared to that of scaffolds **III** and **IV**, within a similar 3D conformational framework.

The further expansion of molecular diversity can be achieved via the combination of various moieties to an isoxazole such as aliphatic and aromatic groups with various substituents in their

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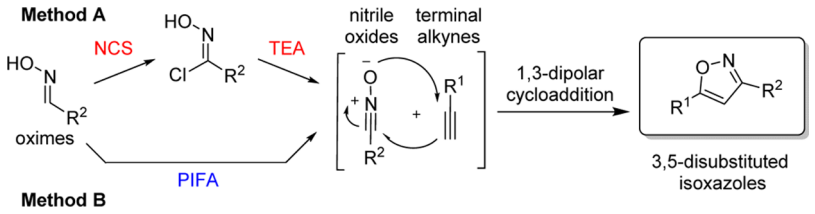
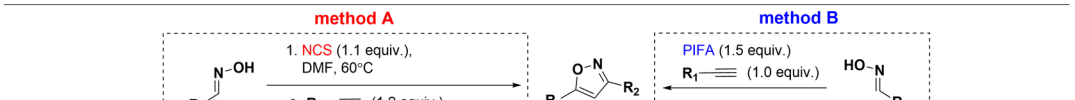


Figure 3. Synthesis of 3,5-disubstituted isoxazoles by 1,3-dipolar cycloaddition from terminal alkynes and nitrile oxides generated from oximes using two complementary method A and B. (R^1 = privileged cores, R^2 = alkyl and aryl groups.)

Table 1. Parallel Synthesis of 3,5-Disubstituted Isoxazoles via Two Complementary Routes for Nitrile Oxides: Methods A and B[c]



R_1	R_2: 1	2	3	4	5[b]	6	7	8	9	10	11	12	13	14	15
a	51	58	58	62	67	60	77	40	41	42	43	47	73	48	49
b[a]	39	57	56	58	(43)	46	25	31	27	28	29	29	47	40	20
c	73	73	60	70	(80)	64	76	49	55	45	58	62	78	49	31
d	71	81	48	51	(64)	57	40	15 (79)	20 (82)	12 (37)	15 (70)	13 (74)	56	18 (71)	18 (38)
e	82	74	40	46	(53)	70	67	38	42	39	40	41	56	54	38
f	70	70	42	51	(52)	53	63	24 (70)	27 (53)	27 (65)	23 (62)	29 (69)	43	27 (68)	14 (24)

[a]The isolated yields of 2 steps including TIPS deprotection (see Supporting Information). [b]Due to the inseparable side products, the desired isoxazole was not obtained by method A. [c]The number in the table is the isolated yield using method A. The isolated yields of method B is in parentheses.

electronic types (electron rich and poor groups) and patterns (ortho, meta, and para). Electron-donating (OCH_3) and electron-withdrawing substituents (CF_3, CN and halogens) were adopted into these scaffolds to diversify the electrostatic surface. In accordance with electronic characters of substituents, scaffold **III** can be differentiated by the introduction of alkyl (**2c**), *p*-methoxyphenyl (**6c**), and *p*-bromophenyl (**15c**) moieties in their display of polar surface area (Figure 2c). In fact, Brown and co-workers pointed out the strong bias toward para substitution of aryl rings in the drug design by performing the population analysis of aryl rings in the drug database.[15] To avoid this unexpected preference for para substitutents, we designed to systematically introduce the identical substituents at the ortho, meta and para positions of aryl groups in our library construction. In fact, the display of polar surface charges is closely related to noncovalent interactions, such as hydrogen bonding, ionic bonding, van der Waals interaction and hydrophobic interactions. Therefore, we hypothesized that our 3,5-disubstituted isoxazole library can ensure the biological relevancy by the combination of privileged substructures and have a potential to perturb various biopolymers through noncovalent interactions.[16]

Our synthetic strategy hinged on a 1,3-dipolar cycloaddition of nitrile oxides and terminal alkynes (Figure 3). Oximes are well-established precursors for nitrile oxides and several synthetic methodologies of nitrile oxides from oximes have been reported. In our study, the oxidation of oximes to nitrile oxides was pursed through two different approaches; method A utilized a halogenating agent in the presence of base and method B used a hypervalent iodine reagent. The in situ generated nitrile oxide underwent a 1,3-dipolar cycloaddition with alkyne affording the corresponding 3,5-disubstituted isoxazoles.

The synthetic results of the isoxazole library are summarized in Table 1. Method A is the most general synthetic route of nitrile oxides and mainly used in this study for the synthesis of each library member. The formation of nitrile oxides for 1,3-dipolar cycloaddition of isoxazole involves two steps: (1) the formation of hydroximoyl chloride by *N*-chlorosuccinimide (NCS) and (2) the subsequent dehydrohalogenation by triethylamine (TEA). The overall tendency of synthetic outcomes in method A indicated that the yields of isoxazoles were dependent on the electronic properties of oximes. In the case of alkyl oximes and aryl oximes containing electron-rich substituents, the 1,3-dipolar cycloaddition reactions with six dipolarophiles (**a**–**f**) afforded the desired isoxazoles in good yields, while aryl oximes containing electron-deficient groups suffered from relatively low yields. In the case of some alkyne

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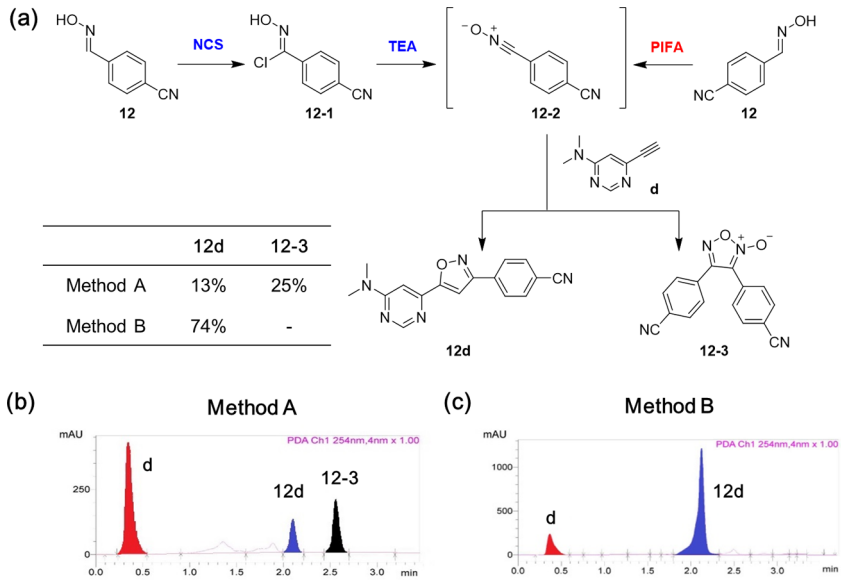

Figure 4. (a) Investigation of 1,3-dipolar cycloaddition of acetylene **d** with *p*-cyanophenyl nitrile oxide **12-2** generated from **12** via either method A or B. (b) LC chromatogram of reaction of **d** and **12-2** generated from **12** via method A and the formation of the product **12d** and the major side product **12-3**. (c) LC chromatogram of reaction of d with **12-2** generated from **12** via method B and the formation of product **12d** (see Supporting Information).

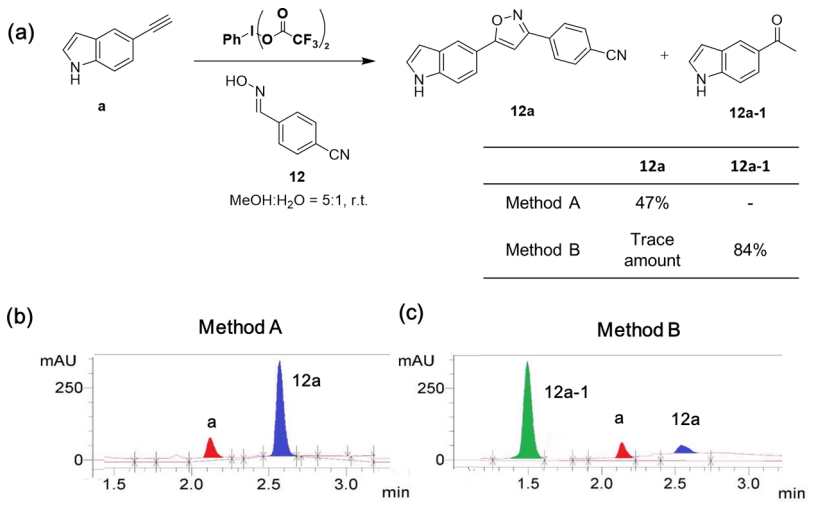

Figure 5. (a) Investigation of 1,3-dipolar cycloaddition of acetylene **a** with *p*-cyanophenyloxime **12** via either method A or B. (b) LC Chromatograms of reaction of **a** and **12** via method A and the formation of the product **12a**. (c) LC Chromatograms of reaction of **a** and **12** via method B and the formation of the product **12a** and major side product **12a-1**.

dipolarophiles (**a**, **c**, and **e**), the desired isoxazoles can be synthesized in moderate yields even with electron-deficient oximes. However, other alkynes (**b**, **d**, and **f**) were transformed to the corresponding isoxazoles in poor yields ranging 12−31% with nitrile oxides containing electron-deficient substituents, probably due to the mismatch of electronic states in 1,3-dipolar cycloaddition between nitrile oxides and dipolarophiles. When we applied method A for the preparation of nitrile oxides containing electron-poor substituents, we observed the formation of side products despite the full conversion of oximes in LC/MS chromatogram. NMR spectral analysis revealed the isolated side products as furoxans which is a well-known dimer of nitrile oxides (Figure 4a and Supporting Inforamtion). It has been reported that nitrile oxide intermediates readily undergo the dimerization pathway either when unreactive dipolarophiles were used or when a 1,3-dipolarophile was absent.[17] To minimize this dimerization, nitrile oxides can be prepared from oximes via a single step transformation using other halogenating reagents,[18] metal oxidents,[19] and hypervalent iodine reagents.[20] On the basis of our reaction condition screening of this transformation, the efficient synthesis of isoxazoles was achieved without significant dimerization of nitrile oxides via the direct oxidation of oximes with a hypervalent iodine reagent—phenyliodine bis-(trifluoroacetate) (PIFA).[21] This hypervalent iodine reagent has advantages including low toxicity, easy handling, commercial availability and high efficiency. In method B, oximes were converted directly into nitrile oxide intermediates upon treatment with PIFA and the subsequent 1,3-dipolar cycloaddition of the in-situ-generated nitrile oxides with alkynes afforded 3,5-disubstituted isoxazoles. As shown in Table 1, we obtained the desired products using method B with moderate

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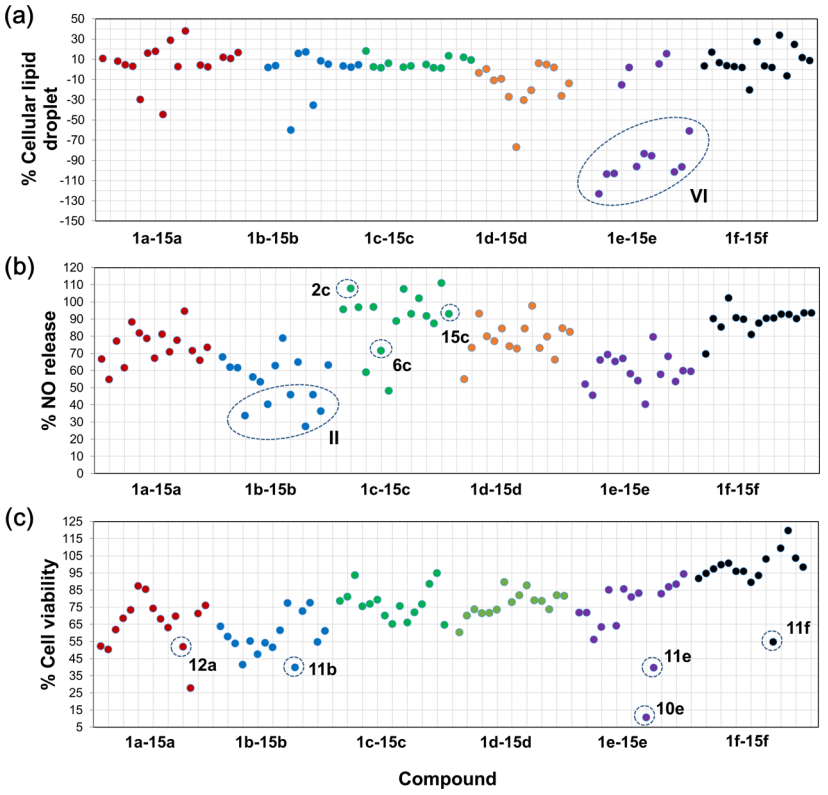

Figure 6. Different patterns of bioactivities among each molecular framework of 3,5-disubstituted isoxazoles in a series of phenotypic assays. Colored circles of each assay depict hit compounds that exhibited phenotypic changes. (a) Image-based high throughput assay via monitoring cellular lipid droplets in HeLa human cervical cancer cells with fluorogenic SF44 probe. Cells treated with 10 μM oleic acid were used as a positive control, and cells in serum-free RPMI media were used as a negative control. (b) Phenotypic screening of cellular nitric oxide release in Raw264.7 murine macrophage cells. LPS-treated and DMSO-treated cells were used as a positive and negative control, respectively. (c) Cell viability assay in HeLa cells. DMSO-treated cells were used as a positive control.

to good yields (in parentheses), even in the case of electron-deficient oximes.

To probe the effects of PIFA on improving the synthetic yields of isoxazoles, we investigated the direct comparison between method A and B for the synthesis of a representative compound **12d** using electron-deficient oxime **12** and electron-poor dipolarophile **d**. As shown in Figure 4a, nitrile oxide **12-2** was generated from oxime **12** via either method A or B, and the resulting **12-2** was subject to 1,3-dipolar cycloaddition with dipolarophile **d**. In method A, the treatment of **12** with NCS allows the formation of chloroxime intermediate **12-1**, which transforms to nitrile oxide **12-2** in the presence of TEA. However, the isolated yield of the desired isoxazole **12d** was only 13%. Instead, furoxan **12-3**, a dimer of **12-2**, was isolated in 25% yield and a large amount of acetylene d remained in the reaction mixture (Figure 4b). In contrast, when **12-2** was generated directly from **12** by PIFA, the clean conversion of d to **12d** in 74% yield was observed without formation of **12-3** by LC-MS analysis (Figure 4c). This result indicated that the dimerization of nitrile oxide can be minimized by its in situ formation using PIFA.

Using method B, we could improve the yields of 1,3-dipolar cycloaddition of terminal alkynes **d** and **f** with electron-deficient oximes ranging 24−82%, compared to that of method A (Table 1). In addition, we observed the multiple chlorination of 3-methoxyphenyl oxime **5** along with the desired chloroxime intermediate upon treatment with NCS. Consequently, the cycloaddition with acetylenes led to the inseparable mixtures (**5b**−**5f**) of the desired products and chlorinated products, except for **5a**. By employing PIFA for the in situ formation of nitrile oxide from oxime **5**, the NCS-mediated chlorination step was avoided and only the corresponding isoxazoles were synthesized in moderate to good yields ranging 43−80%. However, method B also has a limitation as a general protocol for nitrile oxide preparation because of its undesired oxidation of alkyne partners, especially electron-rich alkynes. Therefore, we cannot apply method B in some alkyne dienophiles. For example, the electron-rich alkyne **a**, 5-ethynyl-1*H*-indole, was spontaneously converted to the hydrated form, 5-acetyl-indole, in the presence of trifluoroacetic acid, the side product of PIFA, which significantly lowered the overall yield of 1,3-dipolar cycloaddition (Figure 5). We also observed the similar unexpected side product in the case of benzopyranyl alkyne **b** in method B. In this regards, we applied both method A and B as complementary synthetic routes to access nitrile oxides from various oximes for the cycloaddition with a diverse scope of dipolarophiles.

The library of 3,5-disubstituted isoxazoles containing unique privileged substructures and diverse substituents are expected to have a different pattern of biological activities depending on their distinctive arrangement of polar surface area. To examine their bioactivity, all synthesized 3,5-disubstituted isoxazoles were subject to three independent phenotypic screenings and showed interesting patterns of bioactivity on the basis of privileged substructures (Figure 6a and 6b). As a result of cell-based phenotypic assay by monitoring lipid droplet (LD)[22] with a fluorogenic bioprobe SF44,[23] the cellular LDs in HeLa human cervical cancer cells were significantly decreased upon

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treatment with a series of compounds involving scaffold **VI**, such as **3e**−**5e**, **6e**−**8e**, and **13e**−**15e**, compared to other isoxazole derivatives. In the case of Griess assay in Raw264.7 murine macrophage cells, compounds of scaffold **II**, such as **4b**, **6b**, **10b**, and **12b**−**14b**, showed the significant reduction of cellular nitric oxide (NO) release as a marker of anti-inflammatory activity. Along with the difference in molecular frameworks, we also observed the different patterns in phenotypic changes according to the type of substituents. Among the isoxazole derivatives containing quinoline substructure, compounds of scaffold **III** (**2c**, **6c**, and **15c**) showed a distinct display of polar surface area according to the type of substituent in Figure 2 and inhibited the cellular NO release at the different levels in the Griess assay (Figure 6b). In addition, as shown in Figure 6c, isoxazoles containing cyanophenyl moiety, such as **12a**, **11b**, **10e**, **11e**, and **11f**, revealed the cytotoxic effect against HeLa cells, while other isoxazoles containing other moieties did not influence the cell viability.

CONCLUSION

In conclusion, we designed and synthesized a library of 3,5-disubstituted isoxazoles via incorporating privileged substructures and various substituents, respectively, to make a diverse arrangement of polar surface area within a similar 3-dimensional molecular framework. We visualized the difference of polar surface area in scaffolds **I**−**VI** using in silico analysis. To access 3,5-disubstituted isoxazoles, we adopted 1,3-dipolar cycloaddition between terminal acetylenes and nitrile oxides. For the synthesis of nitrile oxides, we applied two complementary routes and completed the library construction in good to moderate yields. Through the biological evaluation of our isoxazoles via three independent phenotypic assays, we observed the different pattern in the biological activity that exhibited the importance of molecular design via incorporating privileged substructures and various substituents in a 3-dimensionally similar conformer to maximize the diversity in polar surface area.

EXPERIMENTAL PROCEDURES

General Procedure for the Synthesis of Representative Compound 8d, 10d, 12d, 8f, 10f, and 12f. *Method A.* To a solution of oxime (1.0 equiv) in dimethylformamide (DMF), *N*-chlorosuccinimide (1.1 equiv) was added. The mixture was stirred at 60 °C until the starting materials were fully consumed, which was monitored by thin layer chromatography (TLC); then, TEA (1.2 equiv) and terminal acetylene (1.2 equiv) were added to the reaction mixture. After 2 h (the reaction completion was monitored by TLC), the reaction mixture was diluted with dichloromethane (DCM) and washed with deionized water and brine. The combined organic layer was dried with anhydrous Na_2SO_4(s). After the removal of solvent under the reduced pressure, the residue was purified by silica-gel flash column chromatography to obtain the desired compounds.

Method B. To a solution of oxime (1.5 equiv) in MeOH:H_2O (5:1), PIFA (1.5 equiv), and terminal acetylene (1.0 equiv) were added. The mixture was stirred at room temperature until starting materials were consumed (the reaction completion was monitored by TLC); then, the reaction mixture was diluted with DCM and washed with deionized water and brine. The combined organic layer was dried with anhydrous Na_2SO_4(s). After the removal of solvent under the reduced pressure, the residue was purified by silica-gel flash column chromatography to obtain the desired compounds. Characterization data for representative compounds follow; for the full data set, see the Supporting Information.

N,N-Dimethyl-6-(3-(3-(trifluoromethyl)phenyl)isoxazol-5-yl)pyrimidin-4-amine ***8d***. Yield: 79%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.66 (s, 1H), 8.14 (s, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.73 (d, J = 8.0 Hz, 1H), 7.62 (t, J = 7.6 Hz, 1H), 7.33 (s, 1H), 7.05 (s, 1H), 3.22 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.6, 162.5, 162.2, 158.7, 150.8, 131.8, 131.5, 130.1, 129.7_6, 129.7_0, 127.0 (q, J = 3.8 Hz), 123.8 (q, J = 3.8 Hz), 101.7, 98.8, 37.5

2-(5-(6-(Dimethylamino)pyrimidin-4-yl)isoxazol-3-yl)-benzonitrile ***10d***. Yield: 37%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.67 (s, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.84 (d, J = 7.6 Hz, 1H), 7.73 (t, J = 7.6 Hz, 1H), 7.59 (t, J = 7.6 Hz, 1H), 7.50 (s, 1H), 7.06 (s, 1H), 3.22 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.4, 161.0, 158.7, 150.8, 134.4_9, 134.4_5, 133.3, 131.9, 130.3, 129.6, 117.9, 111.2, 103.2, 98.9, 37.5.

4-(5-(6-(Dimethylamino)pyrimidin-4-yl)isoxazol-3-yl)-benzonitrile ***12d***. Yield: 74%, white solid. ^{1}H NMR (400 MHz, acetone-d_6): δ 8.66 (s, 1H), 7.99 (d, J = 8.8 Hz, 2H), 7.79 (d, J = 8.0 Hz, 2H), 7.86−7.75 (m, 3H), 7.32 (s, 1H), 7.05 (s, 1H), 3.22 (s, 6H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 169.3, 161.9, 161.6, 158.4, 150.0, 132.2, 132.4, 127.6, 118.4, 112.9, 102.7, 99.0, 36.9.

N,N-Dimethyl-4-(6-(3-(3-(trifluoromethyl)phenyl)isoxazol-5-yl)pyrimidin-4-yl)aniline ***8f***. Yield: 70%, yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.19 (s, 1H), 8.17−8.07 (m, 5H), 7.65 (d, J = 7.2 Hz, 1H), 7.64 (d, J = 7.2 Hz, 1H), 7.74 (s, 1H), 6.80 (d, J = 8.8 Hz, 2H), 3.09 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.2, 165.5, 162.3, 159.3, 152.9, 152.2, 130.2, 129.8, 129.5, 128.8, 127.8, 127.1, 127.0, 123.9 (q, J = 4.6 Hz), 122.9, 121.7, 111.9, 110.9, 102.7, 40.2.

2-(5-(6-(4-(Dimethylamino)phenyl)pyrimidin-4-yl)-isoxazol-3-yl)benzonitrile ***10f***. Yield: 65%, yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.20 (s, 1H), 8.18 (s, 1H), 8.13 (d, J = 8.8 Hz, 2H), 8.01 (d, J = 7.6 Hz, 1H), 7.86 (d, J = 7.6 Hz, 1H), 7.75 (t, J = 8.0 Hz, 1H), 7.65−7.59 (m, 2H), 6.81 (d, J = 8.8 Hz, 2H) 3.09 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 168.6, 164.5, 129.0, 161.3, 159.7, 159.4, 125.3, 145.2, 134.5, 133.4, 130.6, 129.6, 1298.8, 121.8, 117.8, 112.4, 111.3, 103.8, 30.3.

4-(5-(6-(4-(Dimethylamino)phenyl)pyrimidin-4-yl)-isoxazol-3-yl)benzonitrile ***12f***. Yield: 69%, yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.19 (s, 1H), 8.17−8.11 (m, 3H), 8.02 (d, J = 8.0 Hz, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.42 (s, 1H), 6.80 (d, J = 8.8 Hz, 1H), 3.90 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5, 162.0, 159.3, 152.9, 152.1, 133.0, 128.8, 127.6, 122.8, 120.7, 118.4, 114.1, 111.9, 110.9, 102.2, 40.2.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscombsci.7b00032.

Detailed experimental procedures, full characterization data, and the ^{1}H and ^{13}C NMR spectra for all synthesized compounds (PDF)

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Notes

The authors declare no competing financial interest.

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