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Rapid Synthesis of *N*-Tosylhydrazones under Solvent-Free Conditions and Their Potential Application Against Human Triple-Negative Breast Cancer

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Some *N*-tosylhydrazone derivatives were effectively synthesized under solvent-free conditions by using a grinding method at room temperature. The short reaction time, clean and mild process with simple workup and easy purification of the target compounds were salient features of the present protocol, which enables straightforward access to *N*-tosylhydrazones. Among the tosylhydrazone derivatives evaluated, compound **3l** exhibits excellent apoptosis-promoting and anticancer potential against triple-negative breast cancer (TNBC) cell lines. This research shows that our synthesized compound **3l** may be a desirable and effective therapeutic drug against TNBC.

1. Introduction

Hydrazones possessing the active moiety (–NH–N=CH–) are an important class of organic molecules for drug development. In past decades, these compounds have been synthesized and their biological activities have also been evaluated. Some hydrazone derivatives have proven to possess antidepressant,[1] antimicrobial,[2] antimalarial,[3] antiviral,[4] antischistosomiasis,[5] antiplatelet,[6] vasodilator,[7] anti-inflammatory,[8] analgesi,[9] anticonvulsant,[10] or antituberculosis[11] activities.

On the other hand, other synthetic hydrazones have demonstrated antitumoral activity against various cancer cells. For example, several hydrazone derivatives **I**, bearing the 3,4,5-trimethoxyphenyl moiety (Figure 1) have shown good antiproliferative activity against PC3 cells with an IC_{50} range of 0.2–1.8 μM.[12] *N*′-(4-Bromo-5-methyl-2-oxoindolin-3-ylidene)-3,4,5-trimethoxybenzohydrazide **II**, which also has a 3,4,5-trimethoxyphenyl backbone, exhibits EC_{50} = 0.24 μM against human colorectal carcinoma HCT116 cells.[13] More recently, nine hybrid compounds with a coumarin hydrazide–hydrazone backbone were synthesized by Abdel-Aziz et al.;[14] of which, compound **III a** exhibited significant activity against colon cancer cells (HT29, $IC_{50} = 7.98 \pm 0.05$ μM), whereas compound **III b** displayed the best antiproliferative activity against leukemia cells (K562, $IC_{50} = 9.44 \pm 0.02$ μM). Moreover, isatin-quinazoline hybrids, such as compound **IV**, have been reported to have a selective and potent growth inhibitory effect towards the liver cancer cell line (HepG2, $IC_{50} = 1.0 \pm 0.2$ μM).[15]

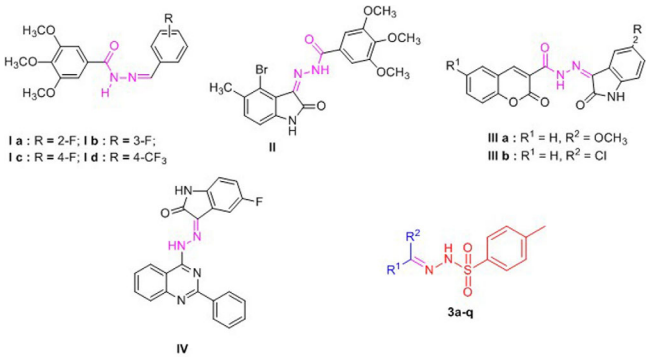

Figure 1. Structures of some reported anticancer hydrazones and target tosylhydrazones **3a–q**.

Tosylhydrazones, which are special class of hydrazones, have been used widely as versatile and useful partners in organic synthesis.[16] In particular, under basic conditions, the tosylhydrazone units are easily converted into diazo compounds,[17] which can undergo insertion reactions leading to the construction of various chemical bonds. For example, the formation of C–C,[18] C–N,[19] C–S,[20] C–P,[21] C–O,[22] C–Sn,[23] C–Si,[24] C–B,[25] and N–N[19d,e] bonds has been reported in the past few years.

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It is well known that the conventional procedure for the synthesis of tosylhydrazones remains the reaction of tosylhydrazides with corresponding aldehydes or ketones. However, most of these methods possess limitations such as the use of organic solvents, elevated temperature, lower yields and a longer reaction time. Recently, green and sustainable reaction systems have been a focus of interest, because of their advantages of being environmentally friendly and atom economical.[26] Among these, solvent-free reactions are more attractive due to their high selectivity, simple handling and easy purification.[27] Furthermore, mechanochemical synthesis conducted by grinding technique has been considered to be a versatile strategy for chemical synthesis, and plays an important role in various solvent-free reactions.[28]

Triple-negative breast cancer (TNBC) is a complex and aggressive subtype of breast cancer, in which exhibits low expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2).[29] In spite of the rapid advanced on clinical therapeutic strategies, a lack of effectively individualized treatments and the discouragement of existing overall prognosis remain troublesome for this subtype of breast cancer patient.[30] In addition, the side effects of anticancer agents and drug resistance of cancer cells can lead to recurrence of the disease.[31] Thus, development of new and high-efficiency anticancer drugs for TNBC is urgently needed.

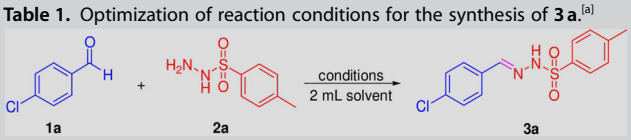
To date, there has been no report on an investigation into the anticancer activity of tosylhydrazone derivatives against TNBC cell lines. Thus, in this study, we aim to determine whether the tosylhydrazone derivatives possess antitumor activity against TNBC. Herein, we report a remarkably rapid and environmentally friendly protocol for the preparation of *N*-tosylhydrazones under solvent-free conditions by using a grinding technique, which yielded 17 *N*-tosylhydrazones (**3a**–**q**). Of these, compound **3l** exhibited the best IC_{50} value of 30.7 μg mL^{-1}, and we then demonstrated its antiproliferation and pro-apoptosis effect in TNBC cells. Taken together, we expect to find some new compounds to overcome the resistance of TNBC, in order to bring new hope for cancer patients.

2. Results and Discussion

2.1. Chemistry

In our initial study, 4-chlorobenzaldehyde (**1a**) and 4-methylbenzenesulfonohydrazide (**2a**) were selected to test the tosylhydrazone formation (molar ratio, **1a**/**2a** = 1:1). As shown in Table 1, seven solvents (CH_3CN, EtOH, EtOAc, toluene, $CHCl_3$, THF, and DMF) were tested at room temperature for 2 h, whereby using CH_3CN as a solvent afforded the highest yield (Table 1, entries 1). Next, with CH_3CN as the solvent, we investigated the effect of reaction temperature. It was found that the yield underwent no significant change when the reactants were refluxed in CH_3CN for 30 min (Table 1, entries 8). Inspired by recent advances in solvent-free reactions, we performed the above reaction in a mortar under solvent-free conditions by using a grinding method. Tosylhydrazone derivative **3a** was obtained in 95% yield within 1 min (Table 1, entry 9). However, a further increase in grinding time resulted in no further improvement in the yield (Table 1, entries 10 and 11). The above experiments clearly demonstrated that the solvent-free grinding technique afforded a higher yield of product in less time than with other conditions.

Table 1. Optimization of reaction conditions for the synthesis of **3a**.[a]



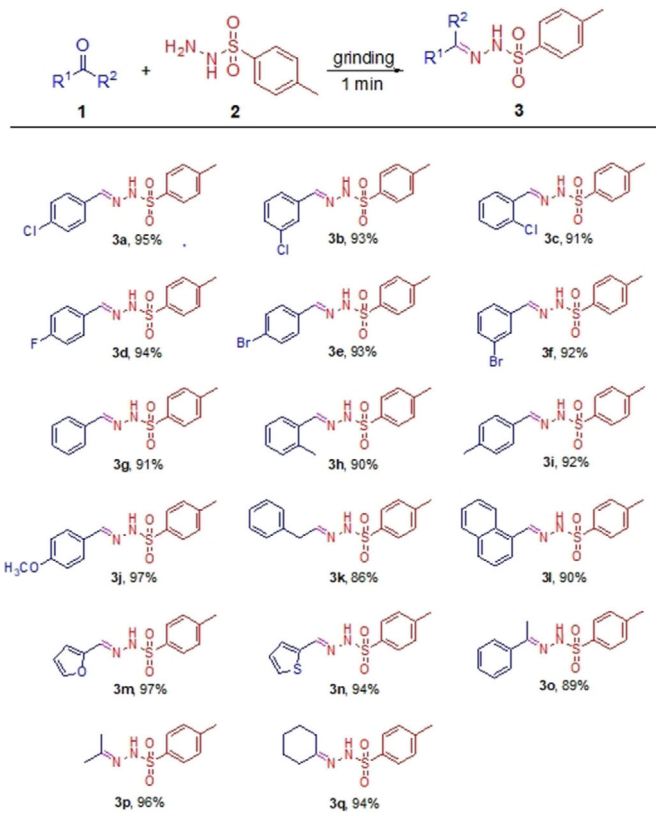
1a 2a 3a

Entry	Solvent	Condition[b]	Time [min]	Yield[c] [%]
1	CH_3CN	RT[c]	120	92
2	EtOH	RT[c]	120	89
3	EtOAc	RT[c]	120	84
4	toluene	RT[c]	120	80
5	$CHCl_3$	RT[c]	120	82
6	THF	RT[c]	120	79
7	DMF	RT[c]	120	71
8	CH_3CN	reflux	30	93
9	–[d]	grinding	1	95
10	–[d]	grinding	5	95
11	–[d]	grinding	10	95

[a] Reaction conditions: **1a** (1 mmol) and **2a** (1 mmol) in 2 mL of solvent. [b] RT = room temperature. [c] Isolated yields. [d] Solvent-free conditions.

On the basis of the above-optimized reaction conditions, we investigated the substrate scope of this protocol, and the results are summarized in Scheme 1. Various aldehydes (ketones) **1** were reactive and the corresponding target products **3** were obtained in good-to-excellent yields. It was noteworthy that this protocol exhibited excellent functional group tolerance, both electron-rich and electron-deficient aromatic aldehydes can be effectively coupled to the benzenesulfonohydrazide (Scheme 1, **3a**–**j**). Most interestingly, phenylacetaldehyde (aliphatic aldehyde) was proved to be a suitable coupling partner, which delivered product **3k** in 86% yield. We also found the reaction system was not sensitive to the steric hindrance effect of groups on the aromatic aldehydes and gave good yield (Scheme 1, **3l**). In addition, this methodology could be applicable to the reactions of heteroaromatic aldehydes, and the corresponding products being obtained in satisfactory yields (Scheme 1; **3m**, **3n**). Furthermore, acetophenone can also be coupled to **2** under our experimental conditions to afford product **3o**. Finally, the reactions could occur under mild conditions with aliphatic ketones, and ideal yields were achieved when acetone and cyclohexanone were used as the substrates (Scheme 1; **3p**, **3q**).

The spectra for the synthesized compounds (**3a**–**q**) are given in the Supporting Information. As a representative case, the IR spectra of **3a** shows a C=N stretching band at about 1595 cm^{-1}. In the ^{1}H NMR spectra, the characteristic PhCH=N and SO_2NH protons appeared as singlet peaks at 7.91 and 11.50 ppm, respectively. The protons belonging to the substituent groups and aromatic ring were observed according to the expected chemical shift values.



Scheme 1. Synthesis of *N*-tosylhydrazones under solvent-free conditions by using a grinding method. The reaction was performed with **1** (1 mmol) and **2** (1 mmol) under solvent-free conditions. Yields refer to isolated yields.

2.2. Biological Evaluation

The IC_{50} value of *N*-tosylhydrazones (**3a–q**) were detected in MDA-MB-231 cells using CCK8 reagents. Among the 17 *N*-tosylhydrazone compounds (**3a–q**), compound **3l** was the selected candidates that displayed strong cytotoxic activity against MDA-MB-231 cells ($IC_{50} = 30.7\ \mu g\,mL^{-1}$) (Figure 2), exhibiting a better IC_{50} value than any other compounds (see the Supporting Information, Table 1). Unfortunately, some compounds failed to record an IC_{50} value, which could be attributed to the solubility of *N*-tosylhydrazone compounds or other unknown factors. Next, compound **3l** was selected for the further detection of cell proliferation and apoptosis ability against TNBC.

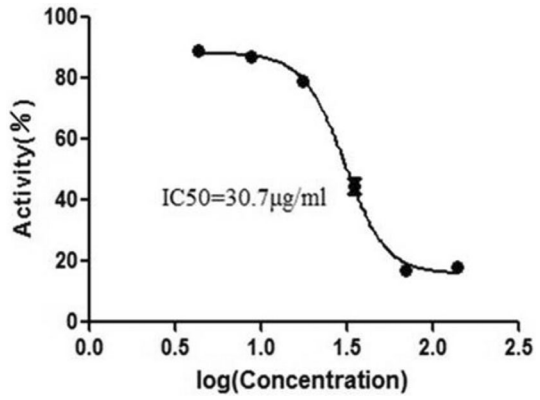


Figure 2. The IC_{50} values for *N*-tosylhydrazones in MDA-MB-231 cells.

Compound **3l** was selected to evaluate the antiproliferation activity of MDA-MB-231 cells. The results showed that compound **3l** (at the concentrations of 30, 60, and 100 $\mu g\,mL^{-1}$) successfully inhibited cell proliferation over six consecutive days, which was superior to the control group as shown in Figure 3 ($P < 0.05$). The above results reveal that *N*-tosylhydrazone compounds displayed significant cytotoxic activity and may be a potential drug for the treatment of TNBC.

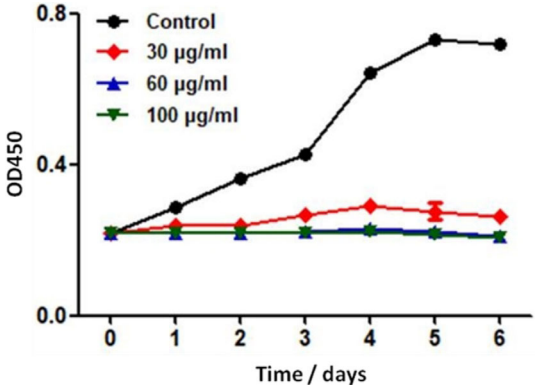


Figure 3. Compound **3l** efficiently suppress the proliferation of MDA-MB-231 cells.

The effect of *N*-tosylhydrazone **3l** in promoting the apoptosis of MDA-MB-231 cells was evaluated by AnnexinV FITC/PI staining and using a flow cytometer. As shown in Figure 4A, after treating with *N*-tosylhydrazone **3l** (at concentrations of 30 and 100 $\mu g\,mL^{-1}$) for 24 h in MDA-MB-231 cells, the percentage of apoptosis, including both early (from 1.71 to 6.27/7.71%) and late apoptotic phases (from 4.57 to 8.33/11.96%), was significantly increased compared with untreated control cells. The results of immunofluorescence staining (Figure 4B) were in accordance with the previous flow cytometer studies that equally exhibited the strongly pro-apoptosis ability of *N*-tosylhydrazone **3l**. The above findings prove that compound **3l** exerts pro-apoptotic potential, which contributes to its antiproliferative activity.

3. Conclusions

In summary, we have developed a new, mild, and environmentally friendly protocol for the synthesis of *N*-tosylhydrazones at room temperature under solvent-free conditions by using a grinding technique. This coupling reaction proceeded rapidly (1 min) and tolerated a wide range of functional groups. The antiproliferative properties of the desired products were also studied. Among these synthetic compounds, compound **3l** was selected to further evaluate the functions against TNBC cell lines according to its regnant IC_{50} value ($IC_{50} = 30.7\ \mu g\,mL^{-1}$). Interestingly, compound **3l** exhibited the highest cytotoxic activity and obvious pro-apoptosis functions against TNBC cell lines, suggesting the novel therapeutic schedule may be accompanied by the synthesis and applica-

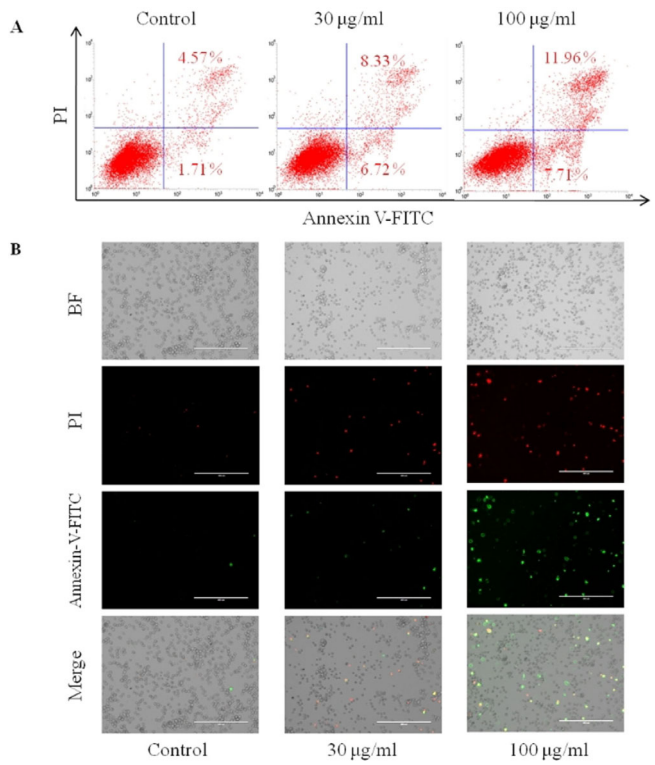


Figure 4. *N*-Tosylhydrazone compound **3l** promotes the apoptosis of MDA-MB-231 cell line. A) AnnexinV FITC/PI staining was used to determine the apoptosis of MDA-MB-231 cells with different concentrations of *N*-tosylhydrazone **3l** (30 and 100 μg mL^{-1}) according to flow cytometry. B) Immunofluorescence staining was used to evaluate cells apoptosis.

tion of compound **3l**. Further research concerning the application of compound **3l** will focus on its functional identification and therapeutic effect evaluation, in order to provide new ideas for the treatment of TNBC.

Experimental Section

General Information

Infrared (IR) spectra were recorded on an IRAffinity-1S spectrometer with KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III HD 400 MHz spectrometer in [*D6*]DMSO solution. ESI mass spectra were collected on a ThermoFisher LCQ Fleet Mass Spectrometer. TLC analyses were performed on commercial glass plates bearing a 0.25 mm layer of Merck Silica gel 60F$_{254}$. Unless otherwise noted, materials obtained from commercial suppliers were used without further purification. Chemical shifts for ^{1}H NMR are expressed in parts per million (ppm) relative to tetramethylsilane (δ 0.0 ppm). Chemical shifts for ^{13}C NMR are expressed in ppm relative to [*D6*]DMSO (δ 39.98 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (Hz), and integration. All reactions were carried out under air unless noted.

Typical Procedure for the Synthesis of *N*-Tosylhydrazones by Grinding

A mixture of aromatic aldehyde (ketone) (1 mmol) and 4-methylbenzenesulfonohydrazide (1 mmol) was thoroughly mixed in a mortar and ground manually with a pestle until the completion of the reaction as indicated by thin-layer chromatography (TLC; 1 min). The resultant material was washed with petroleum ether and filtered to give the desired product.

Cell Cultures

Breast cancer cell line MDA-MB-231 used in this study was cultured by using DMEM media containing 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin and with 10% FBS in a controlled atmosphere of 5% CO_2, 95% humidified air at 37 °C. The cells were subcultivated approximately every 2–3 days at 80% confluence, using 0.25% (w/v) trypsin and then seeded in a proper culture plates for the following study.

Cytotoxic Activity Assays

MDA-MB-231 (1×10^4 cells per well) cells were seeded into 96-well plates for incubation overnight (37 °C with 5% CO_2). The medium was removed and replaced with various concentrations of the 17 *N*-tosylhydrazone compounds (6.25, 12.5, 25, 50, 100, and 200 μg mL^{-1}) for 24 h at 37 °C. After that, 20 μL of CCK-8 solution was added to each well and incubated for 2 h at 37 °C. Finally, the results were detected at 450 nm using a microplate reader. The 50% inhibitory concentration (IC_{50}) represents the concentration of the modulators that was required for 50% inhibition.

Cell Proliferation Assay

A CCK-8 assay was performed to evaluate cell viability after treating with compounds over six consecutive days, according to the manufacturer's instructions. Briefly, MDA-MB-231 cells were seeded in 96-well plates at a density of 2×10^3 cells per well (37 °C with 5% CO_2). Following overnight incubation, compounds were added to each well at a concentration of 0, 30, 60, as well as 100 μg mL^{-1}, and then cultured for 1, 2, 3, 4, 5, and 6 days. At the indicated intervals, 20 μL of Cell Counting Kit-8 (Beyotime) was added to each well and incubated for 2 h at 37 °C. The absorbance was detected at 450 nm by using a PerkinElmer EnSpire® Multimode Plate Reader (PerkinElmer).

AnnexinV FITC/PI Apoptosis Assay

Apoptotic cells were further analyzed by using the AnnexinV FITC/PI assay. Briefly, MDA-MB-231 cells were treated with compound **3l** for 12 h with a concentration of 30.7 and 100 μg mL^{-1}. Cells were then harvested through trypsin and washed twice with PBS before being stained with AnnexinV FITC and PI for 10 min. Then, cells were analyzed by using a flow cytometer BD FACS Canto II, and FlowJo7.6.4 software was used to quantify the apoptosis of cells. Cell apoptosis was also evaluated by using immunofluorescence microscopy (EVOS ® FL Auto).

Statistical Analysis

All experiments were performed at least three times. Mean ± standard deviation was used for statistical treatment of data using Graph Pad Prism (5.0, CA, USA) software, with $P<0.05$ considered to be significant difference.

Spectral Data of Products (3 a–q)

N′-(4-Chlorobenzylidene)-4-methylbenzenesulfonohydrazide, 3 a

White solid; Yield: 293 mg (95 %); IR (KBr) υ 3183, 1595, 1466, 1331, 1167, 1086, 814 cm^{-1}; ^1H NMR (400 MHz, [*D6*]DMSO) δ 11.50 (s, 1 H), 7.91 (s, 1 H), 7.76 (d, J = 8.2 Hz, 2 H), 7.58 (d, J = 8.5 Hz, 2 H), 7.46 (d, J = 8.5 Hz, 1 H), 7.41 (d, J = 8.2 Hz, 1 H), 2.36 (s, 3 H); ^{13}C NMR (100 MHz, [*D6*]DMSO) δ 146.1, 143.9, 136.6, 135.0, 133.1, 130.2, 128.9, 127.7, 21.5; ESI-MS m/z: 309.17 $[M+H]^+$.

N′-(3-Chlorobenzylidene)-4-methylbenzenesulfonohydrazide, 3 b

White solid; Yield: 287 mg (93 %); IR (KBr) υ 3156, 1595, 1439, 1358, 1325, 1175, 1059, 949 cm^{-1}; ^1H NMR (400 MHz, [*D6*]DMSO) δ 11.65 (s, 1 H), 7.91 (s, 1 H), 7.78 (d, J = 8.2 Hz, 2 H), 7.60 (d, J = 9.8 Hz, 1 H), 7.52 (d, J = 6.8 Hz, 1 H), 7.46–7.37 (m, 4 H), 2.35 (s, 3 H); ^{13}C NMR (100 MHz, [*D6*]DMSO) δ 145.7, 144.0, 136.5, 136.3, 134.0, 131.2, 130.2, 127.7, 126.6, 125.8, 21.5; ESI-MS m/z: 309.08 $[M+H]^+$.

N′-(2-Chlorobenzylidene)-4-methylbenzenesulfonohydrazide, 3 c

White solid; Yield: 281 mg (91 %); IR (KBr) υ 3192, 1593, 1452, 1329, 1169, 1078, 955 cm^{-1}; ^1H NMR (400 MHz, [*D6*]DMSO) δ 11.76 (s, 1 H), 8.26 (s, 1 H), 7.81–7.72 (m, 3 H), 7.47–7.35 (m, 5 H), 2.36 (s, 3 H); ^{13}C NMR (100 MHz, [*D6*]DMSO) δ 144.1, 143.1, 136.5, 133.3, 132.0, 131.3, 130.4, 130.2, 128.1, 127.7, 127.0, 21.5; ESI-MS m/z: 309.08 $[M+H]^+$.

N′-(4-Fluorobenzylidene)-4-methylbenzenesulfonohydrazide, 3 d

White solid; Yield: 275 mg (94 %); IR (KBr) υ 3211, 1601, 1510, 1360, 1319, 1159, 1053, 930 cm^{-1}; ^1H NMR (400 MHz, [*D6*]DMSO) δ 11.48 (s, 1 H), 7.93 (s, 1 H), 7.78 (d, J = 8.2 Hz, 2 H), 7.62 (dd, J = 8.6, 5.7 Hz, 2 H), 7.39 (d, J = 8.2 Hz, 2 H), 7.21 (t, J = 8.8 Hz, 2 H), 2.34 (s, 3 H); ^{13}C NMR (100 MHz, [*D6*]DMSO) δ 164.8, 162.3, 146.4, 143.9, 136.6, 130.8, 130.7, 130.1, 129.4, 129.3, 127.7,116.4, 116.2, 21.4; ESI-MS m/z: 293.17 $[M+H]^+$.

N′-(4-Bromobenzylidene)-4-methylbenzenesulfonohydrazide, 3 e

White solid; Yield: 329 mg (93 %); IR (KBr) υ 3184, 1593, 1470, 1331, 1169, 1069, 812 cm^{-1}; ^1H NMR (400 MHz, [*D6*]DMSO) δ 11.57 (s, 1 H), 7.89 (s, 1 H), 7.76 (d, J = 8.2 Hz, 2 H), 7.60 (d, J = 8.5 Hz, 2 H), 7.51 (d, J = 8.5 Hz, 2 H), 7.41 (d, J = 8.2 Hz, 2 H), 2.38 (s, 3 H); ^{13}C NMR (100 MHz, [*D6*]DMSO) δ 146.2, 143.9, 136.6, 133.4, 132.3, 130.2, 129.1, 127.7, 123.8, 21.5; ESI-MS m/z: 353.17 $[M+H]^+$.

N′-(3-Bromobenzylidene)-4-methylbenzenesulfonohydrazide, 3 f

White solid; Yield: 325 mg (92 %); IR (KBr) υ 3142, 1597, 1518, 1362, 1329, 1167, 1057, 949 cm^{-1}; ^1H NMR (400 MHz, [*D6*]DMSO) δ 11.67 (s, 1 H), 7.90 (s, 1 H), 7.78 (d, J = 8.2 Hz, 2 H), 7.72 (s, 1 H), 7.55 (t, J = 6.4 Hz, 2 H), 7.39 (d, J = 8.2 Hz, 2 H), 7.33 (t, J = 7.9 Hz, 1 H), 2.33 (s, 3 H); ^{13}C NMR (100 MHz, [*D6*]DMSO) δ 145.7, 144.0, 136.6, 133.0, 131.4, 130.2, 129.5, 127.7, 126.1, 122.6, 21.5; ESI-MS m/z: 353.08 $[M+H]^+$.

N′-Benzylidene-4-methylbenzenesulfonohydrazide, 3 g

White solid; Yield: 250 mg (91 %); IR (KBr) υ 3223, 1597, 1429, 1360, 1165, 1043, 955 cm^{-1}; ^1H NMR (400 MHz, [*D6*]DMSO) δ 11.40 (s, 1 H), 7.91 (s, 1 H), 7.77 (d, J = 8.2 Hz, 2 H), 7.61–7.51 (m, 2 H), 7.42–7.38 (m, 5 H), 2.36 (s, 3 H); ^{13}C NMR (100 MHz, [*D6*]DMSO) δ 147.4, 143.9, 136.6, 134.1, 130.5, 130.1, 129.3, 127.7, 127.2, 21.5; ESI-MS m/z: 275.17 $[M+H]^+$.

N′-(2-Methylbenzylidene)-4-methylbenzenesulfonohydrazide, 3 h

White solid; Yield: 260 mg (90 %); IR (KBr) υ 3183, 1595, 1458, 1360, 1325, 1167, 1055, 953 cm^{-1}; ^1H NMR (400 MHz, [*D6*]DMSO) δ 11.41 (s, 1 H), 8.15 (s, 1 H), 7.77 (d, J = 8.2 Hz, 2 H), 7.56 (d, J = 7.7 Hz, 1 H), 7.41 (d, J = 8.2 Hz, 2 H), 7.31–7.15 (m, 3 H), 2.35 (s, 3 H), 2.31 (s, 3 H); ^{13}C NMR (100 MHz, [*D6*]DMSO) δ 146.5, 143.9, 137.0, 136.6, 132.1, 131.4, 130.2, 130.1, 127.7, 126.7, 126.6, 21.5, 19.7; ESI-MS m/z: 289.17 $[M+H]^+$.

N′-(4-Methylbenzylidene)-4-methylbenzenesulfonohydrazide, 3 i

White solid; Yield: 265 mg (92 %); IR (KBr) υ 3215, 1595, 1433, 1362, 1165, 1047, 937 cm^{-1}; ^1H NMR (400 MHz, [*D6*]DMSO) δ 11.35 (s, 1 H), 7.87 (s, 1 H), 7.76 (d, J = 8.2 Hz, 2 H), 7.44 (d, J = 7.9 Hz, 2 H), 7.40 (d, J = 8.2 Hz, 2 H), 7.20 (d, J = 7.9 Hz, 2 H), 2.35 (s, 3 H), 2.30 (s, 3 H); ^{13}C NMR (100 MHz, [*D6*]DMSO) δ 147.6, 143.9, 140.4, 136.6, 131.4, 130.1, 129.8, 127.7, 127.2, 21.5, 21.4; ESI-MS m/z: 289.17 $[M+H]^+$.

N′-(4-Methoxybenzylidene)-4-methylbenzenesulfonohydrazide, 3 j

Yellow solid; Yield: 295 mg (97 %); IR (KBr) υ 3225, 1609, 1520, 1423, 1165, 1036, 949 cm^{-1}; ^1H NMR (400 MHz, [*D6*]DMSO) δ 11.28 (s, 1 H), 7.89 (s, 1 H), 7.79 (d, J = 8.2 Hz, 2 H), 7.51 (d, J = 8.7 Hz, 2 H), 7.38 (d, J = 8.2 Hz, 2 H), 6.94 (d, J = 8.7 Hz, 2 H), 3.75 (s, 3 H), 2.35 (s, 3 H); ^{13}C NMR (100 MHz, [*D6*]DMSO) δ 161.2, 147.5, 143.8, 136.7, 130.1, 128.8, 127.7, 126.8, 114.7, 55.7, 21.4; ESI-MS m/z: 305.17 $[M+H]^+$.

4-Methyl-*N*′-(2-phenylethylidene)benzenesulfonohydrazide, 3 k

White solid; Yield: 248 mg (86 %); IR (KBr) υ 3209, 1599, 1435, 1357, 1162, 1013 cm^{-1}; ^1H NMR (400 MHz, [*D6*]DMSO) δ 10.98 (s, 1 H), 7.69 (d, J = 8.1 Hz, 2 H), 7.43–7.38 (m, 3 H), 7.30–7.18 (m, 5 H), 6.99 (d, J = 6.7 Hz, 2 H), 2.41 (s, 3 H); ^{13}C NMR (100 MHz, [*D6*]DMSO) δ 150.6, 143.8, 136.8, 136.5, 130.1, 129.2, 128.9, 127.8, 127.1, 38.5, 21.5; ESI-MS m/z: 289.17 $[M+H]^+$.

4-Methyl-*N*′-(naphthalen-1-ylmethylene)benzenesulfonohydrazide, 3 l

Yellow solid; Yield: 292 mg (90 %); IR (KBr) υ 3150, 1597, 1508, 1321, 1171, 1063, 941 cm^{-1}; ^1H NMR (400 MHz, [*D6*]DMSO) δ 11.57 (s, 1 H), 8.58 (d, J = 8.2 Hz, 1 H), 8.51 (s, 1 H), 7.97 (d, J = 8.0 Hz, 2 H), 7.85 (d, J = 8.2 Hz, 2 H), 7.72 (d, J = 7.1 Hz, 1 H), 7.63–7.51 (m, 3 H), 7.43 (d, J = 8.0 Hz, 2 H), 2.34 (s, 3 H); ^{13}C NMR (100 MHz, [*D6*]DMSO) δ 147.8, 144.0, 136.6, 133.9, 131.1, 130.2, 129.4, 129.2, 128.6, 127.9, 127.8, 126.8, 125.9, 124.7, 21.5; ESI-MS m/z: 325.17 $[M+H]^+$.

N′-(Furan-2-ylmethylene)-4-methylbenzenesulfonohydrazide, 3 m

Yellow solid; Yield: 256 mg (97%); IR (KBr) υ 3196, 1595, 1487, 1346, 1161, 1049, 914 cm^{-1}; ^1H NMR (400 MHz, [*D6*]DMSO) δ 11.45 (s, 1H), 7.79 (s, 1H), 7.77–7.72 (m, 3H), 7.40 (d, J=8.1 Hz, 2H), 6.80 (d, J=3.4 Hz, 1H), 6.57–6.54 (m, 1H), 2.35 (s, 3H); ^{13}C NMR (100 MHz, [*D6*]DMSO) δ 149.0, 145.5, 143.9, 137.4, 136.6, 130.2, 127.6, 114.4, 112.5, 21.5; ESI-MS m/z: 265.17 $[M+H]^+$.

4-Methyl-N′-(thiophen-2-ylmethylene)benzenesulfonohydrazide, 3 n

White solid; Yield: 264 mg (94%); IR (KBr) υ 3167, 1605, 1439, 1341, 1173, 1055, 922 cm^{-1}; ^1H NMR (400 MHz, [*D6*]DMSO) δ 11.40 (s, 1H), 8.11 (s, 1H), 7.75 (d, J=8.2 Hz, 2H), 7.57 (d, J=5.0 Hz, 1H), 7.39 (d, J=8.2 Hz, 2H), 7.35 (d, J=3.4 Hz, 1H), 7.08–7.03 (m, 1H), 2.34 (s, 3H); ^{13}C NMR (100 MHz, [*D6*]DMSO) δ 143.9, 142.7, 138.8, 136.5, 131.2, 130.1, 129.2, 128.3, 127.7, 21.5; ESI-MS m/z: 281.08 $[M+H]^+$.

4-Methyl-N′-(1-phenylethylidene)benzenesulfonohydrazide, 3 o

White solid; Yield: 257 mg (89%); IR (KBr) υ 3219, 1595, 1508, 1400, 1335, 1367,1057, 914 cm^{-1}; ^1H NMR (400 MHz, [*D6*]DMSO) δ 10.52 (s, 1H), 7.82 (d, J=8.2 Hz, 2H), 7.64–7.61 (m, 2H), 7.46–7.34 (m, 5H), 2.37 (s, 3H), 2.18 (s, 3H); ^{13}C NMR (100 MHz, [*D6*]DMSO) δ 153.6, 143.8, 137.9, 136.7, 129.9, 129.8, 128.8, 128.1, 126.4, 21.5, 14.8; ESI-MS m/z: 289.17 $[M+H]^+$.

4-Methyl-N′-(propan-2-ylidene)benzenesulfonohydrazide, 3 p

White solid; Yield: 217 mg (96%); IR (KBr) υ 3224, 1599, 1388, 1333, 1162, 1091, 927 cm^{-1}; ^1H NMR (400 MHz, [*D6*]DMSO) δ 9.99 (s, 1H), 7.74 (d, J=8.1 Hz, 2H), 7.39 (d, J=8.1 Hz, 2H), 2.38 (s, 3H), 1.79 (s, 6H); ^{13}C NMR (100 MHz, [*D6*]DMSO) δ 157.2, 143.5, 136.9, 129.8, 127.9, 25.3, 21.5, 18.1; ESI-MS m/z: 227.17 $[M+H]^+$.

N′-Cyclohexylidene-4-methylbenzenesulfonohydrazide, 3 q

White solid; Yield: 250 mg (94%); IR (KBr) υ 3255, 2935, 1591, 1404, 1333, 1162, 1037, 943 cm^{-1}; ^1H NMR (400 MHz, [*D6*]DMSO) δ 10.10 (s, 1H), 7.72 (d, J=8.1 Hz, 2H), 7.38 (d, J=6.5 Hz, 2H), 2.37 (s, 3H), 2.25 (s, 2H), 2.07 (s, 2H), 1.51 (s, 6H); ^{13}C NMR (100 MHz, [*D6*]DMSO) δ 162.6, 143.4, 136.9, 129.8, 127.9, 35.1, 27.8, 27.2, 25.9, 25.3, 21.5; ESI-MS m/z: 267.17 $[M+H]^+$.

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Conflict of Interest

The authors declare no conflict of interest.

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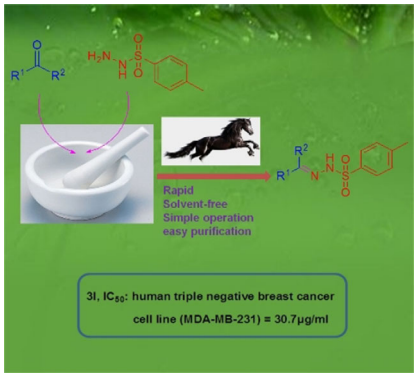
Rapid Synthesis of *N*-Tosylhydrazones under Solvent-Free Conditions and Their Potential Application Against Human Triple-Negative Breast Cancer



Daily grind: Some *N*-tosylhydrazone derivatives are effectively synthesized under solvent-free conditions by using a grinding method. The short reaction time, clean and mild process with simple workup and easy purification of target compounds are salient features of the protocol, which enables straightforward access to *N*-tosylhydrazones. Among the tosylhydrazone derivatives evaluated, one compound exhibits excellent apoptosis-promoting and anticancer potential against triple-negative breast cancer (TNBC) cell lines, indicating that our synthesized compound may be a desirable and effective therapeutic drug for TNBC.