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Nicotinamide-based diamides derivatives as potential cytotoxic agents: synthesis and biological evaluation

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Abstract

A series of diamides derivatives containing nicotinamide unit were designed, synthesized and evaluated for their potential cytotoxic activities against human cancer cell lines. All the synthesized compounds were characterized using spectroscopic methods mainly including ^{1}H NMR, ^{13}C NMR and MS. The bio-evaluation results indicated that some of the obtained compounds (such as **4d**, **4h**, **4i**) exhibited good to moderate cytotoxic effects on lung cancer cell lines (NCI-H460, A549, and NCI-H1975), especially, compound **4d** exhibited the highly potential inhibitory activities against NCI-H460 cell line with the IC_{50} values of 4.07 ± 1.30 μg/mL, which might be developed as novel lead compounds for potential cytotoxic agents.

Keywords: Nicotinamide, Diamides, Synthesis, Lung cancer, Cytotoxic activity

Background

Lung cancer is the leading disease-related cause of deaths of human population, and which has also seriously threaten the health and life of humans for a long period [1–4]. Although substantial advances have been made in the systemic therapy of solid tumors over the past two decades, most patients obtain only modest benefit from treatment, whereas toxicity is common and the drug resistances rising from the tumor cell mutation challenges current medical therapies [5, 6]. So it is still an unmet medical need to development new drugs having broad therapeutic index (risk/efficacy).

Nicotinamide (Fig. 1) is a widely used vitamin [7], and some researches also demonstrated that it might reduce the risk of nonmelanoma skin cancers, and also effective to treat bullous pemphigoid [8]. As a special class of heterocyclic compounds, nicotinamide can be used as accessory reagents for chemotherapy and radiation therapy, and so which received significant attentions for their interesting biological activities. Recently, many nicotinamide derivatives have been reported for their broad pharmacological properties such as anticancer [9–11], anti-angiogenic [9], and antinociceptive effect [12] etc. Meanwhile, some nicotinamide derivatives have also been developed as agrochemicals for their insecticidal, herbicidal, and fungicidal activities [13–18]. In addition, many diamides derivatives have been investigated for their wide range of pharmacological activities including antitumor [19, 20], anti-inflammatory activities, Factor Xa inhibitors [21], CCK1 receptor antagonists [22], and insecticidal and fungicidal activities [23–25] etc., and which all demonstrated this diamide scaffold might be an important pharmacophore for drug discovery. The diverse bioactivity of this class of compounds urges us to construct a series of novel structural variants of diamides derivatives.

Thus, based on the aforementioned description, this work focused on the design, convenient synthesis, and cytotoxic evaluation of a series of novel nicotinamide-based diamides derivatives based on pharmacophores hybridization. The nicotinamide and diamide scaffolds have been integrated in one molecule as shown in Fig. 2, and the potential anticancer effects of these prepared compounds were screened against three lung cancer cell lines (NCI-H460, A549, NCI-H1975) and two normal

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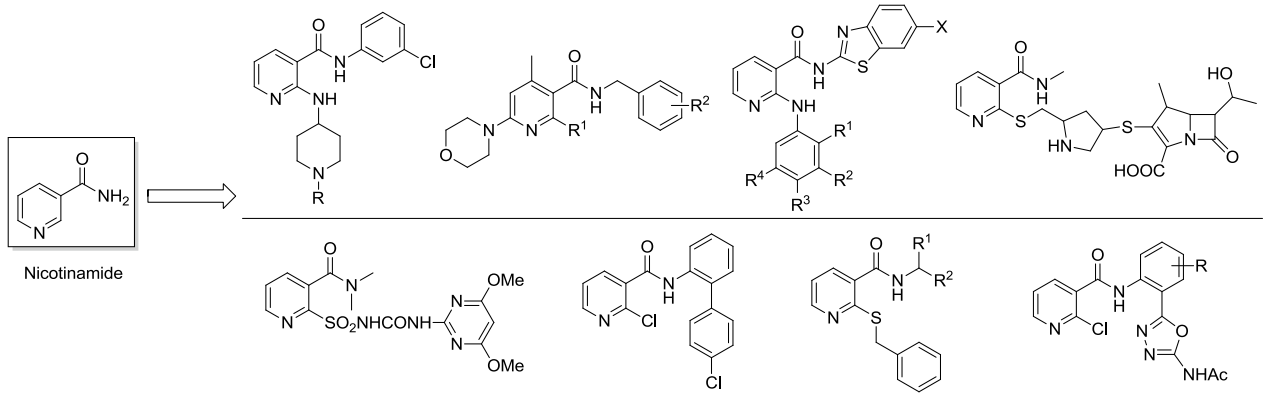


Fig. 1 Structures of nicotinamide and its derivatives

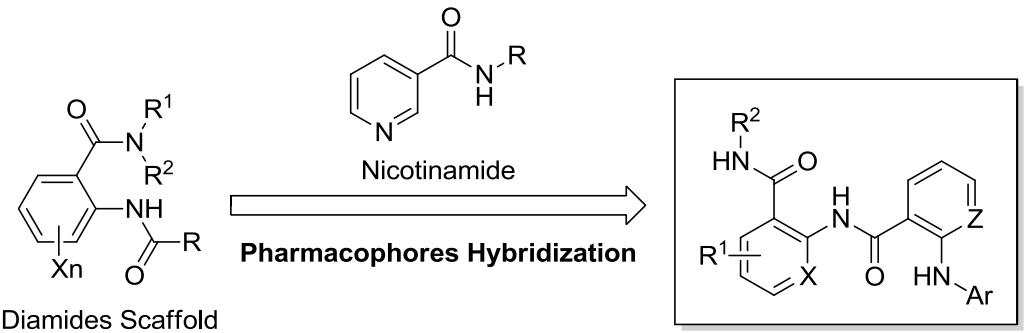


Fig. 2 Design strategy of nicotinamide-based diamides derivatives

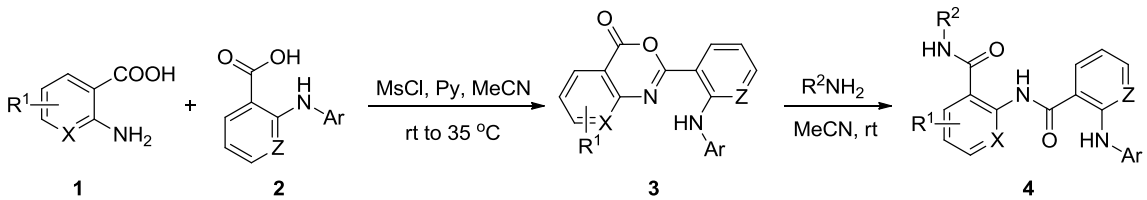
cells (HL-7702, MDCK). The results indicated some of the target molecules might be not a target-specific agent, may behave as a new lead compounds for highly potential cytotoxic agents.

Results and discussion

Chemistry

The general procedures for the preparation of these novel nicotinamide-based diamides derivatives **4a–k** and diamides **4l–o** are outlined in Scheme 1.

The key building blocks ortho-amino aryl acid **1** and **2** were selected as starting materials, and which could be conveniently transferred to the corresponding oxazinone heterocyclic intermediates **3** via a classical heterocyclization reaction [13, 20, 23–25] with simple procedures. Then these oxazinones were treated with various amines resulting in the target nicotinamide-based diamides derivatives **4a–k** via nucleophilic substitution reaction. Furthermore, for a few comparative activity measurements of compounds with no nicotinamide moiety, some similar diamide derivatives (where X = Z = C) as shown in Scheme 1 have also been synthesized using the aforementioned similar method. All title compounds gave satisfactory chemical analyses, and the chemical structures



Scheme 1 Synthesis of nicotinamide-based diamides derivatives

of the synthesized compounds were summarized in the part of experimental, and the typical ^{1}H NMR spectra analyses for compound **4a** have been shown in Fig. 3.

Bioassay

All newly prepared nicotinamide-based diamides derivatives **4a–k** and several similar diamides **4l–o** were evaluated for their in vitro cytotoxic effects against NCI-H460 (Human large cell lung cancer cell line), A549 (Human lung cancer cell line), NCI-H1975 (Human lung cancer cell line), HL-7702 (Human normal liver cells), and MDCK (Madin-Darby canine kidney cells) cell lines by the modified MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay [26, 27] using 5-FU (5-Fluorouracil) as a positive control. The preliminary results were summarized in Fig. 4.

From the Fig. 4, we can find that some of the nicotinamide-based diamides derivatives indicated moderate to good inhibition activities against these three human lung cancer cell lines. Notably, the compounds **4c**, **4d**, **4g**, **4h**, and **4i** exhibited significant inhibitory activities against all tested cell lines at the concentration of 40 μg/mL. Also, it is interesting to note that compounds **4e**, **4k** and **4l** presented selective cytotoxicity for A549 cell line, and the similar diamide **4m** exhibited good inhibition activities for all tested cell lines.

According to the preliminary bioassay results, some of the title molecules have been demonstrated to exhibit good inhibitory activities against all three lung cancer cell lines, so in order to further explore the potential activities, the IC_{50} values were investigated based on the above cell-based method. The in vitro activities expressed as IC_{50} values for these compounds are described in Table 1.

As shown in Table 1, the results further demonstrated that some of the synthesized nicotinamide-based diamides derivatives (**4d**, **4g**, **4h**, **4i**) exhibited higher inhibition activities compared to the control 5-FU under the same conditions. As indicated in Table 1, compound **4d** containing an alpha-aminoketone unit showed the strongest inhibitory effect on all three cell lines, with an IC_{50} values of 4.07 ± 1.30 (NCI-H460), 13.09 ± 2.45 (A549) and 12.82 ± 1.59 (NCI-H1975) μg/mL,

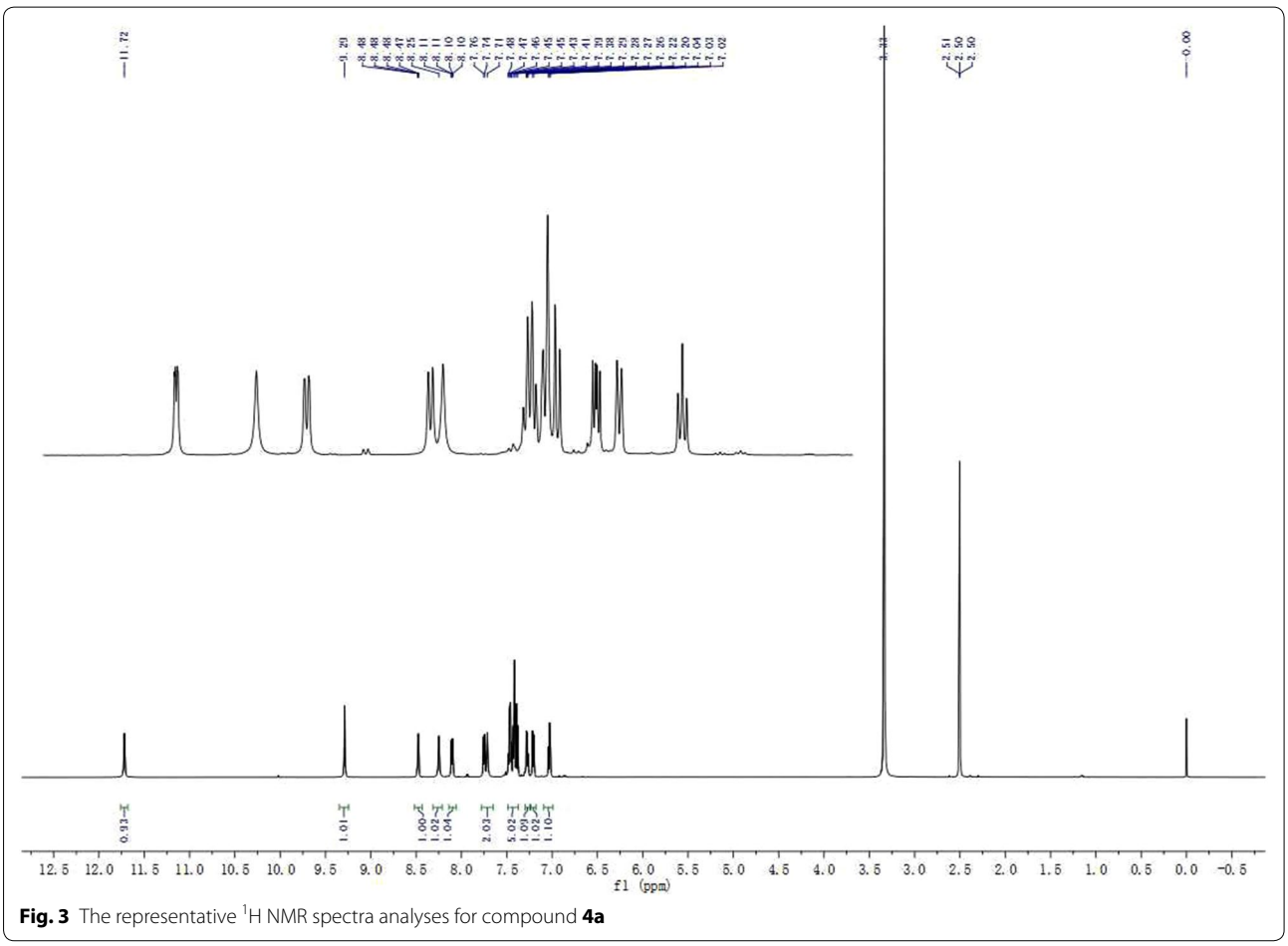


Fig. 3 The representative ^{1}H NMR spectra analyses for compound **4a**

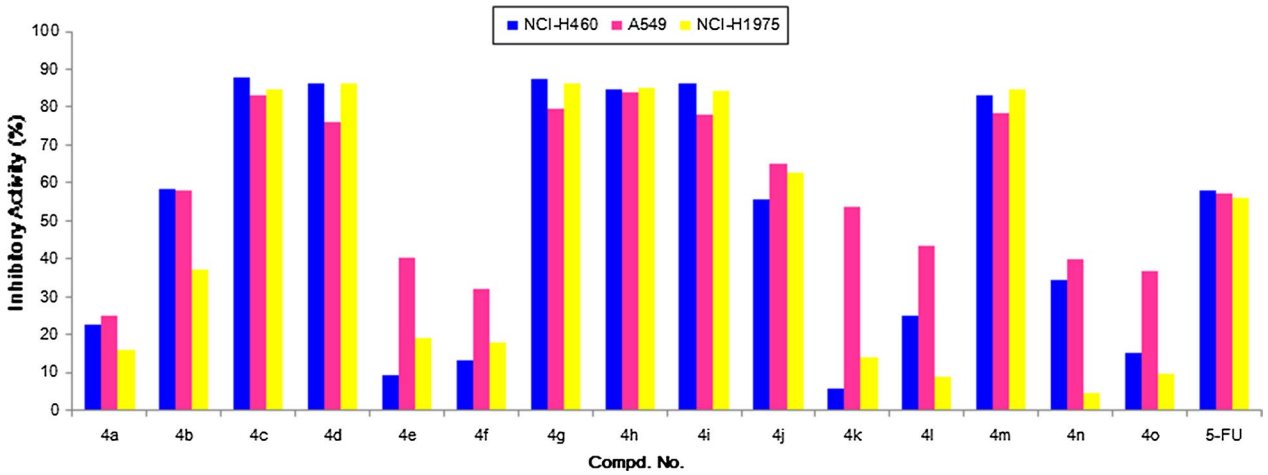


Fig. 4 Cytotoxic activities of compounds **4a–k** and **4l–o** at 40 μg/mL. *NCI-H460* human large cell lung cancer cell line, *A549* human lung cancer cell line, *NCI-H1975* human lung cancer cell line, *5-FU* 5-fluorouracil, used as a positive control

respectively. However, the corresponding compound **4c** with an alpha-aminoalcohol moiety indicated lower inhibition activities. Meanwhile, the two groups of structurally similar compounds (**4a** and **4g**, **4b** and **4h**) exhibited significant activity differences, and the compounds **4g** and **4h** containing two nicotinamide units present good activities. Especially, we also can find that compounds **4g** presented obviously selective cytotoxic activities against the NCI-H460 and NCI-H1975 cell lines except A549 cell line. In addition, most of the similar diamides without nicotinamide moiety (entries 12–15) lost the inhibitory activity, however, compound **4m** exhibited good inhibition activities, which deserve the further studies. Furthermore, the cytotoxic effects on non-cancer cells HL-7702 and MDCK for these compounds have also been tested in our experiment. The results in Table 1 can further confirm that the cytotoxic effect of compound **4d** is more specific to cancer cells NCI-H460 compared to the control 5-FU, and the cytotoxic activities against NCI-H460 and normal cells (HL-7702 and MDCK) demonstrated that compound **4d** exhibited good selective cytotoxicity between NCI-H460 ($IC_{50} = 4.07 \pm 1.30$ μg/mL) and normal cells (HL-7702, $IC_{50} = 26.87 \pm 0.95$ μg/mL; MDCK, $IC_{50} = 13.45 \pm 0.29$ μg/mL). These interesting finds may provide some useful information for developing potential cytotoxicity agents.

In addition, the dose-response analysis of cell growth inhibition activities for representation compounds **4d**, **4h**, **4m** and 5-FU have been displayed in Fig. 5, which identified that these compounds exhibited obvious cytotoxic effects on NCI-H460, A549, and NCI-H1975 cell lines in a concentration-dependent manner.

Experimental

Instrumentation and chemicals

^{1}H-NMR spectra were recorded on a Bruker spectrometer at 600 (Bruker, Bremen, Germany) with DMSO-d_6 as the solvent and TMS as the internal standard; ^{13}C-NMR spectra were recorded on a Bruker spectrometer at 150 MHz with DMSO-d_6 as the solvent. Chemical shifts are reported in δ (parts per million) values, and coupling constants ^{n}J are reported in Hz. Mass spectra were performed on a Waters ACQUITY UPLC® H-CLASS PDA (Waters®, Milford, MA, USA) instrument. Analytical thin-layer chromatography (TLC) was carried out on precoated plates, and spots were visualized with ultraviolet light. All chemicals or reagents used for syntheses were commercially available, were of AR grade, and were used as received.

General synthetic procedures for the key intermediates 3

The key intermediates **3** could be conveniently prepared by the coupling of substituted anthranilic acids with the N-substituted ortho-amino aryl acid according to the modified method. The general procedures are as follows: To a solution of substituted anthranilic acid **1** (1 mmol) and N-substituted ortho-amino aryl acid **2** (1 mmol) in 10 mL anhydrous acetonitrile was added pyridine (3 mmol), and then the reaction mixture was cooled to 0 °C. Where after, methanesulfonyl chloride (1.5 mmol) was added dropwise to the reaction mixture over 15–20 min. After addition, the reaction mixture was then allowed to warm to room temperature and stirred for additional hours, and which was detected by thin-layer chromatography. After completion of the reaction,

Table 1 In vitro cytotoxic activities of the compounds against human cancer cell lines and normal cells

Entry	Compd no.	Substituents					In vitro cytotoxicity IC_{50}^{a} (μg/mL)				
		R^1	R^2	X	Z	Ar	NCI-H460	A549	NCI-H1975	HL-7702	MDCK
1	**4a**	H	H	N	CH	3-CF_3Ph	> 40	> 40	> 40	> 40	> 40
2	**4b**	H	CH_3	N	CH	3-CF_3Ph	36.68 ± 2.80	> 40	> 40	> 40	> 40
3	**4c**	H	$CH_3CH(OH)CH_2$	N	CH	3-CF_3Ph	20.63 ± 1.37	26.31 ± 3.22	27.25 ± 2.76	19.73 ± 0.45	16.98 ± 0.84
4	**4d**	H	CH_3COCH_2	N	CH	3-CF_3Ph	4.07 ± 1.30	13.09 ± 2.45	12.82 ± 1.59	26.87 ± 0.95	13.45 ± 0.29
5	**4e**	3-CH_3-5-Cl	H	C	N	3-CF_3Ph	> 40	> 40	> 40	> 40	> 40
6	**4f**	3-CH_3-5-Cl	CH_3	C	N	3-CF_3Ph	> 40	> 40	> 40	> 40	> 40
7	**4g**	H	H	N	N	3-CF_3Ph	9.17 ± 2.02	20.12 ± 0.48	10.85 ± 2.22	9.86 ± 0.34	13.04 ± 1.27
8	**4h**	H	CH_3	N	N	3-CF_3Ph	9.31 ± 2.66	17.18 ± 3.40	12.44 ± 2.52	11.62 ± 2.08	11.06 ± 0.24
9	**4i**	H	$CH_3CH(OH)CH_2$	N	N	3-CF_3Ph	13.08 ± 4.49	16.53 ± 3.53	9.15 ± 1.64	14.51 ± 2.19	11.62 ± 0.06
10	**4j**	4,5-(CH=CH–CH=CH)–	H	CH	N	3-CF_3Ph	38.44 ± 3.95	24.79 ± 3.00	25.20 ± 3.35	18.09 ± 1.63	14.30 ± 0.66
11	**4k**	4,5-(CH=CH–CH=CH)–	CH_3	CH	N	3-CF_3Ph	> 40	35.30 ± 2.19	> 40	> 40	> 40
12	**4l**	3-CH_3-5-Cl	H	C	CH	3-CF_3Ph	> 40	> 40	> 40	> 40	> 40
13	**4m**	3-CH_3-5-Cl	CH_3	C	CH	3-CF_3Ph	6.85 ± 0.23	6.77 ± 0.86	10.83 ± 1.02	21.44 ± 1.79	21.25 ± 3.59
14	**4n**	4,5-(CH=CH–CH=CH)–	H	CH	CH	3-CF_3Ph	> 40	> 40	> 40	> 40	14.44 ± 0.01
15	**4o**	4,5-(CH=CH–CH=CH)–	CH_3	CH	CH	3-CF_3Ph	> 40	> 40	> 40	> 40	> 40
16	**5-FU**[b]	–	–	–	–	–	8.02 ± 2.35	37.51 ± 3.25	24.75 ± 5.80	11.35 ± 1.67	8.65 ± 0.81

NCI-H460 human large cell lung cancer cell line, *A549* human lung cancer cell line, *NCI-H1975* human lung cancer cell line, *HL-7702* human normal liver cells, *MDCK* Madin-Darby canine kidney cells

[a] IC_{50} compound concentration required to inhibit tumor cell proliferation by 50%

[b] 5-Fluorouracil, used as a positive control

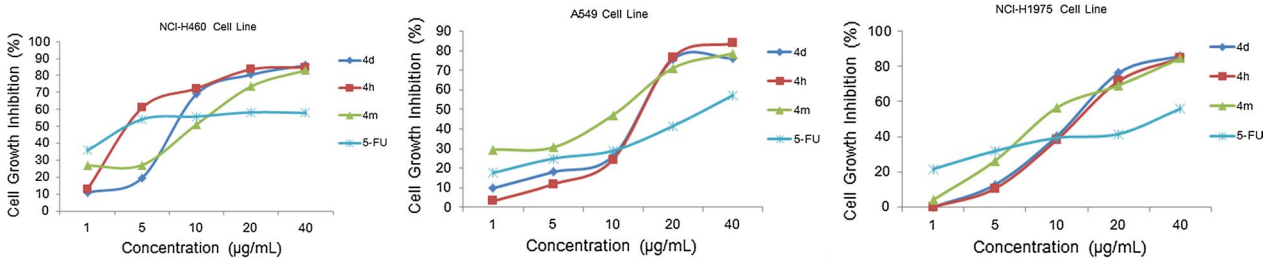


Fig. 5 Dose-response analysis of cell growth inhibition activity for representative compounds **4d**, **4h**, **4m** and **5-FU** (positive control) against NCI-H460 (left), A549 (middle) and NCI-H1975 (right) cell lines

the mixture was quenched by the addition of water and was stirred for 20 min. The suspended solid was collected by filtration and washed with water to afford the key intermediates **3** as pale yellow solids, which can be used directly for the next reaction without further purification.

General synthetic procedures for target compounds 4a–k

The typical process for these nicotinamide-based diamides derivatives is shown as following: To a solution of newly prepared intermediates **3** (1 mmol) in 8 mL of anhydrous acetonitrile was added the corresponding substituted amines (5 mmol) under nitrogen atmosphere. The clear solution was stirred at room temperature for several hours after which time TLC showed no remaining starting material. The reaction mixture was then concentrated in vacuum to remove the partial solvent, and then the residue was filtered and purified by silica gel column chromatography or recrystallization to give the target molecules. Their physico-chemical properties and the spectra data are as follows:

2-(2-((3-(Trifluoromethyl)phenyl)amino)benzamido)nicotinamide 4a

Yellowish powder, ^{1}H NMR (600 MHz, DMSO-d_6): δ = 11.72 (s, 1H), 9.29 (s, 1H), 8.48 (q, J = 7.2 Hz, 1H), 8.25 (bs, 1H), 8.11 (q, J = 6 Hz, 1H), 7.75 (d, J = 8 Hz, 1H), 7.71 (bs, 1H), 7.48–7.38 (m, 5H), 7.28 (q, J = 8.4 Hz, 1H), 7.21 (d, J = 8.4 Hz, 1H), 7.03 (t, J = 6 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 169.59, 167.14, 150.64, 150.39, 143.60, 142.93, 137.89, 132.82, 130.84, 130.42, 129.79, 125.52, 123.72, 122.87, 122.13, 121.10, 120.72, 120.11, 117.44, 114.65; MS (ESI) *m/z* 423.3 $(M + Na)^+$, calcd. for $C_{20}H_{15}F_3N_4O_2$ m/z = 400.1.

N-Methyl-2-(2-((3-(trifluoromethyl)phenyl)amino)benzamido)nicotinamide 4b

White powder, ^{1}H NMR (600 MHz, DMSO-d_6): δ = 12.25 (s, 1H), 9.18 (s, 1H), 8.75 (s, 1H), 8.47 (dd, J = 4.8 Hz, 1H), 8.03 (d, J = 7.8 Hz, 1H), 7.94 (d, J = 7.2 Hz, 1H), 7.74 (d, J = 7.8 Hz, 1H), 7.35–7.10 (m, 6H), 7.06–7.03 (m, 1H), 2.87 (d, J = 4.8 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 167.80, 150.23, 149.96, 144.35, 143.73, 142.59, 137.60, 132.67, 132.49, 130.89, 130.82, 129.78, 123.38, 122.34, 121.81, 120.88, 120.26, 118.67, 117.66, 114.59, 26.68; MS (ESI) *m/z* 437.4 $(M + Na)^+$, calcd. for $C_{21}H_{17}F_3N_4O_2$ m/z = 414.1.

N-(2-Hydroxypropyl)-2-(2-((3-(trifluoromethyl)phenyl)amino)benzamido)nicotinamide 4c

Yellowish powder, ^{1}H NMR (600 MHz, DMSO-d_6): δ = 11.40 (s, 1H), 9.58 (s, 1H), 8.66 (s, 1H), 8.48 (dd, J = 4.8 Hz, 1H), 8.07 (d, J = 7.8 Hz, 1H), 7.75 (d, J = 7.2 Hz, 1H), 7.50–7.02 (m, 9H), 3.80–3.75 (m, 1H), 3.20–3.08 (m, 2H), 1.02 (d, J = 6 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.87, 152.22, 149.32, 143.61, 142.29, 141.58, 135.32, 132.75, 132.21, 130.84, 130.71, 129.34, 122.88, 122.03, 121.82, 120.71, 118.81, 118.12, 117.39, 115.06, 65.34, 47.60, 21.47; MS (ESI) *m/z* 481.3 $(M + Na)^+$, calcd. for $C_{23}H_{21}F_3N_4O_3$ m/z = 458.2.

N-(2-Oxopropyl)-2-(2-((3-(trifluoromethyl)phenyl)amino)benzamido)nicotinamide 4d

Light brown powder, ^{1}H NMR (600 MHz, DMSO-d_6): δ = 11.18 (s, 1H), 9.78 (s, 1H), 8.74 (s, 1H), 8.58 (dd, J = 4.8 Hz, 1H), 8.12 (d, J = 7.8 Hz, 1H), 7.71 (d, J = 7.2 Hz, 1H), 7.58–7.12 (m, 8H), 4.12 (s, 2H), 2.06 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 169.63, 167.68, 156.76, 150.82, 145.13, 144.16, 142.42, 136.29, 134.15, 133.48, 131.32, 130.98, 129.64, 123.26, 122.86, 122.02, 120.96, 119.24, 118.78, 117.67, 114.25, 58.76, 25.35; MS (ESI) *m/z* 479.4 $(M + Na)^+$, calcd. for $C_{23}H_{19}F_3N_4O_3$ m/z = 456.1.

N-(2-Carbamoyl-4-chloro-6-methylphenyl)-2-((3-(trifluoromethyl)phenyl)amino)nicotinamide 4e

Yellow powder, ^{1}H NMR (600 MHz, DMSO-d_6): δ = 10.45 (s, 1H), 10.30 (s, 1H), 8.41 (q, J = 7.2 Hz, 1H), 8.31 (s, 1H), 8.20 (q, J = 6 Hz, 1H), 7.97 (s, 1H), 7.88 (d,

$J = 6$ Hz, 1H), 7.57–7.48 (m, 4H), 7.29 (d, $J = 7.2$ Hz, 1H), 7.01 (q, $J = 7.2$ Hz, 1H), 2.26 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.88, 166.89, 164.67, 154.03, 150.85, 147.17, 141.62, 139.07, 138.19, 133.14, 131.93, 131.20, 130.19, 126.15, 123.30, 118.94, 115.05, 114.37, 114.07, 106.68, 18.26; MS (ESI) *m/z* 471.2 $(M + Na)^+$, calcd. for $C_{21}H_{16}ClF_3N_4O_2$ m/z = 448.1.

N-(4-Chloro-2-methyl-6-(methylcarbamoyl) phenyl)-2-((3-(trifluoromethyl)phenyl)amino)nicotinamide 4f

White powder, ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 10.45$ (s, 1H), 10.16 (s, 1H), 8.68 (q, $J = 7.2$ Hz, 1H), 8.29 (s, 1H), 8.24 (q, $J = 7.2$ Hz, 1H), 7.92 (s, 1H), 7.59 (d, $J = 6$ Hz, 1H), 7.42–7.36 (m, 4H), 7.28 (d, $J = 7.2$ Hz, 1H), 7.01 (q, $J = 7.2$ Hz, 1H), 2.68 (d, $J = 4.8$ Hz, 3H), 2.27 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): $\delta = 171.22$, 167.32, 166.90, 153.99, 150.66, 147.82, 141.67, 139.01, 138.11, 136.01, 133.38, 133.25, 131.77, 130.94, 130.78, 130.23, 128.95, 126.08, 124.79, 123.11, 117.26, 114.37, 24.71, 17.82; MS (ESI) *m/z* 485.2 $(M + Na)^+$, calcd. for $C_{22}H_{18}ClF_3N_4O_2$ m/z = 462.1.

N-(3-Carbamoylpyridin-2-yl)-2-((3-(trifluoromethyl)phenyl) amino)nicotinamide 4g

Yellow powder, ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 11.26$ (s, 1H), 9.24 (s, 1H), 8.57 (q, $J = 7.2$ Hz, 1H), 8.48 (s, 1H), 8.37 (q, $J = 7.2$ Hz, 1H), 7.96 (d, $J = 7.2$ Hz, 1H), 7.75–6.92 (m, 8H); ^{13}C NMR (150 MHz, DMSO-d_6): $\delta = 169.83$, 168.78, 155.46, 153.23, 148.78, 144.02, 142.15, 138.33, 133.54, 131.62, 129.42, 127.55, 126.82, 123.67, 122.18, 121.37, 120.78, 118.46, 115.53; MS (ESI) *m/z* 424.5 $(M + Na)^+$, calcd. for $C_{19}H_{14}F_3N_5O_2$ m/z = 401.1.

N-Methyl-2-(2-((3-(trifluoromethyl)phenyl)amino)nicotinamido)nicotinamide 4h

Yellowish powder, ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 11.22$ (s, 1H), 9.12 (s, 1H), 8.61 (q, $J = 7.2$ Hz, 1H), 8.43 (s, 1H), 8.42 (q, $J = 7.2$ Hz, 1H), 7.84 (d, $J = 7.2$ Hz, 1H), 7.84–6.98 (m, 7H), 2.83 (d, $J = 4.8$ Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): $\delta = 169.54$, 167.42, 156.65, 152.44, 149.56, 145.23, 143.23, 139.76, 133.86, 132.03, 128.62, 127.76, 127.02, 124.21, 122.76, 121.32, 120.22, 119.38, 114.17, 25.43; MS (ESI) *m/z* 438.4 $(M + Na)^+$, calcd. for $C_{20}H_{16}F_3N_5O_2$ m/z = 415.1.

N-(2-Hydroxypropyl)-2-(2-((3-(trifluoromethyl)phenyl)amino) nicotinamido)nicotinamide 4i

Yellowish powder, ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 11.34$ (s, 1H), 9.48 (s, 1H), 8.65 (q, $J = 7.2$ Hz, 1H), 8.43 (s, 1H), 8.32 (q, $J = 7.2$ Hz, 1H), 8.04 (d, $J = 7.2$ Hz, 1H), 7.89–6.98 (m, 8H), 3.86–3.77 (m, 1H), 3.28–3.14 (m, 2H), 1.08 (d, $J = 6$ Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 170.75, 156.35, 151.62, 144.72, 143.86, 141.92, 138.81, 133.42, 132.78, 131.65, 131.24, 129.86, 124.26, 122.77, 122.13, 118.94, 118.58, 116.37, 114.48, 63.38, 45.84, 21.22; MS (ESI) *m/z* 482.5 $(M + Na)^+$, calcd. for $C_{22}H_{20}F_3N_5O_3$ m/z = 459.2.

N-(3-Carbamoylnaphthalen-2-yl)-2-((3-(trifluoromethyl) phenyl)amino)nicotinamide 4j

Yellowish powder, ^{1}H NMR (600 MHz, DMSO-d_6): δ = 12.81 (s, 1H), 10.82 (s, 1H), 8.99 (s, 1H), 8.66 (s, 1H), 8.54 (s, 1H), 8.47 (q, $J = 6$ Hz, 1H), 8.30 (s, 1H), 8.21 (q, $J = 6$ Hz, 1H), 8.00–7.92 (m, 4H), 7.66–7.63 (m, 1H), 7.56–7.54 (m, 2H), 7.33 (d, $J = 7.2$ Hz, 1H), 7.08 (q, $J = 7.2$ Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 171.64, 166.24, 154.66, 151.65, 141.33, 137.07, 135.55, 134.93, 130.36, 130.26, 129.20, 129.07, 127.74, 126.40, 125.84, 123.77, 123.58, 121.66, 118.20, 115.97, 115.39, 113.12; MS (ESI) *m/z* 473.2 $(M + Na)^+$, calcd. for $C_{24}H_{17}F_3N_4O_2$ m/z = 450.1.

N-(3-(Methylcarbamoyl)naphtha-len-2-yl)-2-((3-(trifluoromethyl)phenyl)amino)nicotinamide 4k

Yellowish powder, ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 12.50$ (s, 1H), 10.76 (s, 1H), 9.11 (d, $J = 3.2$ Hz, 1H), 8.94 (s, 1H), 8.47 (q, $J = 6$ Hz, 1H), 8.43 (s, 1H), 8.29 (s, 1H), 8.21 (q, $J = 6$ Hz, 1H), 7.97–7.92 (m, 3H), 7.65–7.63 (m, 1H), 7.56–7.53 (m, 2H), 7.33 (d, $J = 7.2$ Hz, 1H), 7.11 (q, $J = 7.2$ Hz, 1H), 2.87 (d, $J = 4.8$ Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 169.53, 166.21, 154.56, 151.59, 141.37, 137.10, 135.02, 134.69, 130.25, 129.59, 129.20, 129.04, 128.96, 127.77, 123.71, 122.76, 118.55, 118.41, 115.94, 115.49, 113.29, 26.95; MS (ESI) *m/z* 487.2 $(M + Na)^+$, calcd. for $C_{25}H_{19}F_3N_4O_2$ m/z = 464.1.

General synthetic procedures for compounds 4l–o

In order to compare the activity, four similar diamides derivatives with no nicotinamide moiety have also been obtained according to the aforementioned method for target compounds. Their physico-chemical properties and the spectra data are as follows:

5-Chloro-3-methyl-2-(2-((3-(trifluoromethyl)phenyl)amino) benzamido)benzamide 4l

Yellowish powder, ^{1}H NMR (600 MHz, DMSO-d_6): δ = 10.12 (s, 1H), 9.28 (s, 1H), 7.94 (s, 1H), 7.80 (d, $J = 7.2$ Hz, 1H), 7.56 (s, 2H), 7.51–7.38 (m, 6H), 7.22 (d, $J = 7.2$ Hz, 1H), 7.02 (t, $J = 7.2$ Hz, 1H), 2.20 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.92, 167.57, 143.70, 142.61, 139.09, 135.40, 133.55, 132.53, 131.89, 130.88, 130.68, 129.98, 129.14, 126.03, 124.22, 122.71, 122.12, 120.64, 117.18, 114.49, 18.39; MS (ESI) *m/z* 470.2 $(M + Na)^+$, calcd. for $C_{22}H_{17}ClF_3N_3O_2$ m/z = 447.1.

5-Chloro-N,3-dimethyl-2-(2-((3-(trifluoromethyl)phenyl) amino)benzamido)benzamide 4m

White powder, ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 10.13$ (s, 1H), 9.11 (s, 1H), 8.43 (d, $J = 4.8$ Hz, 1H), 7.74 (d, $J = 7.8$ Hz, 1H), 7.51–7.39 (m, 7H), 7.21 (d, $J = 7.2$ Hz, 1H), 7.03 (t, $J = 7.2$ Hz, 1H), 2.64 (d, $J = 4.8$ Hz, 3H), 2.22 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): $\delta = 167.57$, 167.48, 143.80, 142.20, 139.02, 135.63, 133.42, 132.29, 131.79, 130.86, 130.73, 129.80, 125.88, 123.51, 121.76, 120.78, 117.33, 114.50, 26.58, 18.35; MS (ESI) *m/z* 484.3 $(M + Na)^+$, calcd. for $C_{23}H_{19}ClF_3N_3O_2$ m/z = 461.1.

3-(2-((3-(Trifluoromethyl)phenyl)amino)benzamido)-2-naphthamide 4n

Light brown powder, ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 12.32$ (s, 1H), 9.45 (s, 1H), 9.06 (d, $J = 4.8$ Hz, 1H), 8.92 (s, 1H), 8.47 (s, 1H), 7.96 (q, $J = 7.2$ Hz, 2H), 7.84 (dd, $J = 8.4$ Hz, 1H), 7.67–7.54 (m, 1H), 7.48–7.36 (m, 7H), 7.21 (d, $J = 7.8$ Hz, 1H), 7.11–7.05 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): $\delta = 169.12$, 165.55, 144.15, 142.92, 136.82, 134.47, 133.18, 131.25, 130.48, 129.85, 129.24, 128.74, 128.32, 127.75, 125.47, 124.18, 122.92, 121.74, 121.38, 121.07, 118.82, 118.05, 114.51, 99.62; MS (ESI) *m/z* 472.3 $(M + Na)^+$, calcd. for $C_{25}H_{18}F_3N_3O_2$ m/z = 449.1.

N-Methyl-3-(2-((3-(trifluoromethyl)phenyl)amino) benzamido)-2-naphthamide 4o

White powder, ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 12.15$ (s, 1H), 9.35 (s, 1H), 9.01 (d, $J = 4.8$ Hz, 1H), 8.96 (s, 1H), 8.35 (s, 1H), 7.90 (q, $J = 7.8$ Hz, 2H), 7.80 (dd, $J = 9.6$ Hz, 1H), 7.62–7.59 (m, 1H), 7.52–7.40 (m, 6H), 7.19 (d, $J = 7.2$ Hz, 1H), 7.14–7.11 (m, 1H), 2.80 (d, $J = 4.2$ Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): $\delta = 169.34$, 166.86, 143.91, 142.71, 135.31, 134.69, 132.99, 130.79, 129.37, 129.17, 128.91, 128.84, 127.66, 126.18, 123.52, 122.66, 121.93, 121.51, 121.29, 118.75, 117.71, 114.62, 99.95, 26.79; MS (ESI) *m/z* 486.3 $(M + Na)^+$, calcd. for $C_{26}H_{20}F_3N_3O_2$ m/z = 463.2.

In vitro cytotoxicity assays

The in vitro cytotoxicity of the synthesized compounds against different human lung cancer cell lines (NCI-H460, A549, NCI-H1975) and normal cells (HL-7702 and MDCK) were measured with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. All the data of the experiment were analyzed with SPSS software, and the 50% inhibitory concentrations (IC_{50}) of each compound for the different cell lines were determined. A control was run for each test, and all assays were performed in triplicate on three independent experiments, and measurement data were expressed as the mean ± SD.

Conclusion

In summary, a series of diamides derivatives based on nicotinamide scaffold have been conveniently prepared and evaluated as potential cytotoxic agents. The bioassay indicated that some of these newly synthesized compounds exhibited good cytotoxic activities. Especially, the most potent compounds **4d** and **4h** exhibited higher cytotoxic activities against the tested lung cancer cell lines as compared with 5-FU in vitro, and these interesting results might be used to develop novel lead molecules for potential anticancer agents.

Authors' contributions

SK initiated the idea and performed the chemical synthesis and characterization experiments; LS and MP performed the biological assays; MP, LS, and SK analyzed the results, and SK drafted the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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