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Telescoped Synthesis of 2-Acyl-1-aryl-1,2-dihydroisoquinolines and Their Inhibition of the Transcription Factor NF-κB

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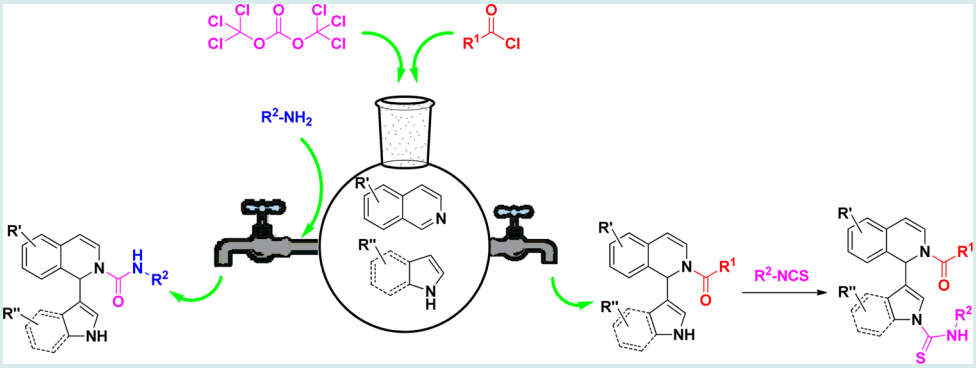
Supporting Information



ABSTRACT: A sequential single-flask multicomponent reactions is highly effective for the synthesis of 1,2-dihydroisoquinolines through amidealkylation from intermediate *N*-acylisoquinolinium salts under mild conditions. *N*-Acylisoquinolinium ions and trichloromethyl-1-(1*H*-indol-3-yl)isoquinoline-2(1*H*)-carboxylate have demonstrated their reactivity toward aromatic and aliphatic π-nucleophiles. One of the 1,2-dihydroisoquinoline derivatives was found to be a potent inhibitor for transcription factor NF-κB by blocking IκBα degradation, p65 nuclear translocation, and NF-κB DNA binding in TNF-α-induced NIH 3T3 cells.

KEYWORDS: *dihydroisoquinolines, transcription factor NF-κB, p65 nuclear translocation, amidealkylation, DNA binding, 3T3 cells*

INTRODUCTION

One of the challenging goals in synthetic chemistry is to develop a new one-pot strategy to construct complex molecules through simultaneous formation and isoquinolines from available starting materials. Multicomponent reactions (MCRs) provide novel methods for the formation of multiple carbon−carbon bonds in a single operational step and represent an innovative approach to the synthesis of polyfunctional molecular scaffolds.[1] Single-flask MCRs enable the rapid construction of small heterocycles that play a significant role in drug discovery research to provide one of the greatest sources of molecular diversity.[2] Recently, one pot, multistep, and successive addition reactions have shown significant potential in the construction of pharmaceutically interesting heterocycles.[3] Among heterocycles, isoquinolines and indoles are important structural motifs in natural products and have received significant attention because of their broad biological activities.[4] Naturally occurring and synthetic analogs of N-substituted indoles are pharmaceutically important because they are inhibitors of enzymes and antihypertensive drugs.[5]

Recently, the stereoselective introduction of 1-arylation to isoquinolines by using a acyl chloride and aryl Grignard reagents was reported by Wanner and co-workers.[6] Furthermore, Wu et al. described silver-catalyzed tandem reactions of *N*-(2-alkynylbenzylidene)-hydrazides with several alkynes to synthesize 1,2-dihydroisoquinolines.[7] Larock and co-workers have synthesized 1,2-dihydroquinolines through the one-pot, multicomponent reaction of 2-(1-alkynyl)benzaldehydes, amines, and ketones in the presence of AgOTf and L-proline.[8]

Nuclear factor-kappa B (NF-κB) is a transcription factor that regulates many cellular processes, including immune responses to infection and inflammation, apoptosis, and embryonic and neuronal development.[9] Constitutively active NF-κB has been identified in malignant hematologic cells and NF-κB is thus a promising target to develop anti-cancer therapeutics in hematologic malignancies.[9] A literature survey revealed that isoquinoline and indole-based natural or synthetic inhibitors are available for NF-κB activation, such as emetine, lestaurtinib, tribromsalan, cycloepoxydon, and Sunitinib malate (Figure 1).[10,11] The lestaurtinib and emetine are cytotoxic to various human acute myeloid leukemia (AML) cell lines and primary human AML

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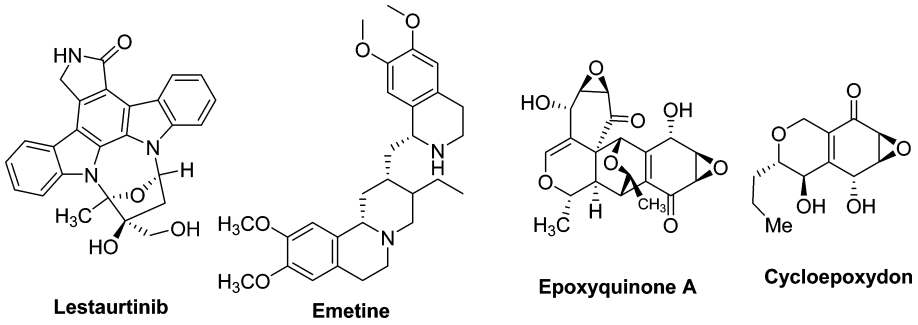
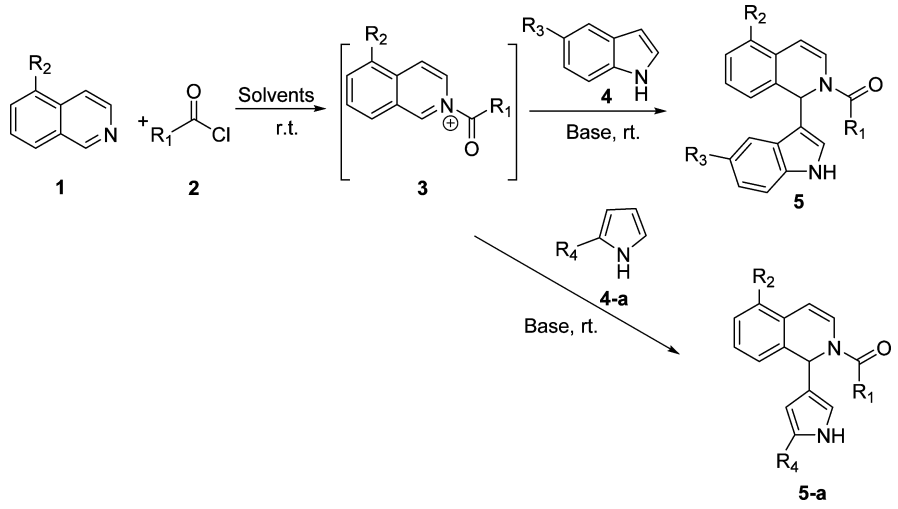


Figure 1. Structure of NF-κB inhibitors.

Scheme 1. A Novel Route toward the Synthesis of 2-Acyl-1-aryl-1,2-dihydroisoquinolines 5



blasts, through inhibition of NF-κB signaling in cancer cells.[10] Epoxyquine A and cycloepoxydon were isolated from fermentation of a deuteromycete strain to inhibit activation of NF-κB that regulates the expression of various cellular genes.[11]

As part of our continued interest in the synthesis of biologically interesting small molecules,[12] we have developed a one-pot, telescoped synthesis for the construction of 2-acyl-1-aryl-1,2-dihydroisoquinolines which represents a new class of compounds to inhibit activation of transcription factor NF-κB.

Our initial effort was focused on one-pot MCRs through N-carboxylation of isoquinoline, followed by C−C bond formation between *N*-acylisoquinolinium ion and indole. *N*-Acylisoquinolinium ions **3** were successfully used as electrophilic reagents in an amidoalkylation reaction for the synthesis of several isoquinolines derivatives and had high reactivity in the heteroarylation reaction of aromatic π-nucleophiles of organometallic compounds.[13]

Herein, we investigated MCRs of isoquinoline **1** with *n*-propyl chloride **2**{*1*} and 1*H*-indole **4** to 2-acyl-1-aryl-1,2-dihydroisoquinolines **5** using various bases and solvents at ambient temperature (Scheme 1), and the results are summarized in Table 1. To enhance the reaction efficiency and improve yields, we conducted the sequential addition of reagents in the same pot without any workup/purification of intermediates (Scheme 1).

A preliminary study shows potassium carbonate in toluene can promote the C−C bond formation between *N*-acylisoquinolinium ion **3** and indole **4**{*1*} to afford the 2-acyl-1-aryl-1,2-dihydroisoquinolines **5**{*1,1,1*} in 15% yield (Table 1, entry 1). The reaction occurs selectively at the C-3 position of indole even when both the C-2 and C-3 positions are unoccupied.[14] We observed that the electrophilic *N*-acylisoquinolinium intermediate **3** underwent selectively heteroarylation at the C-1 position by nucleophilic attack of indoles **4**{*1*}.[15] The same reaction was tested in different solvents (Table 1, entries 1−8), and we found that a polar aprotic solvent such as dimethylformamide (DMF) gave an 88% yield of the corresponding product (Table 1, entry 7). An equimolar amount of sodium hydride gave a smooth and clean reaction to afford the desired products **5**{*1,1,1*} in higher yields (94%) within 30 min at ambient temperature (Table 1, entry 17). Although potassium carbonate (Table 1, entry 7), 4-dimethylaminopyridine (Table 1, entry 15), and sodium hydride (Table 1, entry 17) furnished the product **5**{*1,1,1*} in more or less similar yields, sodium hydride was chosen as a base for further reaction optimization because of its higher yield (94%) and short reaction time (30 min) .

After confirming the viability of the proposed sequential one-pot MCRs to construct diverse dihydroisoquinolines **5**, we explored the scope of this reaction with different isoquinolines **1**{*1*−*3*}, indoles **4**{*1*−*6*} and acyl chlorides **2**{*1*−*9*} (Figure 3). The reactions worked well, tolerating various functional groups at C-4 and C-3 of the acyl chloride (Table 2, **5**{*1,1,5*}−{*1,1,8*}). The acyclic acid chloride **5**{*1,1,1*} gave a better yield than the cyclic acid chlorides (Table 2, **5**{*1,1,2*} and **5**{*1,2,9*}−{*2,4,9*}). The absolute configuration of compound **5**{*1,1,2*} was confirmed by X-ray diffraction (CCDC 935318),[16] in Figure 2. The crystallographic data reveal that the acid chloride and indole moiety are linked to isoquinoline at the N2 and C1 position, respectively. The residue at the C1 indole group distinctly occupies the space perpendicular to the basic skeleton of isoquinoline.

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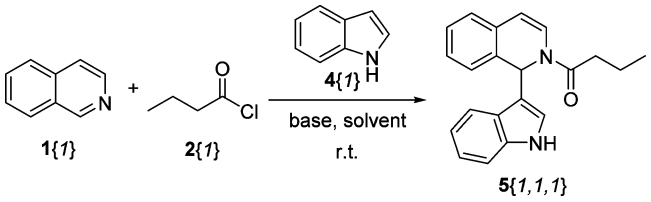
Table 1. Optimization of the Reaction Conditions[a]



entry	base	solvent	yield (%)[b]
1	K_2CO_3	toluene	15
2	K_2CO_3	THF	18
3	K_2CO_3	MeOH	N.R.
4	K_2CO_3	ether	28
5	K_2CO_3	dioxane	37
6	K_2CO_3	DMAC	41
7	K_2CO_3	DMF	88
8	K_2CO_3	EDC	27
9[c]	K_2CO_3	DMF	48
10[d]	K_2CO_3	DMF	63
11	Et_3N	DMF	N.R.
12	Cs_2CO_3	DMF	60
13	CsF	DMF	38
14	DBU	DMF	10
15	DMAP	DMF	86
16	tBuOK	DMF	43
17[e]	NaH	DMF	94

[a]Reactions were performed in the presence of base (1 equiv) at room temp for 12 h. [b]Isolated yield after column purification. [c]Reaction temperature 10 °C. [d]Reaction temperature 40 °C. [e]Reaction time 30 min; N.R.- no reaction.

After reaction optimization, we examined synthesis of 1-(1*H*-indol-3-yl)-*N*-phenylisoquinoline-2(1*H*)-carboxamide **7** by using isocyanate **6**, *N*,*N*′-carbonyldiimidazole (CDI) **10**, and triphosgene, illustrated in Scheme 2. The first approach using pathway A (Scheme 2) did not produce the expected compound in either DMF or toluene after prolonged reaction time at higher temperatures. In path B, the reaction of **1** with CDI **10** and indole **4** followed by addition of amine **11** in DMF did not afford the desired product **7**{*1,1,1*}. Because acid chloride and sulfonyl chloride are more reactive than isocyanate and CDI, the synthesis of a Reissert type adduct is more favorable with more activated electrophiles,[17] therefore, when isoquinoline **1** was reacted with triphosgene and indole **4** (path C), followed by addition of various nucleophiles, such as amines, alcohols, or thiols, to deliver the corresponding 1,2-dihydroisoquinolines **7** derivatives smoothly (Table 3).

Isoquinoline activation by triphosgene followed by nucleophilic attack of indole afforded the corresponding key intermediate trichloromethyl-1-(1*H*-indol-3-yl)isoquinoline-2(1*H*)-carboxylate **16**. The carbon NMR spectrum clearly shows two extra quaternary carbon peaks at ∼148 and ∼132 ppm. Subsequently, compound **16** was employed to expand the library diversity, with various heteroatom nucleophiles, such as amines, alcohols, and thiols, at room temperature, as illustrated in path C (Scheme 2).[17b] For the scope of the nucleophiles, we reacted two amines **11**{*1*−*7*}, an alcohol **13**{*1*−*4*}, and two thiols **14**{*1*−*3*} (Figure 3).

The utility of this stepwise one-pot and multistep synthesis was demonstrated in Table 3. Although more-reactive nucleophiles afforded the highest yields (**9**), poor nucleophiles gave modest yields (**7** and **8**). Not only amine nucleophiles but also alcohol and thiol nucleophiles performed well to afford resultant products **8** and **9** in good yields. The selectivity was also observed with 2-amino thiophenol, in which the thiol is more nucleophilic than the aniline group, furnishing the corresponding compound **9**{*1,1,1,2*} in high yield (92%). But thiol nucleophiles **14**{*3*} gave only a 45% yield for the same condition, and all of the phenols nucleophiles did not react at all. Amine nucleophile **11**{*5*} did not deliver **7**{*1,1,1,5*} but gave 1-(*1H*-indol-3-yl)isoquinoline. It is clear that 4-aminopyridine plays a base instead of a nucleophile because of the electron-withdrawing nature of pyridine N.

As a further extension of this study, we found that the reaction of *N*-sulfonylisoquinolinium **18** with indole **4** furnished the corresponding 1-(1*H*-indol-3-yl)-2-(alkyl or aryl-sulfonyl)-1,2-dihydroisoquinoline **19** in high yields (Scheme 3). We also attempted to increase the diversity of compound **5** or **9** with isothiocyanate **21** to 3-(2-butyryl-1,2-dihydroisoquinolin-1-yl)-*N*-phenethyl-1*H*-indole-1-carbothioamide **22**. Delighted with this observation, we treated a number of 2-acyl-1-aryl-1,2-dihydroisoquinolines **5**{*1,1,1*} or **19**{*1,1,3*}, **19**{*1,1,4*} with isothiocyanate **21**{*1*−*7*} by this protocol. When the C-3 position of the indole moiety is occupied by substituents other than hydrogen, then the indole nitrogen is the most reactive toward electrophiles.[18] Thus, the nitrogen of indole was reacted with isothiocyanate to afford the N-alkylated indole motif; for example, substituted thioureas.

Initially, the reactions of **5**{*1,1,1*} with (2-isothiocyanatoethyl)-benzene **21**{*1*} were carried out with NaH in THF at room temperature to deliver substituted thiourea **22**{*1,1*} in 82% yield (Table 4). N-Alkylation reaction of 2-acyl-1-indole-1,2-dihydroisoquinoline with isothiocyanate was first reported in the presence of base under mild reaction conditions. We tested the reactions on 2-acyl-1-indole-1,2-dihydroisoquinoline **5**{*1,1,1*} (Table 4, entries 1−2) and 2-sulfonyl-1-indole-1,2-dihydroisoquinolin **19**{*1,1,3*}, **19**{*1,1,4*} to provide N-alkylated product **22**{*2,1*}−{*3,7*} in good to moderate yields (Table 4, entries 3−10).

Earlier studies have shown that various isoquinoline- and indole-containing compounds are inhibitors of NF-κB.[7−10] Because NF-κB is a transcription factor involved in the regulation of numerous genes,[19] we proceeded to determine whether 2-acyl-1-aryl-1,2-dihydroisoquinolines could inhibit NF-κB activity. NIH 3T3 cells were either untreated (Figure 4A, lanes 1 and 2) or treated with 20 μM **19**{*1,1,1*} (lanes 3−7) and then stimulated with TNF-α an indicated number of times. The level of IκBα was then analyzed by Western blotting using a polyclonal antibody against the carboxyl terminus of IκBα. Treatment with 20 μM **19**{*1,1,1*} effectively inhibited IκBα degradation at various times (5, 10, 15, and 40 min) post-TNF-α induction (Figure 4A, lanes 3−7). In contrast, treatment with TNF-α for 20 min led to apparent IκBα degradation in the control cells (Figure 4A, lane 2). Because phosphorylation of IκBα at serine residues 32 (S32) and 36 (S36) is necessary for TNFα-induced IκBα degradation,[20] we next examined the effect of **19**{*1,1,1*} in IκBα phosphorylation by a Western blot using an antibody specific for detecting S32 phosphorylation in IκBα. As shown in Figure 4B, phosphorylation of IκBα peaked at 5 min following TNF-α stimulation in untreated cells (lanes 2−4). Treatment with 20 μM **19**{*1,1,1*} led to effective inhibition of IκBα phosphorylation at various time periods after stimulation (lanes 6−8). These results indicate that **19**{*1,1,1*} is a potent inhibitor of NF-κB activation by blocking phosphorylation and degradation of IκBα.

After TNF-α-induced IκB degradation, the transcription factor NF-κB undergoes nuclear translocation and binds to targeted genes.[19b] To further investigate whether NF-κB nuclear

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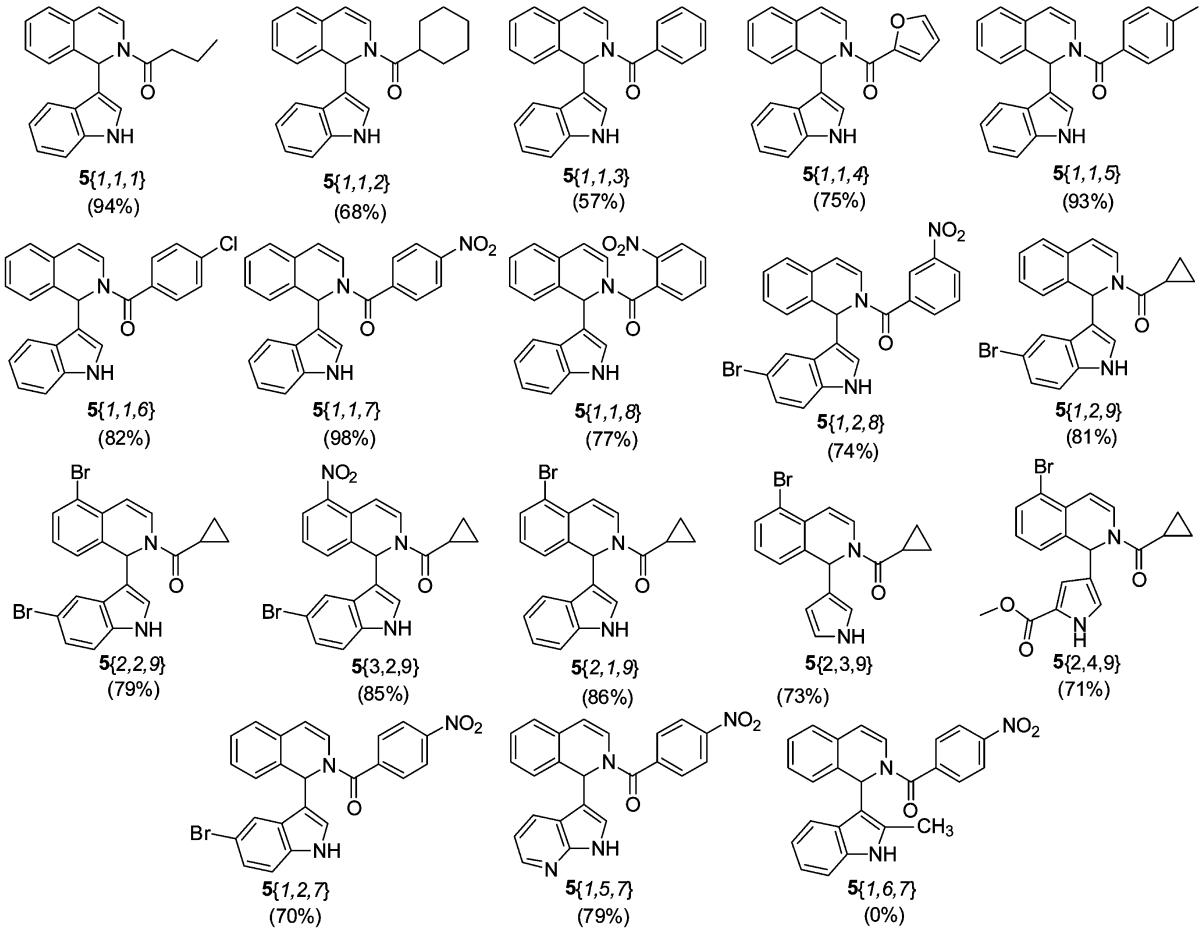
Table 2. Substrate Scope for One-Pot, Two-Step MCRs[a]



[a]Reactions were performed in the presence of NaH (1 equiv) at room temp (25 °C) for 30 min; in parentheses are isolated yield.

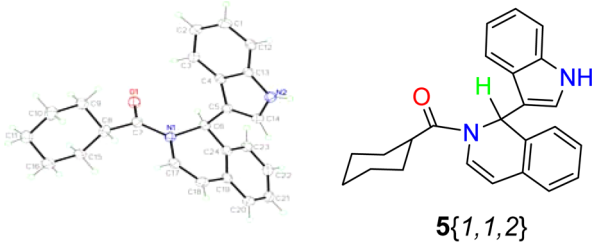


Figure 2. ORTEP diagram of compound 5{*1,1,2*}.

translocation is effectively blocked by **19**{*1,1,1*}, we analyzed nuclear extracts of samples by Western blotting using antibodies against the NF-κB subunit p65. As shown in Figure 4C, apparent nuclear accumulation of p65 was observed in TNF-α-stimulated 3T3 cells (lane 2). In contrast, treatment of **19**{*1,1,1*} resulted in effective inhibition of p65 nuclear translocation (lane 4). In addition, **19**{*1,1,1*} blocked NF-κB DNA binding activity as measured by an electrophoretic mobility shift assay (EMSA) (Figure 4C, lane 4 of upper panel). Taken together, these results suggest that the novel 2-acyl-1-aryl-1,2-dihydroisoquinoline derivative **19**{*1,1,1*} is a potent inhibitor for NF-κB activation, and the inhibition occurs via the inhibition of IκBα phosphorylation and degradation, NF-κB nuclear translocation, and DNA binding activity in TNF-α-induced NIH 3T3 cells (Figure 4).

CONCLUSIONS

We report a telescopic type three-component synthesis of 2-acyl-1-aryl-1,2-dihydroisoquinolines. The efficiency of this novel and bioinspired MCR plays the key role for the synthesis of 2-acyl-1-aryl-1,2-dihydroisoquinolines and 3-(2-butyryl-1,2-dihydroisoquinolin-1-yl)-*N*-phenethyl-1*H*-indole-1-carbothioamide derivatives. The sequential one-pot MCRs show good functional group tolerance and are high-yielding, and the final product isolation is very straightforward. Multiple bonds were conveniently formed in one pot during these telescopic processes and are useful for the assembly of various *N*-heterocycles for pharmaceutical interests. The biological experiments identified that the 1,2-dihydroisoquinoline derivative **19**{*1,1,1*} effectively inhibits NF-κB activation by inhibition of IκBα degradation and NF-κB DNA binding in TNF-α-stimulated NIH 3T3 cells. Further investigation of these novel dihydroisoquinoline derivatives (**5**, **7**, **8**, **9**, and **22**) as potent inhibitors of the transcription factor NF-κB are in progress and will be reported in due course.

EXPERIMENTAL PROCEDURES

General Synthetic Procedures for 1-(1-(1*H*-Indol-3-yl)-isoquinolin-2(1*H*)-yl)butan-1-one (5{*1,1,1*}). Isoquinoline (0.77 mmol, 1 equiv) was dissolved in dimethylformamide (5 mL), then butyryl chloride (0.85 mmol, 1.1 equiv) was added. The reaction mixture was stirred under nitrogen atmosphere at ambient

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Scheme 2. Synthetic Route to the *N*-Ethyl-1-(1*H*-indol-3-yl)isoquinoline-2(1*H*)-carboxamide Derivatives 7, 8, and 9

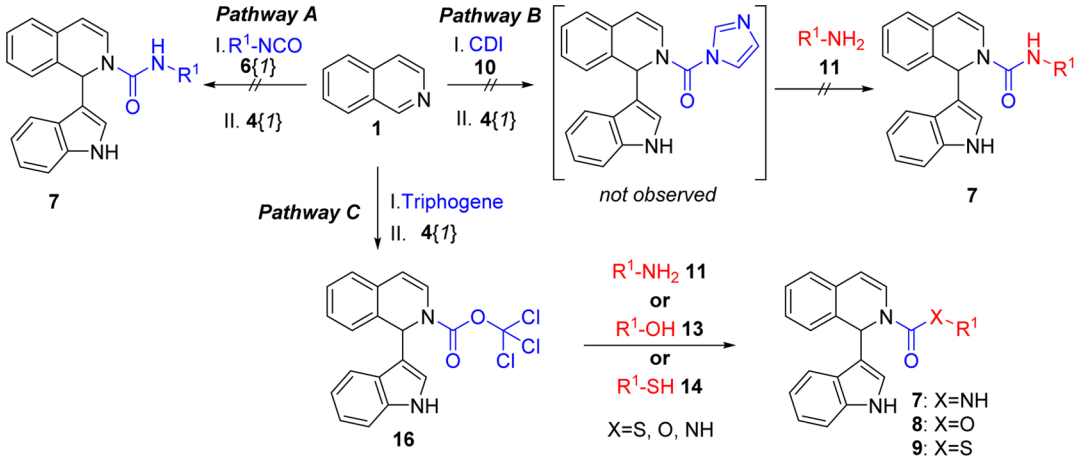
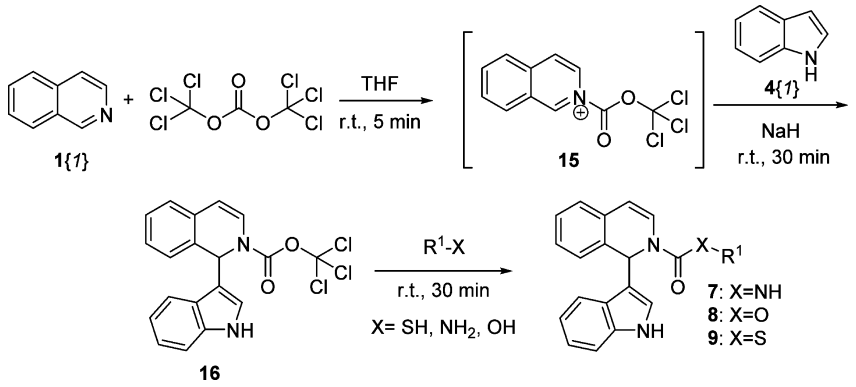
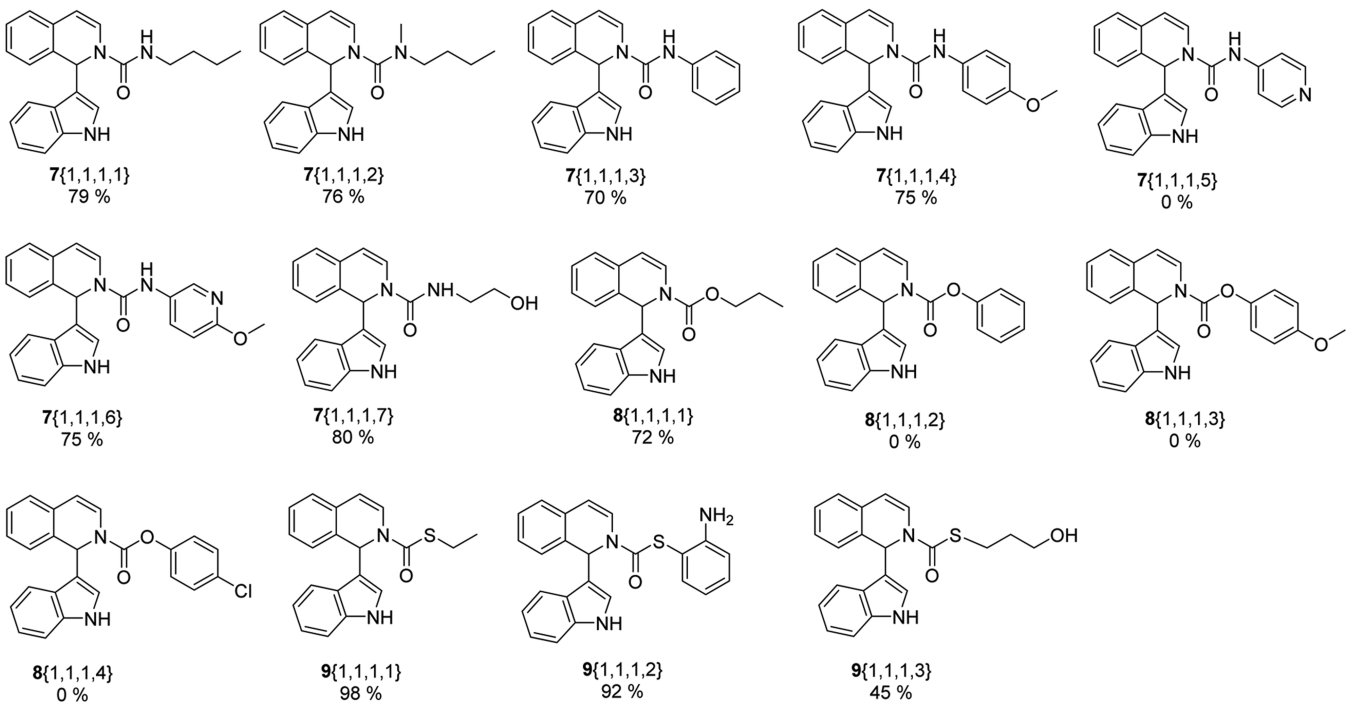


Table 3. One-Flask, Three-Step MCRs Route toward 1-(1*H*-Indol-3-yl)-*N*-phenylisoquinoline-2(1*H*) Derivatives (7, 8, and 9)[a]





[a]Reactions were performed in the presence of NaH (10 equiv) at room temp (25 °C) for 35 min.

temperature for 5 min. The progress of the reaction was monitored by TLC. After complete consumption of isoquinoline, 1*H*-indole (0.77 mmol, 1 equiv) and sodium hydride (0.77 mmol, 1 equiv) were added to the reaction, and the mixture was stirred for 30 min at room

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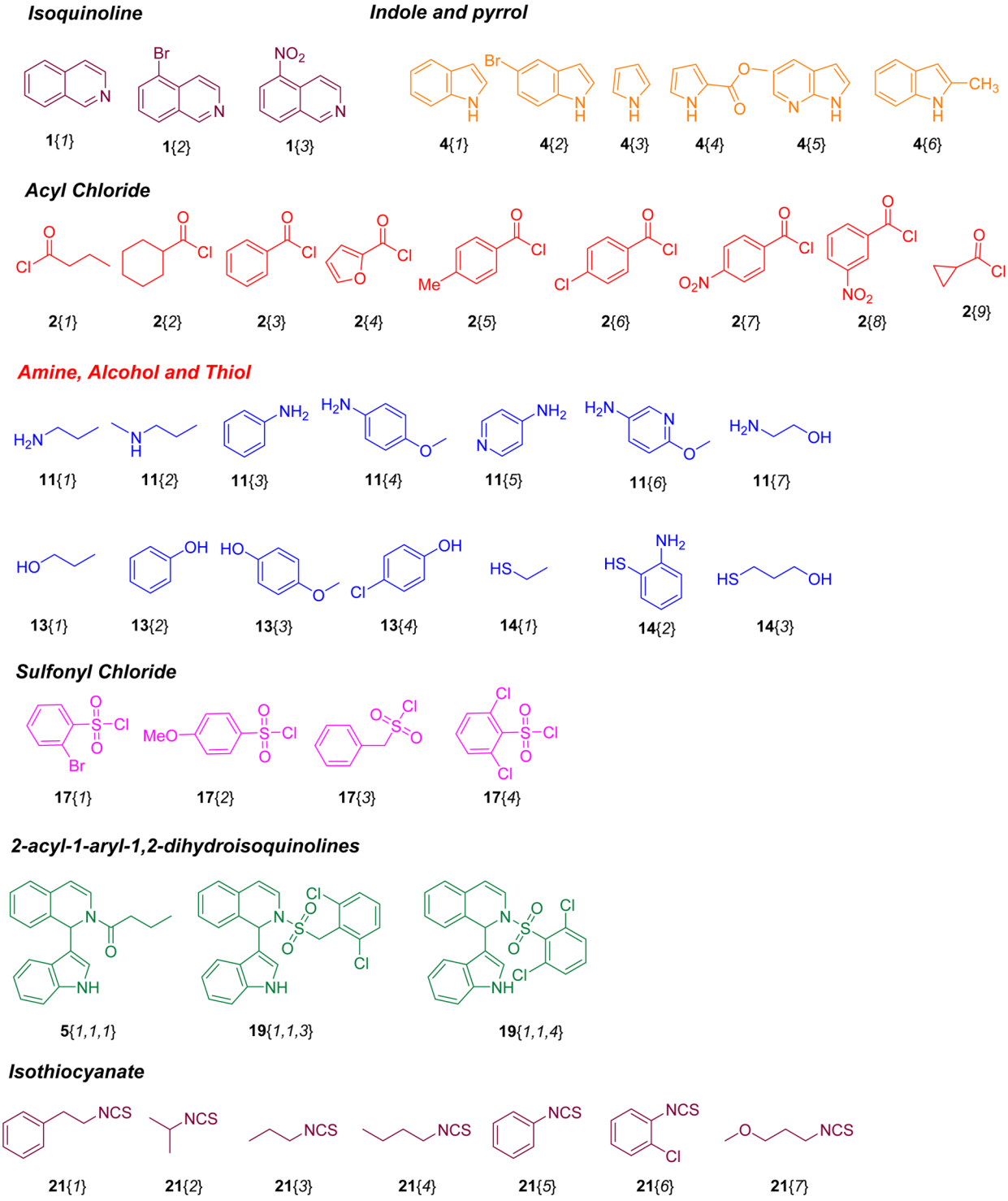

Figure 3. Diversity elements employed for library synthesis: isoquinoline **1**{*1*−*3*}; indoles **4**{*1*−*4*}; acyl chloride **2**{*1*−*9*}; amine **11**{*1*−*7*}; alcohol **13**{*1*−*4*}; thiol **14**{*1*−*3*}; sulfonyl chloride **17**{*1*−*4*}; 2-acyl-1-aryl-1,2-dihydroisoquinolines **5**{*1,1,1*}, **19**{*1,1,3*}, **19**{*1,1,4*}; and isothiocyanate **21**{*1*−*7*}.

temperature. After reaction completion, the reaction mixture was extracted with ethyl acetate (3 × 25 mL), and the collected organic extracts were washed with aqueous sodium carbonate and dried over magnesium sulfate. The solvent was removed by rotary evaporation, and the residue was purified by silica column chromatography (eluent: 20% EA in hexane) to obtain the corresponding compound 1-(1-(1*H*-indol-3-yl)isoquinolin-2(1*H*)-yl)butan-1-one **5**{*1,1,1*} (94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.96 (d, J = 7.7 Hz, 1H), 7.37−7.10 (m, 8H), 6.63−6.58 (m, 2H), 6.13 (d, J = 7.6 Hz, 1H), 2.48−2.36 (m, 2H), 1.83−1.68 (m, 2H), 1.00 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, CD_3COCD_3) δ 170.9, 137.2, 133.9, 131.3, 127.9, 127.5, 127.2, 126.3, 125.6, 125.3, 124.9, 121.8, 120.5, 119.4, 117.3, 111.6, 110.8, 49.4, 35.3, 18.4, 13.6; IR (cm^{-1}, neat) 3243, 2960, 1648, 1617, 746; ESI-MS m/z 339 $[M + Na]^+$. HRMS m/z calcd for $C_{21}H_{20}N_2ONa$, 316.1576; found, 339.1475 $[M + Na]^+$.

The Synthesis of *N*-Butyl-1-(1*H*-indol-3-yl)isoquinoline-2(1*H*)-carboxamide (7{*1,1,1*}). A tetrahydrofuran (THF, 5 mL)

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Scheme 3. Synthetic Pathway for Compound 19

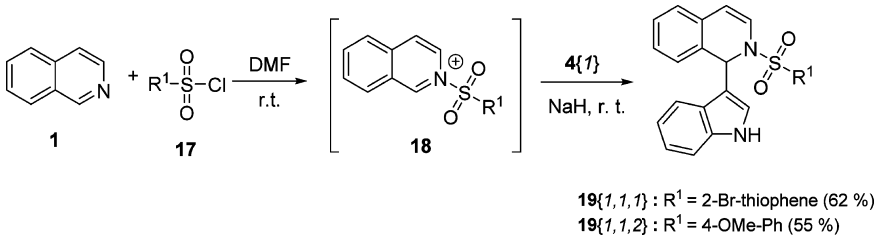
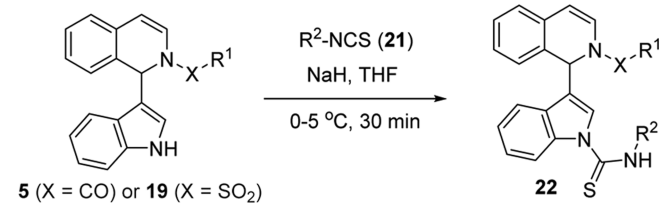
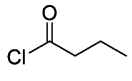
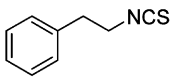
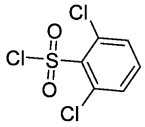
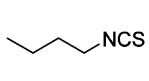
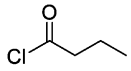
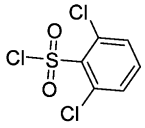
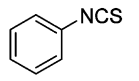
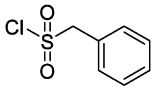
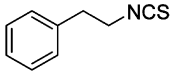
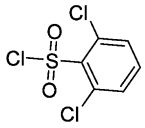
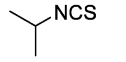
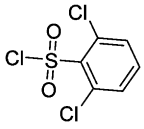
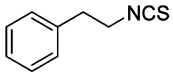
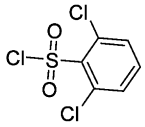
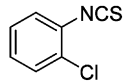
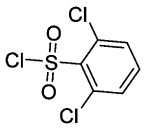
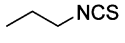
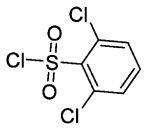
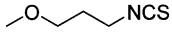


Table 4. Coupling Reaction of Various 1,2-Hydroquinolines with Thioisocyanate[a]



entry	R^1-SO_2Cl or R^1-COCl	R^2-NCS	product	yield (%)[b]	entry	R^1-SO_2Cl	R^2-NCS	product	yield (%)[b]
1			**22**{*1,1*}	82	6			**22**{*3,4*}	43
2			**22**{*1,2*}	67	7			**22**{*3,5*}	43
3			**22**{*2,1*}	65	8			**22**{*3,2*}	26
4			**22**{*3,1*}	60	9			**22**{*3,6*}	49
5			**22**{*3,3*}	40	10			**22**{*3,7*}	33

[a]Reactions were performed with base (10 equiv) in dry THF for 30 min. [b]Isolated yield after column chromatography.

solution of isoquinoline (0.77 mmol, 1 equiv) and bis-(trichloromethyl) carbonate (triphosgene, 1.16 mmol, 1.5 equiv) was stirred at ambient temperature for 5 min. After complete consumption of isoquinoline, 1*H*-indole (0.77 mmol, 1 equiv) and potassium carbonate (1.55 mmol, 2 equiv) were added to the reaction flask and stirred for 30 min, followed by addition of butan-1-amine (11.6 mmol, 15 equiv). After the reaction was complete, the solvent was evaporated under reduced pressure, and the residue was purified by column chromatography (eluent: 20% EA in hexane) to get the corresponding compound *N*-butyl-1-(1*H*-indol-3-yl)isoquinoline-2(1*H*)-carboxamide **7**{*1,1,1*} (79%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (s, 1H), 8.06−7.97 (m, 1H), 7.34−7.27 (m, 1H), 7.21−7.14 (m, 6H), 6.82−6.73 (m, 2 H), 6.78 (s, 1H), 6.74 (d, J = 7.5 Hz, 1H), 6.02 (d, J = 7.5 Hz, 1H), 4.85 (m, 1H), 3.32−3.23 (m, 2H), 1.51−1.37 (m, 2H), 1.31−1.19 (m, 2H), 0.87 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.4, 136.7, 133.1, 130.7, 127.9, 127.4, 127.1, 125.7, 124.9, 124.9, 123.9, 122.7, 120.5, 120.3, 118.4, 111.6, 109.3, 51.9, 41.2, 32.4, 20.4, 14.2; IR (cm^{-1}, neat) 3216, 2925, 1616, 1517, 742; ESI-MS m/z 368 $[M + Na]^+$. HRMS m/z calcd for $C_{22}H_{23}N_3O$, 345.1841; found, 368.1741 $[M + Na]^+$.

The Synthesis of 3-(2-Butyryl-1,2-dihydroisoquinolin-1-yl)-*N*-phenethyl-1*H*-indole-1-carbothioamide (22{*1,1*}). Sodium hydride (6.32 mmol, 20 equiv) was dissolved in tetrahydrofuran (THF, 10 mL), followed by addition of compound 1-(1-(1*H*-indol-3-yl)isoquinolin-2(1*H*)-yl)butan-1-one **5**{*1,1,1*} (0.32 mmol, 1 equiv) with slow addition of (2-isothiocyanatoethyl)benzene (1.58 mmol, 1.5 equiv) in a

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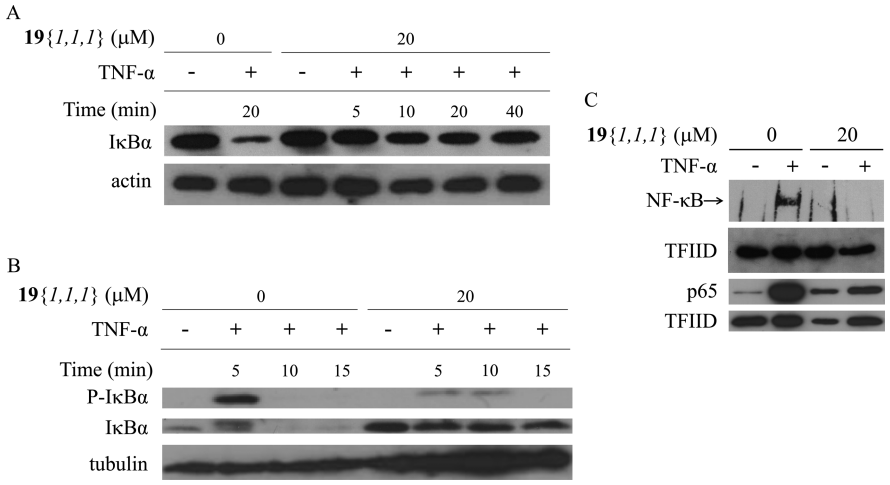

Figure 4. Novel 2-acyl-1-aryl-1,2-dihydroisoquinoline derivative **19** is a potent inhibitor of the transcription factor NF-κB. NIH 3T3 cells were untreated or treated with 20 μM **19**{*1,1,1*} for 2 h and stimulated with or without TNF-α (5 ng/mL) for indicated times before harvesting. Equal amounts of whole cell lysates were analyzed by Western blotting with the indicated antibodies. **19**{*1,1,1*} inhibited TNF-α induced IκBα degradation (A) and phosphorylation (B) in 3T3 cells. (C) **19**{*1,1,1*} inhibited p65 nuclear translocation, and NF-κB DNA binding activity induced by TNF-α. 3T3 cells were either untreated or treated with **19**{*1,1,1*} (20 μM) and stimulated with or without TNF-α (5 ng/mL) as described in parts A and B. EMSA analysis using a biotin-labeled NF-κB binding sequence and Western blots for p65 and TFIID (nuclear protein loading control) were performed using equal amounts of nuclear extracts.

round-bottom flask at 0 °C. The resultant reaction mixture was stirred at 0 °C for 30 min. After the reaction was complete, the solvent was evaporated under reduced pressure. The resulting crude residue was purified by column chromatography (eluent: 10% EA in hexane) to obtain the corresponding 3-(2-butyryl-1,2-dihydroisoquinolin-1-yl)-*N*-phenethyl-1*H*-indole-1-carbothioamide **22**{*1,1*} (82%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, J = 7.9 Hz, 1H), 7.41−7.05 (m, 14H), 6.59 (d, J = 7.5 Hz, 1H), 6.13 (d, J = 7.5 Hz, 1H), 4.04 (q, J = 6.5 Hz, 2H), 3.06 (t, J = 6.5 Hz, 2H), 2.38 (t, J = 7.4 Hz, 2H), 1.77−1.62 (m, 2H), 0.96 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.9, 171.7, 138.4, 134.5, 132.6, 130.5, 129.8, 129.5, 129.2, 129.0, 129.0, 128.5, 128.2, 127.5, 127.3, 125.5, 124.8, 124.5, 123.2, 121.8, 121.3, 112.8, 112.2, 48.9, 47.4, 35.9, 34.2, 18.6, 14.3; IR (cm^{-1}, neat) 3239, 2958, 1656, 1621, 1344, 1174, 1043; ESI-MS m/z 479.32 (M^+). HRMS m/z calcd for $C_{30}H_{29}N_3OS$, 479.2031; found, 478.1974 $[M - H]^+$.

Cell Culture and Treatment. NIH 3T3 fibroblasts were maintained in Dulbecco's modified Eagle's medium (DMEM) (Biowest, Nuaille, France) supplemented with 10% fetal bovine serum (FBS; Biowest), 100 U/mL penicillin, and 100 μg/mL streptomycin. Cells were maintained at 37 °C in a fully humidified incubator with 5% CO_2.

Stock solutions of 2-acyl-1-aryl-1,2-dihydroisoquinoline derivative **19**{*1,1,1*} were dissolved in DMSO and stored at −80 °C before use. Twenty-four hours before drug treatment, 3T3 cells were serum-starved in medium containing 0.5% FBS. After serum starvation, the cells were treated with the vesicle control DMSO or 20 μM **19**{*1,1,1*} for 2 h and stimulated with 5 ng/mL recombinant human TNF-α (R&D Systems, Minneapolis, MN) for the indicated amount of time.

Protein Extraction and Western Blotting. After the indicated treatments, 3T3 cells were washed with ice-cold PBS twice before harvesting. Whole cell lysates or nuclear extracts were prepared as previously described.[21] The protein concentration was then determined by the method of Bradford using the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA) according to the manufacturer's instructions.

Samples containing equal amounts of protein were analyzed by SDS−polyacrylamide gel electrophoresis and transferred to PVDF membranes. Western blotting primary antibodies were purchased from the following companies: rabbit polyclonal anti-IκBα (SC-371, Santa Cruz Biotechnology, Dallas, TX); rabbit monoclonal antiphospho-IκBα (Ser32; 2859S, Cell Signaling Technology, Beverly, MA); rabbit polyclonal anti-NF-κB p65 subunit (SC-372, Santa Cruz Biotechnology); rabbit polyclonal anti-TFIID (SC-204, Santa Cruz Biotechnology); goat polyclonal anti-β-actin (SC-1616, Santa Cruz Biotechnology), and rabbit polyclonal anti-α-tubulin (SC-12462-R, Santa Cruz Biotechnology). The blots with bound primary antibodies were then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Thermo Scientific, Waltham, MA) and visualized using SuperSignal West Dura Chemiluminescent Subtract (Thermo Scientific).

Electrophoretic Mobility Shift Assay. NF-κB activity was measured by an electrophophoretic mobility shift assay (EMSA). Equal amounts of nuclear extracts were incubated with the 26-base-pair biotin end-labeled κB binding site (κB site: 5-GGGAAATTCC-3)[22] using the LightShift Chemiluminescent EMSA Kit (Thermo Scientific) according to the manufacturer's instructions. The complexes were separated by gel electrophoresis using a 5% nondenaturing polyacrylamide gel and transferred to a nylon membrane. The transferred DNA was cross-linked to membrane by UV light and then incubated with streptavidin−horseradish peroxidase conjugate. The biotin-labeled DNA−protein complexes were detected by chemiluminescence.

■ ASSOCIATED CONTENT

Supporting Information

Additional preparation procedures; ^{1}H, ^{13}C, ESI, HRMS, IR data, and spectra for the compounds; X-ray information. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscombsci.5b00001.

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Notes

The authors declare no competing financial interest.

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