Novel Indazole-based MKK7–TIPRL Interaction Inhibitors as TRAIL Sensitizers

Sujin Gu,[†] Myoung Eun Jung,[†] Ji-Yong Yoon,[‡] Sang-Eun Yoon,[†] Jeong-Ju Lee,[‡] Kwangho Lee,[†,§] Gildon Choi,[†,§,*] Nam-Soon Kim,[‡,¶,*] and Moon-Kook Jeon[†,*]

[†]*Therapeutics and Biotechnology Division, Korea Research Institute of Chemical Technology, Daejeon 34114, Republic of Korea. *E-mail: moteta@krict.re.kr*
[‡]*Genome Research Center, Korea Research Institute of Biosciences and Biotechnology, Daejeon 305-333, Republic of Korea*
[§]*Department of Medicinal Chemistry and Pharmacology, Korea University of Science and Technology, Daejeon 34113, Republic of Korea. *E-mail: gchoi@krict.re.kr*
[¶]*Department of Functional Genomics, Korea University of Science and Technology, Daejeon 34113, Republic of Korea. *E-mail: nskim37@kribb.re.kr*
Received May 31, 2018, Accepted July 26, 2018

This work describes the process by which a metabolically unstable TRT-0002 compound exhibiting Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-sensitizing activity for Huh7 cells at a high working concentration (40 μM) is converted to more potent and metabolically improved analogues by modifying the 5-amino group and the 1-aryl moiety in the 1*H*-indazole skeleton. The efforts enabled us to identify 5-sulfonamido derivatives, TRT-0029 and TRT-0173 compounds, working at lower concentrations (10 and 20 μM, respectively) and with improved metabolic stabilities. As reported previously by us, co-treating cultured Huh7 cells with either TRT-0029 or TRT-0173 and TRAIL resulted in TRAIL-induced apoptosis due to the inhibition of the MKK7–TOR signaling pathway regulator-like (TIPRL) interaction and subsequent phosphorylation of MKK7 and JNK. In addition, the injection of TRT-0029 or TRT-0173 compound suppressed tumor growth in combination with TRAIL in an *in vivo* hepatocellular carcinoma (HCC) mouse xenograft model. TRT-0029 and TRT-0173 compounds and the relevant structure–activity relationship can provide an insight into further study on optimization of potency and metabolic stability.

Keywords: Tumor necrosis factor-related apoptosis-inducing ligand, Apoptosis, Sensitizer, TOR signaling pathway regulator-like, Indazole

Introduction

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a member of the TNF superfamily, is considered a promising anticancer agent owing to its anticancer activity and its ability to selectively induce apoptosis in cancer cells, but not in normal cells.[1] TRAIL is well known for binding to five receptors: the death receptor (DR) 4/5, decoy receptor (DcR) 1/2, and osteoprotegerin. Among them, DR 4/5 contain a death domain (DD) that initiates TRAIL-induced apoptosis. The binding of TRAIL to DR 4/5 recruits the FAS-associated DD (FADD) and DD. This leads to apoptosis owing to poly ADP ribose polymerase (PARP) and caspase.[2–4] However, many cancer cells have been demonstrated to exhibit TRAIL resistance owing to several factors such as: (1) overexpression of antagonistic receptors (DcR 1/2), (2) overpexression of anti-apoptotic proteins (Bcl-2 family), (3) defective functional FADD.[5] One strategy that has been considered for sensitizing TRAIL-induced apoptosis is the combination of TRAIL and compounds inhibiting the function of these factors. Natural/small compounds, such as doxorubicin, cisplatin, curcumin, and wogonin, have been reported as TRAIL sensitizers,[6–9] but the mode of action (MOA) of these compounds is not well known.

Recently, we reported that TOR signaling pathway regulator-like (TIPRL) protein plays an important role in TRAIL resistance by enabling the interaction of the mitogen-activated protein kinase kinase 7 (MKK7) and protein phosphatase type 2A (PP2Ac). The MKK7–TIPRL–PP2Ac complex prevents the phosphorylation of MKK7 and c-Jun N-terminal kinase (JNK), inhibiting the apoptosis cascade caused by TRAIL. In addition, TIPRL depletion induces TRAIL-induced apoptosis via the phosphorylation of MKK7 and activation of JNK.[10] To identify TRAIL sensitizers based on these mechanisms, we established an *in vitro* enzyme-linked immunosorbent assay (ELISA) screening system for detecting MKK7–TIPRL interaction. Using this screening system, we identified natural compounds such as *Tussilago farfara* L. (commonly known as

coltsfoot)[11] and *Taraxacum officinale* F. H. Wigg (commonly known as dandelion)[12] that inhibit the MKK7-TIPRL interaction, which we reported as TRAIL sensitizers.

We also identified small chemical compounds that act as TRAIL sensitizers.[13] In the study, we screened over 6000 small molecule compounds using the high-throughput ELISA system and found TRT-0002 (**1**, Figure 1) inhibiting the MKK7–TIPRL interaction as a hit compound. After intensive hit-to-lead optimization, two lead compounds, TRT-0029 (**2**, Figure 1) and TRT-0173 (**3**, Figure 1), were selected from the 280 synthesized analogues of TRT-0002. These two lead compounds were observed to initiate TRAIL-induced apoptosis in Huh7 cells by inhibiting the MKK7-TIPRL interactions and activating MKK7/JNK. The TRAIL-sensitizing activity of the two lead compounds was also confirmed using an *in vivo* xenograft animal model.[13] These results indicate that the pharmacological inhibition of the MKK7–TIPRL interaction is a promising strategy to overcome TRAIL resistance in hepatocellular carcinoma (HCC).

However, in the previous report, the optimization process by which the lead compounds TRT-0029 (**2**) and TRT-0173 (**3**) were obtained from an initial hit compound TRT-0002 (**1**) was only schematically described. Herein, we explain the process in more detail from the medicinal chemistry perspective. During the hit-to-lead stage, we concentrated on variations of substituents around the 5-amino-1-aryl-1*H* indazole core as represented by the general structure **4** in Figure 1. The TRAIL-sensitizing activity of the derivatives was evaluated based on a cell viability assay. Other *in vitro* properties, such as liver microsomal stability and solubility, will be reported for representative compounds.

Experimental

Synthesis of Compounds 5–10

***Synthetic Procedure for 1-(5-Acetyl-2,4-Dichlorophenyl)-5-Amino-1H-Indazole (1)*[13].** A mixture of 5-amino-1*H*-indazole (6.40 g, 48.1 mmol), 1-acetyl-2,4-dichloro-5-fluorobenene (10.0 g, 48.3 mmol) and potassium carbonate (66.8 g, 483 mmol) in DMF (200 mL) was heated at 110°C for 20 h. After cooling to room temperature, the reaction mixture was partitioned between dichloromethane and water, and the organic layer was washed several times with water and dried over magnesium sulfate. The solvent was evaporated *in vacuo* and the residue was separated by chromatography on a silica gel column (*n*-hexane: dichloromethane: ethyl acetate = 1: 5: 1) to give the intermediate **1** (1.00 g, 6%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.11 (s, 1H), 8.05 (s, 1H), 7.94 (s, 1H), 7.09 (d, *J* = 8.5 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 1H), 6.86 (s, 1H), 5.02 (br s, 2H), 2.63 (s, 3H); MS (ESI): *m/z* for $C_{15}H_{11}Cl_2N_3O$, found 320 [M + H$^+$].

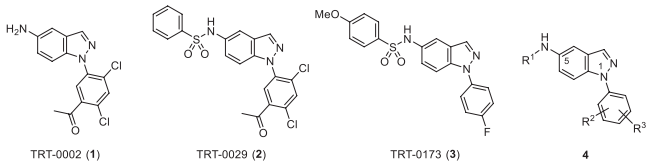


Figure 1. Representation of indazole-based compounds.

Representative Synthetic Procedure for 1-(5-Acetyl-2,-4-Dichlorophenyl)-5-Alkylamino-1H-Indazoles (5). To a solution of the precursor **1** (30 mg, 0.094 mmol) in DMF (2 mL) at room temperature was added 1-bromo-2-methylpropane (26 μL, 0.23 mmol) and potassium carbonate (39 mg, 0.28 mmol). The mixture was heated at 80°C overnight and partitioned between dichloromethane and water after cooling to room temperature. The organic layer was washed several times with water and dried over magnesium sulfate. The solvent was evaporated *in vacuo* and the residue was separated by chromatography on a silica gel column (*n*-hexane: ethyl acetate = 5: 1) to give 1-(5-acetyl-2,4-dichlorophenyl)-5-isobutylamino-1*H*-indazole (**5a**, 4 mg, 10%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06 (s, 1H), 7.74 (s, 1H), 7.68 (s, 1H), 7.06 (d, *J* = 9.5 Hz, 1H), 6.88 (m, 2H), 2.99 (d, *J* = 6.8 Hz, 2H), 2.66 (s, 3H), 1.97 (m, 1H), 1.03 (d, *J* = 6.6 Hz, 6H); MS (ESI): *m/z* for $C_{19}H_{19}Cl_2N_3O$, found 376 [M + H$^+$].

Representative Synthetic Procedure for 1-(5-Acetyl-2,-4-Dichlorophenyl)-5-Dialkylamino-1H-Indazoles (6). To a solution of the precursor **1** (50 mg, 0.16 mmol) in DMF (2 mL) at room temperature was added iodomethane (24 μL, 0.39 mmol) and potassium carbonate (65 mg, 0.45 mmol). The mixture was heated at 60°C overnight and partitioned between dichloromethane and water after cooling to room temperature. The organic layer was washed several times with water and dried over magnesium sulfate. The solvent was evaporated *in vacuo* and the residue was separated by chromatography on a silica gel column (*n*-hexane: ethyl acetate = 1: 1) to give 1-(5-acetyl-2,-4-dichlorophenyl)-5-dimethylamino-1*H*-indazole (**6a**, 11 mg, 20%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.22 (s, 1H), 8.06 (s, 1H), 7.96 (s, 1H), 7.23 (d, *J* = 8.6 Hz, 1H), 7.16 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.01 (d, *J* = 1.9 Hz, 1H), 2.91 (s, 6H), 2.62 (s, 3H); MS (ESI): *m/z* for $C_{17}H_{15}Cl_2N_3O$, found 348 [M + H$^+$].

Representative Synthetic Procedure for 1-(5-Acetyl-2,-4-Dichlorophenyl)-5-Amido-1H-Indazoles (7). To a solution of the precursor **1** (30 mg, 0.094 mmol) in DCM (2 mL) at room temperature was added 3-(1*H*-indol-3-yl) propanoic acid (21 mg, 0.11 mmol) and EDC (22 mg, 0.14 mmol). The mixture was stirred at the same temperature overnight and the solvent was evaporated *in vacuo.* The residue was separated by chromatography on a silica gel column (*n*-hexane: ethyl acetate = 3: 2) to give 1-(5-acetyl-2,4-dichlorophenyl)-5-(3-(1*H*-indol-3-yl)propanoyl)amino-1*H*-indazole (**7a**, 36 mg, 78%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.77 (br s, 1H), 10.04 (br s, 1H), 8.39 (s, 1H), 8.28 (s, 1H), 8.08 (s, 1H), 8.01 (s, 1H),

7.59 (d, J = 7.7 Hz, 1H), 7.52 (d, J = 8.7 Hz, 1H), 7.34–7.30 (m, 2H), 7.14 (s, 1H), 7.07 (t, J = 7.5 Hz, 1H), 6.98 (t, J = 7.5 Hz, 1H), 3.05 (t, J = 7.6 Hz, 2H), 2.71 (t, J = 7.6 Hz, 2H), 2.62 (s, 3H); MS (ESI): *m/z* for $C_{26}H_{20}Cl_2N_4O_2$, found 491 [M + H^+].

Representative Synthetic Procedure for 1-(5-Acetyl-2,-4-Dichlorophenyl)-5-Oxycarbonylamino-1H-Indazoles (8). To a solution of the precursor **1** (50 mg, 0.16 mmol) in DCM (2 mL) at room temperature was added methyl chloroformate (14 μL, 0.18 mmol) and pyridine (19 μL, 0.24 mmol). The mixture was stirred at the same temperature overnight and the solvent was evaporated *in vacuo*. The residue was separated by chromatography on a silica gel column (*n*-hexane: ethyl acetate = 3: 1) to give 1-(5-acetyl-2,4-dichlorophenyl)-5-methoxycarbonylamino-1*H*-indazole (**8a**, 52 mg, 86%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.73 (br s, 1H), 8.38 (s, 1H), 8.08 (s, 1H), 8.03–8.00 (m, 2H), 7.47 (dd, J = 9.0, 1.7 Hz, 1H), 7.32 (d, J = 9.0 Hz, 1H), 3.69 (s, 3H), 2.62 (s, 3H); MS (ESI): *m/z* for $C_{17}H_{13}Cl_2N_3O_3$, found 378 [M + H^+].

Representative Synthetic Procedure for 1-(5-Acetyl-2,-4-Dichlorophenyl)-5-Ureido-1H-Indazoles (9). To a solution of the precursor **1** (50 mg, 0.16 mmol) in DCM (2 mL) at room temperature was added ethyl isocyanate (15 μL, 0.19 mmol) and pyridine (19 μL, 0.24 mmol). The mixture was stirred at the same temperature overnight and the solvent was evaporated *in vacuo*. The residue was separated by chromatography on a silica gel column (*n*-hexane: ethyl acetate = 1: 1) to give 1-(5-acetyl-2,4-dichlorophenyl)-5-ethylaminocarbonylamino-1*H*-indazole (**9a**, 35 mg, 56%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.51 (br s, 1H), 8.31 (s, 1H), 8.07 (s, 1H), 7.99 (s, 2H), 7.36 (dd, J = 9.0, 1.8 Hz, 1H), 7.25 (d, J = 9.0 Hz, 1H), 6.10 (br t, J = 5.4 Hz, 1H), 3.12 (m, 2H), 2.62 (s, 3H), 1.07 (t, J = 7.1 Hz, 3H); MS (ESI): *m/z* for $C_{18}H_{16}Cl_2N_4O_2$, found 391 [M + H^+].

Representative Synthetic Procedure for 1-(5-Acetyl-2,-4-Dichlorophenyl)-5-Sulfonamido-1H-Indazoles (10)[13]. Benzenesulfonyl chloride (14 μL, 0.11 mmol) was added to a solution of compound **1** (30 mg, 0.094 mmol) and pyridine (11 μL, 0.14 mmol) in dichloromethane (2 mL) at room temperature and the resulting mixture was stirred at the same temperature for 15 h. The reaction mixture was partitioned between dichloromethane and water, and the organic layer was dried over magnesium sulfate. The solvent was evaporated *in vacuo*, and the residue was separated by chromatography on a silica gel column (*n*-hexane: ethyl acetate = 4: 1) to give 1-(5-acetyl-2,4-dichlorophenyl)-5-benzenesulfonamido-1*H*-indazole (TRT-0029, **2**, 25 mg, 58%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.18 (s, 1H), 7.76 (t, J = 7.5 Hz, 3H), 7.72 (s, 1H), 7.57–7.54 (m, 2H), 7.46 (t, J = 8.00 Hz, 2H), 7.13 (d, J = 1.5 Hz, 2H), 6.69 (s, 1H), 2.70 (s, 3H); MS (ESI): *m/z* for $C_{21}H_{15}Cl_2N_3O_3S$, found 460 [M + H^+].

1-(5-Acetyl-2,4-dichlorophenyl)-5-(4-methoxybenzenesulfonamido)-1*H*-indazole (**10a**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.19 (s, 1H), 8.05 (s, 1H), 7.75 (s, 1H), 7.69 (d, J = 8.9 Hz, 2H), 7.53 (s, 1H), 7.15 (m, 2H), 6.91 (d, J = 8.9 Hz, 2H), 6.60 (s, 1H), 3.95 (s, 3H), 3.86 (s, 3H); MS (ESI): *m/z* for $C_{22}H_{17}Cl_2N_3O_4S$, found 490 [M + H^+].

1-(5-Acetyl-2,4-dichlorophenyl)-5-phenylmethanesulfonamido-1*H*-indazole (**10b**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.87 (br s, 1H), 8.42 (s, 1H), 8.10 (s, 1H), 8.02 (s, 1H), 7.72 (s, 1H), 7.39–7.29 (m, 7H), 4.45 (s, 2H), 2.63 (s, 3H); MS (ESI): *m/z* for $C_{22}H_{17}Cl_2N_3O_3S$, found 474 [M + H^+].

Synthesis of Compounds 15

Synthetic Procedure for 5-Amino-1-(2,4-Dichloro-5-Methoxycarbonylphenyl)-1H-Indazole (11a). A mixture of 5-amino-1*H*-indazole (3.46 g, 26.0 mmol), methyl 2,4-dichloro-5-fluorobenzoate (4.00 mL, 26.0 mmol), and potassium carbonate (35.9 g, 260 mmol) in DMF (40 mL) was heated at 100°C for 3 days. After cooling to room temperature, the reaction mixture was partitioned between dichloromethane and water, and the organic layer was washed several times with water and dried over magnesium sulfate. The solvent was evaporated *in vacuo* and the residue was separated by chromatography on a silica gel column (*n*-hexane:dichloromethane:ethyl acetate = 1:1:1) to give the intermediate **11a** (430 mg, 5%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05 (s, 1H), 8.04 (s, 1H), 7.72 (s, 1H), 7.07 (d, J = 8.8 Hz, 1H), 7.00 (d, J = 1.4 Hz, 1H), 6.89 (dd, J = 8.8, 1.4 Hz, 1H), 3.92 (s, 3H), 3.70 (br s, 2H); MS (ESI): *m/z* for $C_{15}H_{11}Cl_2N_3O_2$, found 336 [M + H^+].

Synthetic Procedure for 5-Amino-1-(3-Methoxycarbonylphenyl)-1H-Indazole (11b). A mixture of 5-amino-1*H*-indazole (1.00 g, 7.51 mmol), methyl 3-iodobenzoate (1.97 g, 7.51 mmol), cuprous iodide (140 mg, 0.75 mmol), *trans*-cyclohexane-1,2-diamine (450 μL, 3.76 mmol), and tripotassium phosphate (2.90 g, 1.35 mmol) in 1,4-dioxane (40 mL) was heated at 100°C overnight. After cooling to room temperature, the reaction mixture was partitioned between dichloromethane and water, and the organic layer was dried over magnesium sulfate. The solvent was evaporated *in vacuo* and the residue was separated by chromatography on a silica gel column (*n*-hexane: ethyl acetate = 1: 1) to give the intermediate **11b** (1.25 g, 63%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.28 (s, 1H), 8.12 (s, 1H), 8.06 (dd, J = 7.9, 1.4 Hz, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.71 (t, J = 7.9 Hz, 1H), 7.65 (d, J = 8.9 Hz, 1H), 6.95 (dd, J = 8.9, 2.1 Hz, 1H), 6.89 (s, 1H), 5.08 (s, 2H), 3.92 (s, 3H); MS (ESI): *m/z* for $C_{15}H_{13}N_3O_2$, found 268 [M + H^+].

Representative Synthetic Procedure for 5-((tert-Butoxycarbonyl)amino)-1-(2,4-Dichloro-5-Methoxycarbonylphenyl)-1H-Indazole (12a) and 5-((tert-Butoxycarbonyl) amino)-1-(3-Methoxycarbonylphenyl)-1H-Indazole (12b). To a solution of compound **11a** (100 mg, 0.300 mmol) in DMF (1 mL) at 0 °C was added DIPEA (260 μL, 1.49 mmol) and Boc_2O (190 mg, 0.892 mmol). The mixture was stirred at 0 °C for 2 h and the reaction temperature was elevated to room temperature. In addition, the mixture was stirred at room temperature overnight. The reaction mixture was partitioned between dichloromethane and

water, and the organic layer was washed several times with water and dried over magnesium sulfate. The solvent was evaporated *in vacuo* and the residue was separated by chromatography on a silica gel column (*n*-hexane: ethyl acetate = 4: 1) to give the intermediate **12a** (93 mg, 72%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17 (s, 1H), 8.05 (s, 1H), 7.94 (s, 1H), 7.73 (s, 1H), 7.31 (d, *J* = 8.9 Hz, 1H), 7.13 (d, *J* = 8.9 Hz, 1H), 6.55 (br s, 1H), 3.92 (s, 3H), 1.54 (s, 9H); MS (ESI): *m/z* for $C_{20}H_{19}Cl_2N_3O_4$, found 436 [M + H$^+$].

5-((*tert*-Butoxycarbonyl)amino)-1-(3-methoxycarbonylphenyl)-1*H*-indazole (**12b**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.40 (s, 1H), 8.14 (s, 1H), 8.01 (d, *J* = 7.8 Hz, 1H), 7.96–7.92 (m, 2H), 7.69 (d, *J* = 9.0 Hz, 1H), 7.61 (t, *J* = 7.9 Hz, 1H), 7.32 (dd, *J* = 9.0, 2.0 Hz, 1H), 6.59 (br s, 1H), 3.96 (s, 3H), 1.55 (s, 9H); MS (ESI): *m/z* for $C_{20}H_{21}N_3O_4$, found 368 [M + H$^+$].

Representative Synthetic Procedure for 5-((*tert*-Butoxycarbonyl)amino)-1-(3-Carboxy-4,6-Dichlorophenyl)-1H-Indazole (13a) and 5-((*tert*-Butoxycarbonyl)amino)-1-(3-Carboxyphenyl)-1H-Indazole (13b). A mixture of compound **12a** (160 mg, 0.380 mmol) and lithium hydroxide monohydrate (24 mg, 0.57 mmol) in THF/MeOH (2 mL/1 mL) was stirred at 50°C for 2 h. After completion of the reaction, the reaction mixture was cooled to room temperature and acidified to pH 4 by addition of 1 N aqueous HCl solution. The precipitated solid was filtered, washed with water, and dried to give the intermediate **13a** (140 mg, 90%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.43 (s, 1H), 8.34 (s, 1H), 8.06 (s, 1H), 8.02 (s, 1H), 7.94 (s, 1H), 7.47 (d, *J* = 8.7 Hz, 1H), 7.26 (d, *J* = 8.7 Hz, 1H), 1.50 (s, 9H); MS (ESI): *m/z* for $C_{19}H_{17}Cl_2N_3O_4$, found 422 [M + H$^+$].

5-((*tert*-Butoxycarbonyl)amino)-1-(3-carboxyphenyl)-1*H*-indazole (**13b**). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.48 (br s, 1H), 8.34 (s, 1H), 8.26 (s, 1H), 8.06–8.03 (m, 2H), 7.93 (d, *J* = 7.8 Hz, 1H), 7.79 (d, *J* = 9.1 Hz, 1H), 7.72 (t, *J* = 7.9 Hz, 1H), 7.53 (dd, *J* = 9.1, 1.5 Hz, 1H), 1.50 (s, 9H); MS (ESI): *m/z* for $C_{19}H_{19}N_3O_4$, found 354 [M + H$^+$].

Representative Synthetic Procedure for 5-((*tert*-Butoxycarbonyl)amino)-1-(3-Carbamoylphenyl)-1H-Indazoles (14). To a solution of compound **13a** (13 mg, 0.030 mmol) and aniline (3 μL, 0.03 mmol) in DCM (1 mL) at room temperature was added EDC (7 mg, 0.05 mmol). The mixture was stirred at the same temperature overnight and the solvent was evaporated *in vacuo*. The residue was separated by chromatography on a silica gel column (*n*-hexane: ethyl acetate = 3: 1) to give 5-((*tert*-butoxycarbonyl)amino)-1-(2,-4-dichloro-5-(phenylcarbamoyl)phenyl)-1*H*-indazole (**14a**, 4.4 mg, 29%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (s, 1H), 7.99–7.85 (m, 3H), 7.72 (s, 1H), 7.62 (d, *J* = 7.7 Hz, 2H), 7.38 (m, 2H), 7.28 (s, 1H), 7.18 (m, 2H), 6.58 (s, 1H), 1.54 (s, 9H); MS (ESI): *m/z* for $C_{25}H_{22}Cl_2N_4O_3$, found 497 [M + H$^+$].

5-((*tert*-Butoxycarbonyl)amino)-1-(3-(benzylcarbamoyl)-4,6-dichlorophenyl)-1*H*-indazole (**14b**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (s, 1H), 7.94 (s, 1H), 7.85 (s, 1H), 7.67 (s, 1H), 7.35 (m, 5H), 7.31 (m, 1H), 7.15 (d, *J* = 9.1 Hz, 1H), 6.55 (s, 1H), 6.47 (s, 1H), 4.65 (d, *J* = 5.6 Hz, 2H), 1.54 (s, 9H); MS (ESI): *m/z* for $C_{26}H_{24}Cl_2N_4O_3$, found 511 [M + H$^+$].

5-((*tert*-Butoxycarbonyl)amino)-1-(2,4-dichloro-5-(phenethylcarbamoyl)phenyl)-1*H*-indazole (**14c**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14 (s, 1H), 7.94 (br s, 1H), 7.75 (s, 1H), 7.63 (s, 1H), 7.33–7.21 (m, 6H), 7.14 (d, *J* = 8.9 Hz, 1H), 6.56 (br s, 1H), 6.22 (br t, *J* = 6.8 Hz, 1H), 3.74 (q, *J* = 6.8 Hz, 2H), 2.95 (t, *J* = 6.8 Hz, 2H), 1.54 (s, 9H); MS (ESI): *m/z* for $C_{27}H_{26}Cl_2N_4O_3$, found 525 [M + H$^+$].

Representative Synthetic Procedure for 5-Amino-1-(3-Carbamoylphenyl)-1H-Indazoles (15). A solution of compound **14a** (4.4 mg, 0.0088 mmol) in DCM/TFA (0.5 mL/0.5 mL) at room temperature was stirred for 2 h and the solvent was evaporated *in vacuo* to give 5-amino-1-(2,4-dichloro-5-(phenylcarbamoyl)phenyl)-1*H*-indazole trifluoroacetic acid salt (**15a**, 3.5 mg, 78%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.65 (s, 1H), 8.43 (s, 1H), 8.14 (s, 1H), 7.91 (s, 1H), 7.69 (d, *J* = 7.7 Hz, 2H), 7.64 (s, 1H), 7.42–7.25 (m, 4H), 7.12 (m, 1H); MS (ESI): *m/z* for $C_{20}H_{14}Cl_2N_4O$, found 397 [M + H$^+$].

5-Amino-1-(3-(benzylcarbamoyl)-4,6-dichlorophenyl)-1*H*-indazole trifluoroacetic acid salt (**15b**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.15 (br t, *J* = 5.7 Hz, 1H), 8.40 (s, 1H), 8.06 (s, 1H), 7.73 (s, 1H), 7.58 (s, 1H), 7.34 (m, 5H), 7.25 (m, 2H), 4.45 (d, *J* = 5.7 Hz, 2H); MS (ESI): *m/z* for $C_{21}H_{16}Cl_2N_4O$, found 411 [M + H$^+$].

5-Amino-1-(2,4-dichloro-5-(phenethylcarbamoyl)phenyl)-1*H*-indazole trifluoroacetic acid salt (**15c**). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.71 (t, *J* = 6.8 Hz, 1H), 8.41 (s, 1H), 8.04 (s, 1H), 7.58–7.55 (m, 1H), 7.56 (s, 1H), 7.35 (m, 1H), 7.25 (m, 5H), 7.17 (m, 1H), 3.44 (q, *J* = 6.8 Hz, 2H), 2.82 (t, *J* = 6.8 Hz, 2H); MS (ESI): *m/z* for $C_{22}H_{18}Cl_2N_4O$, found 425 [M + H$^+$].

Synthesis of Compounds 19

Representative Synthetic Procedure for 1-Aryl-2-(2-Fluoro-5-Nitrobenzylidene)hydrazines (16)[13]. To a solution of 2-fluoro-5-nitrobenzaldehyde (50 mg, 0.27 mmol) in ethanol was added 4-fluorophenylhydrazine (37 mg, 0.29 mmol) and toluenesulfonic acid monohydrate (3.0 mg, 0.02 mmol), and the mixture was stirred at reflux for 1 h. After cooling to room temperature, the precipitated solid was collected by filtration to give 1-(2-fluoro-5-nitrobenzylidene)-2-(4-fluorophenyl)hydrazine (**16a**, 65 mg, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.84 (dd, *J* = 6.2, 2.9 Hz, 1H), 8.12 (m, 1H), 7.86 (s, 1H), 7.23 (t, *J* = 9.3 Hz, 1H), 7.08 (m, 4H); MS (ESI): *m/z* for $C_{13}H_9F_2N_3O_2$, found 278 [M + H$^+$].

1-(4-Chlorophenyl)-2-(2-fluoro-5-nitrobenzylidene)hydrazine (**16b**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.84 (dd, *J* = 6.2, 2.8 Hz, 1H), 8.17 (m, 1H), 7.90 (s, 1H), 7.31 (d, *J* = 8.7 Hz, 2H), 7.24 (t, *J* = 9.2 Hz, 1H), 7.12 (d, *J* =

8.7 Hz, 2H); MS (ESI): *m/z* for $C_{13}H_9ClFN_3O_2$, found 294 [M + H^+].

1-(2-Chlorophenyl)-2-(2-fluoro-5-nitrobenzylidene)hydrazine (**16c**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.91 (dd, *J* = 6.2, 2.9 Hz, 1H), 8.46 (br s, 1H), 8.19 (m, 1H), 8.05 (s, 1H), 7.70 (dd, *J* = 8.6, 1.4 Hz, 1H), 7.33 (m, 2H), 7.23 (t, *J* = 9.2 Hz, 1H), 6.91 (dt, *J* = 7.8, 1.4 Hz, 1H); MS (ESI): *m/z* for $C_{13}H_9ClFN_3O_2$, found 294 [M + H^+].

1-(2,4-Difluorophenyl)-2-(2-fluoro-5-nitrobenzylidene)hydrazine (**16d**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.85 (dd, *J* = 6.2, 2.9 Hz, 1H), 8.16 (m, 1H), 7.97 (s, 1H), 7.59 (m, 1H), 7.22 (m, 1H), 6.96–6.82 (m, 2H); MS (ESI): *m/z* for $C_{13}H_8F_3N_3O_2$, found 296 [M + H^+].

1-(2-Fluoro-5-nitrobenzylidene)-2-(2-methylphenyl)hydrazine (**16e**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.90 (dd, *J* = 6.2, 2.9 Hz, 1H), 8.13 (m, 1H), 7.97 (s, 1H), 7.60 (d, *J* = 7.9 Hz, 1H), 7.26 (m, 1H), 7.19 (d, *J* = 9.2 Hz, 1H), 7.12 (d, *J* = 7.5 Hz, 1H), 6.90 (t, *J* = 7.5 Hz, 1H), 2.28 (s, 3H); MS (ESI): *m/z* for $C_{14}H_{12}FN_3O_2$, found 274 [M + H^+].

***Representative Synthetic Procedure for 1-Aryl-5-Nitro-1H-Indazoles (17a–e)*[13].** The mixture of compound **16a** (61 mg, 0.22 mmol) and potassium carbonate (134 mg, 0.97 mmol) in *N,N*-dimethylformamide (2 mL) was heated at 100°C for 15 h. After cooling to room temperature, the reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with water several times and dried over magnesium sulfate. The solvent was evaporated *in vacuo* and the residue was chromatographed on a silica gel column (*n*-hexane: ethyl acetate = 1: 1) to give 1-(4-fluorophenyl)-5-nitro-1*H*-indazole (**17a**, 17 mg, 30%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.80 (d, *J* = 2.0 Hz, 1H), 8.40 (s, 1H), 8.32 (dd, *J* = 9.2, 2.1 Hz, 1H), 7.68 (m, 3H), 7.30 (t, *J* = 6.1 Hz, 2H); MS (ESI): *m/z* for $C_{13}H_8FN_3O_2$, found 258 [M + H^+].

1-(4-Chlorophenyl)-5-nitro-1*H*-indazole (**17b**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.80 (d, *J* = 2.0 Hz, 1H), 8.41 (s, 1H), 8.33 (dd, *J* = 9.2, 2.0 Hz, 1H), 7.74 (d, *J* = 9.2 Hz, 1H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.57 (d, *J* = 8.8 Hz, 2H); MS (ESI): *m/z* for $C_{13}H_8ClN_3O_2$, found 274 [M + H^+].

1-(2-Chlorophenyl)-5-nitro-1*H*-indazole (**17c**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.81 (d, *J* = 1.5 Hz, 1H), 8.44 (s, 1H), 8.29 (dd, *J* = 9.2, 1.9 Hz, 1H), 7.65 (dd, *J* = 7.7, 2.2 Hz, 1H), 7.57–7.42 (m, 3H), 7.30 (d, *J* = 9.3 Hz, 1H); MS (ESI): *m/z* for $C_{13}H_8ClN_3O_2$, found 274 [M + H^+].

1-(2,4-Difluorophenyl)-5-nitro-1*H*-indazole (**17d**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.80 (d, *J* = 2.0 Hz, 1H), 8.44 (s, 1H), 8.33 (dd, *J* = 9.2, 2.1 Hz, 1H), 7.68–7.59 (m, 1H), 7.40 (dd, *J* = 9.2, 3.1 Hz, 1H), 7.16–7.09 (m, 2H); MS (ESI): *m/z* for $C_{13}H_7F_2N_3O_2$, found 276 [M + H^+].

1-(2-Methylphenyl)-5-nitro-1*H*-indazole (**17e**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.81 (s, 1H), 8.41 (s, 1H), 8.26 (d, *J* = 9.2 Hz, 1H), 7.44 (m, 2H), 7.38 (m, 2H), 7.27 (m, 1H), 2.11 (s, 3H); MS (ESI): *m/z* for $C_{14}H_{11}N_3O_2$, found 254 [M + H^+].

Synthetic Procedure for 1-(4-Fluoro-2-Methylphenyl)-5-Nitro-1H-Indazole (17f). The mixture of 5-nitro-1*H*-indazole (50 mg, 0.31 mmol), 4-fluoro-2-methylphenylboronic acid (94 mg, 0.61 mmol), and cupric acetate (50 mg, 0.28 mmol) in pyridine/dichloromethane (0.1 mL/3 mL) was stirred at room temperature overnight. The solvent was evaporated *in vacuo* and the residue was chromatographed on a silica gel column (*n*-hexane: ethyl acetate = 4: 1) to give 1-(4-fluoro-2-methylphenyl)-5-nitro-1*H*-indazole (**17f**, 40 mg, 48%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.84 (s, 1H), 8.44 (s, 1H), 8.30 (dd, *J* = 9.2, 2.1 Hz, 1H), 7.38 (m, 1H), 7.26 (m, 1H), 7.17 (m, 1H), 7.11 (m, 1H), 2.11 (s, 3H); MS (ESI): *m/z* for $C_{14}H_{10}FN_3O_2$, found 272 [M + H^+].

***Representative Synthetic Procedure for 5-Amino-1-Aryl-1H-Indazoles (18a–f)*[13].** To a solution of compound **17a** (17 mg, 0.066 mmol) in dichloromethane (5 mL) was added 10% palladium on carbon (10 mg) at room temperature and the mixture was shaken under hydrogen atmosphere (60~70 psi) at the same temperature for 5 h. The reaction mixture was filtered through Celite and the filtrate was evaporated *in vacuo* to afford 5-amino-1-(4-fluorophenyl)-1*H*-indazole (**18a**, 14 mg, 93%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03 (s, 1H), 7.74 (dd, *J* = 8.1, 5.2 Hz, 2H), 7.55 (d, *J* = 8.9 Hz, 1H), 7.38 (t, *J* = 8.7 Hz, 2H), 6.88 (t, *J* = 8.2 Hz, 2H), 5.00 (br s, 2H); MS (ESI): *m/z* for $C_{13}H_{10}FN_3$, found 228 [M + H^+].

5-Amino-1-(4-chlorophenyl)-1*H*-indazole (**18b**). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.08 (s, 1H), 7.74 (dd, *J* = 8.8 Hz, 2H), 7.63 (d, *J* = 8.9 Hz, 1H), 7.60 (d, *J* = 8.8 Hz, 2H), 6.92 (dd, *J* = 8.9, 2.1 Hz, 1H), 6.87 (d, *J* = 2.1 Hz, 1H), 5.06 (br s, 2H); MS (ESI): *m/z* for $C_{13}H_{10}ClN_3$, found 244 [M + H^+].

5-Amino-1-(2-chlorophenyl)-1*H*-indazole (**18c**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.04 (s, 1H), 7.72 (m, 1H), 7.54 (m, 3H), 6.97 (d, *J* = 9.7 Hz, 1H), 6.83 (m, 2H), 4.95 (br s, 2H); MS (ESI): *m/z* for $C_{13}H_{10}ClN_3$, found 244 [M + H^+].

5-Amino-1-(2,4-difluorophenyl)-1*H*-indazole (**18d**). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.08 (s, 1H), 7.68 (m, 1H), 7.59 (m, 1H), 7.32 (m, 1H), 7.12 (m, 1H), 6.89–6.85 (m, 2H), 5.00 (br s, 2H); MS (ESI): *m/z* for $C_{13}H_9F_2N_3$, found 246 [M + H^+].

5-Amino-1-(2-methylphenyl)-1*H*-indazole (**18e**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.99 (s, 1H), 7.45–7.34 (m, 4H), 6.96 (d, *J* = 8.5 Hz, 1H), 6.84 (s, 1H), 6.82 (m, 1H), 4.93 (br s, 1H), 2.06 (s, 3H); MS (ESI): *m/z* for $C_{14}H_{13}N_3$, found 224 [M + H^+].

5-Amino-1-(4-fluoro-2-methylphenyl)-1*H*-indazole (**18f**). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.00 (s, 1H), 7.40 (dd, *J* = 8.7, 5,6 Hz, 1H), 7.33 (dd, *J* = 9.7, 2.8 Hz, 1H), 7.21 (m, 1H), 6.95 (d, *J* = 8.6 Hz, 1H), 6.85–6.82 (m, 2H), 4.94 (br s, 2H), 2.04 (s, 3H); MS (ESI): *m/z* for $C_{14}H_{12}FN_3$, found 242 [M + H^+].

Representative Synthetic Procedure for 5-Amino-1-Aryl-1H-Indazoles (18 g–i). A mixture of 5-amino-1*H*-indazole (50 mg, 0.38 mmol), 3-chloro-1-fluoro-4-iodobenzene

(100 μL, 0.376 mmol), cuprous iodide (7 mg, 0.04 mmol), *trans*-cyclohexane-1,2-diamine (25 μL, 0.19 mmol), and tripotassium phosphate (140 mg, 0.676 mmol) in 1,4-dioxane (2 mL) was heated at 100°C for 3 days. After cooling to room temperature, the reaction mixture was partitioned between dichloromethane and water, and the organic layer was dried over magnesium sulfate. The solvent was evaporated *in vacuo* and the residue was separated by chromatography on a silica gel column (*n*-hexane: ethyl acetate = 1: 1) to give 5-amino-1-(2-chloro-4-fluorophenyl)-1*H*-indazole (**18 g**, 10 mg, 10%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.05 (s, 1H), 7.76 (dd, *J* = 8.7, 2.8 Hz, 1H), 7.63 (dd, *J* = 8.8, 5.7 Hz, 1H), 7.44 (m, 1H), 6.97 (d, *J* = 9.6 Hz, 1H), 6.86–6.83 (m, 2H), 4.97 (br s, 2H); MS (ESI): *m/z* for $C_{13}H_9ClFN_3$, found 262 [M + H$^+$].

5-Amino-1-(2,4-dichlorophenyl)-1*H*-indazole (**18h**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.06 (s, 1H), 7.92 (d, *J* = 2.0 Hz, 1H), 7.61 (m, 2H), 7.01 (d, *J* = 9.3 Hz, 1H), 6.86 (m, 2H), 4.97 (br s, 2H); MS (ESI): *m/z* for $C_{13}H_9Cl_2N_3$, found 278 [M + H$^+$].

5-Amino-1-phenyl-1*H*-indazole (**18i**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.04 (s, 1H), 7.72 (d, *J* = 7.8 Hz, 2H), 7.63–7.54 (m, 3H), 7.33 (m, 1H), 6.90 (d, *J* = 9.1 Hz, 1H), 6.86 (s, 1H), 5.00 (br s, 2H); MS (ESI): *m/z* for $C_{13}H_{11}N_3$, found 210 [M + H$^+$].

***Representative Synthetic Procedure for 1-Aryl-5-Sulfonamido-1H-Indazoles (19)*[13].** To a solution of compound **18a** (7 mg, 0.03 mmol) and pyridine (4 μL, 0.05 mmol) in dichloromethane (2 mL) at room temperature was added 4-methoxybenzenesulfonyl chloride (8 mg, 0.04 mmol), and the mixture was stirred at the same temperature for 15 h. The reaction mixture was partitioned between dichloromethane and water. The organic layer was dried over magnesium sulfate. The solvent was evaporated *in vacuo* and the residue was chromatographed on a silica gel column (*n*-hexane: ethyl acetate = 4: 1) to give 1-(4-fluorophenyl)-5-(4-methoxybenzenesulfonamido)-1*H*-indazole (TRT-0173, **3**, 8 mg, 70%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.69–7.64 (m, 4H), 7.61 (d, *J* = 9.0 Hz, 1H), 7.50 (d, *J* = 1.8 Hz, 1H), 7.25 (t, *J* = 8.6 Hz, 2H), 7.17 (dd, *J* = 9.0, 2.0 Hz, 1H), 6.91 (dd, *J* = 7.2, 1.7 Hz, 2H), 6.55 (s, 1H), 3.85 (s, 1H); MS (ESI): *m/z* for $C_{20}H_{16}FN_3O_3S$, found 398 [M + H$^+$].

1-(2,4-Dichlorophenyl)-5-(4-methoxybenzenesulfonamido)-1*H*-indazole (**19a**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (s, 1H), 7.67 (d, *J* = 8.9 Hz, 2H), 7.61 (s, 1H), 7.49 (s, 1H), 7.42 (s, 2H), 7.11 (s, 2H), 6.88 (d, *J* = 8.9 Hz, 2H), 6.62 (s, 1H), 3.83 (s, 3H); MS (ESI): *m/z* for $C_{20}H_{15}Cl_2N_3O_3S$, found 448 [M + H$^+$].

1-(2,4-Dichlorophenyl)-5-(2-fluorobenzenesulfonamido)-1*H*-indazole (**19b**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.16 (s, 1H), 7.79 (m, 1H), 7.63–7.55 (m, 3H), 7.42 (s, 2H), 7.25–7.19 (m, 3H), 7.12 (d, *J* = 8.9 Hz, 1H), 6.90 (br s, 1H); MS (ESI): *m/z* for $C_{19}H_{12}Cl_2FN_3O_2S$, found 436 [M + H$^+$].

5-(2-Chlorobenzenesulfonamido)-1-(2,4-dichlorophenyl)-1*H*-indazole (**19c**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.94 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.60–7.53 (m, 3H), 7.47 (m, 1H), 7.38 (s, 2H), 7.31 (m, 1H), 7.20 (m, 1H), 7.15 (s, 1H), 7.08 (d, *J* = 8.9 Hz, 1H); MS (ESI): *m/z* for $C_{19}H_{12}Cl_3N_3O_2S$, found 451 [M + H$^+$].

1-(2,4-Dichlorophenyl)-5-(3-methylbenzenesulfonamido)-1*H*-indazole (**19d**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.18 (s, 1H), 7.63 (s, 1H), 7.58 (s, 1H), 7.53–7.48 (m, 2H), 7.46–7.43 (m, 2H), 7.37–7.33 (m, 2H), 7.13 (s, 2H), 6.61 (br s, 1H), 2.36 (s, 3H); MS (ESI): *m/z* for $C_{20}H_{15}Cl_2N_3O_2S$, found 432 [M + H$^+$].

1-(2,4-Dichlorophenyl)-5-(2-methylbenzenesulfonamido)-1*H*-indazole (**19e**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.14 (s, 1H), 7.93 (d, *J* = 7.9 Hz, 1H), 7.62 (s, 1H), 7.47 (m, 2H), 7.42 (s, 2H), 7.33 (m, 1H), 7.28 (m, 1H), 7.11 (s, 2H), 6.80 (s, 1H), 2.70 (s, 3H); MS (ESI): *m/z* for $C_{20}H_{15}Cl_2N_3O_2S$, found 432 [M + H$^+$].

1-(2,4-Dichlorophenyl)-5-(2-trifluoromethylbenzenesulfonamido)-1*H*-indazole (**19f**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.16 (s, 1H), 7.96–7.92 (m, 2H), 7.68 (m, 1H), 7.63 (s, 1 h), 7.55 (m, 1H), 7.53 (s, 1H), 7.43 (s, 2H), 7.13–7.11 (m, 2H), 6.75 (br s, 1H); MS (ESI): *m/z* for $C_{20}H_{12}Cl_2F_3N_3O_2S$, found 486 [M + H$^+$].

1-(2,4-Dichlorophenyl)-5-(2-thiophenesulfonamido)-1*H*-indazole (**19g**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.20 (s, 1H), 7.64 (s, 1H), 7.60–7.56 (m, 2H), 7.48–7.44 (m, 3H), 7.20–7.17 (m, 2H), 7.04–7.02 (m, 1H), 6.77 (br s, 1H); MS (ESI): *m/z* for $C_{17}H_{11}Cl_2N_3O_2S_2$, found 424 [M + H$^+$].

5-Benzenesulfonamido-1-(2,4-difluorophenyl)-1*H*-indazole (**19h**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (s, 1H), 7.73 (m, 2H), 7.58–7.41 (m, 5H), 7.20–7.15 (m, 1H), 7.15–7.03 (m, 3H), 6.61 (br s, 1H); MS (ESI): *m/z* for $C_{19}H_{13}F_2N_3O_2S$, found 386 [M + H$^+$].

1-(2,4-Difluorophenyl)-5-(4-methoxybenzenesulfonamido)-1*H*-indazole (**19i**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (s, 1H), 7.66 (d, *J* = 8.9 Hz, 2H), 7.55 (m, 1H), 7.49 (s, 1H), 7.26–7.19 (m, 1H), 7.15–7.03 (m, 3H), 6.88 (d, *J* = 8.9 Hz, 2H), 6.49 (br s, 1H), 3.82 (s, 3H); MS (ESI): *m/z* for $C_{20}H_{15}F_2N_3O_3S$, found 416 [M + H$^+$].

1-(2,4-Difluorophenyl)-5-(4-fluorobenzenesulfonamido)-1*H*-indazole (**19j**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.16 (s, 1H), 7.74 (m, 2H), 7.61–7.52 (m, 1H), 7.51 (s, 1H), 7.23–7.20 (m, 1H), 7.14–7.03 (m, 5H), 6.60 (br s, 1H); MS (ESI): *m/z* for $C_{19}H_{12}F_3N_3O_2S$, found 404 [M + H$^+$].

1-(2,4-Difluorophenyl)-5-(3-fluorobenzenesulfonamido)-1*H*-indazole (**19k**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17 (s, 1H), 7.62–7.38 (m, 5H), 7.30–7.20 (m, 2H), 7.13–7.03 (m, 3H), 6.61 (br s, 1H); MS (ESI): *m/z* for $C_{19}H_{12}F_3N_3O_2S$, found 404 [M + H$^+$].

1-(2,4-Difluorophenyl)-5-(2-fluorobenzenesulfonamido)-1*H*-indazole (**19l**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.77 (t, *J* = 7.7 Hz, 1H), 7.56–7.48 (m, 3H), 7.25–7.15 (m, 4H), 7.08–7.00 (m, 2H), 6.89 (br s, 1H); MS (ESI): *m/z* for $C_{19}H_{12}F_3N_3O_2S$, found 404 [M + H$^+$].

5-(4-Chlorobenzenesulfonamido)-1-(2,4-difluorophenyl)-1*H*-indazole (**19m**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17 (s, 1H), 7.66 (d, *J* = 8.6 Hz, 2H), 7.66–7.51 (m, 1H), 7.51 (s, 1H), 7.41 (d, *J* = 8.6 Hz, 2H), 7.26–7.20 (m, 1H), 7.18–7.02 (m, 3H), 6.62 (br s, 1H); MS (ESI): *m/z* for $C_{19}H_{12}ClF_2N_3O_2S$, found 420 [M + H$^+$].

5-(2-Chlorobenzenesulfonamido)-1-(2,4-difluorophenyl)-1*H*-indazole (**19n**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.93 (dd, *J* = 7.9, 1,4 Hz, 1H), 7.56–7.43 (m, 4H), 7.29–7.25 (m, 1H), 7.20 (m, 2H), 7.12 (br s, 1H), 7.08–7.00 (m, 2H); MS (ESI): *m/z* for $C_{19}H_{12}ClF_2N_3O_2S$, found 420 [M + H$^+$].

1-(2,4-Difluorophenyl)-5-(4-methylbenzenesulfonamido)-1*H*-indazole (**19o**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14 (s, 1H), 7.62 (d, *J* = 8.3 Hz, 2H), 7.59–7.49 (m, 1H), 7.49 (s, 1H), 7.22 (d, *J* = 8.3 Hz, 2H), 7.21–7.18 (m, 1H), 7.18–7.02 (m, 3H), 6.64 (br s, 1H), 2.38 (s, 3H); MS (ESI): *m/z* for $C_{20}H_{15}F_2N_3O_2S$, found 400 [M + H$^+$].

1-(2-Chloro-4-fluorophenyl)-5-(4-methoxybenzenesulfonamido)-1*H*-indazole (**19p**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14 (s, 1H), 7.66 (d, *J* = 8.9 Hz, 2H), 7.50–7.44 (m, 2H), 7.34 (m, 1H), 7.16 (m, 1H), 7.11 (m, 2H), 6.88 (d, *J* = 8.9 Hz, 2H), 6.58 (s, 1H), 3.83 (s, 3H); MS (ESI): *m/z* for $C_{20}H_{15}ClFN_3O_3S$, found 432 [M + H$^+$].

1-(4-Chlorophenyl)-5-(4-methoxybenzenesulfonamido)-1*H*-indazole (**19q**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.14 (s, 1H), 7.70–7.68 (m, 1H), 7.65 (d, *J* = 8.5 Hz, 2H), 7.59 (d, *J* = 9.0 Hz, 1H), 7.53–7.50 (m, 3H), 7.19 (m, 1H), 6.90 (d, *J* = 8.9 Hz, 2H), 3.84 (s, 3H); MS (ESI): *m/z* for $C_{20}H_{16}ClN_3O_3S$, found 414 [M + H$^+$].

1-(2-Chlorophenyl)-5-(4-methoxybenzenesulfonamido)-1*H*-indazole (**19r**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (s, 1H), 7.67 (d, *J* = 8.9 Hz, 2H), 7.61–7.57 (m, 1H), 7.51–7.46 (m, 2H), 7.46–7.41 (m, 2H), 7.11 (s, 2H), 6.88 (d, *J* = 8.9 Hz, 2H), 6.75 (br s, 1H), 3.82 (s, 3H); MS (ESI): *m/z* for $C_{20}H_{16}ClN_3O_3S$, found 414 [M + H$^+$].

5-(4-Fluorobenzenesulfonamido)-1-(4-fluorophenyl)-1*H*-indazole (**19s**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12 (s, 1H), 7.76–7.70 (m, 2H), 7.66–7.60 (m, 2H), 7.55 (d, *J* = 8.9 Hz, 1H), 7.50 (s, 1H), 7.23–7.19 (m, 2H), 7.14–7.07 (m, 3H), 6.51 (br s, 1H); MS (ESI): *m/z* for $C_{19}H_{13}F_2N_3O_2S$, found 386 [M + H$^+$].

1-(4-Fluorophenyl)-5-(4-methylbenzenesulfonamido)-1*H*-indazole (**19t**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.12 (s, 1H), 7.67–7.62 (m, 4H), 7.56 (d, *J* = 8.9 Hz, 1H), 7.51 (s, 1H), 7.27–7.23 (m, 4H), 7.19–7.15 (m, 1H), 6.59 (br s, 1H), 2.40 (s, 3H); MS (ESI): *m/z* for $C_{20}H_{16}FN_3O_2S$, found 382 [M + H$^+$].

1-(4-Fluorophenyl)-5-(3-methylbenzenesulfonamido)-1*H*-indazole (**19u**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.65 (m, 2H), 7.61 (s, 1H), 7.58–7.51 (m, 3H), 7.37–7.32 (m, 2H), 7.25 (m, 2H), 7.17 (d, *J* = 9.0 Hz, 1H), 6.62 (br s, 1H), 2.38 (s, 1H); MS (ESI): *m/z* for $C_{20}H_{16}FN_3O_2S$, found 382 [M + H$^+$].

1-(4-Fluorophenyl)-5-(3-trifluoromethylbenzenesulfonamido)-1*H*-indazole (**19v**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.15 (s, 1H), 8.03 (s, 1H), 7.85 (m, 2H), 7.65 (m, 2H), 7.61–7.57 (m, 2H), 7.53 (s, 1H), 7.28–7.23 (m, 2H), 7.14 (m, 1H), 6.63 (br s, 1H); MS (ESI): *m/z* for $C_{20}H_{13}F_4N_3O_2S$, found 436 [M + H$^+$].

5-(4-Cyanobenzenesulfonamido)-1-(4-fluorophenyl)-1*H*-indazole (**19w**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.16 (s, 1H), 7.85 (d, *J* = 8.5 Hz, 2H), 7.76 (d, *J* = 8.5 Hz, 2H), 7.68–7.64 (m, 2H), 7.58 (d, *J* = 9.0 Hz, 1H), 7.55 (s, 1H), 7.28–7.24 (m, 2H), 7.12 (d, *J* = 8.9 Hz, 1H), 6.96 (br s, 1H); MS (ESI): *m/z* for $C_{20}H_{13}FN_4O_2S$, found 393 [M + H$^+$].

1-(4-Fluorophenyl)-5-(2-thiophenesulfonamido)-1*H*-indazole (**19x**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.16 (s, 1H), 7.69–7.65 (m, 2H), 7.61–7.56 (m, 3H), 7.46 (m, 1H), 7.28–7.20 (m, 3H), 7.02 (m, 1H), 6.63 (br s, 1H); MS (ESI): *m/z* for $C_{17}H_{12}FN_3O_2S_2$, found 374 [M + H$^+$].

5-(4-Methoxybenzenesulfonamido)-1-(2-methylphenyl)-1*H*-indazole (**19y**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.11 (s, 1H), 7.67 (d, *J* = 8.9 Hz, 2H), 7.48 (s, 1H), 7.39–7.32 (m, 4H), 7.08 (m, 2H), 6.88 (d, *J* = 8.9 Hz, 2H), 6.66 (s, 1H), 3.83 (s, 3H), 2.09 (s, 3H); MS (ESI): *m/z* for $C_{21}H_{19}N_3O_3S$, found 394 [M + H$^+$].

5-Benzenesulfonamido-1-phenyl-1*H*-indazole (**19z**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.11 (s, 1H), 7.74–7.60 (m, 5H), 7.57–7.34 (m, 7H), 7.12 (d, *J* = 8.9 Hz, 1H), 6.48 (br s, 1H); MS (ESI): *m/z* for $C_{19}H_{15}N_3O_2S$, found 350 [M + H$^+$].

5-(4-Methoxybenzenesulfonamido)-1-phenyl-1*H*-indazole (**19aa**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.14 (s, 1H), 7.72–7.63 (m, 5H), 7.52 (m, 1H), 7.51 (s, 1H), 7.40 (t, *J* = 7.6 Hz, 1H), 7.15 (dd, *J* = 9.0, 2.0 Hz, 1H), 6.91 (dd, *J* = 7.0, 1.9 Hz, 2H), 6.48 (s, 1H), 3.85 (s, 3H); MS (ESI): *m/z* for $C_{20}H_{17}N_3O_3S$, found 380 [M + H$^+$].

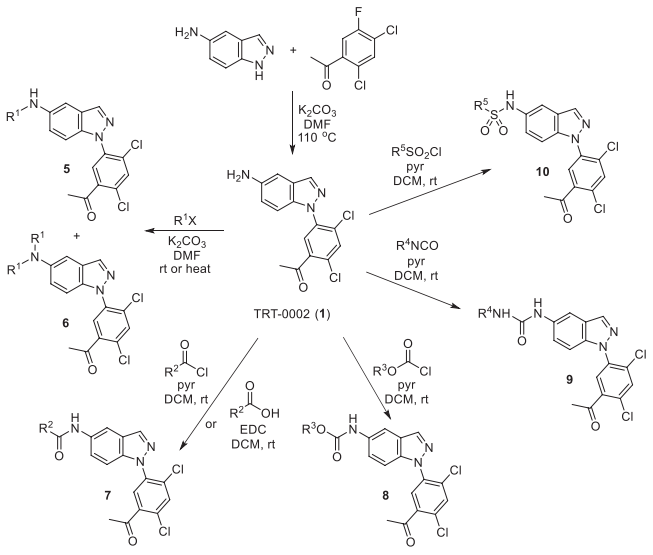
Cell Viability Assay. Cell viability was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, St. Louis, MO, USA) assay. The Huh7 cells were seeded at a density of 1×10^4 cells per well and treated with the compounds and/or TRAIL. After 24 or 48 h, MTT (2 mg/mL) was added to each well, and the absorbance was measured using a microplate reader (Bio-Rad, Hercules, CA, USA) at 570 nm. The cell viability was calculated as the percentage of viable cells in the drug-treated group versus the untreated control using the following equation: cell viability (%) = (OD [drug] − OD [blank])/(OD [control] − OD [blank]) × 100. The cell viability difference between the compound/TRAIL combination treatment and the treatment of only the compound is considered as the TRAIL sensitizing activity of the compound. The term working concentration is defined as the minimum concentration of a compound exhibiting the largest TRAIL sensitizing activity.

Liver Microsomal Stability Assay. Liver microsomal stability was determined in human, rat, and mouse liver microsomes. The compound (1 μM) was mixed with human, rat, or mouse liver microsomes (0.5 mg/mL; BD Biosciences Gentest, CA) in 100 mM potassium phosphate buffer

(pH 7.4) and incubated at 37°C for 5 min. The reaction was initiated by NADPH regeneration solution (BD Biosciences) and terminated by three times volume of ice-cold acetonitrile with imipramine (80 ng/mL) as internal standard at single-time-point 30 min. After pre-treatment of biological samples with vortex and centrifuge, the samples were analyzed by LC/MS/MS system.

Results and Discussion

The hit compound TRT-0002 (**1**) exhibited low metabolic stability in the liver microsomal stability test (0.54%, 0.54%, and 26.8% remaining after 30 min for the mouse, rat, and human specimens, respectively). Firstly, the 1,4-diaminobenzene moiety in the TRT-0002 compound was expected to be vulnerable to oxidative metabolism. Hence, as an initial trial for overcoming the metabolic instability and exploring the chemical space beyond the 5-amino group, we attempted to transform the amino group in the 5 position in TRT-0002 into various amino-related functionalities such as alkylamino, dialkylamino, amido, carbamate, ureido, and sulfonamido groups. The derivatives of TRT-0002 were synthesized as shown in Scheme 1. The precursor TRT-0002 (**1**) was prepared via the reaction of 5-amino-1*H*-indazole and 2′,4′-dichloro-5′-fluoroacetophenone under basic conditions.[13] The structure of TRT-0002 was discriminated from its 2-aryl-2*H*-indazole regioisomer based on HMBC and NOE experiments as described in the previous report.[13] The alkylation of TRT-0002 with alkyl halides resulted in the formation of mono-alkyl (**5**) and/or bis-alkyl (**6**) derivatives depending on the amount of alkyl halides and the reaction temperature. The acylation of TRT-0002 with acid chlorides or acids afforded 5-amido derivatives **7**. Carbamate (**8**), ureido (**9**), and sulfonamido (**10**)[13] derivatives were obtained from the reactions with chloroformates, isocyanates, and sulfonyl chlorides in an analogous manner. The building blocks used in the derivatization reactions of TRT-0002 (**1**) are shown in Figure 2. The TRAIL-sensitizing activity of the derivatives was assessed based on the cell viability using an MTT assay in Huh7 cells. The results for compounds exhibiting a cell viability difference greater than 25% between the compound/TRAIL combination treatment and the treatment of only the compound, together with the working concentration and treatment time are shown in Table 1. The effective working concentration was determined based on the responses of Huh7 cells treated with various doses in the presence and absence of TRAIL. The data of the TRT-0002 compound (**1**) are listed for reference (a cell viability difference of 52% at a 40-μM working concentration).[13] The synthetic procedures for the compounds listed in Table 1 are described in Experimental section. In addition, the representative procedures for the synthesis of derivatives **8** and **9** are described in Experimental section. Among the synthesized alkylamino (**5**) and dialkylamino (**6**) derivatives, 5-isobutylamino (**5a**) and 5-dimethylamino (**6a**) analogues satisfied the criterion, with cell viability differences of 26% and 39%, respectively. The compound with only the 3-(1*H*-indol-3-yl)propanoylamino group (**7a**) results in a cell viability difference of more than 25% in the case of 5-amido derivatives **7**. The carbamate (**8**) and ureido (**9**) analogues did not meet the criterion of the cell viability difference. Noticeably, 5-benzenesulfonamido derivative **2** (TRT-0029) showed a 58% cell viability difference at a lower working concentration of 10 μM.[13] In addition, in the case of 5-sulfonamido derivatives **10**, 5-(4-methoxybenzenesulfonamido) (**10a**) and



Scheme 1. Derivatization reactions of TRT-0002 (**1**).

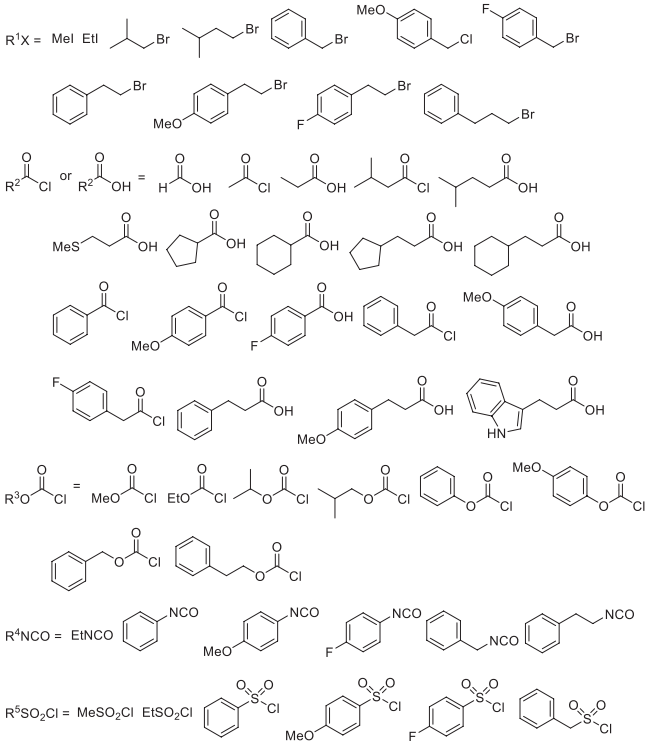
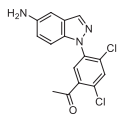
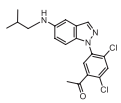
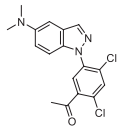
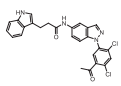
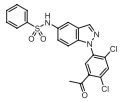
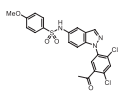
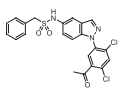


Figure 2. Building blocks used in derivatization reactions of TRT-0002 (**1**).

Table 1. Results of cell viability assay for derivatives **5**, **6**, **7**, **8**, **9**, and **10**.

Compound	Working concentration (μM)	Treatment time (h)	Compound only (%)[a]	Compound + TRAIL (%)[a]	Difference (%)
 TRT-0002 (**1**)	40	48	81	29	52
 (**5a**)	40	24	54	28	26
 (**6a**)	40	24	62	23	39
 (**7a**)	40	24	61	25	36
 TRT-0029 (**2**)	10	24	69	11	58
 (**10a**)	10	48	59	7	52
 (**10b**)	20	24	51	23	28

[a] Cell viability assays were performed in duplicate or triplicate. Errors for the individual values were found to be 0.5 ~ 9.6%.

5-phenylmethanesulfonamido (**10b**) analogues displayed a 52% and 28% cell viability difference at 10 μM and 20 μM, respectively. In particular, compound TRT-0029 (**2**) caused TRAIL-induced apoptosis by activating MKK7/JNK via the inhibition of the MKK7–TIPRL interaction, as described in our previous report.[13] Further, the TRT-0029 compound exhibited a slightly improved metabolic stability in the liver microsomal stability test (5.4%, 2.1%, and 42.6% remaining after 30 min for the mouse, rat, and human specimens, respectively) compared with that of the TRT-0002 compound (**1**).

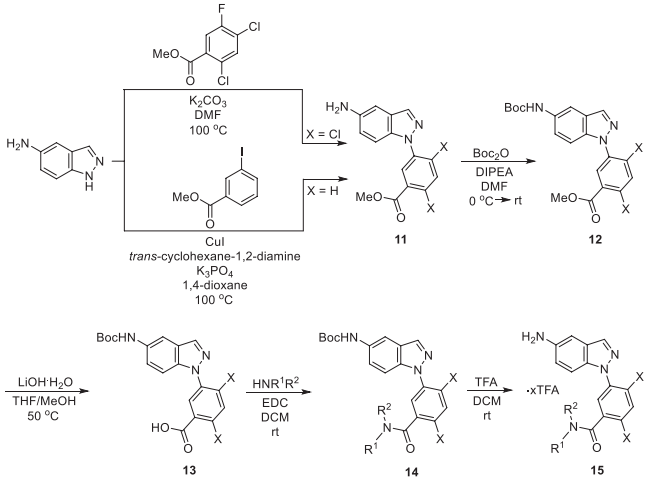
Next, we focused on replacing the acetyl group on the 1-aryl moiety in TRT-0002 (**1**) with a carbamoyl group, as represented by the target compounds **15** in Scheme 2, because we thought that the acetyl group is potentially liable to reductive metabolism and we intended to explore the chemical space near it. The intermediate 5-amino-1-(2,-4-dichloro-5-methoxycarbonylphenyl)-1*H*-indazole (**11a**, X = Cl) was synthesized from the reaction of 5-amino-1*H*-indazole and methyl 2,4-dichloro-5-fluorobenzoate under basic conditions. The dechlorinated analogue **11b** (X = H) was prepared by a copper-catalyzed reaction[14] involving 5-amino-1*H*-indazole and methyl 3-iodobenzoate. Protection of the 5-amino functionality in compounds **11** with the Boc group and subsequent hydrolysis of the ester group in compounds **12** afforded the carboxylic acid intermediates **13**. Amide coupling reactions of intermediates **13** with various primary and secondary amine building blocks listed in Figure 3 provided Boc-protected carbamoyl derivatives **14**, which were deprotected to give the final target products **15**. The TRAIL-sensitizing activities of the analogues are shown in Table 2 for compounds exhibiting a cell viability difference of more than 25% between the compound/TRAIL combination treatment and the compound alone treatment; the working concentrations and treatment times are also shown. Unfortunately, only three compounds fulfilled the cell viability difference requirement. Further they exhibited less potent TRAIL-sensitizing activities (of 33%,

25%, and 29% for **15a**, **15b**, and **15c**, respectively, at a working concentration of 40 μM) compared with those of compounds TRT-0002 (**1**) and TRT-0029 (**2**) (52% and 58% at working concentrations of 40 and 10 μM, respectively). The synthetic procedures for the compounds listed in Table 2 are described in Experimental section.

On the basis of the above-mentioned results, we speculated that the replacement of the 5-amino group in TRT-0002 compound **1** by a 5-sulfonamido group and the introduction of an aryl moiety without the acetyl group at the 1 position on the indazole ring system, as represented by the general structure **19** in Scheme 3, would give compounds with superior TRAIL-sensitizing activity and metabolic stability. The precursors **18** were prepared through three different routes. In one approach, the condensation reaction of 2-fluoro-5-nitrobenzaldehyde with arylhydrazines (Ar = 4-Cl-C_6H_4, 2-Cl-C_6H_4, 4-F-C_6H_4, 2,4-di-F-C_6H_3, 2-Me-C_6H_4) in the presence of toluenesulfonic acid resulted in the formation of the hydrazones **16a**–**e**, which were then cyclized in the presence of potassium carbonate to give 1-aryl-5-nitro-1*H*-indazoles (**17a**–**e**).[13] In another approach, compound **17f** (Ar = 4-F-2-Me-C_6H_3) was obtained from the copper-catalyzed reaction[15] of 5-nitro-1*H*-indazole with 4-fluoro-2-methylphenylboronic acid. The nitro reduction of compounds **17a**–**f** yielded the intermediates **18a**–**f**.[13] The 5-amino-1-aryl-1*H*-indazole compounds (**18g**–**i**) were also synthesized directly from copper-catalyzed reactions[14] of 5-amino-1*H*-indazole with aryl iodides (Ar = 2,4-di-Cl-C_6H_3, 2-Cl-4-F-C_6H_3, C_6H_5). The precursors **18** were sulfonylated under typical conditions to give 1-aryl-5-sulfonamido-1*H*-indazole compounds **19**.[13] The sulfonyl chloride building blocks used in the derivatization reactions are shown in Figure 4. The TRAIL-sensitizing activities of the analogues **19** are shown in Table 3 for compounds exhibiting a cell viability difference of more than 25% between the compound/TRAIL combination treatment and the compound alone treatment, together with their corresponding working concentrations and treatment times. The synthetic procedures for the compounds listed in Table 3 are described in Experimental section. In the case of 1-(2,4-dichlorophenyl) derivatives **19a**–**g**, 5-(2-fluorobenzenesulfonamido) (**19b**) and 5-(3-methylbenzenesulfonamido) (**19d**) analogues showed relatively good TRAIL-sensitizing activities (of 51% and 40% at a working concentration of 20 μM, respectively). Among the 1-(2,4-difluorophenyl)-1*H*-indazole compounds **19h**–**o**, derivatives with 5-(4-methoxybenzenesulfonamido) (**19i**), 5-(2-fluorobenzenesulfonamido) (**19l**), and 5-(4-methylbenzenesulfonamido) (**19o**) groups exhibited enhanced sensitivity for TRAIL (54%, 55%, and 50% at a working concentration of 20 μM, respectively). Compounds **19p** (1-(2-chloro-4-fluorophenyl)-5-(4-methoxybenzenesulfonamido)-1*H*-indazole), **19q** (1-(4-chlorophenyl)-5-(4-methoxybenzenesulfonamido)-1*H*-indazole), and **19r** (1-(2-chlorophenyl)-5-(4-methoxybenzenesulfonamido)-1*H*-indazole) exhibited 35%, 43%, and 54% cell viability differences at a working concentration of 20 μM, respectively. In the case of the 1-(4-fluorophenyl)-1*H*-indazole compounds **3** (TRT-0173) and **19s**–**x**, analogues with 4-methoxybenzenesulfonamido (**3**, TRT-0173), 3-methylbenzenesulfonamido (**19u**), and 3-trifluoromethylbenzenesulfonamido (**19v**) groups at the 5 position on the indazole ring system displayed clearly high cell viability differences of 80%,[13] 86%, and 83% at a working concentration of 20 μM, respectively. These compounds (**3**, **19u** and **19v**) also exhibited significant TRAIL-sensitizing activities (cell viability differences of 31%, 41%, and 40%, respectively) even at 10 μM concentration of the compounds. In addition, the 5-(4-cyanobenzenesulfonamido) derivative (**19w**) showed a 40% TRAIL-sensitizing activity at a working concentration of 20 μM. Finally, compounds **19y** (5-(4-methoxybenzenesulfonamido)-1-(2-methylphenyl)-1*H*-indazole), **19z** (5-benzenesulfonamido-1-phenyl-1*H*-indazole), and **19aa** (5-(4-methoxybenzenesulfonamido)-1-phenyl-1*H*-indazole) exhibited 44%, 29%, and 37% TRAIL-sensitizing activities at a working concentration of 20 μM, respectively. Through MOA studies, it was realized that TRT-0173 (**3**) enhances TRAIL-induced apoptosis in Huh7 cells by inhibiting MKK7–TIPRL interactions and activating MKK7/JNK.[13] Although the TRT-0173 compound operated at a higher working concentration (20 μM) than that of TRT-0029 (10 μM), TRT-0173 demonstrated an improved metabolic stability in liver microsomal stability tests (mouse, 32.1%; rat, 34.6%; and human, 66.4% remaining after 30 min) compared with those of TRT-0002 (**1**) and TRT-0029 (**2**). The metabolic stability data for compounds **19u** and **19v** were 6.2% and 34.6% for mouse, <1% and 0.42% for rat, and 36.7% and 65.3% for human (remaining after 30 min), respectively.

Conclusion

We attempted to convert a metabolically unstable TRT-0002 compound exhibiting TRAIL-sensitizing activity for Huh7 cells at a high working concentration (40 μM) to more potent and metabolically improved analogues by modifying the 5-amino group and the 1-aryl moiety in the 1*H*-indazole skeleton. The first trial that varied the 5-amino group to obtain amino-related functionalities such as alkylamino, dialkylamino, amido, carbamate, ureido, and sulfonamido groups resulted in the identification of a 5-sulfonamido derivative TRT-0029 compound working at a lower concentration (10 μM) with a slightly improved metabolic stability. Next, we replaced the acetyl group in the 1-aryl group of the TRT-0002 compound with various carbamoyl groups. The effort was fruitless and no analogues with improved potency were obtained. Subsequently, we focused on the synthesis of 5-sulfonamido derivatives having a 1-aryl moiety without an acetyl group. The resulting TRT-0173 compound was found to exhibit TRAIL-sensitizing activity at a working concentration 20 μM and improved metabolic stability compared with



Scheme 2. Synthesis of derivatives **15**.

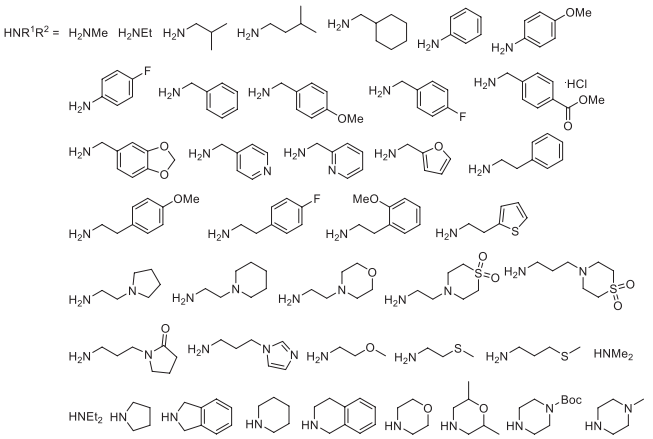
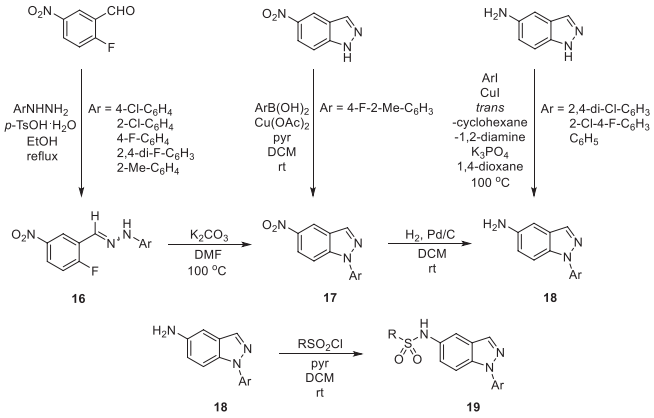


Figure 3. Building blocks used in the synthesis of derivatives **15**.



Scheme 3. Synthesis of derivatives **19**.

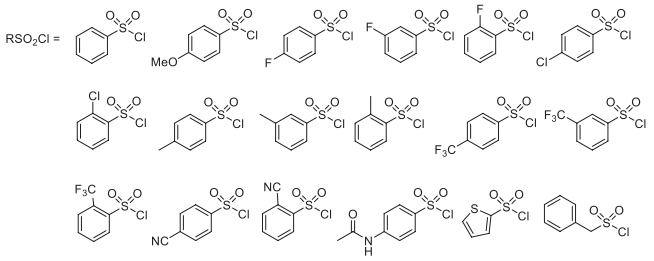
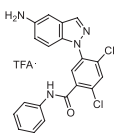
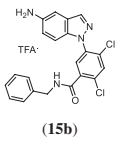
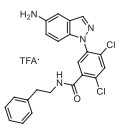


Figure 4. Building blocks used in the synthesis of derivatives **19**.

that of the TRT-0029 analogue. As reported previously,[13] co-treatment of cultured Huh7 cells with either TRT-0029 or TRT-0173 and TRAIL resulted in TRAIL-induced apoptosis owing to the inhibition of the MKK7–TIPRL interaction and subsequent phosphorylation of MKK7 and JNK. In addition, the injection of these two compounds suppressed tumor growth in combination with TRAIL in an *in vivo* HCC mouse xenograft model. With regard to the physicochemical aspect, the TRT-0029 and TRT-0173 compounds seemed to be somewhat lipophilic based on

Table 2. Results of cell viability assay for derivatives **15.**

Compound	Working concentration (μM)	Treatment time (h)	Compound only (%)[a]	Compound + TRAIL (%)[a]	Difference (%)
 (**15a**)	40	24	80	47	33
 (**15b**)	40	48	56	31	25
 (**15c**)	40	48	48	19	29

[a] Cell viability assays were performed in duplicate or triplicate. Errors for the individual values were found to be 0.6 ~ 7.0%.

Table 3. Results of cell viability assay for derivatives **19.**

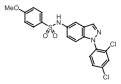
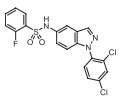
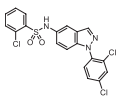
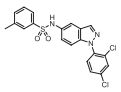
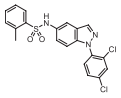
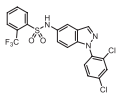
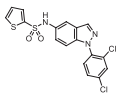
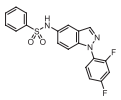
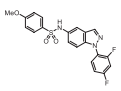
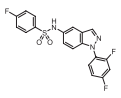
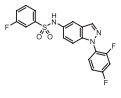
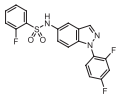
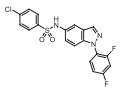
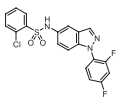
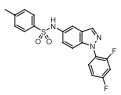
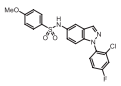
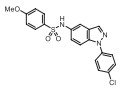
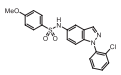
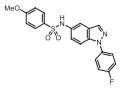
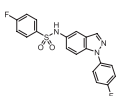
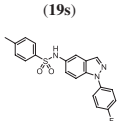
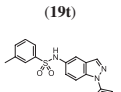
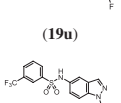
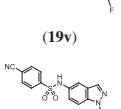
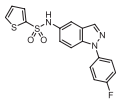
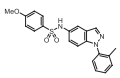
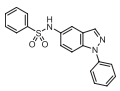
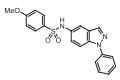
Compound	Working concentration (μM)	Treatment time (h)	Compound only (%)[a]	Compound + TRAIL (%)[a]	Difference (%)
 (**19a**)	20	24	37	10	27
 (**19b**)	20	24	62	11	51
 (**19c**)	20	24	68	37	31
 (**19d**)	20	24	48	8	40
 (**19e**)	20	24	43	12	31
 (**19f**)	20	24	48	15	33
 (**19g**)	20	24	34	7	27
 (**19h**)	20	24	61	24	37
 (**19i**)	20	24	66	12	54
 (**19j**)	20	24	57	31	26
 (**19k**)	20	24	50	11	39
 (**19l**)	20	24	62	7	55
	20	24	32	3	29

Table 3 (*continued*)

Compound	Working concentration (μM)	Treatment time (h)	Compound only (%)[a]	Compound + TRAIL (%)[a]	Difference (%)
 (**19m**)	20	24	45	13	32
 (**19n**)	20	24	62	12	50
 (**19o**)	20	24	63	28	35
 (**19p**)	20	24	47	4	43
 (**19q**)	20	24	66	12	54
 (**19r**)	20	24	92	12	80
 TRT-0173 (**3**)	20	24	46	7	39
 (**19s**)	20	24	94	69	25
 (**19t**)	20	24	92	6	86
 (**19u**)	20	24	87	4	83
 (**19v**)	20	24	53	13	40
 (**19w**)	20	24	68	41	27

(*continued overleaf*)

Table 3 (*continued*)

Compound	Working concentration (μM)	Treatment time (h)	Compound only (%)[a]	Compound + TRAIL (%)[a]	Difference (%)
 (**19x**)	20	24	63	19	44
 (**19y**)	20	24	61	32	29
 (**19z**)	20	24	69	32	37
 (**19aa**)					

[a] Cell viability assays were performed in duplicate or triplicate. Errors for the individual values were found to be 0.4 ~ 14.0%.

their CLogP values (5.07 and 4.60, respectively), and their solubilities were too low to be detected in an equilibrium solubility test in water. Despite such limitations, it is assumed that two compounds and the relevant structure–activity relationship would provide an insight to enable further study on optimization of potency and metabolic stability.

Acknowledgments. This research was possible thanks to generous financial support from the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (NRF-2012M3A9C) and the Korea Research Institute of Chemical Technology (KK1803-B00). This work was also supported by the Basic Science Research Program of the NRF funded by the Ministry of Education, Science and Technology (NRF-2014M3A9A5034157) and the KRIBB Research Initiative Program. The TIPRL and MKK7 clones were provided by the Korea Human Gene Bank, KRIBB, Korea.

References

1. R. M. Pitti, S. A. Marsters, S. Ruppert, C. J. Donahue, A. Moore, A. Ashkenazi, *J. Biol. Chem.* **1996**, *271*, 12687.
2. S. Wang, W. S. El-Deiry, *Oncogene* **2003**, *22*, 8628.
3. M. Elmallah, O. Micheau, *Mar. Drugs* **2015**, *13*, 6884.
4. H. Wajant, K. Pfizenmaier, P. Scheurich, *Apoptosis* **2002**, *7*, 449.
5. L. Zhang, B. Fang, *Cancer Gene Ther.* **2005**, *12*, 228.
6. Y. Oh, M. Swierczewska, T. H. Kim, S. M. Lim, H. N. Eom, J. H. Park, D. H. Na, K. Kim, K. C. Lee, M. G. Pomper, S. Lee, *J. Control. Release* **2015**, *220*, 671.
7. C. T. Hellwig, M. Rehm, *Mol. Cancer Ther.* **2012**, *11*, 3.
8. D. Deeb, H. Jiang, X. Gao, M. S. Hafner, H. Wong, G. Divine, R. A. Chapman, S. A. Dulchavsky, S. C. Gautam, *Mol. Cancer Ther.* **2004**, *3*, 803.
9. J. Ding, G. Polier, R. Kohler, M. Giaisi, P. H. Krammer, M. Li-Weber, *J. Biol. Chem.* **2012**, *287*, 641.
10. I. S. Song, S. Y. Jun, H.–. J. Na, H.–. T. Kim, S. Y. Jung, G. H. Ha, Y.–. H. Park, L. Z. Long, D.–. Y. Yu, J.–. M. Kim, J. H. Kim, J.–. H. Ko, C.–. H. Kim, N.–. S. Kim, *Gastroenterology* **2012**, *143*, 1341.
11. H.-J. Lee, H.-S. Cho, S. Y. Jun, J.-J. Lee, J.-Y. Yoon, J.-H. Lee, H.-H. Song, S. H. Choi, S.-Y. Kim, V. Saloura, C. G. Park, N.-S. Kim, *Oncol. Rep.* **2014**, *32*, 1117.
12. J.-Y. Yoon, H.-S. Cho, J.-J. Lee, H.-J. Lee, S. Y. Jun, J.-H. Lee, H.-H. Song, S. Choi, V. Saloura, C. G. Park, C.-H. Kim, N.-S. Kim, *Mol. Carcinog.* **2016**, *55*, 387.
13. J.-Y. Yoon, J.-J. Lee, S. Gu, M. E. Jung, H.-S. Cho, J. H. Lim, S. Y. Jun, J.-H. Ahn, J.-S. Min, M.-H. Choi, S.-J. Jeon, Y.-J. Lee, A. Go, Y.-J. Heo, C.-R. Jung, G. Choi, K. Lee, M.-K. Jeon, N.-S. Kim, *Oncotarget* **2017**, *8*, 112610.
14. J. E. Sheppeck, J. L. Gilmore, T. G. Dhar, H.-Y. Xiao, J. Wang, B. V. Yang, L. M. Doweyko, *Chem. Abstr.* **2008**, *148*, 561902.
15. M. Berger, H. Rehwinkel, H. Schaecke, M. Lepistoe, K. Edmann, *Chem. Abstr.* **2008**, *148*, 49593.

