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# Multisubstituted indole-acrylonitrile hybrids as potential cytotoxic agents

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# ABSTRACT

A series of multisubstituted indole–acrylonitrile hybrids were designed, synthesized and evaluated for their potential cytotoxic activities. The bio-evaluation results indicated that some of the target compounds (such as **3a**, **3f**, **3k**, **3n**) exhibited good to moderate cytotoxic effect on HepG2, BCG-823, BEL-7402, and HL-7702 cell lines. Especially, the compounds **3a** and **3k** also exhibited high cytotoxic activities (**3a**, 19.38 ± 3.38  $\mu$ M; **3k**, 15.43 ± 3.54  $\mu$ M) against the BEL-7402 cell line resistant to Taxol (>25  $\mu$ M) and 5-FU (>500  $\mu$ M), which might be developed as novel lead scaffold for potential anticancer agents.

Nitrogen-containing heterocycles always display extensive bioactivities, and which are also extremely versatile structural units serving as important intermediates for the construction of active molecules in drug design and agrochemical industry.<sup>1–3</sup> Among these heterocyclic building blocks, the myriad indole skeleton has emerged as a most promising class of synthons for pharmaceutical drugs and functional materials.<sup>4–7</sup> Up to now, many heterocyclic compounds derived from indole have been identified as potential antibacterial, anticancer, antiviral agents and protein kinase inhibitors.<sup>8–11</sup> In addition, some indole intermediates can also be used as important synthons for further transformation to related fused-heterocycles or various indole alkaloids.

On the other hand, acrylonitrile units have also attracted considerable attention for decades, and which are privileged scaffolds because of their broad applications range from medicinal agents,<sup>12–16</sup> agrochemicals<sup>17–19</sup> to functional materials.<sup>20–22</sup> Recently, many examples bearing this moiety have been reported to confirm that the introduction of this pharmacophores can result in highly potential activity as protein kinase inhibitors or anticancer agents. As shown in Figure 1, the compounds AG-490<sup>23</sup> and AG-18<sup>24</sup> are potential EGFR inhibitors, the compound AG-1024<sup>25</sup> is an IGF-1R autophosphorylation inhibitors, WP1066 is a novel inhibitor of JAK2 and STAT3,<sup>26</sup> WP1130 is a selective deubiquitinase inhibitor.<sup>27</sup> Meanwhile, the flexible properties and itself characteristics of acrylonitrile group make it present important chemical significance, and which is always the focus field to researchers in materials due to its conjugation system.<sup>20–22</sup> Thus, based on the aforementioned statements, this work focused on the design, convenient synthesis, and cytotoxic evaluation of a series of multisubstituted indole–acrylonitrile derivatives based on pharmacophores hybridization. We utilized indole scaffold as key prototype structural unit and planed for the integration of indole skeleton and acrylonitrile pharmacophores to the core structure as shown in Figure 2, which might be developed as lead compounds for high potential cytotoxic agents.

The general procedures for the preparation of novel multisubstituted indole–acrylonitrile derivatives **3a–p** are outlined in Scheme 1.

The key building blocks N-substituted indoles **1b** were obtained by alkylation reaction of indole in the presence of Me<sub>2</sub>SO<sub>4</sub>/K<sub>2</sub>CO<sub>3</sub>, and then the reactants **1a–c** were conveniently transferred to the corresponding 3-(1*H*-indol-3-yl)-3-oxopropanenitriles **2a–c** via classical Friedel–Crafts acylation reaction under the condition of Ac<sub>2</sub>O/NCCH<sub>2</sub>COOH. The following Knoevenagel condensation reactions of intermediates **2a–c** were treated with various aldehydes resulting in multisubstituted indole–acrylonitrile derivatives **3a– p**. All target compounds gave satisfactory chemical analyses, and the general procedures and spectra data were described in Supplementary data.

The newly prepared multisubstituted indole–acrylonitrile hybrids **3a–p** were evaluated for their in vitro cytotoxic effects against HepG2 (hepatocellular liver carcinoma), BCG-823 (gastric cancer), BEL-7402 (hepatocellular carcinoma), and HL-7702 (normal liver cell) cell lines by the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay<sup>28</sup> using 5-FU (5-Fluorouracil) and Taxol as a positive control. The preliminary results were summarized in Table 1.





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Figure 1. Representative compounds containing acrylonitrile unit.

Generally, as shown in Table 1, the newly prepared indole–acrylonitrile hybrids (**3a–p**) displayed moderate to good inhibition activities against the four human cancer cell lines. Notably, the compounds **3a**, **3f**, **3k**, and **3n** exhibited significant inhibitory activities (71.07–87.21%) against all four tested cell lines at 40 µg/mL concentration. Also, it is interesting to note that compound **3b** and **3d** presented selective cytotoxicity for a special human hepatocellular liver carcinoma cell, and the inhibitions against HepG2 cell were up to 72.05% and 71.52%, respectively. However, from the results indicated in Table 1, we also observed that the intermediates **2a–c** exhibited lower activities against the tested



Figure 2. Design strategy of multisubstituted indole-acrylonitrile hybrids.

cancer cell lines. The results of preliminary cytotoxic assay indicated this combination of indole core and acrylonitrile unit lead to the high potential active scaffold, and which can be used as lead compounds for optimization of anticancer agents.

Based on the preliminary results, most of the target compounds displayed good inhibitory activities against the tested cell lines, so in order to further investigate the potential activities, the  $IC_{50}$  values were further evaluated using the above cell-based method. The inhibitory activities expressed as IC<sub>50</sub> values for the target compounds are presented in Table 2. The results further testify that some of the designed indole-acrylonitrile hybrids **3a-p** exhibited higher inhibition activities compared to the commercial 5-FU under the same conditions. As indicated in Table 2, compound 3a showed the strongest inhibitory effect on all four cell lines, with an IC<sub>50</sub> values of  $7.07 \pm 0.92$  (HepG2),  $4.92 \pm 2.46$  (BCG-823), 19.38 ± 3.38 (BEL-7402) and 18.76 ± 5.84 (HL-7702) µM, respectively. Especially, we can find that the most potential compounds 3a, 3f, 3k and 3n also showed higher cytotoxic activities (3a, 19.38 ± 3.38  $\mu$ M; **3f**, 20.99 ± 4.11  $\mu$ M; **3k**, 15.43 ± 3.54  $\mu$ M; **3n**,  $28.12 \pm 5.13 \mu$ M) against the BEL-7402 cell line resistant to Taxol



Scheme 1. General synthetic route for multisubstituted indole-acrylonitrile derivatives.

#### Table 1 Cytotoxic activity (% cell growth inhibition) of the compounds **3a**–**p** and **2a–c** at 40 µg/mL concentration against various human cancer cell lines

Entry	Compd no.	Growth-inhibitory properties (%)				
		HepG2 <sup><i>a</i></sup>	BCG-823 <sup>a</sup>	BEL-7402 <sup>a</sup>	HL-7702 <sup>a</sup>	
1	3a	71.07 ± 3.25	77.51 ± 7.48	84.73 ± 8.82	72.08 ± 5.66	
2	3b	72.05 ± 4.24	$63.94 \pm 6.80$	2.16 ± 1.87	66.51 ± 3.16	
3	3c	23.87 ± 7.49	15.89 ± 6.48	23.91 ± 11.56	23.99 ± 7.01	
4	3d	71.52 ± 1.40	61.53 ± 8.00	$14.10 \pm 5.24$	38.53 ± 6.60	
5	Зе	39.71 ± 3.23	26.19 ± 9.80	32.79 ± 10.86	41.51 ± 4.99	
6	3f	75.71 ± 4.22	81.74 ± 4.07	82.55 ± 8.36	77.79 ± 4.35	
7	3g	72.40 ± 2.03	$76.74 \pm 2.49$	28.50 ± 2.77	69.56 ± 3.41	
8	3h	8.23 ± 6.16	3.66 ± 6.33	44.54 ± 6.82	35.33 ± 9.59	
9	3i	$70.94 \pm 9.01$	81.00 ± 5.84	78.18 ± 12.28	66.03 ± 8.11	
10	3j	73.18 ± 2.01	33.95 ± 12.31	$24.63 \pm 7.66$	42.95 ± 7.49	
11	3k	72.88 ± 6.35	82.37 ± 3.13	87.21 ± 5.85	74.10 ± 3.03	
12	31	36.11 ± 6.49	$41.72 \pm 8.24$	$48.59 \pm 6.44$	37.26 ± 10.42	
13	3m	16.88 ± 5.42	3.90 ± 5.56	49.81 ± 7.96	26.51 ± 7.12	
14	3n	71.53 ± 0.49	76.51 ± 6.41	79.40 ± 6.61	74.13 ± 3.45	
15	30	25.27 ± 5.29	$2.37 \pm 4.10$	25.74 ± 5.36	12.50 ± 6.21	
16	3р	$0.00 \pm 0.00$	$0.00 \pm 0.00$	$20.15 \pm 6.50$	$1.63 \pm 2.82$	
17	2a	$71.74 \pm 5.42$	31.06 ± 2.08	$5.55 \pm 6.39$	31.08 ± 8.58	
18	2b	73.24 ± 2.44	62.61 ± 2.23	26.26 ± 10.64	38.40 ± 2.88	
19	2c	$12.59 \pm 7.47$	21.89 ± 12.09	51.15 ± 9.03	35.09 ± 3.26	

<sup>a</sup> Abbreviations: HepG2-human hepatocellular liver carcinoma cell line; BCG-823-human gastric cancer cell line; BEL-7402-human hepatocellular carcinoma cell line; HL-7702-human normal liver cell line.

# Table 2 Cytotoxic activity of the compounds 3a-p and 2a-c against various cell lines



Entry	Compd no.	Substituents		In vitro cytotoxicity $IC_{50}^{a}$ ( $\mu$ M)				
		$\mathbb{R}^1$	$\mathbb{R}^2$	R <sup>3</sup>	HepG2 <sup>b</sup>	BCG-823 <sup>b</sup>	BEL-7402 <sup>b</sup>	HL-7702 <sup>b</sup>
1	3a	Н	Me	-s NH	7.07 ± 0.92	4.92 ± 2.46	19.38 ± 3.38	18.76 ± 5.84
2	3b	Н	Me		18.61 ± 7.18	90.93 ± 1.60	>100	76.84 ± 12.76
3	3c	Н	Me	s NO	>100	>100	>100	>100
4	3d	Н	Me	-s- -E- N_	24.38 ± 5.57	>100	>100	>100
5	3e	Н	Me	s NC	>100	>100	>100	>100
6	3f	Н	Me		15.79 ± 5.19	12.33 ± 1.29	20.99 ± 4.11	15.58 ± 0.87
7	3g	Н	Н		37.28 ± 4.69	56.89 ± 2.49	>100	55.51 ± 6.63
8	3h	Н	Н	5 2	>100	>100	>100	>100
9	3i	Н	Н	s RO	83.65 ± 15.97	67.34 ± 2.43	72.19 ± 9.02	86.78 ± 14.58
10	3j	Н	Н	5 2 NH	58.07 ± 15.07	>100	>100	>100
11	3k	Н	Н	2 2	38.89 ± 2.89	17.04 ± 4.18	15.43 ± 3.54	$40.50 \pm 8.04$
12	31	Н	Н	-s- -s- N- N- N- N- N- N- N- N- N- N	>100	>100	>100	>100
13	3m	Н	Н		>100	>100	>100	>100
14	3n	Н	Н		22.76 ± 4.24	24.10 ± 8.26	28.12 ± 5.13	15.84 ± 4.69
15	30	CN	Н		>100	>100	>100	>100
16	3р	CN	Н	NH	>100	>100	>100	>100
17	2a	_			44.54 ± 14.67	>200	>200	>200
							(	continued on next page)

Table 2 (continued)

Entry	Compd no.	Substituents				In vitro cytotoxicity $IC_{50}^{a}$ ( $\mu$ M)			
		R <sup>1</sup>	$\mathbb{R}^2$	R <sup>3</sup>	HepG2 <sup>b</sup>	BCG-823 <sup>b</sup>	BEL-7402 <sup>b</sup>	HL-7702 <sup>b</sup>	
18	2b	_			31.80 ± 9.09	87.33 ± 7.07	>200	>200	
19	2c	_			>200	>200	>200	>200	
20	Taxol <sup>c</sup>	_			$0.0064 \pm 0.0045$	0.0028 ± 0.0003	>25	0.0518 ± 0.0395	
21	5-FU <sup>d</sup>	-			86.87 ± 9.23	$67.65 \pm 16.91$	>500	95.36 ± 6.92	

<sup>a</sup>  $IC_{50}$ —compound concentration required to inhibit tumor cell proliferation by 50%.

<sup>b</sup> Abbreviations: HepG2-human hepatocellular liver carcinoma cell line; BCG-823-human gastric cancer cell line; BEL-7402-human hepatocellular carcinoma cell line; HL-7702-human normal liver cell line.

<sup>c</sup> Taxol, used as a positive control.

<sup>d</sup> 5-Fluorouracil, used as a positive control.



**Figure 3.** Morphology image of BEL-7402 cells treated with the compounds **3a**, **3f**, **3k**, **3n**, or Taxol for 48 h (400×). A is the control, the cells treated with DMSO 0.1% (v/v) as a vehicle control. B–F are treated with the compounds **3a**, **3f**, **3k**, **3n**, or Taxol at concentration of 10 µg/mL, respectively.

(>25  $\mu$ M) and 5-FU (>500  $\mu$ M). The most potent compounds **3a** and **3k** all contain two indole cores in single molecule, which may have appropriate hydrophobic–lipophilic interactions with proteins. In contrast, compound **3p** bearing two indole moieties almost lost inhibitory activity due to its poor solubility. By comprehensive consideration of the structure–activity relationships for these compounds, we also can conclude that the compounds bearing indole core (entries 1 and 11) or containing multisubstituted heterocycles (entries 6 and 14) exhibited higher activities. These interesting results might be used to develop novel lead scaffolds for potential anticancer agents.

Based on the aforementioned results, we can find that the compounds **3a**, **3f**, **3k** and **3n** also showed higher cytotoxic activities against the BEL-7402 cell line resistant to Taxol and 5-FU, which are very interesting. Furthermore, during the experiments, significant morphological changes of the cells have been observed under an inverted microscope. So we present the selective morphological image of the cells treated with compounds **3a**, **3f**, **3k** and **3n** at 10 µg/mL for 48 h in the following pictures (Fig. 3). Contrast with the presented images in Figure 3, we found that the quantities of the cells treated with compounds **3a**, **3f**, **3k** and **3n** were obviously decreased greatly for BEL-7402 cell lines. On the other hand, significant volume shrink and changing to globular and fragment of some BEL-7402 cells have also been observed. However, such morphological changes were not apparent in the control cells (Fig. 3A) and the cells treated with Taxol (Fig. 3F).

In conclusion, a series of multisubstituted indole–acrylonitrile hybrids have been designed and synthesized as potential cytotoxic agents. Bio-evaluation indicated that some of the newly synthesized compounds exhibited good cytotoxic activities. Especially, the most potent compounds **3a**, **3f**, **3k** and **3n** also showed higher cytotoxic activities against the BEL-7402 cell line resistant to Taxol and 5-FU, which is characterized by IC<sub>50</sub> values in the low  $\mu$ M range. These interesting results might be used to develop novel lead scaffold for potential anticancer agents.

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# Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl. 2014.03.011.

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