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Synthesis, biological evaluation and molecular docking studies of novel 2-(2-cyanophenyl)-*N*-phenylacetamide derivatives

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Abstract A series of novel 2-(2-cyanophenyl)-*N*-phenylacetamide derivatives **3(a-u)** were designed and synthesized via selective amidation of methyl-2-(2-cyanophenyl)acetates over amidine formation by using $AlMe_3$ as catalyst in good yields. All the newly synthesized derivatives were well characterized by ^1H NMR, ^{13}C NMR, FTIR and HRMS spectral techniques. All the synthesized title compounds were evaluated for their in vitro anticancer activity against three cancer cell lines. Among all compounds, **3i** (IC_{50} = 1.20 μM, IC_{50} = 1.10 μM), **3j** (IC_{50} = 0.11 μM, IC_{50} = 0.18 μM), **3o** (IC_{50} = 0.98 μM, IC_{50} = 2.76 μM) showed excellent inhibitory activity than the standard Etoposide (IC_{50} = 2.11 μM, IC_{50} = 3.08 μM) against MCF-7 and A-549 cell lines, respectively. Docking analysis of all the compounds with the *human* topoisomerase II revealed that the compound **3j** fitted well in the active site pocket, showing the best docking score of 158.072 kcal/mol.

Keywords *N*-phenylacetamide · Trimethylaluminum · Docking studies · Anticancer activity

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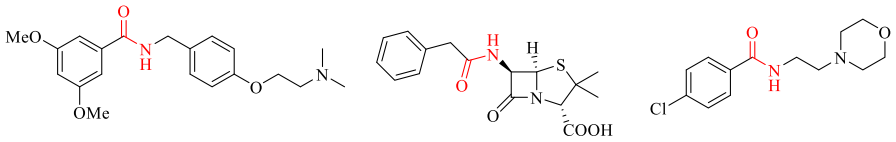
Introduction

Cancer is one of the most crucial health concerns and forms a critical originator of human death [1]. The number of new cases of cancer is 454.8 per 100,000 people per year. The number of cancer deaths (cancer mortality) is 171.2 per 100,000 people per year. From past few decades to date, a plethora of researchers worldwide have made exceptional effort in the development of novel drugs in the world market, and the research is heading toward ultimately saving human lives. Nevertheless, the majority of drugs possess selectivity of action, and hence the treatment of certain types of tumor are yet challenging due to their aggressive nature, resistance to chemicals, malignant cell metastasis and the absence of drug selectivity [2]. Henceforth, the augmentations of new anticancer products that are effective and safer from the commercial materials are always in demand. With respect to a recently conducted survey on developing a new pharmacophore model in order to treat any disease or disorder, a complex of two pharmacophores in a single form is an effective approach in medicinal chemistry. This approach aids in the expedition of highly effective novel compounds.

In this regard, researchers worldwide are putting forth significant effort to discover novel compounds, which help to treat inflammation and cancer. Heterocyclic compounds were found to be crucial in drug design and development [3, 4]. During the past few years, many building blocks have been designed, developed and approved for a range of diseases. Among these, the amide linkage is one of the most fundamental and widespread chemical bonds, underlying the properties of a vast array of organic molecules, polymers, and materials, as well as a wide selection of bioactive natural products including proteins and peptides. Around 25% of amides are present in top-selling medicines and many other pharmaceuticals [5]. These are versatile intermediates for the synthesis of many pharmacologically important nitrogen and oxygen containing heterocyclics [6]. Some of the known drugs containing amide moiety are shown in Fig. 1. Despite the enormous importance of amides in organic chemistry, most of the established methods for the synthesis of amides are reported. In the literature number of recent reviews have covered metal-catalyzed amide bond formation [7, 8] and the use of more traditional coupling reagents [9]. In general, amides are prepared by the reaction of carboxylic acid derivatives with amines [10, 11]. Recently, rare earth metal halides and triflates have been found to be successful Lewis acids for promoting the amination of nitriles [12]. In 1977 Weinreb et al. [13] published $AlMe_3$ promoted amide synthesis from esters and amines. After that, the $AlMe_3$-mediated amide synthesis from esters has found many applications in organic synthesis [14]. These methods suffer from common limitations such as stoichiometric amounts of coupling reagents, poor atomic efficiency and formation of a large amount of byproducts [15–18]. To overcome these problems, novel amide bond forming reactions have been developed in recent years [18–22]. Herein we report a novel series of 2-(2-cyanophenyl)-*N*-phenylacetamide derivatives from methyl-2-(2-cyanophenyl)acetates by selective amidation using $AlMe_3$ as catalyst. All the newly synthesized derivatives were



Trimethobenzamide (Antiemetic) | Penicilline G (antibiotic) | Moclobemide (Antidepressant)

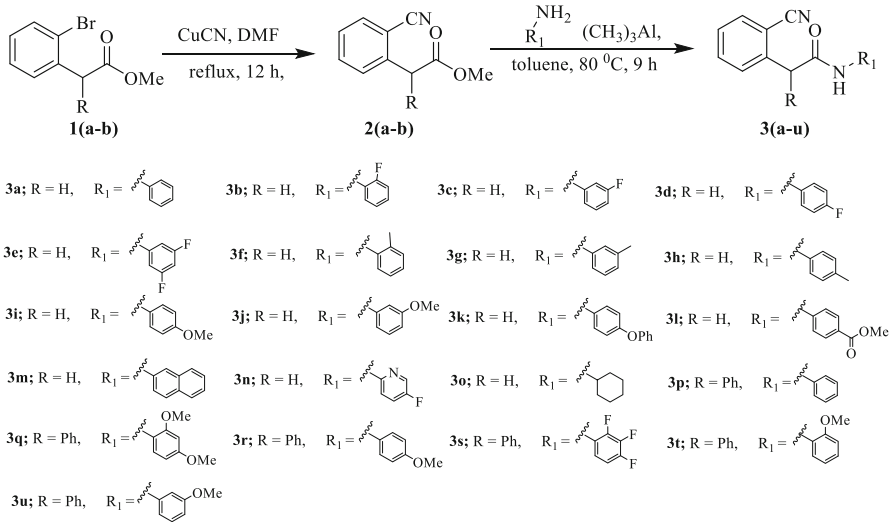
Fig. 1 Some of the known amide moiety containing drugs

evaluated for their in vitro anticancer activity and molecular docking studies were performed with *human* DNA topoisomerase II.

Results and discussions

Chemistry

Here we have developed Me_3Al promoted synthesis of 2-(2-cyanophenyl)-*N*-phenylacetamide derivatives. A series of 2-(2-cyanophenyl)-*N*-phenylacetamide derivatives **3(a-u)** were synthesized in two steps as shown in Scheme 1. In the first step, methyl-2-(2-cyanophenyl)acetate derivatives **2(a-b)** were obtained by the reaction of methyl-2-(2-bromophenyl)acetate derivatives with CuCN in DMF solvent stirring for 10–12 h [23]. In the next step, reaction between methyl-2-(2-cyanophenyl)acetate derivatives **2(a-b)** and various substituted amines in the presence of $AlMe_3$ as catalyst in toluene stirring at 80 °C for 9–10 h. The optimization of the reaction conditions is shown in Table 1. We started the reaction with methyl-2-(2-cyanophenyl)acetate with $AlCl_3$ as catalyst in neat condition. To our delight, 2-(2-cyanophenyl)-*N*-phenylacetamide product was observed in 20% yield (Table 1, Entry 1). Then we tried the reaction in different solvents viz., toluene and THF (tetrahydrofuran) resulting in an improved yield of 28% with toluene and 23% with THF solvents (Table 1, Entries 2–3). We continued our investigation to improve the yield. We also tried the reaction with other catalytic systems such as $FeCl_3$, $TiCl_4$, $Ti(iPrO)_4$, $SnCl_4$, $ZnCl_2$ and Me_3Al, out of which Me_3Al gave surprising results of amide compound 3a exclusively forming over amidine formation up to 80% (Table 1, Entries 4–15). Then we increased catalyst loading with 2.0 mmol resulting in no further remarkable improvement in yield (82%) (Table 1, Entry 16). Still, we tried to increase catalyst loading to 2.5 mmol, but no further improvement in yield was observed (Table 1, Entry 17). On the other hand, we observed that title compound 3a formed over amidine by increasing the amine to 2.0 mmol, but with no further improvement in yield (Table 1, Entry 18). Finally, we explored the reaction conditions as methyl-2-(2-cyanophenyl)acetate (1.0 mmol), aniline (1.5 mmol) and Me_3Al (1.5 mmol) in the toluene solvent system for the conversion reaction of 2-(2-cyanophenyl)-*N*-phenylacetamide derivatives. Based on our present investigation, the reaction proceeds through the formation of intermediate 1. The ester is activated by intermediate 1 and undergoes a loss of $AlMe_2OMe$ to give amide product (Scheme 2).



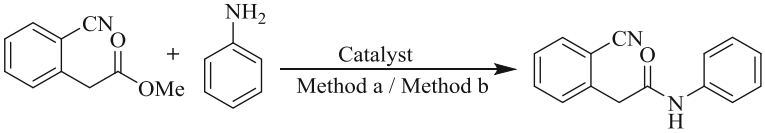
Scheme 1 Synthesis of 2-(2-cyanophenyl)-*N*-phenylacetamide derivatives **3(a-u)**

All the products derived from anilines, which includes electron-rich, electron-deficient and neutral, as well as heteroaromatic and carbocylic amines, participated well in the reaction with good yields (60–85%). All the newly synthesized compounds were elucidated and were proven using spectral methods such as ^{1}H NMR, ^{13}C NMR, FTIR and HRMS. All the newly synthesized compounds were in good agreement with the proposed structures (Supplemental Material). To the best of our knowledge, all the newly synthesized derivatives have not yet been reported in the literature.

Anticancer activity

All the synthesized title compounds **3(a-u)** were evaluated for their in vitro anticancer activity measured by sulforhodamine B (SRB) assay method [24] against three cancer cell lines colo205 (human colon adenocarcinoma cell line), MCF-7 (human breast adenocarcinoma cell line) and A-549 (human lung adenocarcinoma cell line). All tested compounds exhibited excellent to potent inhibitory activity as compared to the standard drug Etoposide and results of anticancer activity are shown in Table 2. Among the series, compounds **3e** (IC_{50} = 1.17 μM), **3i** (IC_{50} = 1.20 μM), **3j** (IC_{50} = 0.11 μM), **3o** (IC_{50} = 0.98 μM) and **3s** (IC_{50} = 1.89 μM) showed excellent inhibitory activity against MCF-7 cancer cell line compared to the standard Etoposide (IC_{50} = 2.11 μM). Similarly, compounds **3e** (IC_{50} = 2.90 μM), **3g** (IC_{50} = 2.99 μM), **3i** (IC_{50} = 1.10 μM), **3j** (IC_{50} = 0.18 μM) and **3o** (IC_{50} = 2.76 μM) also showed excellent inhibitory activity against A-549 cancer cell line compared to the standard Etoposide (IC_{50} = 3.08 μM). Compounds **3f** (IC_{50} = 3.81 μM), **3q** (IC_{50} = 4.76 μM) and **3t** (IC_{50} = 4.66 μM) showed good

Table 1 Optimization conditions for the synthesis of compound **3a**



Entry	Catalyst	Solvent	Reaction temp (°C)/time (h)	% of yield
1	$AlCl_3$	Neat[a]	150 °C, 16 h	20
2	$AlCl_3$	Toluene[b]	90 °C, 14 h	28
3	$AlCl_3$	THF[b]	80 °C, 9 h	23
4	$FeCl_3$	Neat[a]	100 °C, 16 h	10
5	$FeCl_3$	Toluene[b]	90 °C, 14 h	Trace
6	$FeCl_3$	THF[b]	80 °C, 9 h	–
7	$TiCl_4$	Toluene[b]	100 °C, 8 h	–
8	$TiCl_4$	THF[b]	80 °C, 9 h	–
9	$Ti(iPrO)_4$	THF[b]	90 °C, 12 h	–
10	$Ti(iPrO)_4$	Toluene[b]	105 °C, 8 h	8
11	$SnCl_4$	Neat[a]	100 °C, 12 h	5
12	$SnCl_4$	Toluene[b]	105 °C, 8 h	12
13	$ZnCl_2$	Toluene[b]	90 °C, 14 h	–
14	Me_3Al	THF[b]	80 °C, 9 h	40
15	Me_3Al	Toluene[b]	90 °C, 9 h	80
16	Me_3Al	Toluene[c]	90 °C, 9 h	82
17	Me_3Al	Toluene[d]	90 °C, 9 h	82
18	Me_3Al	Toluene[e]	90 °C, 9 h	80

[a]Method a: **2a** (1 mmol), aniline (1.5 mmol), catalyst (1.5 mmol). [b]Method b: **2a**(1 mmol), aniline (1.5 mmol), catalyst (1.5 mmol), in the presence of solvent. [c]Method c: **2a** (1 mmol), aniline (1.5 mmol), catalyst (2.0 mmol), in the presence of solvent. [d]Method d: **2a** (1 mmol), aniline (1.5 mmol), catalyst (2.5 mmol), in the presence of solvent. [e]Method e: **2a** (1 mmol), aniline (2.0 mmol), catalyst (1.5 mmol), in the presence of solvent

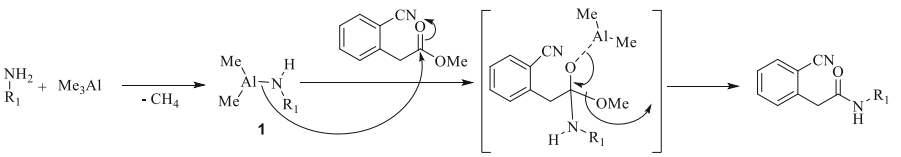
inhibitory activity on MCF-7 cancer cell line, compounds **3a** (IC_{50} = 2.90 μM), **3q** (IC_{50} = 8.34 μM) showed inhibitory good activity on A-549 cancer cell line. The compounds **3a** (IC_{50} = 3.76 μM), **3b** (IC_{50} = 2.87 μM), **3e** (IC_{50} = 2.18 μM), **3i** (IC_{50} = 2.89 μM), **3j** (IC_{50} = 2.33 μM), **3k** (IC_{50} = 3.45 μM), **3l** (IC_{50} = 2.89 μM), **3o** (IC_{50} = 2.21 μM) and **3r** (IC_{50} = 3.78 μM) showed good to moderate inhibitory activity against colo-205 cancer cell line.

Molecular docking studies

To understand the ligand orientation and the inhibitory ability towards *human* DNA topoisomerase II, a molecular docking study was carried out by using the LibDock module in Discovery Studio. The 3D structure of *human* DNA topoisomerase II (PDB ID: 1T8I) was retrieved from the Protein Data Bank (http://www.rcsb.org). We initially carried out docking of the synthesized compounds and later with the

Table 2 Anticancer activity of synthesized compounds **3(a-u)**

Compound	MCF-7	A-549	Colo-205
3a	6.54	4.87	3.76
3b	29.50	–	2.87
3c	13.80	–	–
3d	–	55.7	–
3e	1.17	2.90	2.18
3f	3.81	–	34.7
3g	–	2.99	8.45
3h	–	10.4	–
3i	1.20	1.10	2.89
3j	0.11	0.18	2.33
3k	14.3	11.56	3.45
3l	26.80	–	2.89
3m	–	54.3	–
3n	–	9.45	17.6
3o	0.98	2.76	2.21
3p	12.7	–	9.45
3q	4.78	8.34	13.7
3r	5.80	18.3	3.78
3s	1.89	31.5	16.2
3t	4.66	10.4	–
3u	13.89	9.34	–
Etoposide	2.11	3.08	0.13



Scheme 2 Plausible mechanism for the formation of amides

known inhibitor/anticancer drug Etoposide. The used docking program LibDock produces several poses, each producing their corresponding LibDock scores with different orientations within the defined active site of the protein.

The high LibDock score of the ligand pose was taken into account for the prediction of the best ligand binding conformation. So, the mentioned pre-validated analysis was used to sort out the retrieved hit molecules, and then those are further validated by using the visualization method to find the suitable binding mode of the inhibitors based on the critical interactions with the active site residues. The docked ligands were found to have similar binding poses to the co-crystallized ligand, thus validating the adopted docking methodology. Finally, the Analyze Ligand Poses subprotocol was performed to count H bonds and close contacts (Van der Waals

Table 3 Details of LibDock score of synthesised compounds 3(**a-u**) and Etoposide on human DNA topoisomerase II (PDB: 1T8I)

Compound	LibDockScore	HBOND count 1: 1t8i	Contacts 1: 1t8i
3a	97.333	3	1
3b	132.778	0	4
3c	117.806	4	5
3d	115.2	4	5
3e	122.072	4	5
3f	121.139	2	3
3g	119.326	2	4
3h	120.951	2	2
3i	121.559	2	2
3j	158.072	1	4
3k	150.882	4	2
3l	154.208	0	9
3m	142.368	2	3
3n	121.04	2	1
3o	133.191	3	2
3p	125.042	2	3
3q	132.778	3	4
3r	130.718	2	4
3s	119.605	4	5
3t	147.311	1	8
3u	140.889	2	3
Etoposide	161.438	4	6

clashes) between the poses and *human* topoisomerase II. The summary of docking information of the top ranked poses of each compound are given in Table 3. Docking analysis of all compounds with the *human* topoisomerase II, revealed that compound **3j** fitted well in the active site pocket, showing the best docking score of 158.072 kcal/mol, that is, closest to Etoposide, which has a docking score of 161.438. The best conformation with H-bond interactions obtained for compound **3j** is shown in Fig. 2. From the Fig. 2 it is revealed that three hydrogen bonds and three close contacts formed between compound **3j** with the protein DNA topoisomerse II. The first hydrogen bond is formed with the amino acid Thr718 of the DNA topoisomerse II. A hydrogen bond is formed between hydrogen atom of the oxygen molecule of the amino acid Thr418 with the 11th oxygen atom of compound 3j (A:Thr718:OH-3j:N20) with a hydrogen bond distance of 2.484 Å, and the second hydrogen bond formed in between Arg364:NH2-O10:3j.mol with a hydrogen bond distance 2.049 Å. A third hydrogen bond formed between A:ARG364:NH22-3j.mol:O10 with a distance of 2.080 Å.

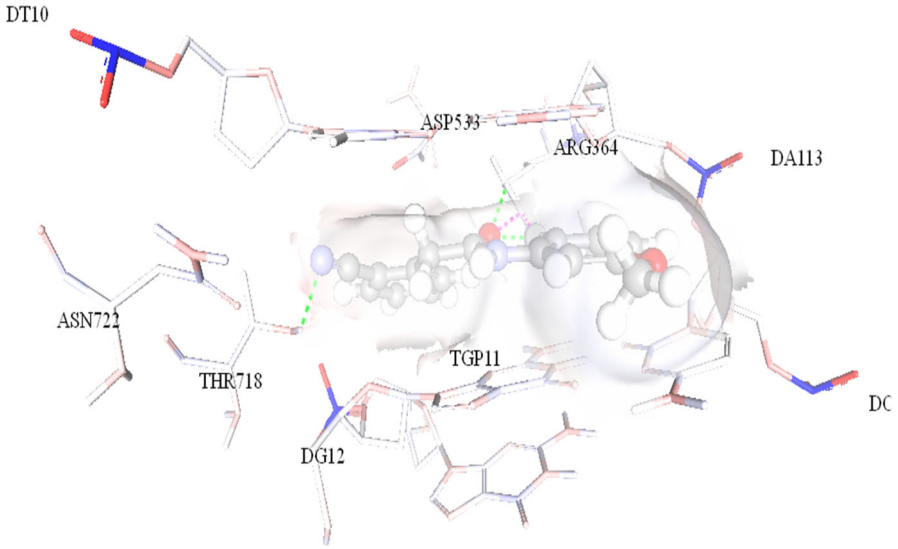


Fig. 2 Receptor-ligand hydrogen bonds (green colour) and bumps (pink colour) of compounds **3j** with active site residues of Human DNA topoisomerase II (PDB: 1T8I)

Conclusion

In conclusion, a series of novel 2-(2-cyanophenyl)-*N*-phenylacetamide derivatives **3(a-u)** were designed as potential anticancer agents. These compounds were obtained in good yields via methyl-2-(2-cyanophenyl)acetates selective amidation using $AlMe_3$ as catalyst. All the newly synthesized derivatives were well characterized by spectral techniques such as ^{1}H NMR, ^{13}C NMR, FTIR and HRMS. All the synthesized title compounds were evaluated for their in vitro anticancer activity and molecular docking studies. Among all compounds, 3i (IC_{50} = 1.20 μM, IC_{50} = 1.10 μM), 3j (IC_{50} = 0.11 μM, IC_{50} = 0.18 μM), 3o (IC_{50} = 0.98 μM, IC_{50} = 2.76 μM) showed excellent inhibitory activity against MCF-7 and A-549 cell lines, respectively. Docking analysis of all compounds with the *human* topoisomerase II, revealed that the compound **3j** fitted well in the active site pocket, showing the best docking score of 158.072 kcal/mol. The potent activity of these *N*-phenylacetamide derivatives suggests that they are potential candidates for the development of new anticancer drugs. Further work is in progress to improve the potency of these compounds.

Experimental section

General information

Reagents were commercially available with analytical grade and used as purchased without further purification. Solvents were purified according to well-known

laboratory methods. All reactions were performed by using oven-dried glassware under an inert atmosphere. Reaction mixtures were monitored by thin-layer chromatography (TLC) using silica gel 60-F_{254} pre-coated glass plates (Merck, Italy). Spots on the TLC plates were visualized with a UV lamp (254 nm) and by spraying with 0.2% ninhydrin in ethanol and charring after elution. Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded on a 400 MHz and 500 MHz spectrometer (Bruker) in DMSO and $CDCl_3$ using TMS as an internal standard. Chemical shifts are reported in parts per million (δ), coupling constants (J values) were reported in Hertz (Hz) and spin multiplicities are indicated by the following symbols: s (singlet), d (doublet), t (triplet), sept (septet), m (multiplet), bs (broad singlet), bd (broad doublet). ^{13}C NMR spectra were routinely run with broadband decoupling. Infrared spectra were taken with a Hitachi 260-30 spectrometer. Mass spectra were recorded in LCQ Fleet mass spectrometer, Thermo Fisher Instruments Limited, US. Electrospray ionisation mass spectrometry (ESI–MS) analysis was performed in the positive ion and negative ion mode on a liquid chromatography ion trap.

*General procedure for synthesis of compound **2(a-b)***

A solution of compound **1(a-b)** (1 mmol) in DMF (5 mL) was added to a stirred solution of CuCN (1.2 mmol) in DMF (10 mL) at room temperature and refluxed for 12 h. After completion of the reaction (monitored by TLC), the reaction mixture was quenched with a solution of iron (III) chloride (1.2 mmol) in H_2O (5 mL) at room temperature and stirred vigorously for 15 min. The aqueous layer was extracted with AcOEt (2 × 15 mL). The combined organic layers were washed with water, dried over anhydrous sodium sulphate and concentrated in vacuo. Crude product was purified by flash chromatography (Hexane/AcOEt 9:1 v/v) to afford compound **2(a-b)** as clear oil.

*General procedure for synthesis of substituted 2-(2-cyanophenyl)-N-phenylacetamide **3(a-u)** derivatives*

Trimethyl aluminium (1.5 mmol, 2.0 M in toluene) was added to a solution of amine (2 mmol) in toluene (10 mL) at 0 °C and stirred at room temperature for 30 min. Compound **4** (1 mmol) was added at 0 °C and stirred at 80 °C for 9 h. After the completion of the reaction (by TLC), the reaction mixture was quenched with water (15 mL), stirred for 10 min, filtered through a pad of Celite, and the Celite bed washed with AcOEt (10 mL). The organic layer was separated from the filtrate, and the aqueous layer was extracted with AcOEt (2 × 15 mL). The combined organic layers were washed with 1 N HCl and brine solution, dried over anhydrous sodium sulphate and concentrated in vaccuo. Crude product was purified by flash chromatography (Hexane/AcOEt 7:3 v/v) to afford 2-(2-cyanophenyl)-*N*-phenylacetamide **3(a-u)** derivatives.

2-(2-cyanophenyl)-N-phenylacetamide ***(3a)***

Pale brown solid, yield 80%, IR (KBr) cm^{-1}: 3283, 2222, 1655, 1539, 765; ^1H NMR (400 MHz, DMSO-d_6) δ: 10.30 (s, 1H), 7.84 (dd, J = 7.82, 0.98 Hz, 1H), 7.66–7.72 (m, 1H), 7.54–7.62 (m, 3H), 7.46–7.52 (m, 1H), 7.32 (t, J = 8.07 Hz, 2H), 7.03–7.09 (m, 1H), 3.96 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ: 167.3, 139.4, 138.9, 133.0, 132.6, 131.0, 128.7, 127.6, 123.3, 119.1, 117.8, 112.6, 41.2; HRMS (ESI): calcd. for $C_{15}H_{13}N_2O$ $[M + H]^+$ 237.1028; found 237.1034.

2-(2-cyanophenyl)-N-(2-fluorophenyl)acetamide ***(3b)***

Off-white solid, yield 75%, IR (KBr) cm^{-1}: 3255, 2224, 1669, 1540, 756; ^1H NMR (400 MHz, DMSO-d_6) δ: 10.12 (brs, 1H), 7.82–7.87 (m, 2H), 7.69 (td, J = 7.58, 1.47 Hz, 1H), 7.57 (d, J = 7.83 Hz, 1H), 7.46–7.52 (m, 1H), 7.23–7.32 (m, 1H), 7.13–7.20 (m, 2H), 4.02 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.8, 153.7, 151.3, 138.0, 133.18, 132.8, 130.8, 128.0, 125.9, 124.8, 122.0, 117.8, 114.9, 113.0, 42.6.; HRMS (ESI): calcd. for $C_{15}H_{12}N_2OF$ $[M + H]^+$ 255.0934; found 255.0922.

2-(2-cyanophenyl)-N-(3-fluorophenyl)acetamide ***(3c)***

Pale brown solid, yield 73%, IR (KBr) cm^{-1}: 3257, 2226, 1659, 1551, 755; ^1H NMR (400 MHz, DMSO-d_6) δ: 10.54 (s, 1H), 7.84 (dd, J = 7.83, 0.98 Hz, 1H), 7.65–7.71 (m, 1H), 7.54–7.60 (m, 2H), 7.48 (td, J = 7.58, 0.98 Hz, 1H), 7.28–7.39 (m, 2H), 6.85–6.92 (m, 1H), 3.97 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.2, 164.0, 161.6, 139.12, 138.3, 133.3, 132.7, 130.9, 129.9, 127.9, 118.1, 115.2, 112.6, 111.3, 42.5; HRMS (ESI): calcd. for $C_{15}H_{12}N_2OF$ $[M + H]^+$ 255.0934; found 255.0904.

2-(2-cyanophenyl)-N-(4-fluorophenyl)acetamide ***(3d)***

Pale brown solid, yield 75%, IR (KBr) cm^{-1}: 3255, 2225, 1654, 1506, 757; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.94 (brs, 1H), 7.64 (d, J = 7.6 Hz, 1H), 7.52–7.59 (m, 2H), 7.36–7.44 (m, 3H), 6.94 (t, J = 8.4 Hz, 2H), 3.87 (s, 2H).; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.0, 160.7, 158.3, 138.5, 133.5, 132.7, 130.9, 127.8, 122.0, 118.1, 115.6, 112.6, 42.4; HRMS (ESI): calcd. for $C_{15}H_{12}N_2OF$ $[M + H]^+$ 255.0934; found 255.0919.

2-(2-cyanophenyl)-N-(3,5-difluorophenyl)acetamide ***(3e)***

Off-white solid, yield 76%, IR (KBr) cm^{-1}: 3272, 2227, 1662, 1563, 757; ^1H NMR (400 MHz, DMSO-d_6) δ: 10.71 (s, 1H), 7.84 (d, J = 7.82 Hz, 1H), 7.65–7.72 (m, 1H), 7.56 (d, J = 7.82 Hz, 1H), 7.49 (t, J = 7.58 Hz, 1H), 7.31 (d, J = 7.34 Hz, 2H), 6.93 (t, J = 9.29 Hz, 1H), 3.98 (s, 2H).; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.0, 164.4, 161.9, 139.57, 137.9, 133.5, 133.8, 131.0, 128.1, 118.2, 112.6, 103.0, 100.1, 42.8; HRMS(ESI): calcd for $C_{15}H_{11}N_2OF_2$ $[M + H]^+$ 273.0839; found 273.0832.

2-(2-cyanophenyl)-N-o-tolylacetamide ***(3f)***

Off-white solid, yield 85%, IR (KBr) cm^{-1}: 3178, 2235, 1680, 1520, 728; ^1H NMR (400 MHz, DMSO-d_6) δ: 9.65 (s, 1H), 7.83 (d, J = 7.82 Hz, 1H), 7.65–7.72 (m, 1H), 7.58 (d, J = 7.34 Hz, 1H), 7.47 (t, J = 7.58 Hz, 1H), 7.38 (d, J = 7.82 Hz, 1H), 7.21 (d, J = 7.83 Hz, 1H), 7.04–7.11 (m, 1H), 7.13–7.19 (m, 1H), 3.98 (s, 2H), 2.22 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.0, 138.7, 135.2, 133.2, 132.7, 130.8, 130.4, 129.6, 127.8, 126.5, 125.5, 123.3, 118.0, 112.7, 42.5, 17.6; HRMS (ESI): calcd. for $C_{16}H_{15}N_2O$ $[M + H]^+$ 251.1184; found 251.1174.

2-(2-cyanophenyl)-N-m-tolylacetamide ***(3g)***

Off-white solid, yield 80%, IR (KBr) cm^{-1}: 3255, 2352, 1659, 1547, 763; ^1H NMR (400 MHz, DMSO-d_6) δ:10.23 (s, 1H), 7.83 (d, J = 7.34 Hz, 1H), 7.65–7.71 (m, 1H), 7.55 (d, J = 7.82 Hz, 1H), 7.47–7.50 (m, 1H), 7.42–7.46 (m, 1H), 7.36 (d, J = 8.31 Hz, 1H), 7.18 (t, J = 7.83 Hz, 1H), 6.87 (d, J = 7.34 Hz, 1H), 3.93 (s, 2H), 2.27 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.0, 160.0, 138.7, 138.5, 133.2, 132.7, 130.9, 129.5, 127.8, 118.2, 112.7, 112.1, 110.5, 105.6, 55.2, 42.6; HRMS (ESI):calcd for $C_{16}H_{15}N_2O$ $[M + H]^+$ 251.1184; found 251.1183.

2-(2-cyanophenyl)-N-p-tolylacetamide ***(3h)***

Off-white solid, yield 81%, IR (KBr) cm^{-1}: 3294, 2224, 1648, 1525, 759; ^1H NMR (400 MHz, DMSO-d_6) δ:10.22 (s, 1H), 7.82 (dd, J = 7.6, 0.8 Hz, 1H), 7.65–7.69 (m, 1H), 7.54 (d, J = 7.6 Hz, 1H), 7.45–7.49 (m, 3H), 7.10 (d, J = 8.4 Hz, 2H), 3.92 (s, 2H), 2.25 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.8, 138.7, 135.0, 134.2, 133.1, 132.7, 130.8, 129.3, 129.3, 127.73, 120.2, 118.1, 112.7, 42.5, 20.8; HRMS (ESI): calcd for$C_{16}H_{15}N_2O$ $[M + H]^+$ 251.1184; found 251.1180.

2-(2-cyanophenyl)-N-(4-methoxyphenyl)acetamide ***(3i)***

Pale green solid, yield 75%, IR (KBr) cm^{-1}: 3265, 2230, 1635, 1541, 1110, 732; ^1H NMR (400 MHz, DMSO-d_6) δ: 10.16 (s, 1H), 7.83 (d, J = 7.82 Hz, 1H), 7.68 (td, J = 7.70, 1.22 Hz, 1H), 7.55 (d, J = 7.34 Hz, 1H), 7.45–7.52 (m, 3H), 6.85–6.91 (m, 2H), 3.90 (s, 2H), 3.72 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.67, 156.67, 138.74, 133.23, 132.73, 130.91, 130.61, 127.82, 121.94, 118.18, 114.10, 112.72, 55.44, 42.67; HRMS (ESI): calcd for $C_{16}H_{15}N_2O_2$$[M + H]^+$ 267.1134; found 267.1123.

2-(2-cyanophenyl)-N-(3-methoxyphenyl)acetamide ***(3j)***

Pale green solid, yield 75%, IR (KBr) cm^{-1}: 3255, 2352, 1659, 1547, 763; ^1H NMR (400 MHz, DMSO-d_6) δ: 10.3 (s, 1H), 7.84 (d, J = 7.82 Hz, 1H), 7.65–7.72 (m, 1H), 7.56 (d, J = 7.82 Hz, 1H), 7.49 (t, J = 7.58 Hz, 1H), 7.31 (d, J = 7.34 Hz, 1H), 7.21 (t, J = 8.0 Hz, 2H), 6.93 (t, J = 9.29 Hz, 1H), 3.93 (s, 2H), 3.71 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.0, 160.0, 138.8, 138.5, 133.2, 132.7, 130.9,

129.5, 127.8, 118.2, 112.7, 112.1, 110.5, 105.6, 55.2, 42.6; HRMS (ESI): calcd. for $C_{16}H_{15}N_2O_2$ $[M + H]^+$ 267.1134; found 267.1128.

2-(2-cyanophenyl)-N-(4-phenoxyphenyl)acetamide ***(3k)***

Pale brown solid, yield 78%, IR (KBr) cm^{-1}: 3278, 2606, 1653, 1532, 762; ^1H NMR (400 MHz, DMSO-d_6) δ: 10.36 (s, 1H), 7.84 (d, J = 7.34 Hz, 1H), 7.66–7.72 (m, 1H), 7.59–7.64 (m, 2H), 7.57 (d, J = 7.82 Hz, 1H), 7.46–7.52 (m, 1H), 7.34–7.40 (m, 2H), 7.11 (t, J = 7.34 Hz, 1H), 6.94–7.03 (m, 4H), 3.95 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.0, 157.4, 153.7, 138.6, 133.2, 133.0, 132.7, 130.9, 129.6, 127.8, 123.0, 121.9, 119.4, 118.4, 118.1, 112.7, 42.4; HRMS (ESI): calcd. for $C_{21}H_{17}N_2O_2$ $[M + H]^+$ 329.1290; found 329.1266.

Methyl 4-(2-(2-cyanophenyl)acetamido)benzoate ***(3l)***

Off-white solid, yield 70%, IR (KBr) cm^{-1}: 3252, 2219, 1715, 1650, 1528, 740; ^1H NMR (300 MHz, DMSO-d_6) δ: 10.68 (s, 1H), 7.91–7.94 (m, 2H), 7.84 (d, J = 7.8 Hz, 1H), 7.66–7.74 (m, 3H), 7.56 (d, J = 7.5 Hz, 1H), 7.46–7.51 (m, 1H), 4.0 (s, 2H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.1, 166.5, 141.7, 138.1, 133.3, 132.8, 131.0, 130.7, 128.0, 126.0, 119.0, 118.2, 112.7, 52.0, 42.8; HRMS (ESI):calcd. for $C_{17}H_{15}N_2O_3$ $[M + H]^+$ 295.1083; found 295.1082.

2-(2-cyanophenyl)-N-(naphthalen-3-yl)acetamide ***(3m)***

Pale brown solid, yield 76%, IR (KBr) cm^{-1}: 3057, 2220, 1614, 1562, 772; ^1H NMR (400 MHz, DMSO-d_6) δ: 10.31 (s, 1H), 8.12–8.20 (m, 1H), 8.00–8.00 (m, 1H), 7.96 (dd, J = 6.36, 2.93 Hz, 1H), 7.86 (d, J = 7.34 Hz, 1H), 7.79 (d, J = 8.31 Hz, 2H), 7.67–7.74 (m, 1H), 7.63–7.66 (m, 2H), 7.45–7.62 (m, 2H), 4.14 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.5, 138.6, 134.0, 133.3, 132.8, 131.9, 131.0, 128.6, 127.9, 127.1, 126.4, 126.2, 126.0, 125.5, 121.2, 120.7, 118.2, 112.8, 42.7; HRMS (ESI): calcd. for $C_{19}H_{15}N_2O$ $[M + H]^+$ 287.1184; found 287.1188.

2-(2-cyanophenyl)-N-(5-fluoropyridin-2-yl)acetamide ***(3n)***

Pale yellow solid, yield 60%, IR (KBr) cm^{-1}: 3252, 2219, 1693, 1547, 759; ^1H NMR (400 MHz, DMSO-d_6) δ: 10.98 (s, 1H), 8.35 (d, J = 2.93 Hz, 1H), 8.07 (dd, J = 9.05, 4.16 Hz, 1H), 7.83 (dd, J = 7.82, 0.98 Hz, 1H), 7.65–7.77 (m, 2H), 7.55 (d, J = 7.34 Hz, 1H), 7.48 (td, J = 7.58, 0.98 Hz, 1H), 4.03 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.0, 157.8, 155.2, 147.1, 137.7, 135.5, 133.2, 130.8, 128.1, 125.3, 117.7, 115.0, 113.2, 42.5; HRMS (ESI): calcd. for $C_{14}H_{11}N_3OF$ $[M + H]^+$ 256.0866; found 256.0877.

2-(2-cyanophenyl)-N-cyclohexylacetamide ***(3o)***

Off-white solid, yield 70%, IR (KBr) cm^{-1}: 3250, 1645, 1532, 741; ^1H NMR (400 MHz, DMSO-d_6) δ: 8.06 (d, J = 8.0 Hz, 1H), 7.77 (dd, J = 7.8, 1.0 Hz, 1H),

7.61–7.65 (m, 1H), 7.43 (q, J = 7.2 Hz, 2H), 3.65 (s, 2H), 3.5–3.53 (m, 1H), 1.66–1.75 (m, 4H), 1.54 (d, J = 12.4 Hz, 1H), 1.11–1.27 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.5, 139.2, 133.1, 132.6, 130.7, 127.5, 118.0, 112.5, 48.6, 42.1, 32.8, 25.3, 24.6; HRMS (ESI): calcd. for $C_{15}H_{19}N_2O$ $[M + H]^+$ 243.1497; found 243.1490.

2-(2-cyanophenyl)-N,2-diphenylacetamide ***(3p)***

Off-white solid, yield 79%, IR (KBr) cm^{-1}: 3225, 1660, 1545, 735;^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.59 (s, 1H), 7.85 (dd, J = 7.9, 0.9 Hz, 1H), 7.67–7.73 (m, 1H), 7.58–7.62 (m, 3H), 7.48 (td, J = 7.5, 0.9 Hz, 1H), 7.35–7.41 (m, 2H), 7.29–7.33 (m, 5H), 7.06 (t, J = 7.3 Hz, 1H), 5.55 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.2, 142.9, 137.5, 137.2, 133.1, 132.9, 129.8, 128.9, 128.8, 128.1, 127.7, 124.7, 120.0, 118.0, 112.7, 57.2; HRMS (ESI): Calcd. for $C_{21}H_{17}N_2O$ $[M + H]^+$ 313.1341; found 313.1339.

2-(2-cyanophenyl)-N-(2,4-dimethoxyphenyl)-2-phenylacetamide ***(3q)***

Off-white solid, yield 83%, IR (KBr) cm^{-1}: 3230, 1670, 1545, 735; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.26 (d, J = 8.8 Hz, 1H), 7.89 (s, 1H), 7.63–7.69 (m, 2H), 7.55–7.61 (m, 1H), 7.30–7.43 (m, 6H), 6.42–6.47 (m, 2H), 5.47 (s, 1H), 3.78 (s, 3H), 3.74 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.6, 156.8, 149.4, 143.1, 137.5, 132.9, 129.7, 129.0, 127.9, 127.6, 120.8, 120.5, 117.8, 113.0, 103.7, 98.6, 57.6, 55.7; HRMS (ESI): Calcd. for $C_{23}H_{21}N_2O_3$ $[M + H]^+$ 373.1552; found 373.1562.

2-(2-cyanophenyl)-N-(4-methoxyphenyl)-2-phenylacetamide ***(3r)***

Off-white solid, yield 80%, IR (KBr) cm^{-1}: 3230, 1677, 1545, 740; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.81 (brs, 1H), 7.61–7.67 (m, 2H), 7.53–7.57 (m, 1H), 7.31–7.42 (m, 8H), 6.79 (dd, J = 6.75, 2 Hz, 2H), 5.45 (s, 1H), 3.75 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 168.0, 156.6, 143.0, 137.4, 133.1, 132.8, 130.6, 129.4, 128.8, 127.9, 121.8, 118.0, 114.0, 112.6, 56.9, 55.4; HRMS (ESI): Calcd. for $C_{22}H_{19}N_2O_2$ $[M + H]^+$ 343.1447; found 343.1433.

2-(2-cyanophenyl)-N-(2,3,4-trifluorophenyl)-2-phenylacetamide ***(3s)***

Pale brown solid, yield 78%, IR (KBr) cm^{-1}: 3230, 1670, 1535, 745; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.91–7.95 (m, 1H), 7.76 (brs, 1H), 7.74 (d, J = 13.2 Hz, 1H), 7.56–7.63 (m, 2H), 7.32–7.46 (m, 6H), 6.91–6.97 (m, 1H), 5.54 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.7, 146.6, 142.3, 141.4, 141.0, 138.4, 136.5, 133.1, 129.2, 128.2, 125.2, 123.2, 117.7, 116.3, 112.9, 111.6, 57.1; HRMS (ESI): Calcd. for $C_{21}H_{14}N_2OF_3$ $[M + H]^+$ 367.1058; found 367.1060.

2-(2-cyanophenyl)-N-(2-methoxyphenyl)-2-phenylacetamide ***(3t)***

Off-white solid, yield 76%, IR (KBr) cm^{-1}: 3230, 1665, 1540, 735; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.44 (d, J = 7.6 Hz, 1H), 8.16 (s, 1H), 7.69 (d, J = 7.3 Hz, 2H), 7.59–7.65 (m, 1H), 7.34–7.47 (m, 6H), 7.05–7.11 (m, 1H), 6.98 (t, J = 7.2 Hz 1H), 6.86 (d, J = 7.8 Hz, 1H), 5.54 (s, 1H), 3.78 (br s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.0, 148.0, 143.0, 137.3, 133.0, 129.7, 129.0, 128.0, 127.7, 127.2, 124.2, 120.9, 119.6, 117.8, 113.0, 110.0, 57.8, 55.6; HRMS (ESI): Calcd. for $C_{22}H_{19}N_2O_2$ $[M + H]^+$ 343.1447; found 343.1432.

2-(2-cyanophenyl)-N-(3-methoxyphenyl)-2-phenylacetamide ***(3u)***

Off-white solid, yield 78%, IR (KBr) cm^{-1}: 3230, 1665, 1540, 740; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.66 (br s, 1H), 7.59–7.64 (m, 2H), 7.56–7.58 (m, 1H), 7.32–7.42 (m, 7H), 7.69 (t, J = 8.0 Hz, 1H), 6.93 (d, J = 8.0 Hz, 1H), 6.65 (d, J = 6.5 Hz, 1H), 5.46 (s, 1H), 3.76 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 168.3, 160.1, 142.9, 138.8, 137.2, 133.2, 132.9, 129.8, 129.6, 129.2, 128.9, 128.1, 127.8, 118.0, 112.7, 112.0, 110.8, 105.4, 57.3, 55.3; HRMS (ESI): Calcd. for $C_{22}H_{19}N_2O_2$ $[M + H]^+$ 343.1447; found 343.1457.

Biological evaluation method

Procedure of the SRB-assay

The synthesized compounds **3(a-u)** have been evaluated for their in vitro cytotoxicity in human cancer cell lines. A protocol of 48 h of continuous drug exposure has been used and a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth. The cell lines were grown in DMEM medium containing 10% fetal bovine serum and 2 mM L-glutamine and were inoculated into 96 well microtiter plates in 90 mL at plating densities depending on the doubling time of individual cell lines. The microtiter plates were incubated at 37 °C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs. Aliquots of 10 mL of the drug dilutions were added to the appropriate microtiter wells already containing 90 mL of cells, resulting in the required final drug concentrations. For each compound four concentrations (0.1, 1, 10 and 100 μM) were evaluated, and each was done in triplicate wells. Plates were incubated further for 48 h and assay was terminated by the addition of 50 mL of cold trichloroacetic acid (TCA) (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 mL) at 0.4% (w/v) in 1% acetic acid was added to each of the cells, and plates were incubated for 20 min at room temperature. The residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on an ELISA plate reader at a wavelength of 540 nm with 690 nm reference wavelengths. Percent growth was calculated on a plate by plate basis for test wells relative to control wells. The above determinations

were repeated three times. Percentage growth was expressed as the (ratio of average absorbance of the test well to the average absorbance of the control wells) × 100. Growth inhibition of 50% (GI_{50}) was calculated from [(Ti − Tz)/(C − Tz)] × 100 1/4 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. Where, Tz 1/4 Optical density at time zero, OD of control 1/4 C, and OD of test growth in the presence of drug 1/4 Ti.

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